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This is the paper that prompted the 4/8/81 article in "Chemical Week" on "long fiber asbestos is the bad actor".

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THE INFLUENCE OF VARYING LENGTHS OF GLASS AND ASBESTOS FIBRES ON TISSUE RESPONSE IN GUINEA PIGS

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Abstract—Intratracheal injection of samples of naturally occurring and man-made mineral fibres into guinea pigs showed that while long fibre samples produced marked fibrosis, short fibre specimens produced only a macrophage reaction. In most cases the long fibre samples were administered in smaller doses than the short. The samples tested were crocidolite asbestos, a synthetic fluoramphibole and two specimens of glass fibre with different mean diameters.

With all the minerals tested some short fibres, but not long fibres, were transported to the hilar lymph nodes. In some instances the numbers of short fibres found in these nodes appeared to be much higher than would be expected from the percentage of short fibres in the original sample, and it is suggested that this may be due to the breakdown of long fibres within the lung.

THERE is the growing conviction that the fibrogenic effect of asbestos is size dependent. An increasing number of publications demonstrate the lack or relative lack of fibrotic response to "short" fibres and the dependence of fibrosis on the presence of "long" fibres, short and long being variously defined.

These observations are strongly supportive of the concept that stimulation of connective tissue proliferation is a function of the physical properties of fibres rather than of their chemical characteristics. If this is so, it would seem reasonable to expect that fibrous materials other than asbestos might evoke similar reactions. The availability of a series of fibres of asbestos and of glass carefully characterized as to size prompted us to examine and compare the biological response to different fibre types. The test system employed was intratracheal instillation into halothane anaesthetized guinea pigs of suspensions of fibre in distilled water.

In the complete study 28 different groups of 30 animals each received injections of 3 to 25 mg of fibre administered in 2 to 8 instillations. The number of injections and the amount of each injection was in large part determined by the quantity of material that could be held in suspension and instilled without too much clumping and aggregation.

For the purposes of this brief presentation, we have chosen to demonstrate the contrasting effects of long and short fibres of 4 pairs of materials, 2 forms of asbestos and 2 of glass. In the course of the study, animals were sacrificed at 6 months, 1 year and 2 years. We shall only present the findings at 2 years after the last injection.

The first pair of samples is of crocidolite fibres. The long fibre sample is one in which more than 80% of the fibres are over 10 μm in length while the short sample is one in which more than 99% are less than 5 μm in length (Fig. 1). In both samples 70% of the diameters range from 0.10 to 0.30 μm. The long-fibre group received a total dose of 4 mg and the short-fibre group received 25 mg.

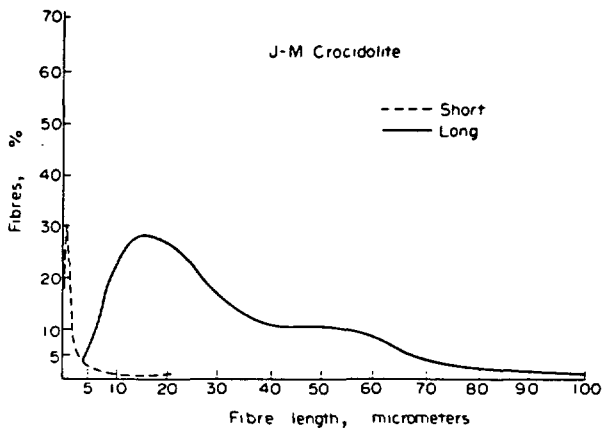

FIG. 1. Schematic representation of fibre lengths of short and long samples of crocidolite.

The long sample produced extensive interstitial fibrosis, most marked in those portions of lung abutting on the terminal bronchioles and involving the respiratory bronchioles and proximal alveoli (Fig. 2a).

In contrast the short sample of crocidolite produced no fibrosis (Fig. 2b).

Higher magnification of the reaction to long fibre reveals an occasional visible fibre in the fibrotic interstitium with asbestos body formation (Fig. 3a).

Some short fibre is retained in the lung within aggregates of macrophages (Fig. 3b).

Within the hilar nodes of the animals exposed to long fibres, there are macrophages containing fibres too small to be resolved (Fig. 4a). None of these are long and thus must represent the result of a "sieving" effect by which the long fibre remains in the lung while the small proportion of fibres which are short are phagocytosed and translocated to the lymph nodes.

It is worthy of note that these short fibres do not lead to fibrosis and in the lymph nodes of animals exposed to short fibres there are many more macrophages with ingested fibre but again without fibrosis (Fig. 4b).

The second set of samples consists of long and short fibres of a synthetic fluoramphibole. Diameters are for the most part less than 1.0 μm although 3.5% of the long fibres are 1 to 2 μm in diameter. The long fibres are such that 16% are longer than

FIG. 2a. Long crocidolite. There are extensive areas of fibrosis abutting on the terminal bronchioles and involving respiratory bronchioles and proximal alveoli (× 10).

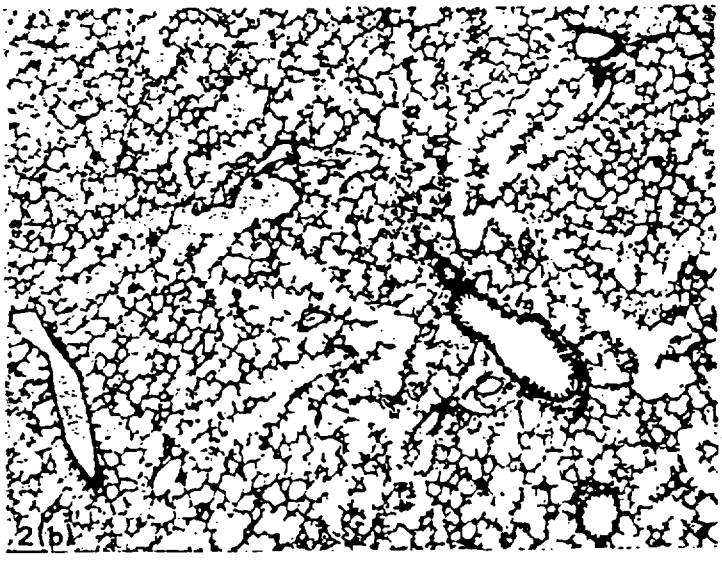

FIG. 2b. Short crocidolite. Fibrosis is absent. Aggregates of macrophages are seen in some alveoli (× 10).

