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Respirable industrial fibres: mechanisms of pathogenicity

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Background

Fibrous materials have much to commend them in industrial applications, where they can offer, in varying degree, reinforcement, thermal and electrical insulation, flexibility, and strength. In the first part of this century the fibrous material that was available, cheaply and in bulk, that could provide these properties to industry was the asbestos group of silicate minerals (table 1). The fibrous, crystalline, asbestiform habit, however, also means that during mining and industrial use fibres are released into the air. Some of these are in the respirable size range and may be deposited in the bronchiolar-alveolar region. Subsequently we have come to realise that asbestos inhalation is associated with several different lung diseases—namely, asbestosis (interstitial fibrosis), carcinoma of the lung, mesothelioma, and small airways disease—and the association is now well documented. This information on the harmful effects of asbestos has led to bans in some countries and considerable litigation and claims for damages throughout the developed world. Regulatory and legal pressures on asbestos have contributed to an increase in the use of alternative materials, both natural and man-made, including some fibrous materials. More importantly, advances in materials science have identified an ever widening range of applications in which the fibrous nature of a material offers positive advantages. Thus in addition to their traditional use in thermal insulation new fibre composites have found new uses, such as metal reinforcement, to which asbestos was never suited (table 2).

The finding that in at least some biological assays some non-asbestos fibres could cause the same types of effect as asbestos has raised the question of whether all fibres have the same pathogenic potential as asbestos. A major approach in present research on fibres is therefore to compare any fibre under study with asbestos and to gauge the potential for causing lung disease in the light of experience with asbestos. Thus the spectre of asbestos permeates all discussion of research into respirable fibres, of whatever origin, leading to a complex interplay of science, politics, and commercial pressure.

This review aims to address only the scientific ideas that dominate research into the mechanisms underlying the development of diseases caused by respirable industrial fibres. The constraints of space and the breadth of the published research prevent this review from being comprehensive. Further details can be obtained from recent reviews on mechanisms of fibre pathology[1] and on non-asbestos fibres and their health effects in general.[2-4]

Role of fibre size, shape, and chemistry

DEPOSITION

A fibre, as defined by the World Health Organisation for measurement of the exposure of workers to airborne fibres in factories and mines, is a structure longer than 5 μm, less than 3 μm in diameter, and with an aspect ratio (length:diameter) greater than 3:1. The deposition of a particle in the lung is a function of the "aerodynamic diameter" (particle size expressed in terms of settling speed). For fibres this is governed predominantly by the actual diameter, density and length being of subsidiary importance. Only fibres with a physical diameter of 3 μm or less may be deposited in the alveolar region, the efficiency of deposition increasing with decreasing diameter to a physical diameter of about 1 μm; beyond this the efficiency of deposition may begin to decrease again. Subsequently thin fibres will be deposited in the alveolar region of the lung even if they are

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Table 1   *The asbestos minerals*

Serpentine group	Amphibole group
Chrysotile	Crocidolite
	Amosite
	Anthophyllite
	Tremolite
	Actinolite

Table 2   *Industrial fibres other than asbestos*

Natural	Manmade
Erionite	Slag wool
Wollastonite	Rock wool
Attapulgite	Glass wool
Sepiolite	Continuous filament glass
Halloysite	Ceramics
	Alumina
	Zirconia
	Silicon carbide
	Graphite
	Boron
	Aromatic amide

very long (up to 30 or 40 μm). In contrast, for compact particles density is more important, and particles with diameters greater than about 5 μm are unlikely to reach the alveolar region. The site of the deposition of fibres in the lungs of rats was elucidated by the elegant studies of Brody *et al*[5] and Warheit *et al*[6] who used microdissection and scanning electron microscopy to follow the fate of chrysotile asbestos fibres delivered to the rat lung during a single exposure. These fibres were found to be deposited almost exclusively around the terminal bronchioles and the first alveolar duct bifurcations, where the fibres could first be seen lying free and later phagocytosed by macrophages. Our own studies, using amosite asbestos and glass fibres, have confirmed this sequence of events and fibres up to 35 μm long can be seen lying on the epithelium of the terminal bronchioles and the alveolar ducts and inside macrophages (fig 1).

