ACOUSTIC IMPEDANCE METHOD FOR DETECTING LUNG DYSFUNCTION

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ABSTRACT

The acoustic impedances of seven rat lungs were measured at frequencies between 100 and 6400 Hz. Rats were divided into two groups: a silica exposed group (N=3) and a control group (N=4). The silica exposed group was injected intratracheally with silica solution. Three of the control group were intratracheally injected with saline. Between four and six weeks after the injections, all lungs were excised and degassed. Lungs were suspended in a pressure chamber, with the trachea canula attached to the end of a tapered impedance tube. The lungs were subjected to transpulmonary pressures between −30 cm H_2O and 6 cm H_2O to simulate deflation and inflation. With transpulmonary pressure being held constant, the impedance tube was excited with random noise. A dual channel analyzer calculated $H_{12}(f)$, the transfer function between the two microphones. This function was used to calculate the lung's impedance at that pressure. The impedance magnitude spectra of both groups typically had peaks at 2000, 3500, and 5500 Hz. Statistically significant differences (90% confidence level or greater) between the two groups occur at the 3500 Hz peak at transpulmonary pressures of 20, 8, 6, 4, and 2 cm of H_2O. This fact seems to confirm that this method can detect lung disease. Further research will indicate whether this method will be able to detect the onset of coal worker's pneumoconiosis.

INTRODUCTION

One of the first studies of impedance of the human lung were conducted by DuBois et al.[2] using the forced oscillation technique. This technique, however, was limited to frequencies below 30 Hz. Further studies by Van Den Berg[6] revealed that the lung reflected higher frequency sounds (100–10,000 Hz), instead of behaving as an anechoic termination. This discovery has lead to several studies of the acoustical properties of both human and animal lungs at high frequencies.[3,4,5]

Ishizaka et al.[4] measured the input impedances of laryngectomized human subjects using a two microphone technique. This study reported peaks in the impedance magnitude at 640, 1400, and 2100 Hz. Fredberg et al.[3] used a transient forced oscillation technique to measure the input impedance of excised canine lungs for frequencies up to 10,000 Hz. Jayaraman and Frazer[5] used a two microphone technique in combination with transmission matrix theory to study changes in the acoustic impedance of excised rat lungs during deflation and inflation.

This study's focus is to determine the differences in the acoustic impedance of excised silicotic and healthy rat lungs. Seven Long Evans Hooded rats, weighing between 200 and 250 g, were divided into two groups. The silica-exposed group (N=3) were intratracheally injected with a silica-saline solution to induce silicosis. Three rats of the control group (N=4) were given a sham exposure of saline. During a period four to six weeks after injection, all lungs were excised and degassed.

METHODS

Figure 1 displays a block diagram of the impedance tube facility used in this study. An excised lung is attached to the end of a tapered tube within a plexiglass pressure chamber. Random noise, produced by a Bruel and Kjaer 2032 dual channel analyzer, is amplified and introduced into the tube via a side-mounted speaker driver (University, type ID-30C-8). The standing waves thus formed in the impedance tube are measured by two Bruel and Kjaer 4136 pressure microphones mounted 2.3 cm apart in a plexiglass cylinder. The signals of these microphones are the inputs to the dual channel analyzer, which calculates the transfer function between the two microphones, H_{12}, and its inverse Fourier transform , h(t). Following Jayaraman and Frazer's example,[5] exponential weighting is applied to h(t) and transmission matrix theory applied to the resulting transfer function to yield the input impedance of the excised lung.

The plexiglass chamber's pressure is controlled by a variable speed pump to produce transpulmonary pressures between 30 and −6 cm of H_2O. The difference between chamber pressure and atmospheric pressure is monitored by a water manometer. A lung is first inflated to 30 cm of H_2O, then deflated to −6 cm of H_2O, pausing at several pressures for impedance measurements. Once fully deflated, the lung is inflated to 30 cm of H_2O, again stopping at various pressures for measurements.

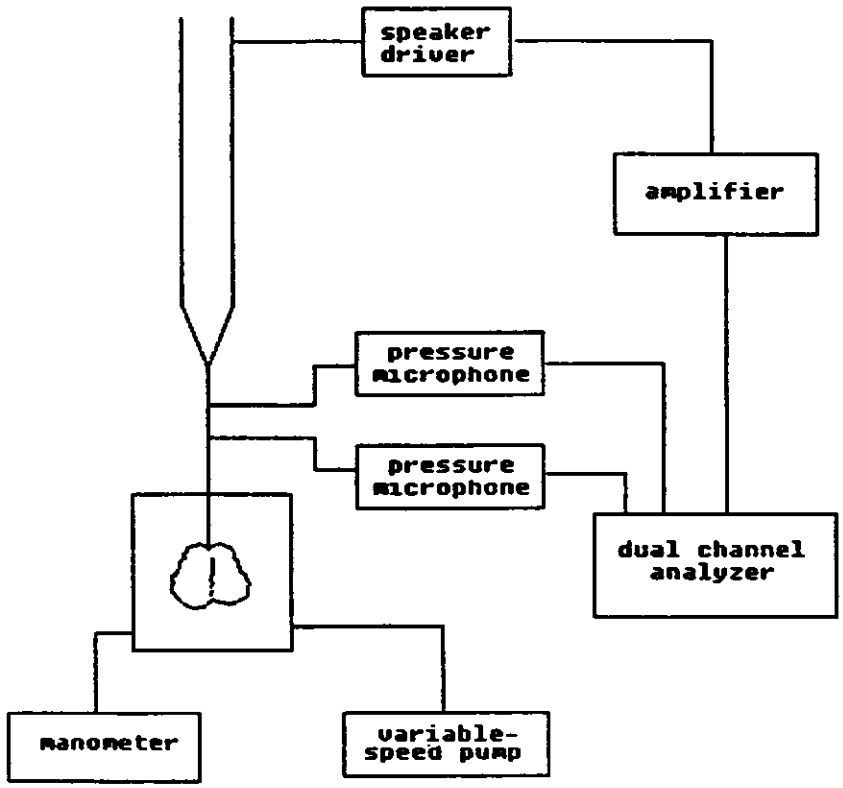

Figure 1. Block diagram of impedance measurement facility.

RESULTS

The average magnitude spectra of the silica and control groups are presented in Figures 2, 3, and 4 for transpulmonary pressures of 30, 8, and 2 cm of H_2O, respectively.

DISCUSSION

The impedance magnitude spectra of all rats have been computed for transpulmonary pressures of 30, 20, 10, 8, 6, 4, 2, 0, and −2 cm of H_2O. Typical rats in both groups had