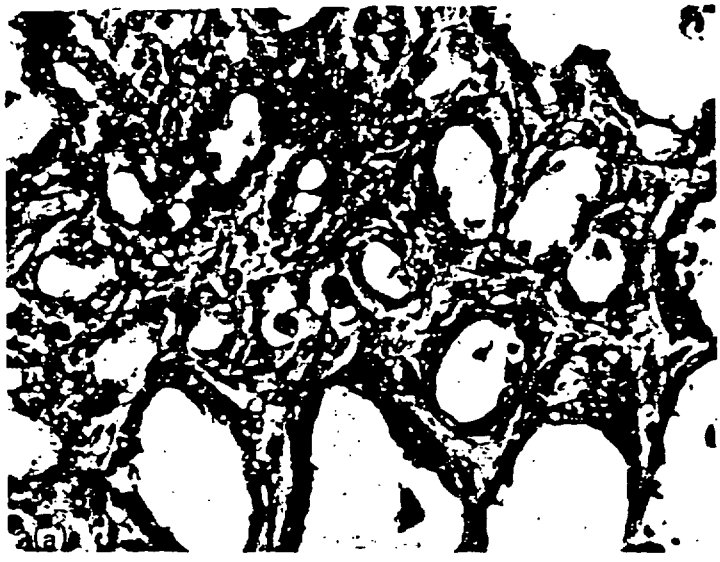

FIG. 3a. Long crocidolite. Dense interstitial fibrosis is seen in the lung. An occasional fibre is visible in the fibrotic interstitium (× 600).

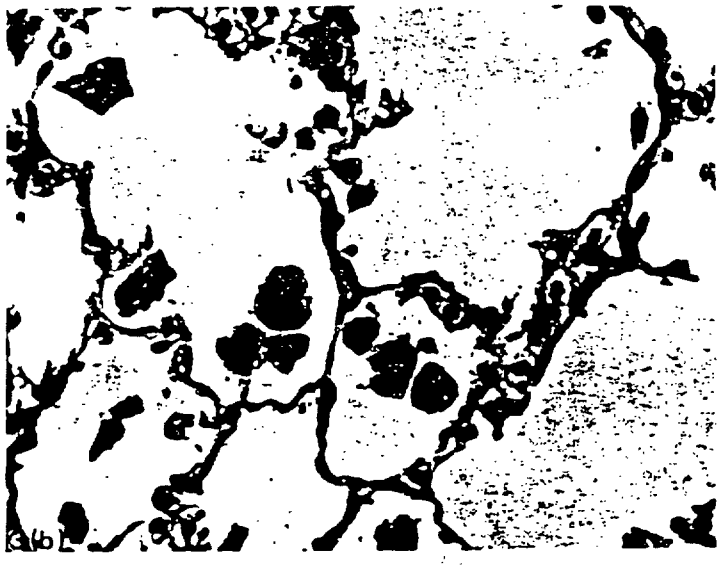

FIG. 3b. Short crocidolite. Short fibres are retained in the lung within macrophages but without fibrosis (× 600).

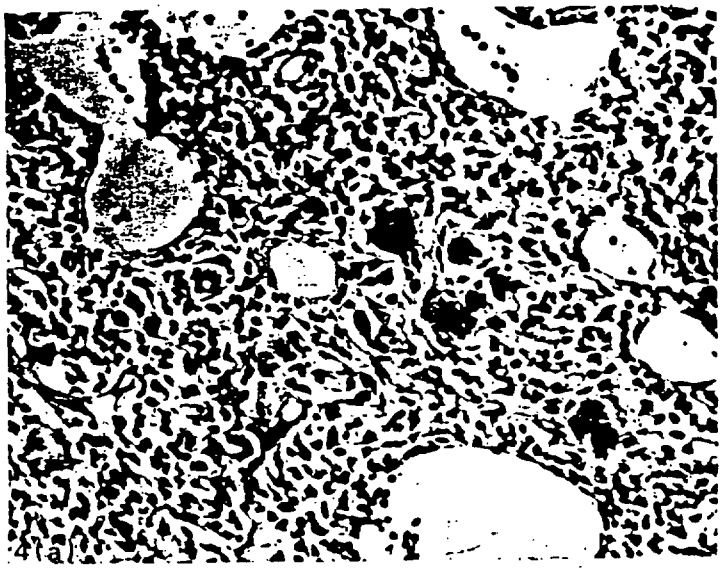

FIG. 4a. Long crocidolite—lymph node. "Sieved" short fibres in macrophages are present within lymph nodes of long-fibre recipients. There is no fibrosis (× 600).

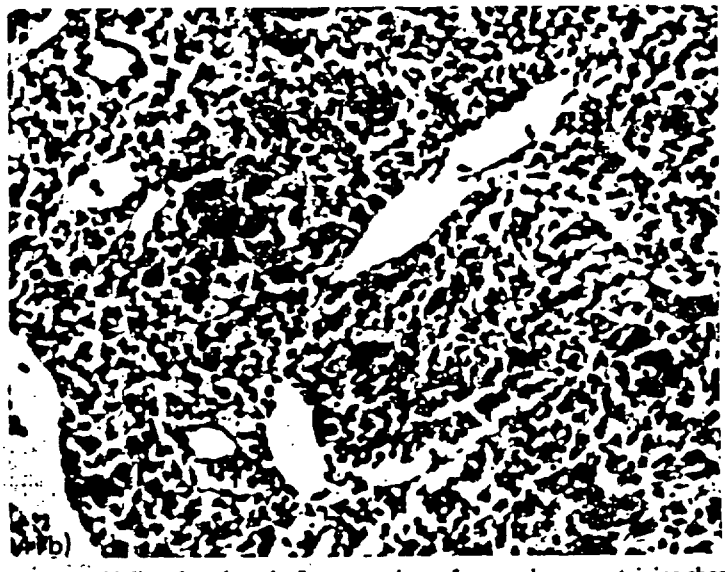

FIG. 4b. Short crocidolite—lymph node. Large numbers of macrophages containing short fibres are seen in the lymph nodes. There is no fibrosis (× 600).