FIBRE LENGTH AND PATHOGENIC POTENTIAL
There has been some progress towards understanding the attributes of fibres that imbue them with the potential for causing pathogenic change. Pioneering studies, such as those of Wright and Kuschner,[7] and Stanton and Layard,[8] showed that short fibres (below 5 μm) are low in pathogenicity and that increasing pathogenicity accompanies increasing fibre length beyond 8–10 μm. In particular, the impressive studies of Stanton and co-workers[8] using implantation of fibres into the pleural space in the rat, showed that a range of quite different fibrous materials could all cause pleural mesotheliomas pro-

vided that there were fibres of the appropriate sizes. The optimum size range for the production of mesotheliomas in this system was over 8 μm long and below 0·25 μm in diameter.[8]

In the early 1980s the Institute of Occupational Medicine in Edinburgh obtained two samples of amosite asbestos, one containing a substantial proportion of long fibres and the other composed exclusively of short fibres. The short fibres were separated from the long by milling and sedimentation. The two samples were very similar in all other respects, such as diameter, elemental composition, and crystallographic features. Milling reduced a substantial proportion (63%) of the particles in the short fibre sample to an aspect ratio of less than 3:1—that is, they could not be defined as fibres. The remaining 37% were fibres by this criterion with an average length of 2·7 μm. These two samples have now been extensively studied by inhalation and instillation and some of the results are summarised in table 3. We believe this to be some of the most compelling evidence showing the importance of fibre size in determining the biological activity of respirable fibres.

One important factor that contributes to the pathogenic potential of long fibres is the evidence from several studies that long fibres are cleared from the deep regions of the lung less effectively than are short fibres.[11 12] This may be a consequence of failure of macrophages to ingest long fibres completely or of difficulty in moving once they have phagocytosed a long fibre. Long fibres may even interact with several macrophages, which may not then be able to act in concert. Such a reaction might mark the onset of a granulomatous response. With regard to the latter possibility, macrophages from the lungs of rats exposed to asbestos by inhalation have been found to have decreased ability to migrate towards a chemotaxin.[13] Thus long fibres could be more harmful to the lung because they are retained, whereas the short fibres are cleared more effectively. But although this presumably contributes to the toxicity of long fibres the evidence from studies using some in vitro assay systems that do not rely on clearance (see, for example, Brown *et al*[14] and Donaldson *et al*[15]) confirms that longer fibres have intrinsically more biological activity.
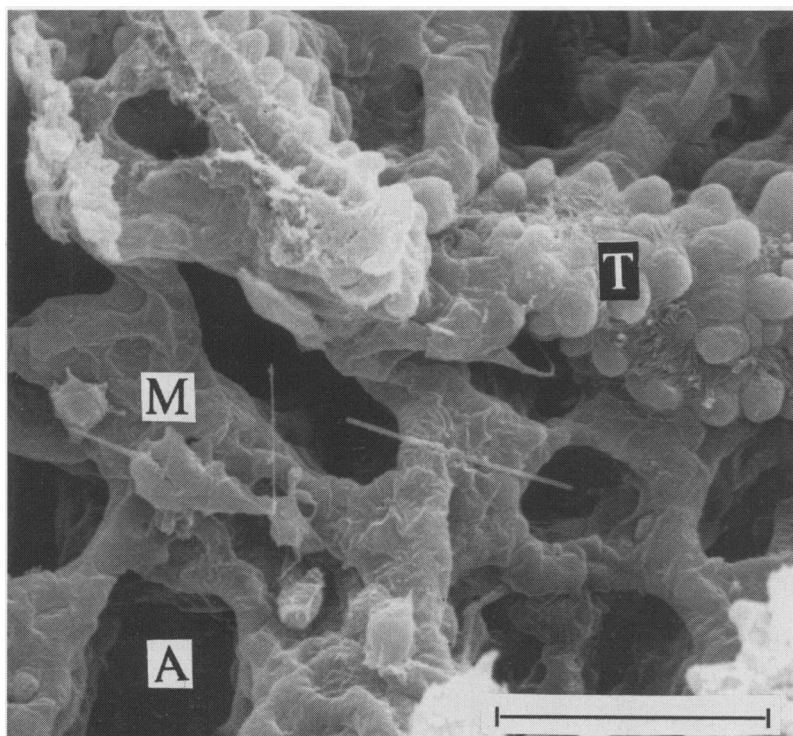

