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PULMONARY EFFECTS OF GLASS FIBRES IN MAN AND ANIMALS

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ABSTRACT

The results of the author's experiments in which animals were exposed to glass microfibres by inhalation are compared with those of other workers who exposed animals to glass microfibres by inhalation, intratracheal instillation, and intrapleural injection.

The author suggests that the fibrogenic properties of inhaled materials are related to both the chemical nature and the physical shape of the particles. In the case of cytotoxic materials such as silica the chemical nature assumes the most significance. In the case of materials which do not appear to cause cell death such as glass, the physical shape of the material may cause a fibrogenic response when the amount present is sufficiently great. The high loading of the lung with long glass microfibres sufficient to cause a marked fibrogenic response is considered to be unlikely to occur by inhalation.

Glass fibre is a generic term used to describe a variety of different fibrous forms of glass which differ mainly in their dimensions. The various processes used in the manufacture of fibrous glass have been reviewed extensively elsewhere[12,13]. These procedures range from steam jet fiberisation, which produces a relatively coarse material known as "mineral wool" to the more recent flame attenuation processes used to produce very fine glass fibres which may be as small as 0.1 μm in diameter. These are commonly referred to as glass microfibres. The larger diameter types of fibrous glass are used for reinforcement of plastic, rubber and paper products and for thermal insulation[3]. Very fine glass "microfibres", which are estimated to form approximately 1% of the total U. S. production of fibrous glass are used chiefly in high temperature insulation and manufacture of high quality filter papers. These fibres may be as small as 0.1 μm in diameter. The very small diameter of these forms allows fibres of up to 100 μm in length to become aerostable and they may be carried into the respiratory tract of man and animals. Fibres of lengths of 100 μm and over are likely to be lodged in the nose or upper respiratory tract and Dement[5] has suggested that a potentially respirable fibre may be defined as being less than 3.5 μm in diameter and less than 50 μm in length.

The results of some animal experiments, discussed later in this text, suggest that the fibrogenic and the carcinogenic properties of certain types of asbestos may be related to their fibrous nature. Consequently concern has been expressed that fibrous glass may be carcinogenic or fibrogenic when inhaled by man.

Human exposure to glass fibre

Although several epidemiological studies have been performed on worker exposure to fibrous glass, it is often difficult to obtain precise information as to the total exposure and perhaps, more importantly, the size of glass fibres to which the subjects were exposed[8,11]. The general picture which emerges from the epidemiological studies performed to date is that fibrous glass workers do not show evidence of any unusual health hazard except perhaps transitory upper respiratory tract irritation[10] and a possible excess of chronic bronchitis[8]. Nevertheless, both asbestosis and induction of mesotheliomas by asbestos exhibit an extremely long latent period of development and very fine glass microfibres have only found wider industrial applications in the last 20 years. Therefore epidemiological surveys of fibrous glass workers carried out during the next 10 years will be of considerable importance.

Animal experiments with glass fibre

Several studies have been carried out in which glass fibres of various dimensions have been introduced into the bodies of experimental animals by intratracheal instillation, inhalation and introduction into the pleural space by injection or surgical intervention.

Wright and Kuschner[18] carried out experiments in which glass fibres were introduced into the lungs of guinea pigs by intratracheal instillation. The results suggested that long fibres of glass (92% longer than 10 μm) produced a fibrotic lesion whereas short fibres of glass (93% less than 10 μm in length) produced only macrophage aggregation. It was suggested that the fibrogenic effects of long glass fibres were related to the incomplete phagocytosis of such fibres by macrophages in the lung which resulted in leakage of "tissue damaging enzymes".

The results of experiments in which materials are administered by intratracheal instillation should be interpreted with caution. The test materials are applied to the surface epithelia of the lung as one massive insult. The reaction of the lung to such an overwhelming insult may well be different from the reaction of the lung to inhalation of the same amount of test material over a longer period. In addition it is possible, particularly in the case of fibrous materials, that the fibres will become entangled during passage through the inoculation needle or down the trachea, producing a bolus of aggregated material. The presence of such an aggregation in the lung may well produce a reaction which would not have occurred if the fibres had been distributed more evenly in the lung by inhalation. In early experiments in my laboratory in which the value of intratracheal instillation was investigated it was found that despite

ultrasonic treatment of suspensions of glass fibre such large masses were not uncommon. An extreme example of this type of phenomenon is shown in Figure 1.