10 μm and 43% are longer than 5 μm. The short-fibre sample consists of more than 99% of fibre less than 5 μm (Fig. 5). Animals exposed to both long and short fibres received a total dose of 12 mg.

The long fibres of this synthetic fluoramphibole again produced striking interstitial fibrosis (Fig. 6a). The short fibres of fluoramphibole left the lung unaltered (Fig. 6b).

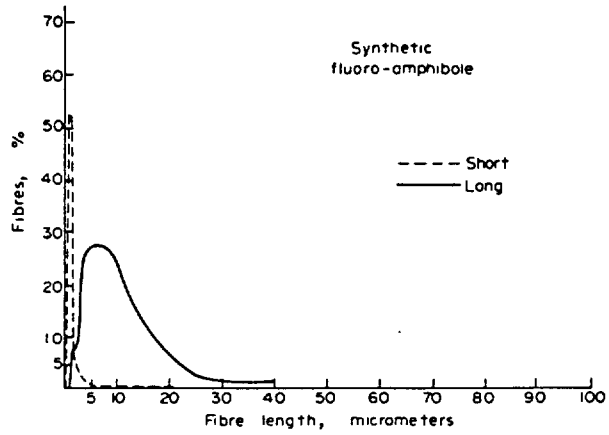

FIG. 5. Schematic representation of fibre lengths of short and long samples of a synthetic fluoramphibole.

In the long-fibre recipients, an occasional asbestos body was observed in association with areas of fibrosis (Fig. 7a). In the short-fibre recipients, an occasional macrophage containing an identifiable fibre was noted (Fig. 7b).

The lymph nodes of the guinea pigs receiving long fibres showed small groups of macrophages containing what must be assumed to be "sieved" smaller fibres (Fig. 8a). The lymph nodes of the short fibre recipients contained large aggregates, indeed sheets, of macrophages but in neither was there fibrosis (Fig. 8b).

Consider the relative effects of a pair of samples of glass fibre of diameters ranging from 0.1 to 1.0 μm. The long fibres are such that 92% are longer than 10 μm while the short-fibre sample contains 93% of fibres which are less than 10 μm in length (Fig. 9). The long-fibre group received a total dose of 12 mg and the short-fibre group received 25 mg.

The long fibre produces a fibrotic lesion which, as in asbestos, involves the area of lung immediately about the terminal bronchiole (Fig. 10a). Although similar in location and character, the quantitative difference between this reaction and that produced by asbestos is striking. The short fibres in this set of samples produce no change other than macrophage aggregation in the alveoli (Fig. 10b).

Occasional fibres can be identified in areas of fibrosis after long-fibre exposure (Fig. 11a). In the short-fibre recipients, the macrophages are stuffed with fibre but unassociated with fibrosis (Fig. 11b).

In the long-fibre recipients the lymph nodes contain large numbers of small fibres in

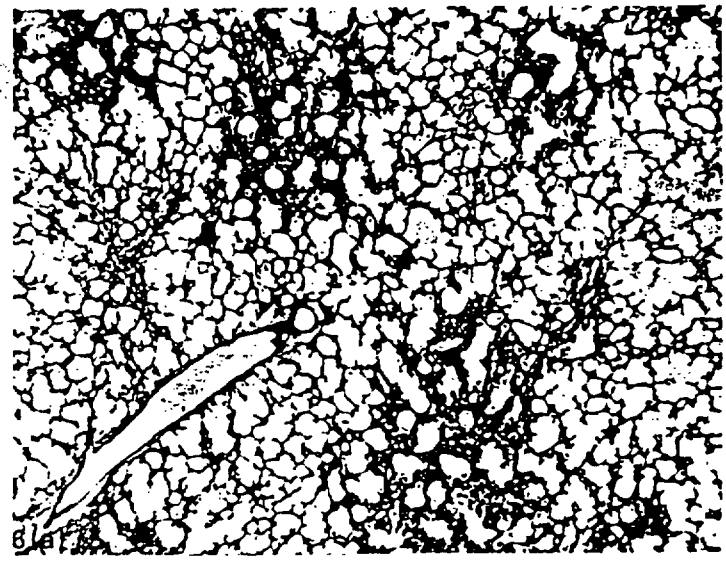

FIG. 6a. Long fluoramphibole. Peribronchiolar fibrosis involves the interstitium about the terminal bronchioles (× 10).

FIG. 6b. Short fluoramphibole. The lung is free of fibrosis. Aggregates of macrophages are present within some alveoli (× 10).

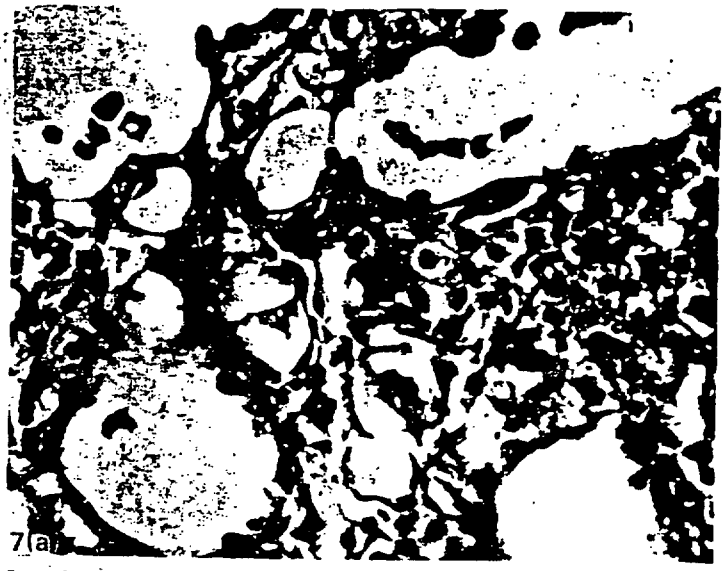

FIG. 7a. Long fluoramphibole. Occasional fibres and "asbestos bodies" are seen in areas of dense interstitial fibrosis (× 600).

