BIOLOGICAL EFFECTS OF SHORT FIBERS

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Evidence implicating fibre diameter in the development of mesotheliomas and pulmonary fibrosis is now generally accepted but the contribution that the length of mineral fibres makes is not so well established. As the difference in length of fibres found in the urban situation, and that in the occupational and para-occupational environments is revealed, so the importance of length may increase. Our investigations, reported here, confirm the concept that fibre length is a significant factor in the development of these asbestos related diseases.

Timbrell[1] illustrated that the maximum diameter of a fibre that could be inhaled into the parenchyma of the lung was 3.0 μm. This has subsequently been confirmed in both man and experimental animal, although fibres above 2.0 μm diameter are uncommon. The length/diameter characteristics were clearly defined by the experimental studies of Stanton,[2] Pott,[3] and Wagner[4] et al. These investigations confirmed that fibres of 0.25 μm in diameter and about 8 μm in length were associated with mesotheliomas, whereas the coarser fibres of between 1.0–3.0 μm in diameter and of 8 μm in length were probably responsible for pulmonary fibrosis.

Our investigations on tremolite exposure in both men and experimental animals[5] have contributed to clarification of the different fibre diameter associated with these different lesions. However, our statement that the shorter fibres physical forms are relatively innocuous was challenged with the request to define "relative." It was hoped that our experiments with Oregon erionite would satisfy the critics.[6] In these studies we showed that the relatively long erionite fibre produced a 100% incidence of mesotheliomas following exposures, both by intrapleural and inhalation exposures compared with no tumours being produced when a non-fibrous synthetic erionite was used. This established that the mineral fibres and not the chemical constituents were responsible for the lesions. The critics pointed out that the control material was non-fibrous and that we had not proved that short fibres within the experimental material were relatively innocuous. Therefore, to answer their queries we would have to produce dust samples of long and short fibre in sufficient quantities for both implantation and inhalation studies. In our investigations in order to produce both tumours and fibrosis by inhalation, the exposure must last twelve months and needs 2 kilograms of dust. Hitherto production of asbestos fibre of specific length has not been successful and milling crocidolite has usually resulted in the short fibre being reduced to non-fibrous particles. Alternatively, in an attempt to produce crocidolite <5.0 in length, for an intrapleural study, it was found that there were no long fibres seen in the pre-inoculation dust, but there were long fibres retained in the granulomas[7] (thus illustrating our theory about the selective retention of fibre).

We decided to use Oregon erionite[6] because of its friable nature and Mr. J.W. Skidmore was successful in producing sufficient quantities by precise milling over a very short time. A similar preparation was made with UICC crocidolite. Final assessments of the success of milling could only be made on fibre measurements at the end of the experiment, on the lungs from the inhalation study, and pleural granuloma from the implantation investigation. The other two samples used were the longer erionite as prepared for the original experiment, and standard UICC crocidolite for long crocidolite. It was accepted that these were mixed length samples, with sufficient long fibre to produce lesions. The dust for the short fibre was prepared by disc milling. By making empirical decisions, only fibres <5 microns in length were found using the following strategy of 10 sessions each of 10 seconds duration, the mill being opened after each session and the dust redistributed. The inhalation[8] and intrapleural[9] methods have previously been described, as have the characterisation of the dust clouds and the respirable inoculated materials. The animals used were SPF Fischer F344 rats (caesarian derived, barrier maintained, and free from disease as shown by random culling). Eighty rats of each sex for the inhalation study and 64 of each sex for intrapleural inoculation, were randomly allocated, in equal numbers, to the 4 treatment groups. Post mortem and histological examination were carried out.[10] The fibrosis grading[11] is standardised on an internationally accepted scale: Grade 1 normal; 2 dust in macrophages; 3 early interstitial reaction; 4 first signs of fibrosis; 5, 6, 7 increasing degrees of fibrosis; 8 severe fibrosis. The only other lesions noted were 1 Bronchiolar hyperplasia; 2 mesothelioma. Finally, characterisation of the fibres was carried out; recovery methods were used on a known weight of lung tissue from the inhalation; and tissue from the granuloma from the intrapleural experiment.

RESULT

Dust Studies

Measurements were carried out on the four dusts recovered after 24 months, i.e. exposure period of 12 months and a further survival time of 12 months. Our findings substantiated the previous experimental results on the retention of the longer fibres after inhalation. It also demonstrated our success in the production of fibres in the size ranges required. Figure 1 and

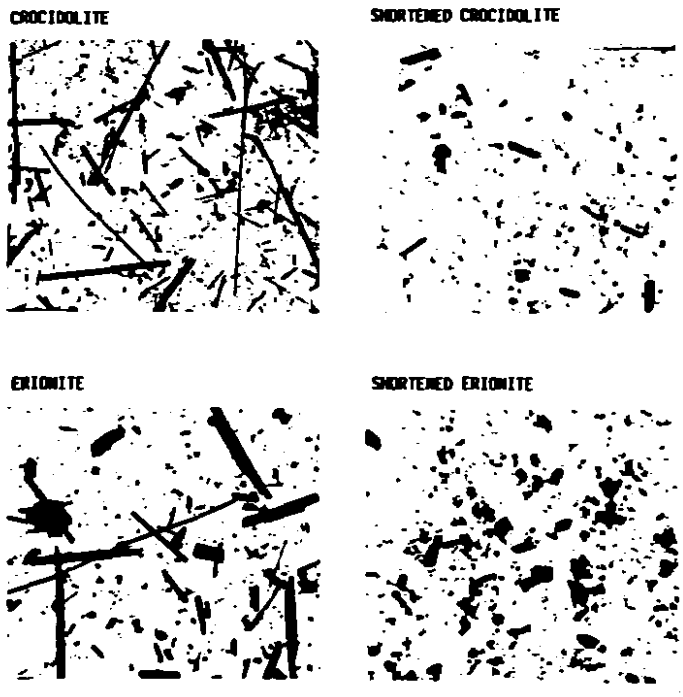

Figure 1. Fibre recovered from rat lung after 24 months.