Introduction of test materials into the pleural cavity of experimental animals may be criticized for similar reasons. The material may aggregate and produce local exposure to relatively large masses which would not have occurred after transpleural migration of inhaled material. However, this technique has stood the test of time and the relationship of asbestos with mesothelioma production was

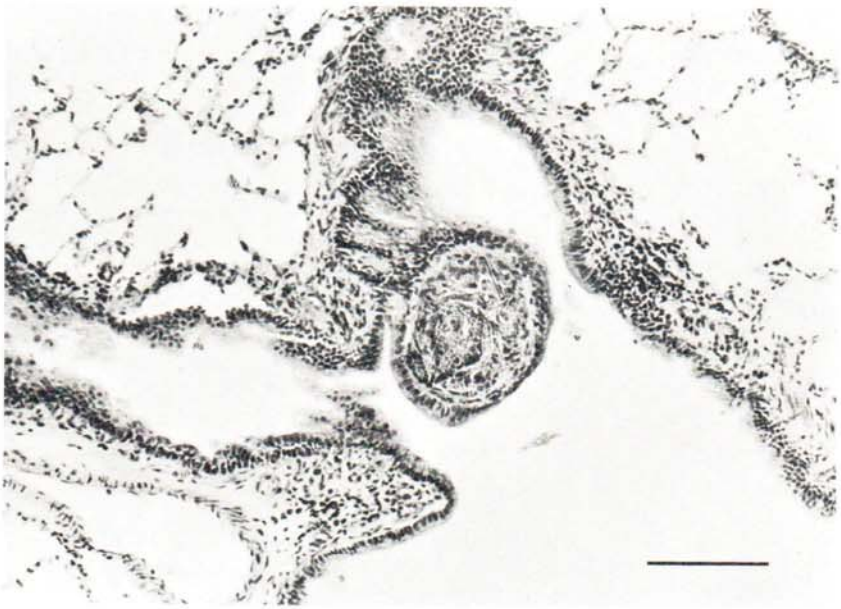

FIG. 1 – Bolus of glass fibre partially blocking the bronchus of a rat. A suspension of glass fibre had been instilled into the trachea 2 months before autopsy. The mass of glass fibre is enclosed by several layers of fibroblasts, which in turn are covered by a layer of respiratory epithelium. Bar = 100 μm.

elegantly demonstrated by Wagner and Berry 10 years ago[16]. The same laboratory has also carried out experiments in which glass microfibres were introduced into the pleural cavity of rats and these experiments indicated that glass microfibres when introduced in this way were also capable of causing mesotheliomas[17]. Stanton and Wrench[14] have reported experiments in which glass fibre was introduced into the pleural cavity of rats by surgical techniques. These studies also indicated that glass fibres of fine diameters (0.06 – 3 μm) were capable of producing tumours which were reported to be mesotheliomas.

Exposure of experimental animals by inhalation of atmospheres containing glass microfibres is the most appropriate route for investigations of the pulmonary effects of this material. However, due primarily to the high cost and technical complexity of inhalation studies relatively few such studies with glass

fibre have been performed. Gross and co-workers[9] have reported an inhalation study in which rats and hamsters were exposed to an aerosol of glass microfibres for 6 months to 2 years. In this study it was found that no gross lesions were produced in the lungs although large numbers of macrophages containing glass fibres were evident in the sections examined.

In our experiments rats were exposed to high concentrations of glass microfibres for four half-hour sessions, one day a week for 8 weeks. Following a 4–week non-exposure period rats were examined as described below.

This work was initiated in order to investigate the relatively short term effects of exposure to high concentration of glass fibre dusts and it is intended to carry out additional longer term inhalation studies with the same material.

METHOD

Rats were exposed to high concentrations of glass microfibres by inhalation. The animals were exposed in a head-only exposure system for four half-hour sessions each week for a total of 8 weeks. A block diagram of the exposure system is presented in Figure 2. The glass fibre used in this study was prepared