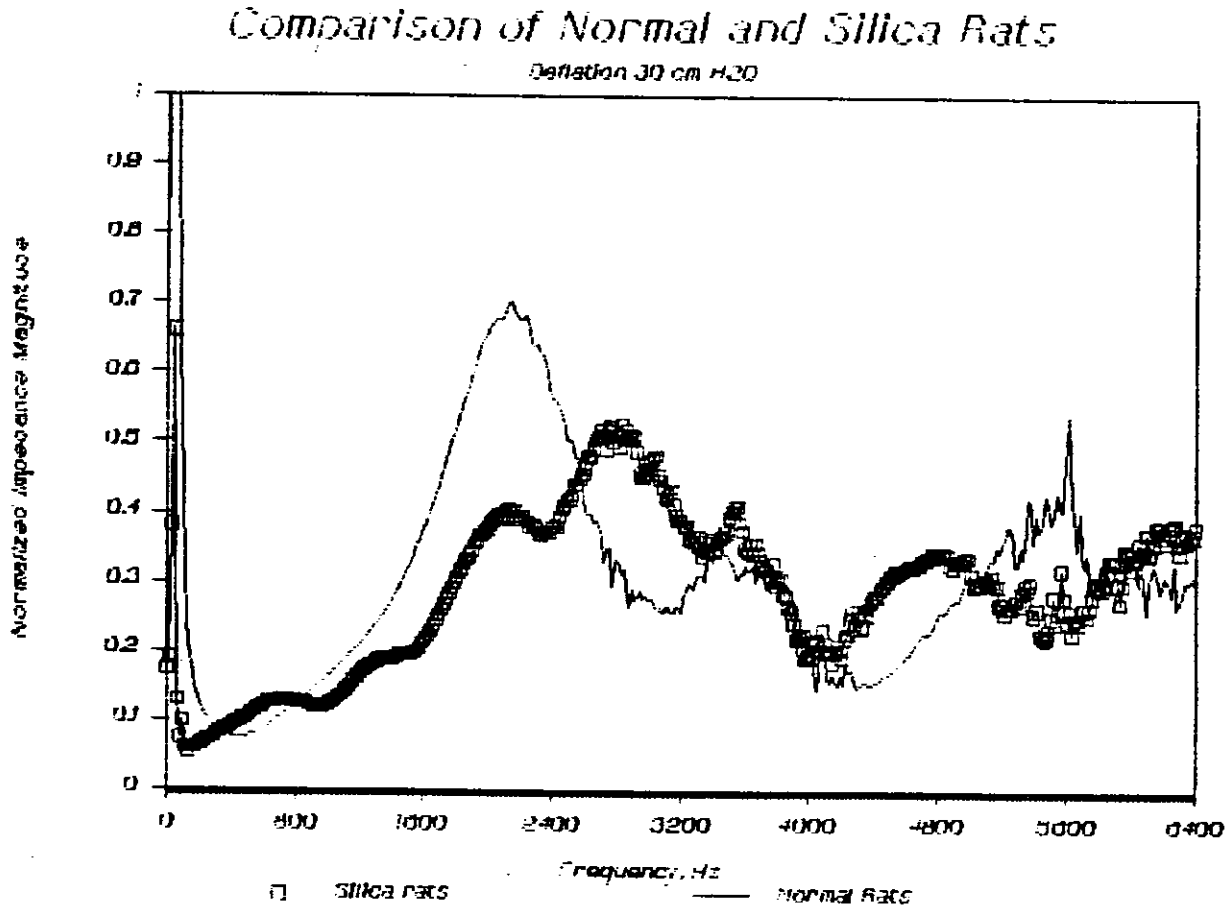

Figure 2. Comparison of average impedance spectra of silica and control groups. Deflation 30 cm H_2O.

peaks at 2000, 3500, and 5500 Hz. The placement of these peaks compare favorably with the study of Jayaraman and Frazer,[5] with the exception that in this study, no peak occurred at 600 Hz.

Significant differences between the silica and control groups occurred at the peak at 3500 Hz. T-tests performed on the peak magnitudes at this frequency showed that the silica group had significantly higher impedance (90% confidence

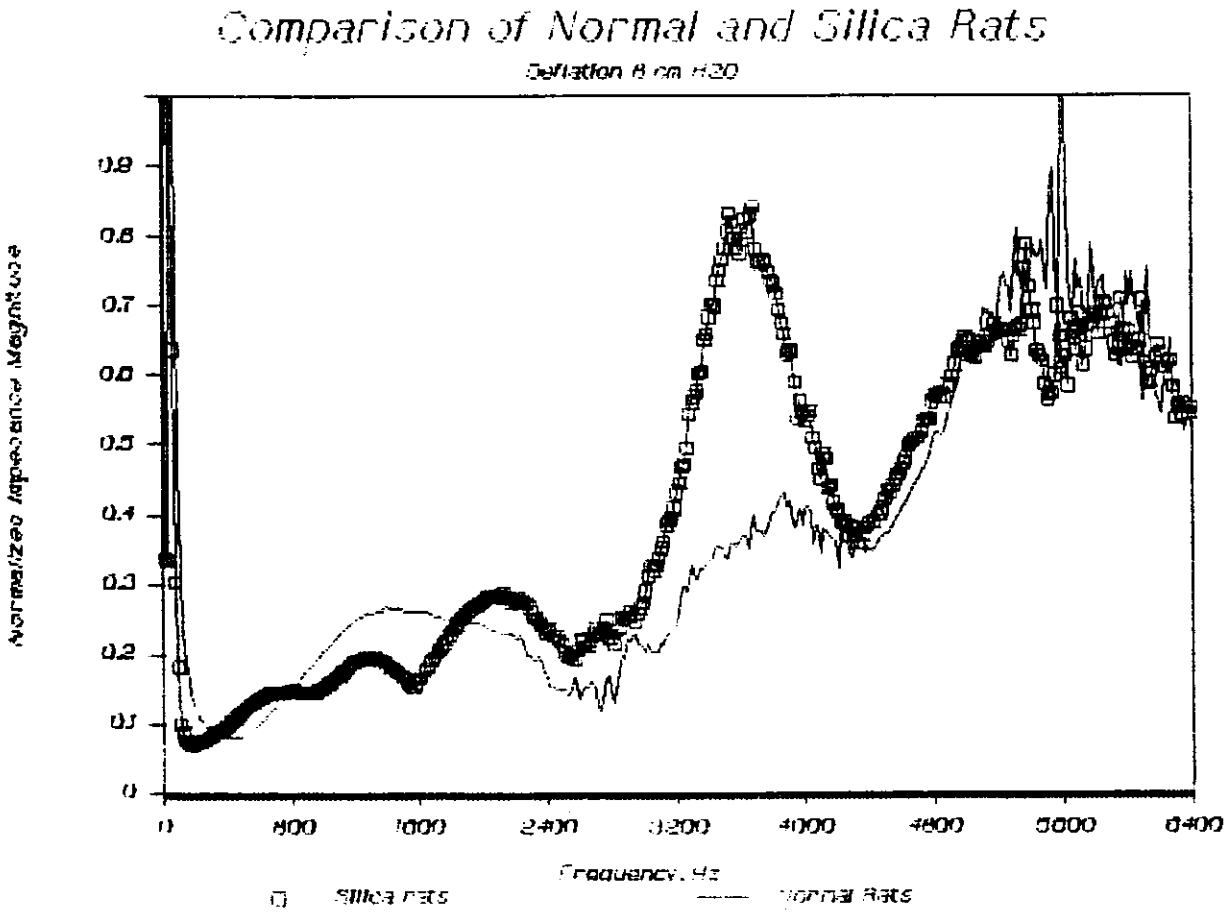

Figure 3. Comparison of average impedance spectra of silica and control groups. Deflation 8 cm H_2O.

level) at transpulmonary pressures of 20 and 8 cm of H_2O during deflation. The silica group also had significantly higher impedance (98% confidence level) at 3500 Hz at pressures of 6 and 4 cm of H_2O during deflation. These findings show that changes in the mechanical properties of lung tissue and the closure of airways occurred at higher pressures in the silica group than with the control group. This finding agrees with the work of Chvalova et al.[1] which found that the pressure-volume curve of silicotic rats was shifted to higher pressures compared to normal rat lungs.

CONCLUSIONS

The above findings indicate that silicosis in rat lungs can be detected by measuring the lung input impedance. The key indicator thus far is the impedance magnitude of the peak at about 3500 Hz. Further studies will determine the effectiveness of the method in detecting the development of lung diseases and if the measurement of acoustic impedance can be an effective clinical tool for the treatment of lung diseases.