FIG. 7b. Short fluoramphibole. There is no evidence of fibrosis. An occasional fibre or intracellular "asbestos body" is seen within macrophages (× 600).

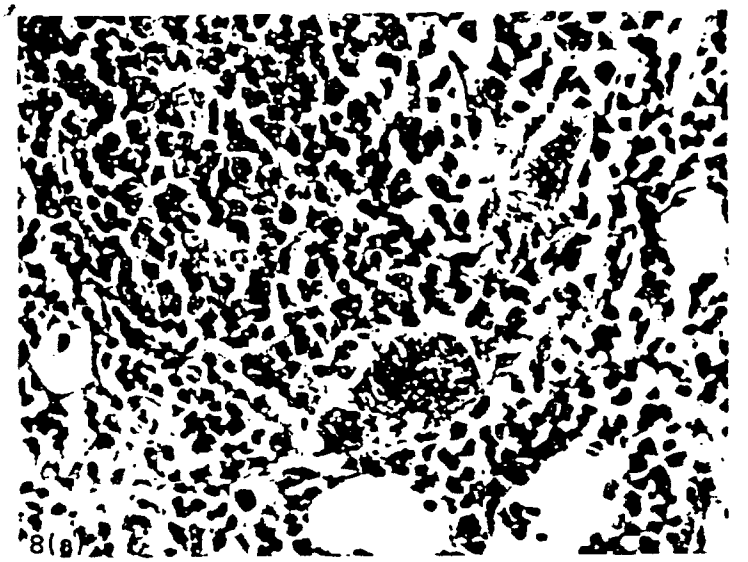

FIG. 8a. Long fluoramphibole—lymph node. "Sieved" shorter fibres are seen within the macrophages of the lymph nodes of long-fibre recipients. There is no fibrosis (× 600).

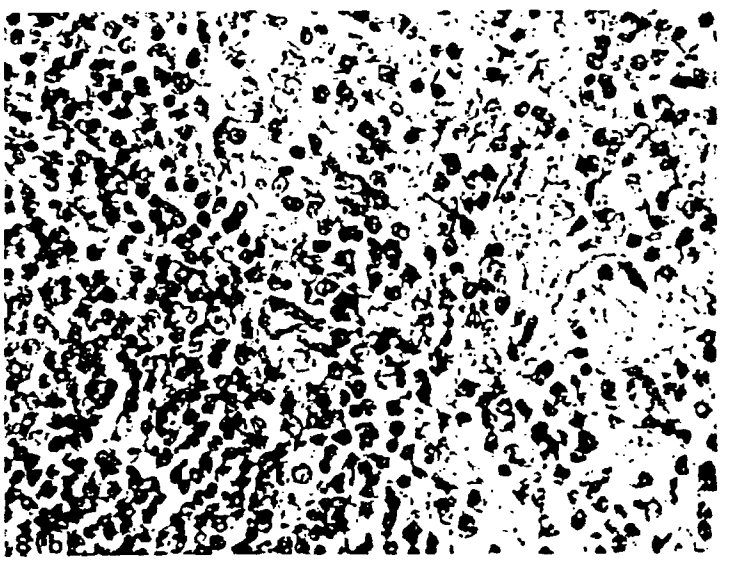

FIG. 8b. Short fluoramphibole—lymph node. Sheets of macrophages are present within the lymph nodes of the short fibre recipients. No fibrogenic reaction is evoked (× 600).

amounts which appear out of proportion to the numbers of small fibres in the original sample (Fig. 12a). The lymph nodes of the small-fibre recipients contain, as one might expect, large numbers of fibre-laden macrophages (Fig. 12b). In neither case is there significant fibrosis.

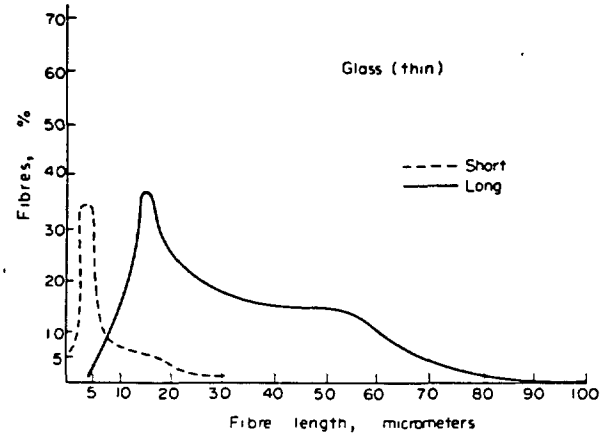

FIG. 9. Schematic representation of fibre lengths of short and long samples of thin glass fibres.

Another pair of samples of very thin glass fibre was used in which mean diameters were less than 0.10 μm. Long fibres were such that 50% were longer than 10 μm and the short fibres were all less than 5 μm in length (Fig 13). Long fibres were administered to a total dose of 12 mg while the total dose of short fibres was 25 mg.

The long fibre again produced a minimal but definite lesion in the same vulnerable area, the zone abutting on the terminal bronchiole (Fig. 14a). The instillation of short fibre did not result in fibrosis of any degree (Fig. 14b).

Fibres of this very fine glass were, of course, not visible in the small but definite areas of cell proliferation and fibrosis found in the long-fibre recipients (Fig. 15a). There was a macrophage reaction in the short fibre recipients (Fig. 15b).

Sheets of macrophages were present in the "long-fibre lymph nodes" (Fig. 16a) and were more marked in the "short-fibre lymph nodes" (Fig. 16b) but fibrosis was not seen in either.

We believe the following conclusions may be drawn from these observations.