Figure 1 Scanning electron microscope image of the distal airspace of a rat that had been inhaling a cloud of 1000 fibres/ml of amosite asbestos for seven hours. An alveolar duct is shown with a portion of terminal bronchiolar epithelium present (T) and openings into alveoli (A). Asbestos fibres can be seen on the alveolar duct surface, both free and partially phagocytosed inside alveolar macrophages (M). (Scale bar = 40 μm.)

Table 3 Biological activity of long and short fibre samples of amosite asbestos in animal studies*

Response measure	Amosite fibres	
	Short	Long
Inhalation % with lung tumours (rats)	0	32·5
Mean fibrosis score (rats)	0	15·6
Intraperitoneal instillation		
% with mesotheliomas (rats)	0	88
Inflammation score (mice)	−/+	+ + + +

*Data summarised from experiments described in Davis *et al*[9] and Donaldson *et al*.[10]

ROLE OF CHEMICAL CHARACTERISTICS OF THE FIBRE SURFACE

Thus the role of fibre size has been well investigated and shown to be important, but less attention has been given to the chemistry of fibres. Of particular interest in this respect is erionite. This naturally occurring fibrous mineral of the zeolite family has been implicated in a high prevalence of mesothelioma in the small Turkish village of Karain, where it is present in the air owing to the weathering of rock.[16] In animal studies erionite produced an incidence of mesothelioma far higher than any other fibre, even though the dimensions of the fibres were not greatly different from those of fibres with less pathological potential.[17] Possibly therefore erionite has a composition that contributes unusually to its pathogenicity. Further evidence that the fibre surface can contribute to the biological activity comes from a study by Brown *et al*,[18] who substituted hydrocarbon chains of different length on the surface of amosite fibres without altering the number or length of the fibres. This treatment appears to alter the ability of the fibres to cause mesothelioma after instillation into the pleura.

There is also clear evidence in vitro that simply altering the protein that is attached to the fibre surface will change the biological activity. Treating asbestos fibres with rat immunoglobulin G in vitro dramatically increased its ability to stimulate macrophages to release the important inflammatory mediators superoxide anion[19] and tumour necrosis factor.[15] Presumably this is a direct effect mediated via the macrophage membrane receptors for the Fc portion of immunoglobulin G. Further research on the chemical nature of the surface of fibres and of the receptors responsible for the interactions of fibres with the cell surface are clearly warranted. In this regard Brown *et al*[20] recently implicated the fibronectin tripeptide receptor in the binding of amosite asbestos to cells in vitro.

The postulated role of free radicals in the toxicity of fibres[21 22] has focused interest in the role of iron at the fibre surface. Because of the ability of iron to enhance and catalyse the reactions of free radicals it has been suggested that fibres may act as a source of iron in tissue, leading to injury by free radicals. The evidence that iron is important has come from studies where iron chelators have ameliorated the toxicity of fibres to cells in vitro.[21 23 24] Recently iron from crocidolite asbestos has been shown to be important in the causation of breaks in DNA strands[25] and in the formation of 8-hydroxydeoxyguanosine (RC Brown, unpublished findings). Although iron may indeed be important in the toxic effects of types of fibre with a substantial content of iron, other fibres, such as chrysotile asbestos and some fibrous glasses, have little or no iron. In other cases it is the iron, mobilised from the fibre, that causes a short term effect; the relevance of this to the long term effect of fibres in vivo is not clear as such fibres become coated with biological

material, which itself may contain iron. Indeed, many fibres form ferruginous (asbestos) bodies, at least in man, so that iron is deposited on the fibre rather than leached from it. Iron derived from fibres is therefore unlikely to be an important unifying factor in fibre toxicity, but the relative importance of the surface and the dimensions of fibres has yet to be determined fully.