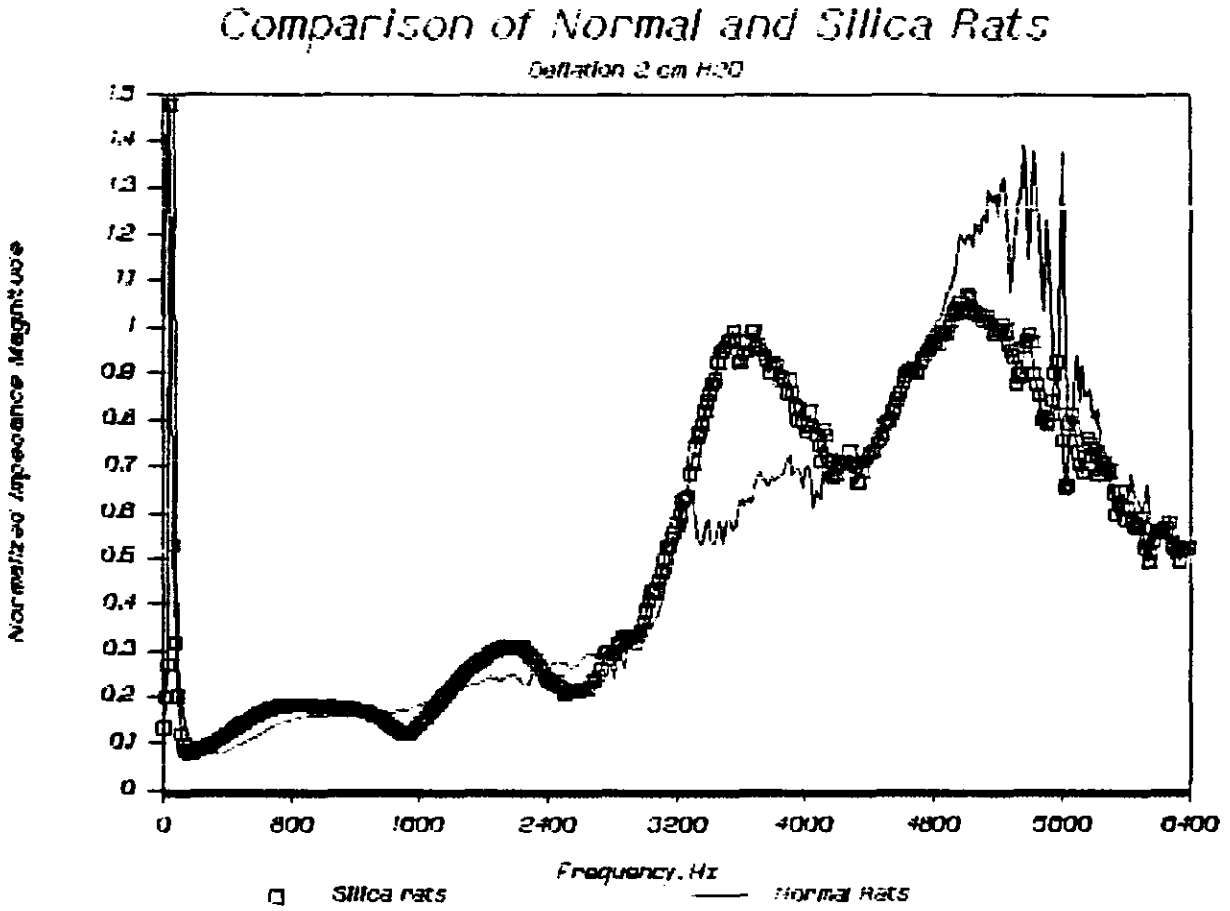

Figure 4. Comparison of average impedance spectra of silica and control groups. Deflation 2 cm H_2O.

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ACKNOWLEDGMENTS: The authors wish to thank the Division of Respiratory Disease Studies, NIOSH, Morgantown, WV for the use of their facilities during this study. This research has been supported by the Department of the Interior's Mineral Institute Program administered by the Bureau of Mines through the Generic Mineral Technology Center for Respirable Dust under grant number G1135142.

CONNECTIVE TISSUE COMPONENTS AS STRUCTURAL BASIS IN LUNG RESEARCH

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ABSTRACT

The connective tissue of the lung is a complex structure influencing its functional properties in health and disease. Under normal circumstances, the connective tissue helps to evenly distribute mechanical forces over the entire surface of the lung, contributes to the overall elastic properties of the parenchyma and mediates intercellular communications as well as cell-matrix-interactions. Components of the connective tissue are collagen molecules, proteoglycans, glycoproteins and elastin. In lung fibrosis an accelerated accumulation of interstitial matrix molecules, predominantly collagen, has been reported. To achieve a morphological analysis of the composition of the lung connective tissue, collagen types I, III, IV and V, the glycoproteins fibronectin and laminin, and proteodermatan-sulfate were isolated. Polyclonal antibodies directed against these antigens were used in indirect immunofluorescence studies of the lung tissue. The results revealed that under normal conditions the matrix molecules participated in the development of specific structural properties of the lung. However, irritation of the lung resulted in a disorder of connective tissue components. The present results indicate that the morphological analysis of matrix molecules may be able to explain functional properties of the lung.

INTRODUCTION

A fundamental prerequisite for understanding convulsive procedures at the alveolar wall is the knowledge about its structural composition in correlation to its functions. The functional structures of the alveolar-capillary region of the lung are the epithelial cells of the alveolar space, the surfactant, the alveolar macrophages, the components of the interstitial connective tissue and the endothelial cells of the capillaries.

The distortion of the genetically defined functional structure by exogeneous or endogeneous noxes obviously induces a metabolic disorder of lung collagens and other components of the extracellular matrix. The changes to matrix components may result in an enhanced accumulation of collagen and fibrous material like elastin leading to lung fibrosis.[1] Since collagen (60–70%), elastin (25–30%), proteoglycans (1%) and fibronectin (0.5%) are major components of the interstitial structure, their inappropriate distribution is of important interest. Moreover, the development of basement membranes, which can be studied by the distribution of laminin, might be a key feature of fibrotic lung disorders.[1] The distribution of different collagen types has been made with immuno-histochemical markers.[2,3] However, these studies showed different results concerning the ratio of collagen type I and type III. In our study we focused on the distribution of these collagen types and of fibronectin and laminin.

MATERIAL AND METHODS

Tissue samples of human lungs were obtained after autopsy and stored immediately at −70°C.

IMMUNOLOGICAL REAGENTS

Antibodies directed against collagen types, fibronectin or laminin were of the same origin as previously described.[4] Antibodies against the core protein of proteodermatan sulfate were kindly provided by Dr. H. Kresse, Physiologisch-Chemisches Institut of the University of Münster/FRG. The specificity of these antibodies was shown in an earlier publication.[5]

IMMUNOFLUORESCENCE

Tissue section 6–8 µm were cut with a SLEE (Mainz,FRG) cryomicrotome, mounted on glass slides, and fixed by air-drying for up to 12 hrs. The fixed tissue sections underwent two different staining procedures: for collagen types, fibronectin and laminin they were stained directly with the first antibody, for proteoglycan they were pre-incubated with chondroitin ABC lyase (Sigma). For pre-incubation, sections were treated with 5 mU chondroitin ABC lyase for 5 minutes at room temperature. The enzyme was inactivated by washing with distilled water for 1 minute. Pre-incubated and untreated tissue sections were allowed to react with antibodies either

directed against the core protein of proteodermatan sulfate or against collagen type I, type III, fibronectin or laminin for 30 minutes of room temperature. Excess antibody was removed by washing five times with phosphate buffered saline, pH 7.5. Bound antibodies were labelled for visualization in a fluorescence microscope (Orthoplan, Leitz, FRG) with anti-rabbit IgG conjugated with fluorescin-isothiocyanate or anti-goat IgG in case of collagen types, also isolated with FITC (Behringwerke, Marburg, FRG). After washing again five times with phosphate buffered saline, the stained tissue sections were preserved by embedding in Entelan (MERCK, Darmstadt, FRG). For all tissues, controls were performed by treatment with whole pre-immune serum or chromatographically purified IgG.

RESULTS

The structural components of the normal alveolar septa are deposited in a thin filamentous backbone. Collagen types I and III were found to be co-distributed. Light microscopically elastin was observed in similar structures as revealed by collagen staining. Collagen type I and type III obviously were associated with elastin fibres. The glycoprotein fibronectin was additionally visible in laminar structures representing either the epithelial or the subendothelial basement membranes. Proteodermatan sulfate was present not only in the interstitial spaces but was also observed close to basement membranes and all surfaces. In lung fibrosis the alveolar septa become enlarged due to higher amounts of connective tissue components. Collagen type I and type III now enveloped the larger elastic fibres. These fibres were additionally covered with fibronectin. The denser connective tissue matrix reduced the capillaries in the alveolar walls visuable by the reduction of basement membrane material laminus. In this stage of fibrosis, sometimes fibronectin was accumulated in alveolar spaces, opsoniering carbon or other dust particles. In further development of lung fibrosis especially in pneumoconiosis granuloma formation is visible. Although the connective tissue exhibited a dense structure, collagen type I and type III were decreased in pericentral areas while elastin seemed to be increased. Also fibronectin or laminin were either diminished or not further recognizable in central parts of granulomas.

DISCUSSION

The present results show the participation of different connective tissue components, especially collagen type I, type III, elastin, fibronectin and laminin in the formation of fibrous material in alveolar walls. Collagen types IV and V were not separately shown. Both collagen types are either in basement membranes (type IV) or co-distributed with collagen type I and type III or with fibronectin. In further development of fibrosis collagen type I and type III depositions were increased. However, in granuloma formation their central parts contermined predominantly elastin. These results indicate that analysis of collagen content of a tissue sample depends partially on the stage of disease.[6] The distribution of the glycoprotein laminin was mainly visible in either epithelial or subendothelial basement membranes. The specific fluorescence for laminin was diminished in later stages of fibrosis indicating a reduced microcirculation. Thus, the functional properties of the lung are totally disturbed. The increase and decrease of connective tissue components reflect their regulation by different cells. In recent years the *in vitro* investigations elaborated different cytokines which in cooperation with proteases may influence the formation of fibrous material.[7,8] New therapeutic approaches should include these cellular factors.