Long fibres (longer than 10 μm) of asbestos produce fibrosis. Short fibres (those less than 10 μm) do not produce fibrosis in either lung or lymph nodes. This is confirmatory of a number of investigations of the comparative fibrogenicity of asbestos fibres of various lengths introduced into the lung, the pleural cavity and the peritoneal cavity (VORWALD *et al.*, 1951; TIMBRELL and SKIDMORE, 1973; BURGER and ENGELBRECHT, 1970; HILSCHER, 1970; WEBSTER, 1970; GROSS, 1974; BECK *et al.*, 1971).

Although the fibrogenicity of glass has been questioned, it would appear that long fibres of this material are also fibrogenic. The uncertainty as to its fibrogenicity in past experiments (GROSS *et al.*, 1970) and the marked quantitative difference between its

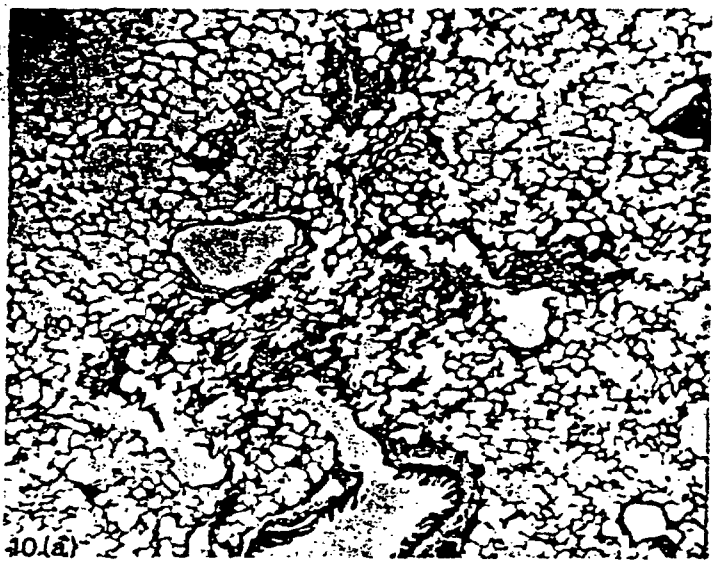

FIG. 10a. Long thin glass. This area represents the zone of most marked fibrotic reaction among all the animals so exposed. The reaction was much less marked in most animals (× 10).

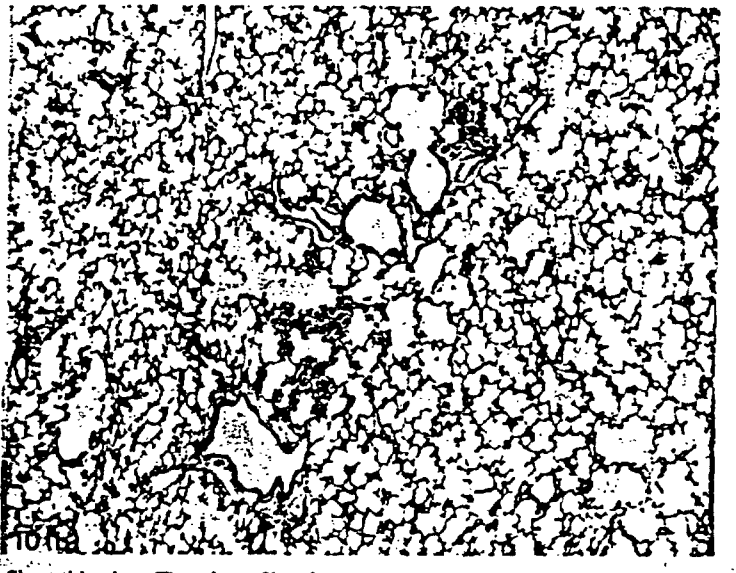

FIG. 10b. Short thin glass. There is no fibrosis. Aggregates of macrophages are seen in groups of alveoli (× 10).

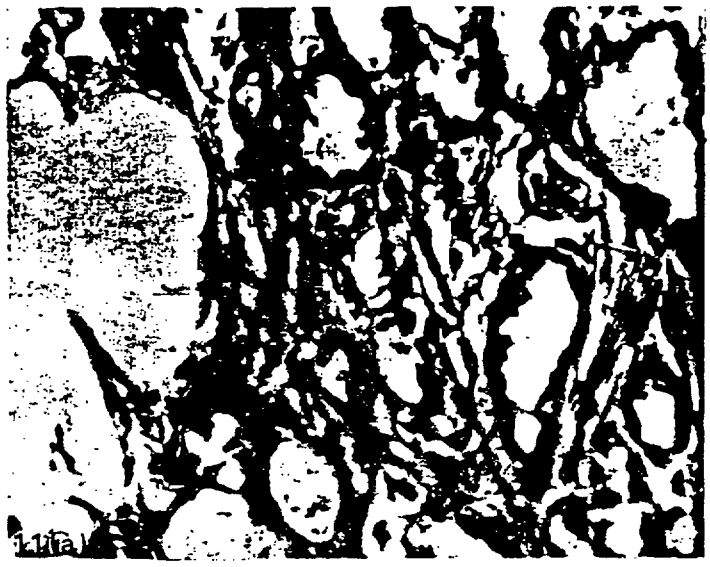

FIG. 11a. Long thin glass. Glass fibres are seen in those interstitial areas which are the site of fibrosis (× 600).

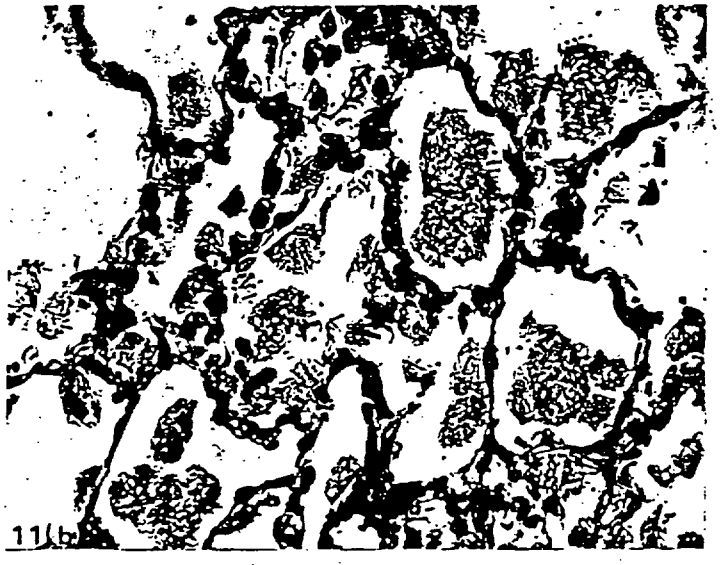

FIG. 11b. Short thin glass. Large numbers of glass fibre are seen within aggregated short fibres within the alveoli. No fibrosis has resulted (× 600).

