Some etiological considerations of fibre carcinogenesis

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The exogenous agents which contribute to the cause of cancer generally fall into one of three major groups: ionising radiation, chemicals and viruses. There is a wealth of speculation as to how the members of these groups act to induce cancer, but the mechanisms of their action remain unknown. Asbestos, in all its forms, contains chemicals that are carcinogenic under certain conditions, however, it is of particular interest as a carcinogen because it has attributes of two of the above groups. Firstly, the presence of various metallic ions and polycyclic hydrocarbons, that are either inherent or acquired through processing, would seem the best explanation for the carcinogenicity of asbestos. On the other hand, particles that are within the dimensional range of viruses are abundant in all forms of asbestos; and it is conceivable that these submicroscopic particles could act in a fashion similar to that of viruses, whatever that may be.

On the other hand, there is reasonably good evidence that neither of these attributes is related to the carcinogenicity of asbestos. The evidence for this conclusion can be summarised as follows:

(1) There is no indication that any of the asbestoses are sufficiently contaminated with known carcinogenic hydrocarbons to account for their carcinogenicity; and rigorous extraction of those hydrocarbons present in asbestos does not affect its carcinogenicity for the pleura of the rat (Wagner *et al.*, 1970).

(2) Variations in the inherent metal content of various types of asbestos are great, yet these various types of asbestos show only slight differences in carcinogenicity (Harington, 1965; Timbrell, 1970; Wagner, 1970; Stanton & Wrench, 1972).

(3) Finely particulate metallic nickel, stainless steel or non-crystalline silicon dioxide applied to the pleura of the rat are not sufficiently carcinogenic to account for the carcinogenicity of asbestos by mill contamination (Stanton & Wrench, 1972).

(4) Reduction of fibre size by the partial pulverisation of asbestos, a process which increases contamination by metallic particles and increases the number of submicroscopic fibrils in asbestos, reduces its carcinogenicity (Stanton & Wrench, 1972).

(5) Hand-cobbed crocidolite ore, hand-milled without metallic contamination, is equal in carcinogenicity to machine-milled crocidolite (Stanton & Wrench, 1972).

(6) Non-asbestiform fibres such as fibrous glass are increasingly carcinogenic as they approach the size range of milled asbestos fibres (Stanton & Wrench, 1972).

One must therefore consider that it is the structural features of asbestos that may be the critical factor in its carcinogenicity, and it is toward this hypothesis that we have directed our attention. If the structural features of asbestos are important, then it follows that similar fibres, if sufficiently durable, should also induce tumours. On the basis of this reasoning, we have assessed the distribution of particles by size in a variety of fibrous materials and applied these to the pleura of rats for a period of two years. These experiments are still in progress; only preliminary results of part of them are available and interpretations are limited. However, estimates of final tumour incidences have been made from the data currently at hand, and the various materials have been segregated into four groups which cause high, moderate, low and negligible tumour incidence (Tables 1-4). The analysis of fibre distribution by size is subject to some error, nevertheless it is sufficient to characterise the general distribution of fibres in the materials.

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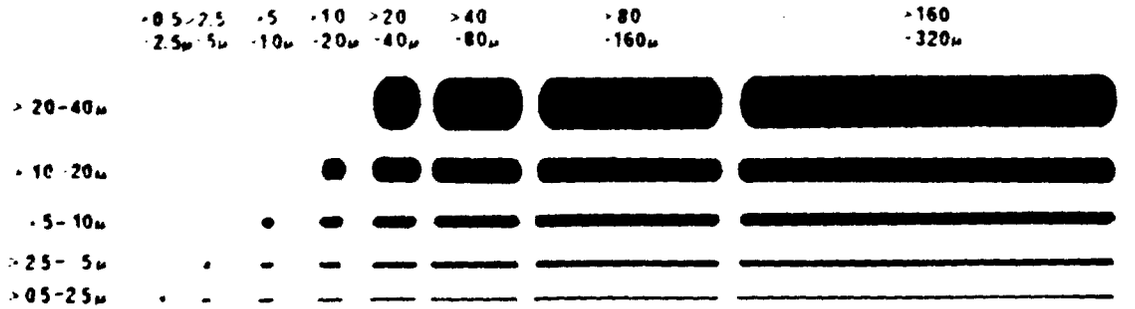

Fig. 1. Graphic illustration of the categories of size used to classify particles in the test samples. Illustrated particles represent mean dimensions (μm).