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STUDY OF FIBROGENIC EFFECTS OF POLYPROPYLENE AND POLYTHENE ON RAT LUNGS

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ABSTRACT

Polypropylene and polythene are macromolecular compounds and typical synthetic organisms. In order to research their fibrogenic effects, 128 rats (half males and half females) were selected and 50 mg of the polypropylene or polythene dusts was injected intrachacheally into each rat. The observation was made for 18 months.

The results showed that in the early stage the prominent histopathological changes in the lungs were foci of dust-granuloma (polythene group showed polynucleo-macrophage granuloma) and hyperplasia of reticular fibers. At the 18th month after injecting the dust, in experiment groups pronounced hyperplasia of reticular fibers as well as collagen fibers were seen in these foci and around bronchi. Collagen content of the lungs in experiment groups was higher than that in control groups (treated with normal saline). The author indicated that the slight fibrogenic effect on the rat lung was caused by both polypropylene and polythene dust.

See Table of Contents, Part II, for Paper.

CHEMOTACTIC RESPONSES OF LEUKOCYTES FROM THE BRONCHOALVEOLAR SPACE OF RATS EXPOSED TO AIRBORNE QUARTZ, COALMINE DUSTS OR TITANIUM DIOXIDE

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INTRODUCTION

Studies on humans and in laboratory animals have revealed that bronchoalveolar deposition of dusts commonly associated with pneumoconiosis, results in recruitment of leukocytes to the lung parenchyma.[1,2] In view of the important role of the leukocytes in mediating both injury and mesenchymal cell proliferation, the resulting alveolitis is considered to be an important factor in determining the progress of disease.[3,4] During inflammation leukocytes are known to marginate and then migrate from the capillaries to the interstitium and alveolar space under the influence of chemotactic factors generated in this region. As part of a study on leukocyte recruitment into the lungs of rats exposed, by inhalation, to pneumoconiosis-producing dusts,[5] we examined the chemotactic activity of bronchoalveolar leukocytes lavaged from these animals. We report here on the chemotactic activity of bronchoalveolar leukocytes from the lungs of rats exposed to 10 mg/m^3 or 50 mg/m^3 airborne mass concentration of; (a) the pathogenic particulate quartz, which causes silicosis; (b) dusts collected from the air of coalmines mining anthracite, high rank coking coal and low rank bituminous coal; (c) as a negative control, titanium dioxide, a fine particulate of respirable size which is not associated with pneumoconiosis.

MATERIALS AND METHODS

Rats

Syngeneic, PVG rats, SPF maintained and fifteen weeks of age at commencement of exposure, were used.

Minerals

The dusts used in the study were (a) titanium dioxide (Rutile), obtained from Tioxide Limited, Stockton-on-Tees; (b) the quartz standard DQ_{12}; (c) coalmine dusts collected from the air of British collieries mining anthracite, high rank (coking) coal and low rank (bituminous) coal. Airborne coalmine dust samples were collected on dry Bondina socks mounted in the return roadway of a single face at each of the three collieries; full details of this procedure are given elsewhere.[5] Details of the mineralogical composition of the samples used are shown in Table I.

Inhalation Exposure

Groups of 48 rats were exposed to airborne dust for 5 days per week, seven hours per day in exposure chambers described by Beckett.[6] The dusts were dispensed using either Wright or the dust dispensers. The concentration of dust in the chambers was monitored as the mass concentration of respirable dust defined by the BMRC Johannesburg sampling criterion[7] using a Casella MRE 113A dust sampler. Full details of the exposure system are described in full elsewhere.[5]

Bronchoalveolar Lavage

At 8, 32 and 75 days into exposure, groups of four rats, and two control rats maintained in room air, were removed from the chamber and subjected to bronchoalveolar lavage. The method is described in detail elsewhere[5] but involved removal of the lungs, exsanguination, followed by lavage of the bronchoalveolar space with 4 × 8 ml volumes of saline at 37°C. The bronchoalveolar leukocytes, so obtained, were concentrated by centrifugation, counted and the proportions of the different leukocyte types assessed by differential counting of May-Grunwald Giemsa stained cytospin preparations.

Assay of Bronchoalveolar Leukocyte Chemotaxis

Chemotaxis was assessed using Blindwell chambers. Three hundred microlitres of 10% zymosan-activated serum (ZAS) (high in the chemotactic complement component C5a), were placed in the lower compartment and a filter (Nuclepore, Pleasanton, California) placed on top. The top compartment was screwed down and 6 × 10^5 alveolar macrophages in 400 µl of RPMI medium (Gibco, Paisley) were placed in the upper compartment. The filters used were 5 µm pore size and incubation was for 3.5 hours at 37°C in 5% CO_2 to allow migration of cells through the filter towards the chemotactic material in the lower compartment. At the end of the incubation period the filter was removed from the chamber, washed, stained and allowed to dry before being mounted on a slide in plastic mountant. Two chambers were set up for each condition and the number of migrated cells in 5 high power fields (x1000) were assessed for each filter.

Table I
Mineralogical Composition of Dusts Used in the Study

DUST	CLASSIFICATION	% ASH	% IN DUST KAOLIN	MICA	QUARTZ
Coalmine dust A	Anthracite	10.6	0.8	4.3	1.4
Coalmine dust H	High rank	13.2	0.6	0.5	1.0
Coalmine dust L	Low rank	53.2	18.1	0.0	6.7
Quartz	DQ_{12} Standard	NOT APPLICABLE - PURE QUARTZ			
Titanium dioxide (TiO_2)	Rutile	NOT APPLICABLE - PURE TiO_2			

Statistical Analysis of Results

Results were obtained from four experimental and two control rats at each time point. Data were analysed by analysis of variance using the Genstat computer package and comparisons made using a 't' test.[5]

RESULTS

Chemotaxis versus Chemokinesis in Leukocyte Migration

To ensure that chemotaxis was the dominant activity being measured in each sample, and not chemokinesis, we used a modified "checkerboard" method:—(mean ± standard deviation migrated cells/high power field); spontaneous migration 0.0 ± 0.0; chemokinesis (measured as migration with 5% ZAS in both the upper and lower compartments) 14.8 ± 6.6; chemotaxis (measured with 5% ZAS in the lower compartment) 46.4 ± 4.8. These results confirm that the majority of the migration was in fact chemotaxis and migration in the Blindwell Chambers will henceforth be referred to as chemotaxis.

Effect of Dust inhalation on Chemotaxis of Bronchoalveolar Leukocytes

Figure 1 shows typical data obtained for chemotaxis experiments with leukocytes from rats exposed, by inhalation, to 10 mg/m^3 of the five dusts. This data clearly shows that inhalation exposure to TiO_2 had very little effect whereas exposure to quartz and the coalmine dust was associated with a marked reduction in the ability of the bronchoalveolar leukocytes to chemotact.

Figure 2 shows the data, from all experiments at 10 mg/m^3 airborne mass concentration, expressed as percentage inhibition of chemotaxis compared to the controls on that day, to more clearly highlight the effect of dust exposure. It is clear that, although inhibition of 30% was present with TiO_2 at 8 days, thereafter the inhibitory effect of TiO_2 did not exceed 17%. In the case of quartz, however, this was as great as 89.4% by day 75. All three coalmine dusts tended to show a gradual rise in the impairment of chemotaxis shown by the bronchoalveolar leukocytes as time of exposure progressed, reaching 50–70% inhibition by day 75.

Figure 3 documents the effects of increasing airborne mass concentration of coalmine dust, on the inhibition of chemotatic activity. The increase from 10–50 mg/m^3 airborne mass concentration was associated with a marked increase in the impairment of chemotaxis, observable in the coalmine dust-exposed bronchoalveolar leukocytes, reaching 70–90% at 50 mg/m^3.