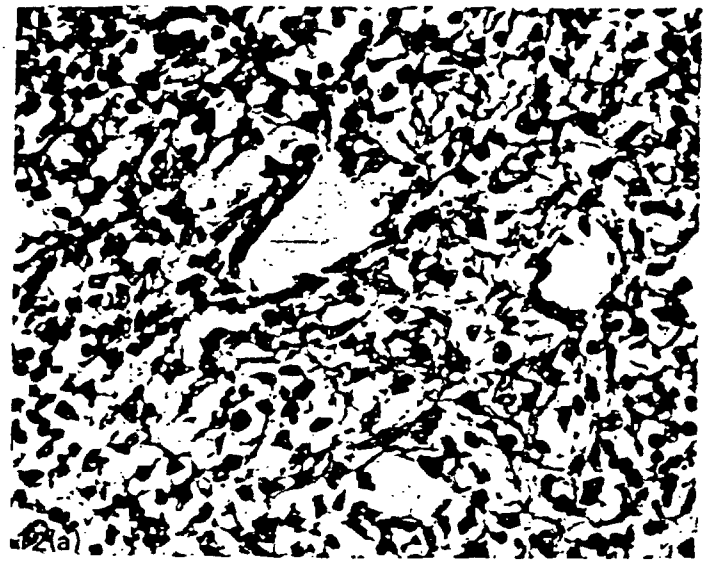

FIG. 12a. Long thin glass—lymph node. A surprisingly large number of short fibres are seen in the lymph nodes of long-fibre recipients. These may be the result of fragmentation of long fibres as well as of the presence of some short fibre in the sample. There is no fibrosis (× 600).

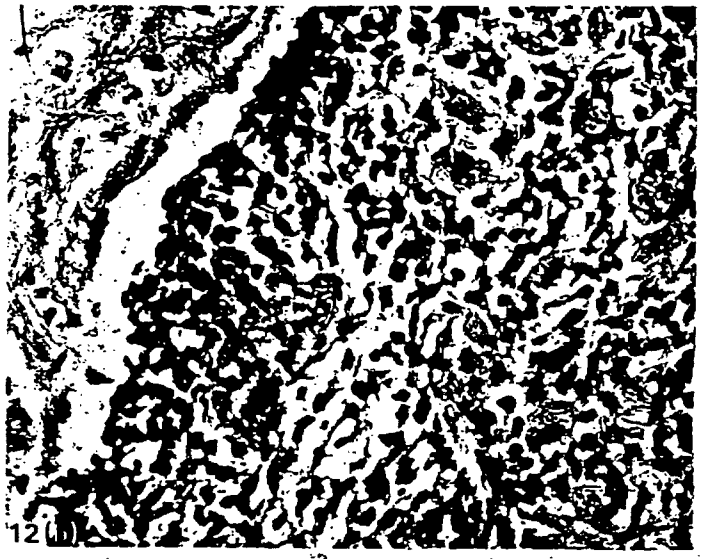

FIG. 12b. Short thin glass—lymph node. Large numbers of fibre-laden macrophages are seen within the lymph nodes. No fibrogenic reaction is evoked (× 600).

effects and those of asbestos in the present series of experiments, we believe to be a consequence of the lesser durability of a long glass fibre as compared to the durability of asbestos. In the experiments in which long glass fibre was introduced, a surprising amount of short fragments of fibres appear in the lymph nodes. This fragmentation is confirmed by electron micrographic studies of ashed lung and lymph nodes of animals in which long fibres had been introduced.

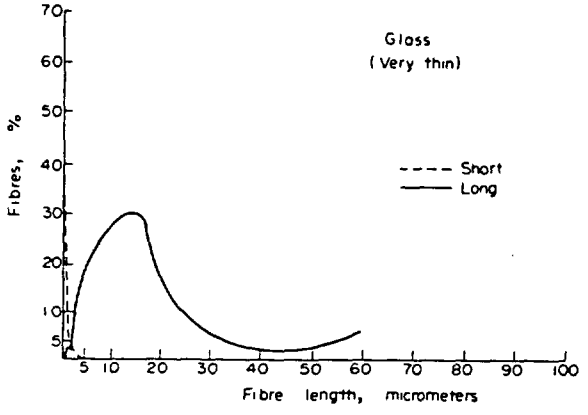

FIG. 13. Schematic representation of fibre lengths of short and long samples of very thin glass fibres.

The mechanism which best explains the similarity of response to a variety of asbestos fibres and to glass is one well worked out for granulocytes (HENSON, 1972) and has been extended to macrophages (BECK *et al.*, 1971; BRUCH, 1974). Cells attempting to engulf long fibres are involved in incomplete or "frustrated" phagocytosis. The process known as exocytosis results in leakage of tissue damaging enzymes from the cell without being specifically toxic to the phagocyte. It is reasonable to presume that tissue damage so produced is the ultimate inciter of fibrosis.

The experiments described and the foregoing discussion relate only to fibrosis. All of this may not, however, be unrelated to tumour induction. Here, too, a specific size dependence of carcinogenicity appears to be emerging (STANTON and WRENCH, 1972; SMITH *et al.*, 1972; MAROUDAS *et al.*, 1973). There is the strong suggestion that the co-carcinogenicity of asbestos with cigarette smoking is the result of the induction of a particularly vulnerable cellular substrate on which the tobacco-derived carcinogen can operate. This substrate is the proliferating peripheral epithelial reaction that accompanies the fibrosis of asbestosis. This may account for the excessive incidence of adenocarcinoma in the lung cancer of asbestotic workers (WHITWELL *et al.*, 1974).