One area where fibre chemistry is likely to be an important factor in determining biological activity relates to the ability of fibres to persist in the lung once deposited there. Fibres that dissolve or break down in the lung are less likely to be dangerous because they may become shorter; short fibres are less active than longer fibres and are more efficiently cleared. In addition, epidemiological studies in workers exposed to the relatively soluble chrysotile asbestos suggest that this particular fibre is much less harmful in man than are other forms of asbestos.

Experiments with chrysotile fibres that have been treated to mimic the dissolution effects of residence in the lungs have shown that they do indeed have decreased biological activity[26] and are removed faster by the clearance systems of the lung.[11] As most glassy fibres are likely to be more soluble in the lung than even chrysotile asbestos possibly these will represent less hazard.

A substantial body of research at present is concerned with the persistence of various fibre types within the lung. In animal experiments even "soluble" fibres may persist in the lungs of rodents for a substantial portion of the animal's lifespan and thus are able to cause disease, whereas the same fibres persisting for the same time may not have the same effect in humans with their longer lifespan. This raises the possibility that "false positive" results might occur in animals because fibres are cleared by purely physicochemical means unrelated to the animals' lifespan. Negative results in such animal experiments should therefore be more reassuring than similar results with purely chemical toxins.

Mechanisms of disease caused by fibres

A substantial body of research has been directed at the interactions between fibres and cells in the hope of understanding the mechanism whereby deposited fibres cause the principal diseases associated with exposure.

EPITHELIAL CELLS

Brody *et al*[27] showed that inhaled chrysotile asbestos fibres could be found inside epithelial cells, and this was interpreted as a mechanism whereby fibres were translocated to the interstitium. Hobson *et al*[28] have also shown the uptake of fibres by airway epithelial cells in vitro. The effect of the interaction of fibres with epithelial cells is both frank toxicity[14 24] and, in some studies, a low level of sublethal genetic damage.[29 30] As well as acting as an initiator in causing direct DNA damage, asbestos is able to increase the frequency of

transformation effects caused by other carcinogens at doses at which asbestos alone has no effect.[31][32] These observations lead to the contention that asbestos acts as a tumour promoter, and indeed several different studies have found that asbestos has activities similar to those of classical promoters.[33]

Increased proliferation of epithelial cells is an early event following inhalation exposure to chrysotile asbestos in rats.[34] Increased cell proliferation is a common effect of promoters[33] and is likely to represent the restorative response to epithelial damage, combined with the response to local accumulation of inflammatory cell derived growth factors. A role for epithelial hyperplasia in the development of neoplasia associated with fibres therefore appears likely.

MACROPHAGES AND INFLAMMATION
Most deposited fibres are likely to be phagocytosed by alveolar macrophages if they remain on the lung surface for long enough (fig 2). Early research showed the ability of fibres to injure macrophages in vitro, and shortening of the fibres by milling reduced the toxic effects.[35] At that time fibre mediated death of macrophages was seen as an important pathogenic step. But, although fibres (mostly asbestos) were able to kill macrophages after phagocytosis a dose-response relationship could be seen, and low concentrations of fibre were not frankly toxic. As the roles of the macrophage became better understood and the subtleties of its interactions with other cells via various mediators became clear, research converged on the sublethal effects of low concentrations of fibres. The idea that fibres at low dose could stimulate macrophages to change their secretory repertoire has therefore come to predominate.

Particular attention has been given to proteases, cytokines, and oxidants because these have an obvious potential for mediating inflammation and pathological change. Table 4 shows the various secretory products of macrophages that have been found to be increased by exposure to fibres in vitro or in vivo.

Stimulation of macrophages by fibres to secrete their products, along with the epithelial events outlined above, culminates in inflammation, characterised by recruitment of more macrophages and neutrophils.[41] A hypothesis can be proposed about the events that occur as a consequence of this localised accumulation of inflammatory cells at the terminal bronchiolar and proximal alveolar region. Oxidants cause local injury and so contribute to loss of epithelial integrity, allowing passage of cells and proteins from the interstitium to the alveolar space; fibres and macrophages and their products may also gain access to the interstitium. Macrophage molecules that stimulate the mesenchymal cells (platelet derived growth factor, tumour necrosis factor, etc) can then signal an increase in production of fibroblasts and their matrix products, leading to fibrosis of the small airway walls and the alveolar walls. Oxidants and growth factors may contribute to "promoting" effects, such as proliferation, and so contribute to neoplastic change.