Attempts to Elucidate the Mechanism of Dust-Related Impairment of Leukocyte Chemotaxis

Limited experiments were carried out to try and elucidate the mechanism whereby dust deposition in the lungs of rats, as described above, caused loss of ability to chemotact. (a) Effect of ingested dust on macrophage chemotaxis. Control rat alveolar macrophages were allowed to adhere to filters and then incubated with quartz or TiO_2 for 1 hour to allow phagocytosis. A chemotaxis gradient was then set up by placing the filters in a chamber with ZAS in the bottom compartment. We then allowed chemotaxis to proceed:—all data given as migrated cells/high power field mean ± standard deviation; untreated macrophages, with no phagocytic burden 54.4 ± 11.3, TiO_2-exposed 51.8 ± 6.2, quartz-exposed 59.8 ± 6.0. Clearly merely having a phagocytic burden inside the macrophages was not sufficiently detrimental to cause impairment of chemotaxis. (b) Effect of incubation for 4 hours on chemotaxis. Allowing dust-exposed macrophages with impaired chemotaxis (obtained after 75 days of exposure to coalmine dust L) to incubate for 4 hours in medium to allow recycling of chemotaxin receptors had no effect on the impaired ability of the cells to chemotact:—control alveolar macrophages, freshly derived 55.0 (7.0)—incubated for 4 hours 48.2 (11.0); dust-exposed bronchoalveolar leukocytes, freshly derived 12.6 (3.6)—incubated for 4 hours 9.1 (2.4). (c) Relationship between % neutrophils in the lavage and % inhibition of chemotaxis. Since neutrophils were present to substantial proportion in some samples of bronchoalveolar leukocytes we assessed whether the presence of neutrophils was related to impairment of chemotaxis. There was no clear relationship between the proportion of neutrophils present in any bronchoalveolar leukocyte sample and impairment of chemotaxis—10–60% inhibition was caused with <10% neutrophils while increasing the percentage of neutrophils

to between 10 and 50%, only caused a maximum further 20% inhibition.

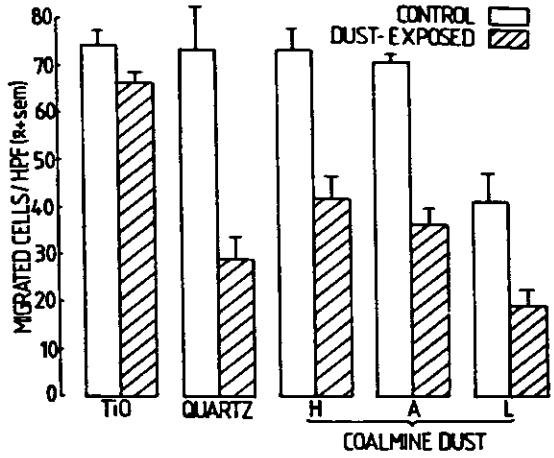

Figure 1. Chemotactic response of bronchoalveolar from rats exposed to the indicated dusts at 10 mg/m^3. Data derived as mean + sd of pooled results obtained for days 8, 32 and 75 (6–12 rats per group). Significant differences dust-exposed v control for all except TiO_2.

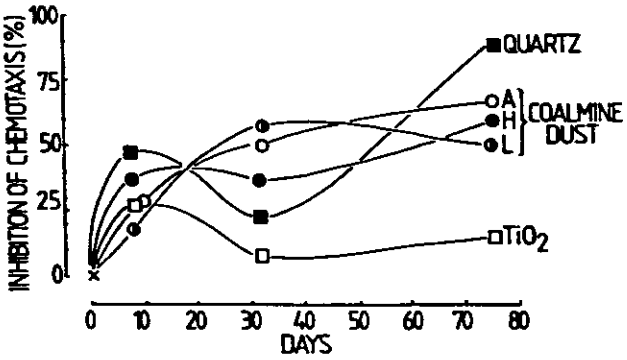

Figure 2. Mean percentage inhibition of chemotaxis shown by bronchoalveolar leukocytes from dust-exposed compared to control rats exposed to 10 mg/m^3 of the indicated dusts. Mean percentage inhibition obtained as:

$$100 - \frac{\text{mean migration of dusted bronchoalveolar leukocytes}}{\text{mean migration of control bronchoalveolar leukocytes}} \times 100$$

Raw data obtained from 2 control and 4 dust-exposed rats.

DISCUSSION

TiO_2 is a fine particulate used extensively in industry and is not associated with pneumoconiosis in exposed populations.[8] It causes minimal response in rats when given by inhalation or intraperitoneal injection.[2,9] Coalmine dust and quartz both cause pneumoconiosis and 3 coalmine dusts of

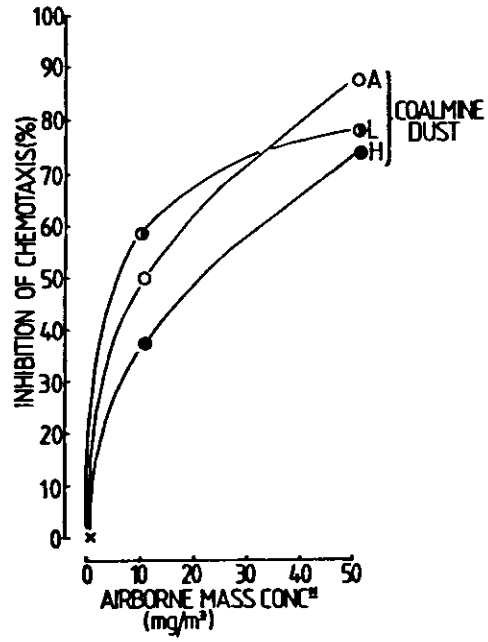

Figure 3. Airborne mass concentration dependence of the chemotaxis inhibition present in bronchoalveolar leukocytes from rats exposed for 32 days to the indicated dusts; no 50 mg/m^3 data available for quartz or TiO_2. Data derived as described in the legend to Figure 2.

different mineralogical composition, including quartz content, were included in order to test whether such differences would contribute to differences in leukocyte recruitment. These studies are reported in detail elsewhere[5] but revealed alveolitis in rats exposed to quartz and all 3 coalmine dusts and failure of TiO_2 to elicit any substantial leukocyte response except at high dose following a long period of exposure.

The studies on the chemotactic activity of bronchoalveolar leukocytes reported here show impairment of chemotaxis in line with the ability of the dust to cause inflammation, i.e., (a) titanium dioxide which caused minimal inflammation caused least impairment of leukocyte chemotaxis; (b) quartz, caused large scale inflammation and the bronchoalveolar leukocytes of the alveolitis had impaired chemotactic activity; (c) coalmine dusts were intermediate in response between TiO_2 and quartz in ability to cause inflammation and impair chemotactic responses. There were no well defined differences between the three coalmine dusts with different mineralogical composition, in terms of their ability to impair chemotaxis.

The results described here do show that chronic deposition of titanium dioxide, a dust not associated with pneumoconiosis did cause a measure of loss of impairment of chemotactic activity. In the cases where quartz and coalmine dusts caused impairment of chemotaxis there was

clear dose dependency in terms of the airborne mass concentration to which the rats were exposed. The data described here was obtained as counts of all migrated leukocytes which included both macrophages and neutrophils in inflammatory populations. However the decreased number of migrated leukocytes present in dust-exposed populations could not be explained on the basis of the neutrophils present, either as different migration characteristics compared to macrophages or effects of neutrophils on macrophage ability to migrate. This was evident since (a) profound inhibition was present even with low percentages of neutrophils;[5] (b) in a limited number of cases differential count of the migrated cells were carried out (data not included) revealing, in some cases, similar proportions of macrophages and neutrophils in the migrated cells to those in the cells as lavaged; in some cases the proportion of neutrophils was decreased but this was never sufficient to explain the overall reduction in migration shown by the inflammatory population and impairment of macrophage chemotaxis must have been present. From this it is clear that macrophages from dust-exposed lung have impaired chemotactic activity and that neutrophils from dust-exposed lung have less chemotactic activity than control alveolar macrophages, at least under the conditions of the assay. The net effect of this is that the ability of the leukocytes to clear dust from dust-inflammed alveoli is likely to be severely impaired.