Further, the development of experimental mesothelioma seems to occur *pari passu* with the fibrosis engendered by tumorigenic fibres and argues for a form of malignant transformation not unlike that induced by plastic films, so-called "solid state" carcinogenesis (BRAND, 1975).

FIG. 14a. Long very thin glass. A mild interstitial fibrosis is noted in the walls of the respiratory bronchioles and adjacent alveoli. Macrophages are also seen within alveoli (× 10).

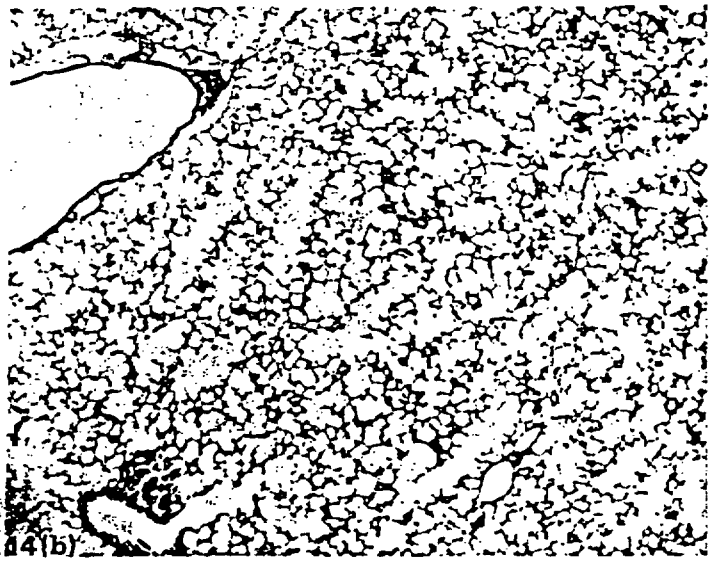

FIG. 14b. Short very thin glass. Except for focal aggregates of intra-alveolar macrophages, the lung is unaltered (× 10).

FIG. 15a. Long very thin glass. There is minimal interstitial fibrous thickening together with increased cellularity in alveoli abutting on respiratory bronchioles. Macrophages are also present within air spaces (× 600).

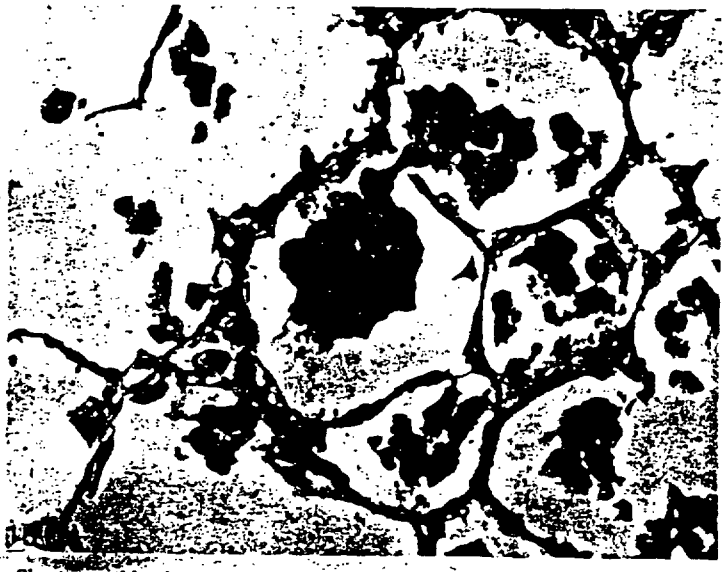

FIG. 15b. Short very thin glass. Aggregates of macrophages are present in focal groups of alveoli. There is no fibrosis (× 600).

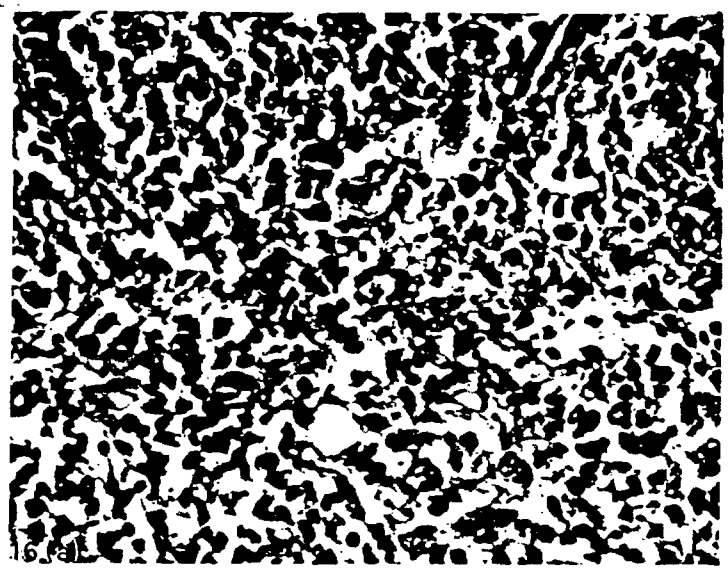

FIG. 16a. Long very thin glass—lymph node. Sheets of macrophages are present within the lymph nodes of the long fibre recipients. There is no fibrosis (× 600).