Tables I and II show the fibre recovered from the lungs of animals after a period of 24 months. It can be seen from the electron micrographs taken of the shortened material the complete absence of any fibres with lengths greater than 5 microns.

DISCUSSION

We were endeavouring to answer two questions:—

1. Is it possible to show by animal experimentation that below a minimum length, fibre of standard diameter does not produce mesotheliomata of significant pulmonary fibrosis, whereas longer fibre of similar type are capable of their production?
2. What is the critical length of fibre?

Minimum Length (1)

We have succeeded in answering the first point, but have not been able to define the critical length of fibre as required in (2).

In the intrapleural inoculation study we were able to produce over 90% of tumours in the animals exposed to either of the long fibre dusts. Using the short fibre a single mesothelioma was produced with the crocidolite sample, no tumours occurred in the animals exposed to the erionite.

The inhalation experiment produced the expected tumour incidence of over 90% of the animals, exposed to the erionite, which had survived for a sufficient time period to develop mesotheliomas. It must be remembered that there had been a serial killing of animals at an earlier stage for the inhalation study which substantially reduced the number surviving for more than one year. The long crocidolite only produced a single tumour. This confirmed the importance of using the Oregon erionite material, as the crocidolite produced too few tumours for comparison with the short fibre results. No tumours occurred in the animals exposed to either of the short fibres.

The inhalation studies demonstrate that in the animals exposed to the long fibres minimal fibrosis occurs, whereas the short fibres only produce a tissue reaction.

Critical Length (2)

Production of fibre below 5 microns and to include a sufficient number of fibres in the 3–5 micron range proved to be extremely difficult. This is because a decrease in the milling time led to the appearance of fibres greater than 5 microns in length. The result of this difficulty was that the majority of the fibre used in the short samples was below 3 microns whilst still retaining the fibrous nature of the material. This was also apparent in the material recovered from the lungs of the animals. Very occasional longer fibre was found in the short crocidolite. This could account for the single mesothelioma seen in an animal exposed to this dust. These results proved that the milling was successful. In a previous experiment with milled dusts a large number of mesotheliomas were induced.[7] This was thought to be due to a long fibre component

Pathology

Intrapleural Inoculation

Dust	Mesothelioma	Non-mesothelioma
UICC Crocidolite	24	8
Shortened Crocidolite	1	31
Erionite	30	2
Shortened Erionite	0	32

Inhalation

	Fibrosis Gradings 4 rats/sacrifice				Tumours		Total Excl. Sacrif.
	3 mths.	6 mths.	12 mths.	24 mths.	Meso.	BAH	
UICC Crocidolite	2.9	3.0	4.1	3.9	1	2	24
Shortened Crocidolite	2.0	2.0	3.3	2.8	0	0	24
Erionite	2.4	3.0	4.0	4.0*	24	2*	27
Shortened Erionite	2.9	3.0	3.0	3.1	0	1	24

* 1 animal only remained for sacrifice

* 2 bronchiolar alveolar hyperplasia with mesotheliomas

remaining in the dust.

As in previous investigations, the animals treated with the long fibre dusts tended to selectively retain the longer fibres. In the inhalation study, it was of importance to see how little long erionite was retained in the lungs of the animals, as others with this exposure developed the tumours.

Attempts should be made to produce a more satisfactory short erionite sample closer to 5.0 microns in length. All investigations have failed to produce a satisfactory sample from amphibole asbestos. It is possible that by using more complex methods a small sample sufficient for the implantation study could be produced. When this was done using glass-micro fibre the cost of production was extremely high, and no attempt was made to produce a larger sample of inhalation experiments.

Table I

Inhalation Experiment

Percentage and Number of Fibres in Defined Categories per Gram of Dried Lung Tissue ($\times 10^6$)

	Fibre Size (μm) L.	Fibre Size (μm) D.	3 months	6 months	12 months	24 months
SHORT CROCIDOLITE	3 - 5	< 0.5	0.3 % 7.0 *	1.2 % 42.6 *	0.6 % 47.9 *	1.0 % 63.0 *
	> 1	< 0.5	10.3 % 231.3 *	29.1 % 1033.1 *	15.3 % 1221.6 *	15.1 % 948.6 *
LONG CROCIDOLITE	> 6	< 0.5	1.6 % 425.5 *	2.8 % 991.7 *	3.2 % 1427.1 *	5.8 % 2529.3 *
	3 - 6	< 0.5	3.0 % 797.9 *	4.8 % 1712.9 *	5.2 % 2319.0 *	8.4 % 3663.1 *

* No. of fibres

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Table II

Inhalation Experiment

Percentage and Number of Fibres in Defined Categories per Gram of Dried Lung Tissue ($\times 10^6$)

	Fibre Size (μm) L.	D.	3 months	6 months	12 months	24 months
SHORT ERIONITE	3 - 5	< 0.5	1.0 %	1.7 %	0.3 %	0.6 %
			2.4 *	8.5 *	9.1 *	6.3 *
	> 1	< 0.5	32.0 %	37.2 %	37.9 %	39.7 %
			77.1 *	186.0 *	1154.4 *	449.3 *
LONG ERIONITE	> 6	< 0.5	7.0 %	5.8 %	5.0 %	7.0 %
			130.0 *	350.9 *	419.1 *	350.8 *
	3 - 6	< 0.5	15.5 %	16.3 %	17.0 %	15.3 %
			287.9 *	991.6 *	1424.8 *	764.3 *

* No. of fibres

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