We have shown that the biological mechanisms underlying the loss of ability to chemotact do not include mere difficulty encountered by dust-loaded cells in trying to pass through the pores of the filter towards the source of chemotaxin. Since the leukocytes lavaged from the bronchoalveolar space have exudated in response to a chemotactic stimulus, it seemed possible that chemotaxin receptors might already be occupied. However, experiments allowing chemotaxin receptors to regenerate, by incubation for 4 hours, produced no effect and impairment was maintained. Other studies from our Institute have suggested that neutrophils could cause some inhibition of the chemotactic activity of macrophages.[10] However plotting % inhibition against % neutrophils in the lavage failed to show any clear relationship between numbers of neutrophils and loss of chemotaxis.[5]

We believe that leukocytes from dust-exposed lung have impaired expression of chemotaxin receptors or inhibition of the cytoskeletal proteins involved in cell movement, or their energy supply. Myrvik[11] reported inhibition of migration of rabbit alveolar macrophages which had phagocytosed asbestos *in vitro*; whilst the impairment of chemotaxis could not be attributed to toxicity in this study, unfortunately inert control dusts were not included. Following exposure *in vivo* and bronchoalveolar lavage, Warheit et al.[12] reported impairment of chemotaxis with asbestos whilst Dauber et al.,[13] and Martin et al.,[14] both described impairment of leukocyte chemotaxis following inhalation exposure to silica. The present response is the first, to our knowledge, showing that coalmine dusts also cause profound impairment of bronchoalveolar macrophage chemotaxis. It was noteable that inhibition of chemotaxis was present following chronic inhalation exposure at an airborne mass concentration of 10 mg/m^3 approximating to the maximum allowable level in British collieries (7 mg/m^3).

The fact that impairment of migration in bronchoalveolar leukocytes was much less with the inert dust TiO_2 than with the two pneumoconiosis-producing dusts suggests that this phenomenon may be important in contributing to lung damage and pathological change in pneumoconiosis. This could be brought about by the pneumoconiosis-producing dusts being allowed to persist in the bronchoalveolar region within alveolar macrophages chronically stimulated by the ingested pathogenic dust. Such stimulated macrophages, refractory to the normal chemotactic gradients which govern their movements, could persist in the alveolar region, releasing injurious agents such as proteases and oxidants and growth factors such as interleukin 1 and tumour necrosis factor, leading to fibrosis.

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ACKNOWLEDGEMENT: This research was funded by the Council of the European Communities.

PATHOPHYSIOLOGICAL EVIDENCE IN MODIFICATION OF COAL-INDUCED LESIONS BY JAGGERY IN RATS

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INTRODUCTION

Inspite of rapid technological advancements made during the twentieth century, occupational lung diseases due to inhalation of airborne particulate or fibrous matters continue to constitute exceptionally high incidence among industrial workers. Pulmonary dust diseases, in general, are not curable in true sense because the usual therapy of destroying or removing the morbific agent from lung has not proved efficaceous as is possible with bacterial or viral infections of the lungs. Apart from protective devices, as designed by engineers and put into actual use, various experimental approaches for the treatment of pulmonary dust diseases were also extensively made in the past with partial success using different types of aerosol therapy, hormonal therapy, vitamin therapy and other substances including dietetic factors.[17] The discovery of antisilicotic drug-PVNO and its efficacy both under *in vitro* and *in vivo* situations demonstrated its usefulness not only against experimental silicosis but also against the pathogenic effects of a mixture of coal and quartz dust in the lungs of rats.[11] In 1975, Chinese investigators demonstrated the therapeutic and preventive effect of Tetrandrine—an alkaloid of bisbenzyl isoquinoline (*Stephania tetrandra* S.) on experimental silicosis in rats and monkeys as well as marked improvement in symptoms and chest X-ray of human silicotics seen with tetrandrine treatment.[3] Beletsky and Coworkers[1] have reported some success with alkali inhalation (aerosol of potassium carbonate) by industrial workers before and after work shift, indicating reduction of silicosis incidence up to 28%. Hydrolysed dextran or glutamate have also helped in bringing about significant prevention of silicosis.[8,13] However, all these agents have also shown to be associated with various harmful side effects.

Many therapeutic properties have been attributed to jaggery in the Ayurvedic system of medicine. Regular consumption of jaggery conferred great symptomatic relief of industrial mine workers in mining and industrial establishments of India. Our earlier work[9,10] and this report indicates the beneficial effects of jaggery, a nutritive substance of considerable potential and without any harmful side effects, on coal mine dust induced lesions in rats.

MATERIALS AND METHODS

Jaggery

A solidified form of the product obtained on boiling in open pan and concentrating sugarcane juice (*Saccharum sinense* Roxb.) is an indigenous edible item which is produced in all parts of India and is consumed as such or as confectionaries. The production of gur involves extraction of juice from cane, purification by straining, boiling and treating with vegetable clarificant followed by further boiling and concentration into a thick semi-solid mass which solidifies on cooling. Usually jaggery contains all the soluble constituents of sugarcane but the exact composition depends upon the variety and age of canes and nature of the soil on which they are cultivated. The chemical composition of jaggery[6,7,12] is given in Table I.

Coal Mine Dust

The respirable size (5 μm diameter) with fairly well defined chemical composition was obtained from Environmental Monitoring Section of Industrial Toxicology Research Centre, Lucknow. To increase fibrogenic potential of coal mine dust, it was supplemented with quartz up to 10% of the total mixture.

Experimental

Male albino rats (108) were procured from ITRC animal breeding facility and maintained on normal laboratory conditions, on standard pellet diet (Gold Mohur, Lipton India Ltd.) and water *ad libitum*. Animals were divided into 5 groups as shown in Experimental Protocol (Table II). The dose of jaggery in rat was based on its average daily consumption of mine workers in India. Following treatment from first day, 6 animals from each group were weighed individually and sacrificed at the end of 30, 60 and 90 days.

Hematology

Blood was collected from jugular vein in heparinized tubes for RBC and WBC counts and estimation of hemoglobin. RBC and WBC were counted on Cell Counter 2041 Labora Manheim GmBH, Germany. Haemoglobin was estimated according to the method of Dacie and Lewis.[4] Fresh blood

Table I

General Composition of Indian Jaggery (Gur) Compiled from Reference 6, 7 and 12

Content	Value (range)
Moisture (%)	3.9–7.2
Protein (%)	0.35–0.40
(a) Non-protein nitrogen (mg/100 g)	19.6–42.9
(b) Protein nitrogen (mg/100 g)	13.7–17.6
Carbohydrate (%)	83.5–95.0
(a) Sucrose (%)	72.8–80.3
(b) Reducing sugar (%)	6.8–14.2
Minerals (%)	0.6–2.6
(a) Calcium (%)	0.2–0.36
(b) Chloride (%)	0.2–0.34
(c) Phosphorus (%)	0.03–0.22
(d) Potassium (%)	0.10–0.16
(e) Sodium (%)	0.006–0.025
(f) Iron (%)	0.005–0.020
(g) Magnesium (%)	0.008–0.105
(h) Copper (%)	0.007–0.010
(i) Cobalt, Nickel and Molybdenum (%)	0.001–0.008
Vitamins	
(a) Thiamine (mg/100 g)	0.018–0.030
(b) Riboflavin (mg/100 g)	0.042–0.046
(c) Nicotinic acid (mg/100 g)	3.92–4.50
(d) Vitamin C (mg/100 g)	5.20–30.00
Carotene (µg/100 g)	155.0–168.0
Phenolics (mg/100 g)	280.0–320.0
Fat, Wax, Pectin and Organic acids (%)	0.10–0.60

Table II
Experimental Protocol

Group	Number of rats used	Treatment	
		Coal[a]	Jaggery[b]
I	22	+	-
II	22	-	+
III	22	+	+
IV[c]	22	+	+
V[d]	20	-	-

[a]Coal dust (50 mg/1 ml of 0.15 M NaCl solution, sterile suspension) was injected intratracheally.

[b]Jaggery (500 mg/1 ml of sterile distilled water) given orally (po) 5 days/week for 90 days (termination of the study).

[c]Animals were first treated with gur (po) for 2 weeks followed by coal dust (intratracheally) and gur (po) treatment for 90 days (termination of the study).

[d]The control animals were treated orally (po) 1 ml of sterile waters 5 days/week for 90 days (termination of the study).

was used for making blood films to enmurates differential leukocytes after Leishman's staining.