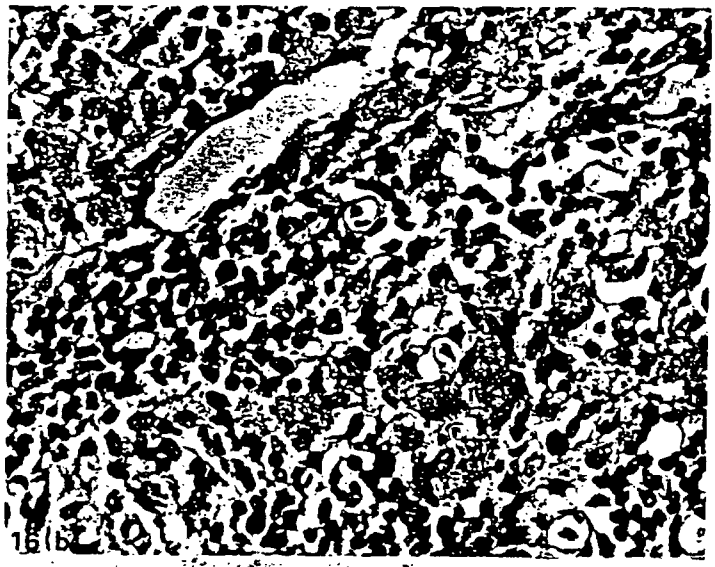

FIG. 16b. Short very thin glass. Large numbers of macrophages are present and have a refractible granular appearance. Individual fibres are not resolvable (× 600).

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DISCUSSION

M. LIPPMANN: For equal masses of fibre instilled in a pair of tests, there would be far greater numbers of the shorter fibres, which would reinforce the conclusion that long fibres are the most important.

Dr KUSCHNER: The masses were not always equal, but when unequal, the short fibre mass was larger. For instance, in the first crocidolite sample, there were 4 mg of long and 25 mg of short fibres, so that you are correct about the number of fibres.

J. C. WAGNER: Why did you not use inhalation experiments rather than intratracheal methods which completely overpower the defence mechanisms of the lung?

Dr KUSCHNER: All animal experiments are unrealistic; one must be quite clear what question one is asking of the experiment. We were not asking about the comparative effects of materials inhaled under natural conditions; but about the differences between the biological effects of series of fibres differing in size which were sure to get into the lung.

G. V. COLES: Were any investigations made of pleural effects after the investigations of fibrogenicity?

Dr KUSCHNER: The pleural reaction was examined with the remainder of the lungs. There was no parietal pleural plaque formation. In animals with a massive fibrosis produced by asbestos there was pleural fibrosis but not mesothelioma. There were no tumours produced in this series of guinea pigs, all of which were sacrificed after 2 years.

R. HUNT: Human studies confirm the fibrogenicity of long asbestos fibres, i.e. 25 μm, as opposed to shorter fibres which are transported away and dealt with by the R. E. system.

Dr KUSCHNER: In an earlier publication, Timbrell and his group made the point that one of the differences between long and short fibres was residence time in the lung. Even if the differing effect these materials have on cell populations is ignored the question of residence time in the lungs must still be considered.

C. J. GÖTHE: The photomicrographs seem to show a considerably larger difference between the histological tissue reactions caused by long-fibred asbestos and long-fibred glass than between the tissue reactions caused by long-fibred and short-fibred glass. How do you explain this difference between asbestos and glass with similar fibre type? Is it due to differences in the physical or chemical properties of your asbestos and glass fibre samples?

Dr KUSCHNER: There was no reaction to the short-fibred glass, but there is, as you say, an extraordinary difference between short- and long-fibred asbestos. This relates to the fact that asbestos is a much more efficient fibrogenic agent and I attribute this in part to its durability. As to the differences between long-fibred asbestos and long-fibred glass, I think that the difference is perhaps based on the fact that glass does not persist in its long-fibred form. If it did, it might be much more like asbestos. A difference can be seen between chrysotile fibrils and other forms of asbestos; possibly because the fibrils are more fragile.

J. BRUCH: Five years ago we tested the fibrogenicity of short and long asbestos fibres and found a strict correlation between the fibre length and the degree of fibrosis. The short fibres were preferentially transported into the lymph nodes. Four months after a single i.p. injection of U.I.C.C. crocidolite and amosite, a relative increase of the longer fibres in the fibrotic lesions compared to the original frequency distribution of the fibre lengths could be observed, the shorter fibres on the other hand predominated in the lymph nodes.

Dr KUSCHNER: Yes, your very clear description of this phenomenon is acknowledged in our paper. I believe that you made the point that the small fibres in the lymph nodes did not produce fibrosis.

F. F. HAHN: Have you made any attempt to quantitate the pulmonary fibrosis, by collagen determinations, or by morphometric or other techniques?

Dr KUSCHNER: No, we did not, but I think it would be very useful. This effect was so remarkable that we presented it without collagen or hydroxy-proline determination.

Dr. J. C. GILSON: Can you give us information about changes in mass and size distribution of fibres within the animals' lungs during the 2 years of observation? You hinted that the glass fibres were being altered.

KUSCHNER: We are just beginning to do that and the concept I advanced was based on an impression derived from the low-temperature ashing of a series of lungs which are now ready to be quantitated. It will be important to do counts at 6 months, 1 year and 2 years after inhalation of fibres of which we have detailed characteristics and see how they compare. Our strong impression, based on the visual observation of electromicroscopy at 1 year and 2 years after inhalation, is that the glass fibres do indeed fragment and that we will have a much larger number at the end of the experiment. We are digesting the lung and the lymph nodes together. It could be useful to make a distinction

because a lot of the short fibre that we demonstrated in the total sample may be in nodes even in the animals that received long fibres as a result of fragmentation and carriage to lymph nodes.

L. MAGOS: I would like to suggest that you present the size distribution of fibres in columns and not by continuous lines which give a false impression.

Dr KUSCHNER: We tried it that way. It would have been difficult to superimpose the two. I think the proper way to do it is to plot a cumulative size distribution.

M. CORN: We performed a laboratory study to determine changes in lengths and diameters of chrysotile asbestos fibres and glass fibres with milling (ASSUNCAO, J. and CORN, M., *Am. ind. Hyg. Ass. J.*, 1975, 36, 811–819). We found that the asbestos fibres became shorter and diameters were reduced but, in the case of glass fibres, diameters remained unaltered and lengths of fibres were reduced. If a 3 : 1 aspect ratio is used to define a fibre, then the percentage of fibres relative to total particles (fibres and non-fibres) decreased with milling. Your experiments suggest a similar shortening of glass fibres after deposition in the lung.

Dr KUSCHNER: I should be surprised to see them reduced to particles, but I am reasonably confident that they are being reduced in size.