MATERIALS AND METHODS

Various specimens of crocidolite, chrysotile, fibrous glass, and fibrous aluminium oxide were applied by open thoracotomy to the left pleural surface of 30, 11- to 14-week-old, female Osborne-Mendel rats at a single standard 40 mg dose level by a method previously described (Stanton *et al.*, 1969). The unique aspect of these experiments is that all test materials were applied to small 45 mg fibrous glass pledgets prior to application. The glass pledgets are composed of large-diameter fibrous glass which, when intact, has no apparent carcinogenicity in itself. We use it simply as a convenient and accurate means of uniformly applying the test material to a wide surface area of the pleura. The test materials are listed in Tables 1–4. The UICC standard reference samples of crocidolite and chrysotile A have been previously described (Timbrell, 1970). Reduction of particle size was accomplished by grinding in a stainless-steel

Table 1 High incidence groups (> 40% mesotheliomas) Percentage of mass occupied by fibres in each range of size

Diameter μm	Length μm							
	·0 5 2 5	·2 5 5	·5–10	>10–20	>20–40	>40 ·80	·80 160	·160 ·320
>20 ·40								
>10 20	Crocidolite UICC							
·5 10	+ + + + *		1			11	22	
>2 5-5		1	<1	1	3	3	17	11
>0 5-2 5	3	5	7	4	4	2	1	4
>20 40								
>10-20	Chrysotile UICC							
>5-10	+ + + +		11					
>2 5-5		4		4				
>0 5-2 5	8	15	14	11	17	7	5	3
>20-40								
>10-20	AAA glass crude fibre							
>5–10	+ + + +							
>2 5–5		1					3	7
>0 5–2 5	<1	<1	1	2	2	5	13	65
>20–40								
>10–20	AAA glass separated (>10 × 1 μm)							
>5–10	+ + + +							
>2 5–5		1			3			
>0 5–2 5	8	3	6	17	18	10	23	12
>20–40								
>10–20	Aluminium oxide whiskers			4	4			
>5–10	+ + + +		3	1	4	8		26
>2 5–5		1	1	1	5	2	12	6
>0 5-2 5	<1	<1	1	2	5	4	4	6

* + 's indicate extent of pleural fibrosis most commonly observed

ball mill to produce partially or fully pulverised samples. The crude fibres were stripped by hand from hand-cobbed ore specimens, and bundles of fibres were retained as long as possible without contamination by extraneous mineral.

The fibrous glasses were obtained from both the Owens-Corning Fiberglas Corporation, Toledo, Ohio and the Johns-Manville Research and Engineering Center, Manville, New Jersey. We are particularly indebted to the latter institution for the size separation of fibrous glasses, which was carried out through a series of millings and the sedimentation of exceptionally fine-diameter glass fibres. All of the glasses were of the usual borosilicate type, whose mineral oxide contents have been previously recorded (Stanton & Wrench, 1972).

The non-fibrous aluminium oxide and aluminium oxide whiskers were commercial products obtained from the Artech Corporation, Falls Church, Virginia. These are single crystal fibres that are more than 99% pure Al_2O_3. The method of counting fibres has been described previously (Stanton & Wrench, 1972).

Samples of the materials, suspended in Formvar, were air-dried on glass slides and photographed at 1000× magnifications. From the photographs, 1000 consecutively counted particles were assigned to the 30 ranges of dimension indicated in Figure 1. Assuming that the particles in a given range were normally distributed around the mean size of that range, the total mass of all particles could be calculated; and it is the percentages of the total mass occupied by particles in a given range or size compartment that are presented in Tables 1–4.

In the tables, the first entry for each diameter and half of the diameters in the second entry in each row represent particles that are non-fibrous by optical standards. The plus figures below the designated specimen indicate the extent of pleural fibrosis most commonly observed in the rats in each experiment.