Relative Organ Weights and Lymphoid Cell Counts

At 30, 60, and 90 days post treatment, body weight, weights of thymus, spleen, peripheral (axial a popliteal lymph nodes) and tracheobronchial lymph nodes, adrenal glands, kidney, liver and lung were determined from individual rats and relative organ weights calculated. Thymus, spleen, peripheral and tracheobronchial lymph nodes cell counts were counted as number of nucleated cell per organ in Counter 2041 after making cell suspension. Viability of cells was determined by Trypan Blue exclusion method.

Histological

Lungs were inflated *in situ* with 10% buffered formalin while other organs were used for estimation of collagen content. After preliminary fixation, the blocks were selected on the fixed positions along with the long axes of both the lung at the level of the hilum to include the maximum area of the lung. Tracheobronchial lymph nodes were excised carefully and fixed in Bouin's solution. Other visceral organs were also fixed in formalin. The paraffin section of 5 μm thickness were prepared and stained with hematoxylin and eosin, silver impregnation for reticulin and Van Gieson's stain for collagen.

Collagen and Sulphydrol Content

Lung Collagen content was estimated by measuring hydroxyproline levels. In brief, dry lung tissues were hydrolysed with 6 N HCl in sealed glass tubes at 110°C for 16 hrs. The hydrolyate was titrated to pH 7 and diluted with distilled water. Hydroxyproline content was then assayed according to Woessner.[15] Concentration of total and free Sulphydryls in lung homogenates prepared in 0.25 M sucrose containing 0.005 M EDTA and free Sulphydryls in blood was determined using the Ellman procedure.[3]

RESULTS

Gross Observation

The visceral organs of rats of different groups did not reveal any significant macroscopic changes up to 90 except in lungs and tracheobronchial lymph nodes of rats receiving coal dust. The lungs of group 1 at 60 days showed black pin point patches of coal dust on the lobes and by 90 days these patches

became more prominent. Black patches of the coal dust were also seen in group III and IV at 90 days but they were less prominent than in group I judged from visual observation. In the early phase in group I, III and IV, the tracheobronchial lymph nodes became prominent and black in colour. However, in group III and IV at 90 days, a marked lymphadenopathy was observed. Tracheobronchial lymph nodes of Group I animals showed some macroscopic changes.

Relative Weight

The relative weight of lungs at 30 days showed marked increase in groups I, III and IV. At 60 and 90 days relative weight of lungs in group I increased significantly (51% and 41% respectively) in comparison to controls while relative lung weight of lungs in group III and IV were almost similar to that of control. The relative weight of tracheobronchial lymph nodes (TLN) of coal dust exposed group (I) showed 61-74% increase. In general the relative weight of lung, TLN, liver and spleen showed an increase at 30 days and a decline at 60 and 90 days.

Hematological Changes

RBC counts increased significantly in all the experimental groups. Concomitantly jaggery treatment in group III and IV significantly increased the RBC counts at different periods. At 90 days the values were higher than those of group I.

At 30 days in coal exposed and jaggery treated group (III and IV) the increase in WBC count was over 50%. A time dependent increase was further observed in these groups with maximum increase of 76% ($P < 0.001$) at the end of 90 days.

The haemoglobin content in group I was more or less the same at all periods but slightly higher than those of controls. However, the percent haemoglobin increased (45–50%; $P < 0.05$) in all the three jaggery receiving groups (II, III and IV) at 30, 60 and 90 days.

Cellularity of Lymphoid Organs

Animals treated with coal and jaggery group III and IV indicated 18-20% (P 0.05) increase in thymocyte counts at all periods. At the termination of experiment (90 days), a decline in splenocyte counts (16% and 33%; P 0.05 respectively) was observed in group I and II animals. The cell counts of tracheobronchial lymph node indicated an increase in group I, III and IV in a time dependent manner at the end of 90 days. Peripheral lymph nodes cell counts indicated significant increase in group III and IV at 60 and 90 days (25–45%; $P < 0.05$).

Histological findings

In group I at 90 days typical coal-induced focal areas of fibrosis was seen which upon silver impregnation showed presence of thick reticulin fibres enclosing coal dust mass. Lung of animals treated orally with jaggery did not reveal any significant histopathological alteration at 30, 60 or 90 days. In group III (coal and jaggery simultaneously) at the termination of study (90 days), the alveolar parenchyma, in general, showed widely scattered and partly unphagocytosed coal particles with minimal cellular reaction and less fibrogenic response upon silver impregnation in comparison to group I.

In group IV (pretreatment with jaggery followed by coal plus jaggery) at 90 days there was hardly any indication of the development of characteristic coal-induced lesions except there was mild thickening of alveolar Septa (Figure 1). Moreover, minimal cellular proliferation around coal deposits in the parenchyma as well as small aggregates of coal particles in the lumen of alveolar duct respiratory and terminal bronchioles as was seen in group III. The changes in tracheobronchial lymph nodes: the nodes were slightly enlarged with diffuse distribution of coal particles in the paracortical regions. Silver impregnation revealed presence of dense fibres along with thick branches of reticulin enclosing coal aggregates. In group III at 90 days there was minimal reaction provoked by coal aggregates which were rather focally distributed and not diffusely as in group I. Focal areas of coal aggregates did not reveal any significant fibroblastic reaction. In group IV and 90 days, the enlarged lymph nodes demonstrated many scattered focal areas of coal aggregates with minimal reaction (Figure 1). In spleen the fibroblastic reaction was seen in red pulp in group I at 90 days while very mild reaction was observed in group III and IV at same period. Histological examination of other organs did not show any significant alterations in their structures in various treatment groups at 90 days.

Lung Collagen

Lung collagen as measured by hydroxyproline content in various treatment groups (I to V) up to 90 days is shown in Figure 2. A time dependent increase was seen in hydroxyproline content of group I animals. Animals of group II, however, did not show any alteration in the lung hydroxyproline content up to 90 days. Group III animals also did not exhibit any initial increase in hydroxyproline content at 30 days but at later periods (60 and 90 days) 10% increase (P 0.001) did occur. Interestingly, hydroxyproline content of lung from animal of group IV remained unaltered and were within the limits of normal variation up to 90 days.

Total and Free Sulphydryl Content of Lung

The jaggery treatment group III and IV showed an elevated level of their lung-SH content. Free SH-contents of the jaggery treated animals (group III and IV) were exceptionally high at 90 days.

Free Sulphydryl Content of Blood

Coal dust instillation and oral treatment of jaggery (group I and II) did not change the free SH-content of blood. In group III and IV the SH-content increased in initial stage (30 days) but an increase became evident at 60 and 90 days in group IV.

DISCUSSION

The result of the present experiments showed significant inhibition of fibrotic changes in the lungs at 90 days in coal treated rats which received prior treatment of jaggery. Moreover the initial reaction of the coal induced cytotoxicity, phagocytosis as well as fibroblastic reaction in lungs remained less prominent and did not damage the lung archi-

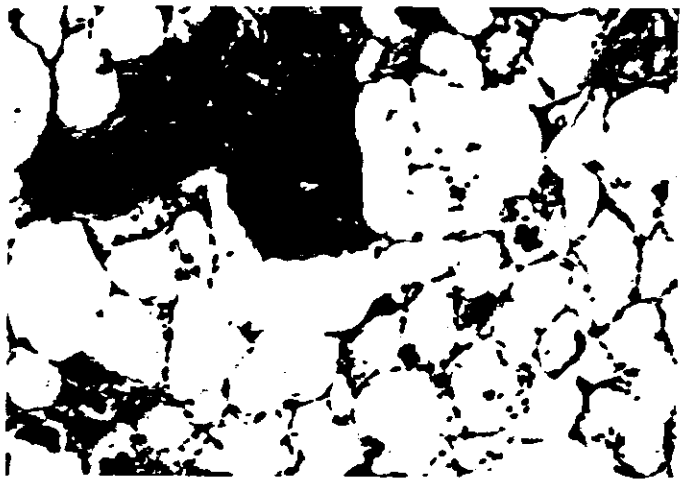

Figure 1A. Rat lung after treatment of Coal alone (group I) at 90 days, thick reticulin fibrosis upon silver impregnation, X 128.