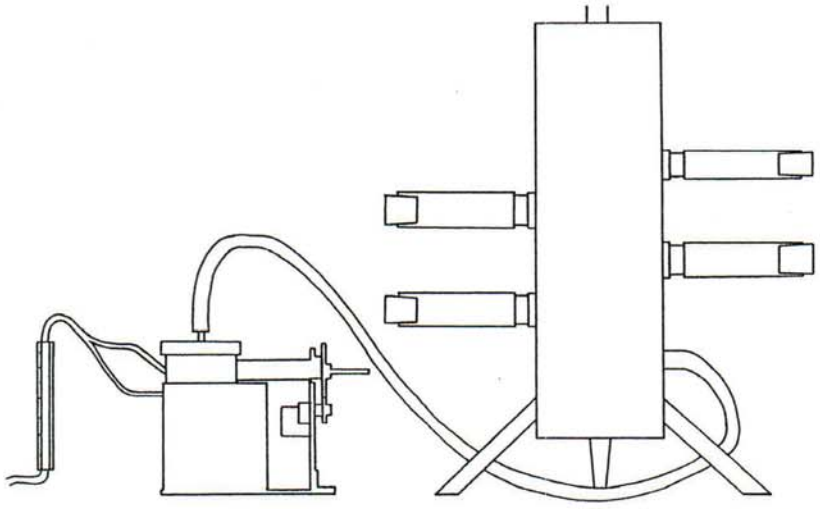

FIG. 2–Block diagram of apparatus for exposure of rats to aerosol of glass microfibres.

by ball-milling Johns Manville code 102 glass microfibres for 75 minutes. The aerosol was generated by means of a modified Timbrell[15] aerosol generator and led through a 1 meter length of tubing which removed larger non-respirable flocs of material by sedimentation.

During each exposure session the total aerosol concentration was determined gravimetrically on two occasions. In addition, a sample of the aerosol

was obtained on a millipore filter for subsequent particle size determination. Particle size determinations were carried out from both light and scanning electron micrographs. The average aerosol concentration was 1.2 mg/litre (1200 mg/m^3). Preliminary examination of selected micrographs revealed that 57% of the particles characterised were classified as fibres ($>3:1$ length to diameter ratio). The fibres were all less than one micrometer in diameter and 46% were less than 0.5 μm. Approximately 20% of the fibres were between 9 and 20 micrometers in length and the majority of the remainder were less than 9 micrometers.

Forty rats (20 male and 20 female) were exposed to the aerosol and the same number of control animals were exposed to air alone under the same conditions. At the end of the 8-week exposure period the animals were retained for 4 weeks and then examined. The lungs from 20 rats (10 male + 10 female) from each group were processed for histopathological examination.

After standard fixation and embedding procedures, sections of lungs, kidneys and spleen from each animal were stained with haematoxylin and eosin. Lung sections were also stained with van Gieson's stain for collagen. Five male and five female rats from exposure and control groups were used for assessment of dynamic pulmonary function parameters. The assessment of dynamic pulmonary function was based on a modification of published techniques[2] with an on-line computer system. The remaining 10 (5 male and 5 female) animals from both groups were assessed for lung hydroxyproline content according to standard techniques.

RESULTS

The results obtained from the dynamic lung function measurements revealed no treatment related effects but it should be noted that such tests are relatively insensitive to changes in the small airways of the lung.

The hydroxyproline analyses revealed no treatment related differences between the exposed and control animals.

The pathological examination of the lungs provided no evidence of collagen deposition and the integrity of the pulmonary architecture was preserved. The main finding was that large numbers of macrophages containing glass fibres accumulated in the alveoli and at the bronchoalveolar junctions (Figure 3).

This observation is in agreement with data published elsewhere[9]. The accumulations of macrophages did not appear to be associated with a pathological reaction in the interstitial regions of the lung. The macrophages in some areas formed clusters in the region of the junction of bronchiole and alveolus. The membranes of the macrophages were not always distinct but there was no evidence of cell death in the sections examined. Occasionally there was evidence of slight proliferation of type II pneumonocytes in the region of the clusters of macrophages. The electron microscopical appearance of a macrophage containing glass fibre from a different series of experiments is shown in Figure 4. Some of the fibres are membrane bound and the ultrastructure of the cell is apparently normal.