THE PLEURA AND MESOTHELIAL CELLS
Because of the association between asbestos exposure and mesothelioma, the effects of fibres on pleural mesothelial cells have been the subject of many investigations. Mesothelial cells in culture have been reported to be particularly susceptible to the toxic effects of fibres, far more so than epithelial cells or fibroblasts.[42] Mesothelial cells phagocytose fibres, leading to chromosomal abnormalities[43] and an increase in unscheduled DNA synthesis as evidence of DNA repair following damage.[44]

With regard to pleural fibrosis, the macrophage events described above in relation to alveolar fibrosis could occur in the most peripheral alveolar units. This would be in close proximity to, and may even be continuous with, the subpleural connective tissue and could lead to localised pleural fibrosis.

Although no clear role for the interactions between mesothelial cells and the leucocyte population of the pleural cavity has emerged, changes in the activity of the pleural
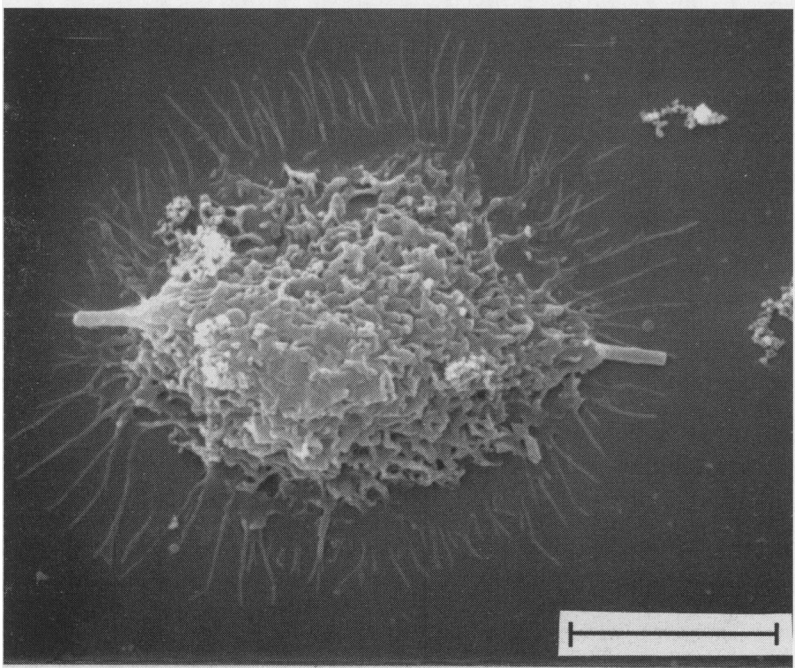

Figure 2 Scanning electron microscope image of an alveolar macrophage from the lungs of a rat exposed to amosite asbestos (see legend to fig 1). A single long fibre, or two shorter fibres, can be seen protruding from the alveolar macrophage, which appears to be activated. (Scale bar = 7·5 μm.)

Table 4 Some secretory products produced by macrophages exposed to asbestos

Asbestos fibre	Secretory product	Reference
Chrysotile	Lysosomal hydrolase	36
Crocidolite	Chemoattractant	37
Chrysotile	Platelet derived growth factor	38
Chrysotile	Superoxide anion	39
Chrysotile	Arachidonic acid metabolites	40
Amosite	Tumour necrosis factor	15

leucocytes after deposition of asbestos fibres in the lung have been described.[45] Such changes could contribute to the development of pleural disease.

FIBRES IN COMBINATION WITH OTHER AGENTS
The clear multiplicative effects of smoking and asbestos exposure in causing lung cancer,[46] and the activity of asbestos as a promoter as seen experimentally, raise the question of combined exposures to fibres and other agents. This has not yet been studied in depth but Pinkerton *et al*[47] have shown that low dose exposure to ozone results in a dramatic slowing down of the clearance of asbestos from the lung. Davis *et al*[48] have shown that inhaling asbestos fibres in combination with other dust, such as titanium dioxide or quartz, may produce substantially more lung and pleural tumours than inhaling fibres alone. Thus we need to consider fibre exposure in relation to concomitant environmental exposures to oxidants and dusts.