The rats are being observed for two years following application. A necropsy is performed on all dead or sick rats, and histologic sections are taken from the site of treatment and from any other abnormal lesion.

CONCLUSIONS

The data are arranged in four tables according to our estimates of mesothelioma incidence. In Table 1 are shown the five specimens which produced tumour incidences greater than 40%. These are the UICC standard reference samples of crocidolite and chrysotile A, two samples of very fine fibrous glass with diameters of 3 μm or less, and the aluminium oxide whiskers. All of these samples are composed almost entirely of fibres, and further have in common a predominance of fibres below 5 μm in diameter. The Al_2O_3 fibres are of particular interest because they are totally different from those of asbestos and glass, both in internal structure and in chemical composition; yet their size distribution is remarkably like that of UICC crocidolite. However, one-third of the fibres are slightly longer and thicker than are the crocidolite fibres and, since the density of Al_2O_3 is greater than asbestos, approximately one-sixth as many fibrous particles are present. The Al_2O_3 fibres are most durable and do not fragment into submicroscopic particles in the manner of asbestos. Nevertheless, electron microscope study reveals an abundance of submicroscopic fibrils of less than 0.1 μm in diameter with lengths comparable to those of fibres in the lowest optical range.

Tables 2 and 3 list the six samples of asbestos and glass that fall in the middle ground of carcinogenicity. These groups show several outstanding differences in fibre distribution from those shown in Table 1; both extremes in the dimensional range of fibres are represented. For example, the two crocidolite samples show similar decreases in carcinogenicity; however, one is composed almost entirely of long, large-diameter fibre bundles (Table 2); while the other (Table 3) has nearly half its mass reduced to fibres that are at the very lowest optical range of diameter and length and the rest reduced to particles too small to be recognised as fibres by optical standards. This latter fraction is of particular importance, since it consists of particles that are aggregates of submicroscopic fibrils with diameters below 0.2 μm and with lengths of 5 to 10 μm. Since these very short fibrils of submicroscopic size account for the bulk of this material and occur in far greater numbers than in the non-pulverised crocidolite, it follows that their role in carcinogenesis is probably negligible (Stanton & Wrench, 1972).

The distributional array of fibres in the three glass samples of these groups indicates the second dimensional parameter of carcinogenicity. Here it is apparent that carcinogenicity decreases as more fibres exceed 2.5 μm in diameter and as the fibre length decreases to less than 10 μm. Again, the submicro-

Table 2 Moderate incidence groups (30–20% mesotheliomas). Percentage of mass occupied by fibres in each range of size

Diameter μm	Length μm: 0.5–2.5	2.5–5	5–10	10–20	20–40	40–80	80–160	160–320
20–40								42
10–20	Crocidolite (coarse fibre)							22
5–10							1	10
2.5–5							2	12
0.5–2.5	+	+	+	+	2	2	3	2
20–40								20
10–20	Chrysotile (long fibre)							16
5–10							10	12
2.5–5		2						
0.5–2.5	+	2	1	4	2	7	6	14
20–40								
10–20	AAA glass stretched fibre			12				
5–10			4					
2.5–5		4	2	2	11	9	8	
0.5–2.5	2	2	4	9	10	2	1	8
20–40								
10–20	AAA glass separated	10–3 μm		2	4			
5–10			1	1	15	10		
2.5–5		3	2	9	22	9	8	12
0.5–2.5	+	+	+	1	2	+	+	+
20–40								
10–20								
5–10								
2.5–5								
0.5–2.5								

* + s indicate extent of pleural fibrosis most commonly observed

scopic fractions of these samples contain an abundance of fibres below 0.2 μm in diameter, with distributions of length similar to those of fibres present in the lowest optical range.