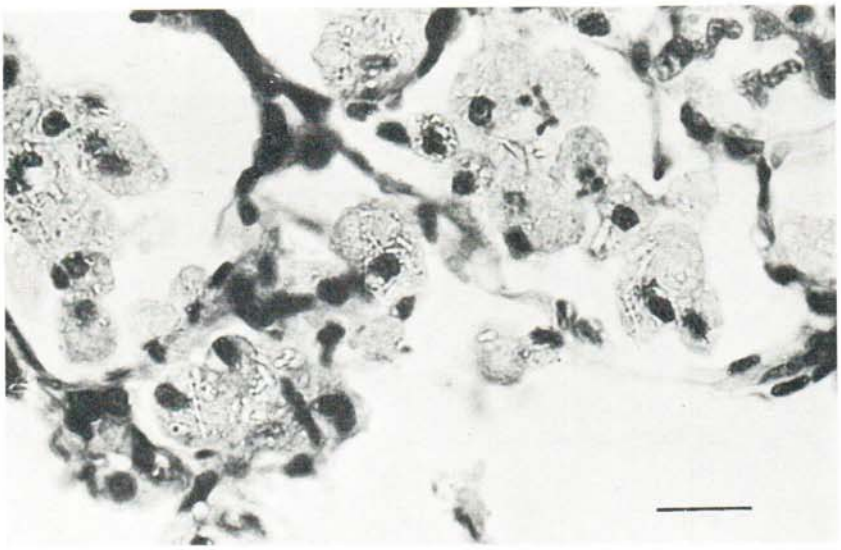

FIG. 3 – Macrophages containing glass microfibres after inhalation of an aerosol of fibres for four half-hour exposures each week for 8 weeks. Bar = 20 μm.

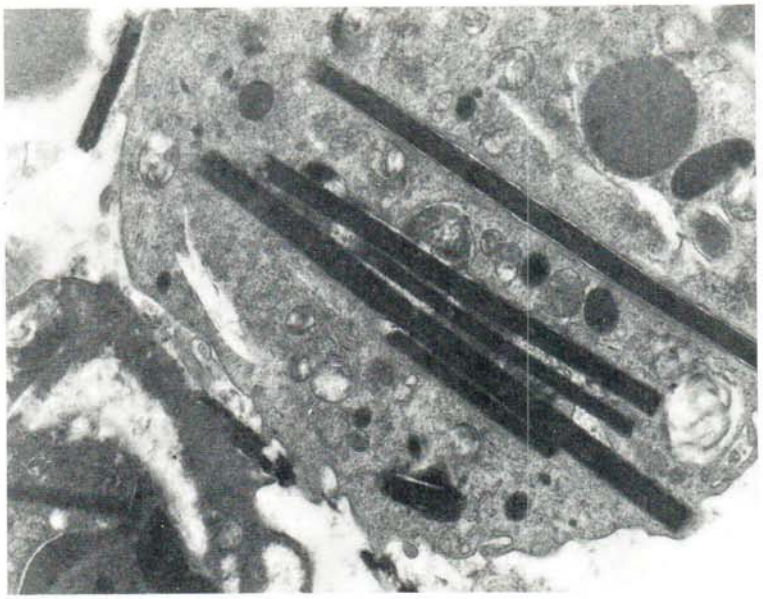

FIG. 4 – Electron micrograph of a lung macrophage containing glass microfibres from the lung of a rat. Some of the fibre appears to be membrane bound. The rat was dosed by intratracheal instillation 3 days before autopsy. TEM × 21750.

DISCUSSION

The results of the small number of published studies and our own work would suggest that the initial response of the animal lung to inhaled glass microfibres is similar to that exhibited by a "nuisance dust". The presence of small quantities of glass fibres in the lungs causes an increase in the number of free macrophages and these are seen to contain phagocytosed glass fibres. Small numbers of microfibres are found in the interstitial tissue and there is evidence of some translocation to regional lymph nodes. Up to 3 months after the initial inhalation of glass microfibres there does not appear to be any reaction in the interstitial tissue of the lung and the lung architecture is of normal appearance.

Clearance of glass microfibres from the lung is believed to occur mainly by way of the trachea. Macrophages which have engulfed the material are carried up the trachea on the mucociliary transport system. However, observations made during our experiments suggest that there may be a considerable involvement of the lymphatic system in clearance of fibrous particulate material from the lungs. That glass microfibres enter the lymphatic system of the lung is evidenced by the appearance of fibres in the regional lymph nodes. In addition, large macrophage--like cells containing fibrous material have been observed in the bronchus associated lymphoid tissue following inhalation of the material. Such a formation is shown in Figure 5. At this stage it is not clear what the significance of these