Future research on respirable industrial fibres

Almost all that is known about the mechanisms of the diseases caused by inhaled fibres is based on experience with asbestos. The dissemination of a set of standard samples of asbestos (Unione Internationale Contre Cancer (UICC) samples produced by the British and South African Medical Research Councils) was a vital step in allowing interlaboratory comparisons to be made of the biological activity of at least these asbestos samples. Whereas elucidation of the cell biology of asbestos bioeffects has moved ahead rapidly no standard samples of non-asbestos fibres have been generally available to the scientific community. Such a standard set should be well characterised with regard to dimensions and surface chemistry. The lack of standard non-asbestos fibres has meant that progress has stalled with regard to very basic questions on the similarities and differences between asbestos and other fibres. The UICC samples were made in 1969 before the

role of fibre length was fully appreciated; they were made by milling commercial asbestos to ensure that each sample contained mostly respirable fibres. These fibres, however, were mostly short and the resulting activity of the UICC series is therefore far from optimal for biological research. Nearly all the experiments carried out so far have used samples containing less than 1% of the long active fraction.

Both this dimensional problem and the absence of standard samples of the manmade fibres have been partially remedied by the United States Thermal Insulation Manufacturer's Association (TIMA). This organisation and the equivalent European bodies have sponsored a series of animal experiments on their products. To do this they isolated fibres with target mean dimensions of 20 μm length and 1 μm diameter from commercial mineral wools. Such fibres could be expected to be extremely biologically active if dimension is the main determinant of activity. As well as carrying out their own programme of research with these materials the Thermal Insulation Manufacturers Association has established a repository, which is making a range of respirable vitreous and ceramic fibres and one long fibre sample of crocidolite available to bona fide researchers. This generous step should greatly improve fibre research.

The asbestos experience has been the driving force for most research into the newer fibres and this has engendered a cautious approach to any respirable fibre. Currently, the idea that any long, thin, durable fibre has the potential to be pathogenic dominates the study of fibre pathology (fig 3) but there is no general consensus on secure definitions of long, thin, or durable; the question of the role of chemical composition, particularly as it affects durability, continues to be vexing. The next 20 years of research into the physico-chemistry of fibres and their interactions with cells should reveal whether "guilt by association" with asbestos is justice for respirable industrial fibres. A ban on fibres would be impractical with the present state of knowledge, as non-fibrous substitutes do not exist for all known applications. In addition, the energy efficiency of domestic and other buildings and industrial processes would be substantially reduced, with consequent increases in air pollution. A thorough understanding of the characteristics of fibres that control their toxicity may allow industry to design safer fibres, to the benefit of themselves, the workforce, and society.
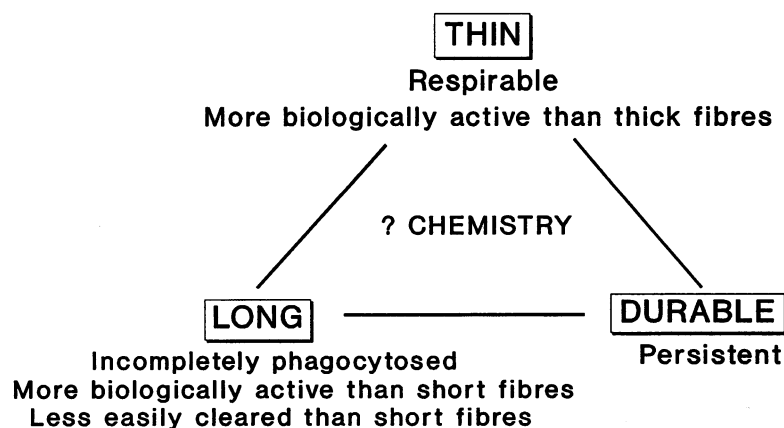

Figure 3 The pathogenicity of fibres: diagram showing the three properties that are currently considered to underlie pathogenicity, indicating how each property is likely to have its biological effects.

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