Finally, Table 4 lists those materials that have yielded less than 5% incidence of mesotheliomas. No tumours have developed in rats exposed to either crocidolite or chrysotile pulverised to the extent of reducing all of the optically visible particles to non-fibrous form. Since these excessively milled materials contain all of the elemental components of asbestos and are contaminated by metallic erosion of the mill far more than are the other samples, it follows that neither of these factors is a likely source of asbestos carcinogenicity and that structural integrity of the fibre is essential. The two glass samples in this group again represent both extremes in structure, namely either fibres of great length with diameters greater than 10 μm or particles that are short, thick and essentially non-fibrous by optical standards. In the non-fibrous Al_2O_3 experiment, a single tumour has been observed among 30 rats. This tumour is of doubtful significance, but it may reflect the low background incidence of less than 5% induced by partial fragmentation of the vehicle.

In summary, the experiments may be compared among single types of material. The results from the seven samples of asbestos indicate that none of the three extremes in fibre distribution yield as high an incidence of mesotheliomas as do the more evenly distributed UICC standard reference samples. Either progressive pulverisation to non-fibrous form or preservation of the test sample in large bundles of fibres clearly reduces carcinogenicity. In comparing the seven glass samples it is apparent that samples composed over 90% by weight of fibres with diameters of 2.5 μm or less are the most carcinogenic; and that as length is reduced in these small fibres carcinogenicity is also reduced. Finally, the contrasting results obtained using fibrous and non-fibrous forms of Al_2O_3 re-emphasise the importance of structure in carcinogenicity. These exceptionally pure, inert fibres, composed of materials foreign to asbestos and glass, seem to carry the same carcinogenic hazard for the pleura as do those materials.

It would seem therefore that carcinogenicity is in

Table 3 Low incidence groups (20–10% mesotheliomas) Percentage of mass occupied by fibres in each range of size

Diameter μm	Length μm							
	0.5–2.5	2.5–5	5–10	10–20	20–40	40–80	80–160	160–320
20–40								
10–20	Crocidolite partly pulverised							
5–10								
2.5–5								
0.5–2.5	<1	20	17	9				
20–40								
10–20								
5–10								
2.5–5								
0.5–2.5								
20–40								
10–20	AAA glass partly pulverised							
5–10	+ *		<1	2		16	32	
2.5–5		4	4	2	8	6	4	8
0.5–2.5	1	2	3	2	2	1	1	3
20–40								
10–20								
5–10								
2.5–5								
0.5–2.5								
20–40								
10–20								
5–10								
2.5–5								
0.5–2.5								

* + 's indicate extent of pleural fibrosis most commonly observed

Table 4 Negligible incidence groups (< 5% mesotheliomas) Percentage of mass occupied by fibres in each range of size

Diameter μm	Length μm							
	0.5–2.5	2.5–5	5–10	10–20	20–40	40–80	80–160	160–320
20–40								
10–20	Crocidolite fully pulverised			20				
5–10	+ *		14					
2.5–5		26						
0.5–2.5	40	<1						
20–40					30			
10–20	Chrysotile fully pulverised			28				
5–10	+		25	5				
2.5–5		5	2	<1				
0.5–2.5	3	<1	<1	<1				
20–40								47
10–20	Commercial glass whole fibre							24
5–10	+							21
2.5–5						<1	<1	7
0.5–2.5						<1	<1	1
20–40								
10–20	AAA glass separated (< 5 × 3 μm)				29			
5–10	+		8	2	4			
2.5–5		5	9	20	14	2		
0.5–2.5	3	2	3	1	<1			
20–40					28			
10–20	Aluminium oxide non-fibrous			31	19			
5–10	+		10	4				
2.5–5		5	1					
0.5–2.5	2	<1						

* + 's indicate extent of pleural fibrosis most commonly observed

some are related to the presence of durable particles of fibrous configuration that are particularly long at perhaps below the smallest diameter which can be recognised optically and that the carcinogenicity of these fibres has little relation to their chemical composition or to their potential contaminants.

SUMMARY

Various specimens of forms of asbestos, fibrous glass and aluminium oxide have been tested for carcinogenicity on the pleura of rats. Results from all three materials indicate that carcinogenicity is related primarily to fibrous structure rather than to chemical properties. A comparison of the dimensional distribution of fibres in those samples of asbestos and glass producing high and low tumour incidence indicate that carcinogenicity may be related to fibres [illegible] in diameter and between [illegible] in length.

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