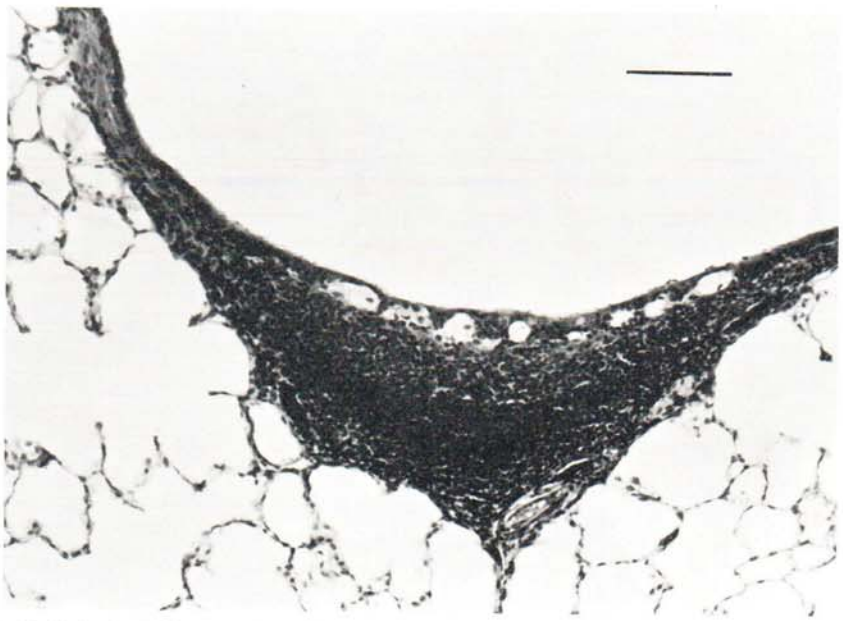

FIG. 5—Collection of "macrophage-like" cells in the bronchus associated lymphoid tissue of a rat lung after inhalation of glass microfibres. The cells contain accumulations of fibrous material. Bar = 100 μm.

cells is but it has been suggested by Green[7] that collections of lymphoid tissue at the bronchoalveolar junction provide an exit for dust laden macrophages which have entered the interstitium.

The fibrogenic activity of asbestos and silica has been related to the chemical nature of these materials[1]. In both cases the mineral dusts are thought to react in a specific way with the membrane of the phagolysosome which is formed in the macrophage during its attempt to "digest" the material. In the case of silica there may be leakage of enzymes from the phagolysosome causing destruction of the cell. In the case of asbestos, under certain circumstances, selective release of lysosomal enzymes has been demonstrated[6]. The release of certain factors from the macrophage may lead directly or indirectly to stimulation of collagen production by fibroblasts.

Although long glass microfibres when phagocytosed by macrophages in culture cause a general slow leakage of the cell contents[4] no specific interaction of the glass fibre with the phagolysosomal membrane has yet been demonstrated. It is becoming clear that long fibres are more capable of producing fibrosis in the lung than short fibres of the same material[18] but it does not appear that the physical shape of the particle is the only factor in determining its fibrogenicity. Surface chemistry may well be an important contributing factor and with non-fibrous particles of silica, for example, it may be of much greater importance than the shape.

Another factor which may influence the apparent fibrogenic potential of a material is the rate at which it is cleared from the lung. It is possible that the fibrous nature of asbestos and glass fibre lead to less efficient clearance and hence a longer residence time in the lung. In the case of glass fibre which appears to be of low cytotoxicity the relatively innocuous nature of the material may allow extremely high loadings in the macrophage. Consequently the macrophage may be impeded in its movement out of the lung, particularly if fibres of greater than 15 micrometers are ingested, and aggregations of macrophages containing glass fibres may form. The presence of large aggregations of macrophages may lead to granuloma formation and subsequent localised collagen deposition. This type of response would only occur when relatively large amounts of glass fibre are present in the lung, for instance, after intratracheal instillation into experimental animals. This type of response has not yet been seen after inhalation of glass fibres by experimental animals.

It would be of value to investigate, in depth, the specific interaction between pulmonary macrophages and long glass fibres and, in particular, to determine whether loading of the macrophage with such fibres reduces their mobility or increases their tendency to form aggregations. Such studies, carried out on cultured cells, would provide valuable insight into the behaviour of the loaded macrophages in the lung. The carcinogenic effects of fibrous materials are outside the scope of the present investigation but the literature would tend to indicate that the physical shape of the particles may be of more significance than the chemical nature in determining the carcinogenic potential of fibres. In summary therefore it is considered that there are two properties of fibres which

relate to their fibrogenic potential; the chemical nature and the physical shape. It is an interaction of the two properties which determines the fibrogenic potential of an inhaled particle. In the case of cytotoxic particles such as silica the chemical nature would appear to be the most important. In the case of fibrous particles of apparently innocuous materials such a glass fibre the physical shape of the fibre may assume greater importance. If long thin fibres gain access to the lungs in large amounts they may be capable of causing immobilisation of the macrophages and subsequent localised pathological lesions. The results of animal experiments would suggest that it is unlikely that such high concentrations of glass microfibres of sufficient lengths for this reaction to occur could be achieved by inhalation. The results of intratracheal instillation experiments carried out with glass microfibres are of limited relevance to human experience.

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