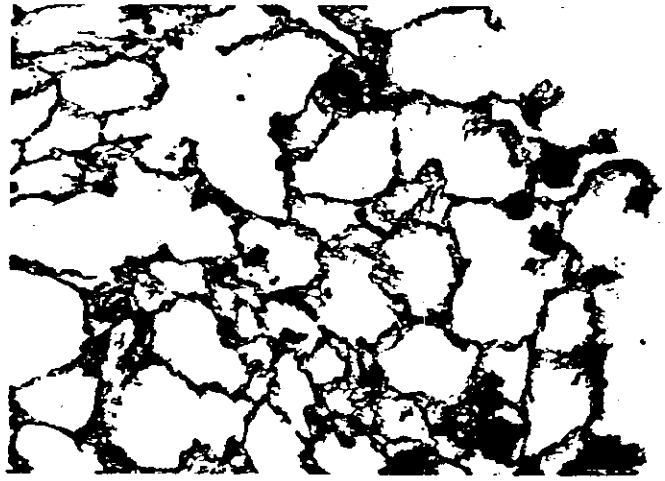

Figure 1B. Rat lung, pretreatment with Jaggery and Coal + Jaggery (group IV) at 90 days, upon silver impregnation showing mild thickening of alveolar septa, X 104.

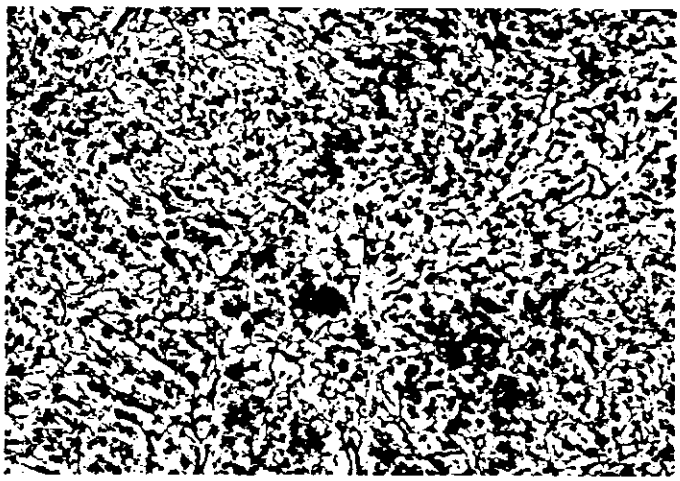

Figure 1C. Tracheobronchial lymph node after treatment of Coal alone (group I) at 90 days upon silver impregnation showing dense reticulin fibres and coal particles, X 104.

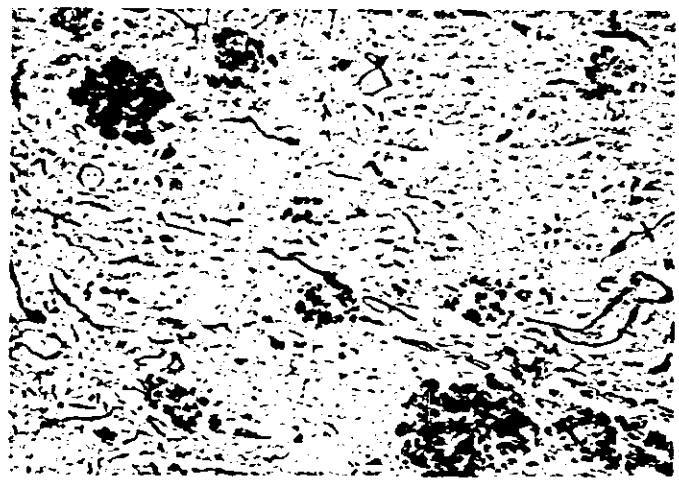

Figure 1D. Tracheobronchial lymph node, pretreated with Jaggery and Coal + Jaggery (group IV) upon silver impregnation, few reticulin fibres along with coal particles, X 104.

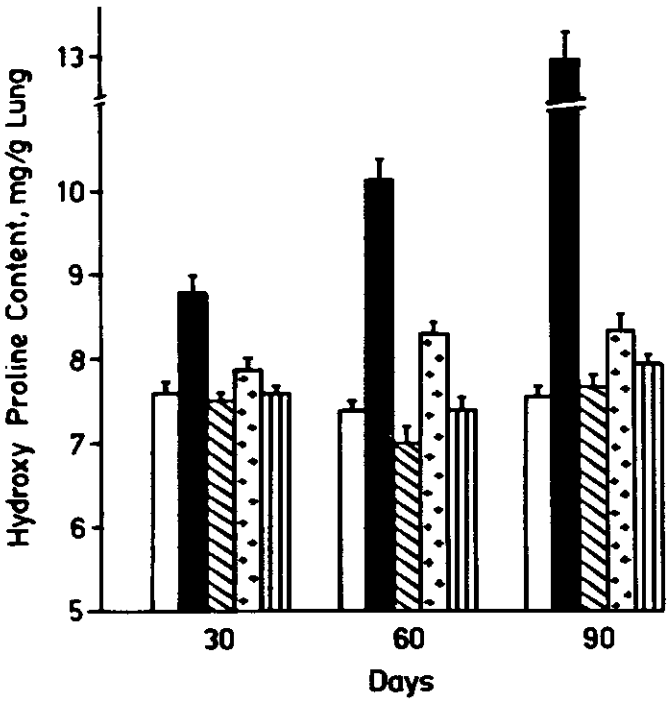

Figure 2. Changes in Hydroxyproline content of rat lung of different groups at 30, 60 and 90 days. □ — Control; □ — Coal alone (group I); ▨ — Jaggery alone (group II); ▦ — Coal and Jaggery simultaneous treatment (group III); ▥ — Pretreatment with Jaggery and Coal + Jaggery (group IV).

tecture. In addition to histologic evidence in lungs, pretreatment of jaggery prevented the increase in the hydroxyproline content (collagen) of lungs. Significant changes in the relative weights of lungs, liver, TLN and spleen of coal exposed rats were observed following jaggery treatment. The WBC counts and haemoglobin contents were significantly higher in all the jaggery treated groups. Concomitantly, the cell counts of lymphoid organs were also elevated. These findings indicate that jaggery treatment presumably increased the physiological status of almost every important organ and cells of the body suggesting that dust clearance from lung could be by the enhanced physiological pathway.

Our earlier studies[9] following jaggery treatment have shown that pathological pathway of clearance is also effectively operative in these groups as dust can be observed in TLNs.

Although it has been reported that protein is essential for fibrogenesis,[2,14] multideficient or protein deficient diets did not modulate silicotic fibrogenesis.[17] In the pre-fibrogenesis stage, there is likely to be proliferation of endoplasmic reticulum leading to enhanced-SH levels. If fibrosis is retarded, as in the jaggery treated groups as evident histopathologically, hydroxylation is initiated with affecting the endoplasmic reticulum. In that case SH is likely to increase. The enhancement of sulphydryl content of coal exposed rat lung following jaggery treatment suggest that jaggery or its microingredient(s) play some protective role in the release of these toxic biologically active substances.

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IMMUNOLOGIC FEATURES OF THE BRONCHOALVEOLAR LAVAGE FLUID OF RATS WITH SILICO-PROTEINOSIS

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ABSTRACT

We provoked silico-proteinosis in specific pathogen free Fisher 344 rats by exposing them to 10 mg/m^3 respirable free crystalline silica for 3 months (6 hrs./day, 5 days/wk) and compared the immunologic features to a group of non-exposed rats. Bronchoalveolar lavage fluid (BALF) return, total cell count, differential cell count, and BALF and serum IgG, albumin, and IgA levels were measured and expressed as mean ± S.E.M.

Percent fluid recovered was no different in the silica exposed and non-exposed rats (51.6±6 v. 64±8). Total cells recovered (x10^6)(64±17 v. 3.1±1.0), % macrophages (67.4±3.0 v. 94.8±0.5), % neutrophils (5.9±1.4 v. 1.3±0.8), and % lymphocytes (26±2 v. 4.5±0.6) were significantly different in the silica versus non-exposed rats, respectively.

IgG (g/dl) was significantly increased in the serum (2.0±0.2 v. 1.2±0.3) and BALF (0.7±0.01 v. 0.02±0.01) of the exposed rats. Serum albumin (g/dl) was not significantly different in the 2 groups but BALF albumin (g/dl) was significantly increased in the silico-proteinosis group (0.20±0.02 v. 0.03±0.01). IgA levels did not significantly differ in the serum and were below detectable limits in both of the groups.

A dramatic influx of humoral and cellular components occurs into the bronchoalveolar lavage fluid of rats with silicoproteinosis, reflecting the extensive inflammatory response associated with this disease.

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