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THE CARCINOGENICITY OF FIBROUS MINERALS

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Abstract

The carcinogenicities of 37 different dimensional distributions of seven different durable fibrous materials were correlated with fiber dimension. Optimum correlation was attained with fibers that measured <0.25 μm x >8 μm. Morphologic studies suggested that fibers in this dimensional range lie free in interstitial tissues, while fibers of smaller dimension are readily phagocytosed and fibers of larger dimension are sequestered by adherent phagocytes and fused phagocytic giant cells. Fibers that are fine and long may be more carcinogenic than others, simply because they are uncompromised by phagocytic activity.

Keywords: Aluminum oxide; asbestos; carcinogenicity; Dawsonite; fibers; fibrous glass; phagocytosis; potassium octatitanate.

For the past several years we have been interested in the question of how asbestos causes cancer once a fiber reaches susceptible tissues. We have approached this problem with the simple device of introducing various types of particles into the pleural space of rats and observing the resultant tumors during the subsequent two years. The methods that we have used can be summarized briefly [1,2,3].[1] A standard 40 mg dose of particles is applied by open thoracotomy directly to the left pleural surface of young female Osborne-Mendel rats. In each experiment, 30 to 50 rats are followed for two years and those surviving at two years are killed. All rats are necropsied and all pathological lesions examined histologically. Tumors that resemble the mesenchymal mesotheliomas of man generally develop after the first year. For the sake of precision we have called these tumors pleural sarcomas. During the second year, rats die at various times with and without pleural sarcomas; consequently, we have used actuarial computation to arrive at a valid estimate of the incidence of pleural sarcomas which takes into account differences in life-span [4]. Probability of pleural sarcoma has ranged from 0 to 100 percent depending on the materials used. Pleural sarcomas have not been observed in several thousand untreated controls; however, pleural sarcomas have occurred in rats treated only by simple thoracotomy. Our best estimate of these non-specific, background pleural sarcomas in treated controls is in the range of 2-4 percent. This is important to keep in mind since it makes interpretation of low level response unreliable with small numbers of animals.

[1]Figures in brackets indicate the literature references at the end of this paper.

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There are two separate features of asbestos particles that merit consideration as potentially carcinogenic. First, the chemical nature of their constituents and contaminents, especially those with a known potential for carcinogenicity such as the polycyclic hydrocarbons and heavy metals. Secondly, the physical structure of asbestos particles which in their fibrous fineness are somewhat unique in the natural world. It is our contention that it is the latter property, namely the simple quality of being an exceptionally fine, long, durable fiber, that is most critical to carcinogenicity. The supporting evidence for this hypothesis is derived primarily from the type of experiments described above, as carried out by us and others [1,2,3,5,6,7]. It can be summarized briefly as follows:

(1) Vigorous extraction of natural and contaminating hydrocarbons from asbestos does not alter its carcinogenicity.

(2) Hand-cobbed, hand-milled asbestos that is free of metallic mill contamination is no less carcinogenic than machine-milled asbestos.

(3) Naturally occurring or contaminating carcinogenic metals such as nickel, cobalt, chromium, iron, magnesium, and silica, or hydrocarbons such as benzo(a)pyrene, of comparable quantity to that in asbestos, when attached to inert non-fibrous particles of a size comparable to asbestos, do not show the carcinogenicity of asbestos.

(4) The carcinogenicity of asbestos is greatly reduced if implanted as whole unseparated sheets of fibers or implanted as very short submicroscopic fibrils.

(5) The carcinogenicity of asbestos is greatly reduced if asbestos is heated sufficiently to increase its fragility or if pulverized to non-fibrous particles.

(6) Finally, between the various types of asbestos, particularly crocidolite, amosite, tremolite, anthophyllite, and chrysotile, there are wide variations in chemical, crystalline, and molecular structure. Nevertheless, when similar dimensional distributions of these asbestoses are applied directly to the pleura their carcinogenic response is similar.

If the carcinogenicity of asbestos depends on its dimensional configuration, two corollary hypotheses are suggested. First, durable fibers of other materials if in the same dimensional range as asbestos should be as carcinogenic as asbestos. Secondly, there should be an optimal dimensional range of fibers relevant to carcinogenicity.

The data which I would like to present today relates to these two corollaries. Table 1 lists 37 experiments with seven different durable fibrous materials, each of differing dimensional distributions, but at or near the size distribution of asbestos. We have listed these in order of their probability of inducing pleural sarcomas, and as you can see the range runs the gamut from 0 to 100 percent. Asbestos fibers of a standard size characterized by a working group of the Unio Internationale contra Cancer would fall in the range of 65-80 percent [8].

The problem that follows is that of determining the dimensional distribution of the particles in each sample. To do this we used the straightforward method of measuring length and diameter from montage photographs of typical samples of the particles implanted. A minimum of 1000 particles were tabulated at magnification of 3000X to 29,000X. Subsequently, in samples containing large particles, magnifications of 1000X were used to tabulate fibers inadequately represented on electron micrograph grids. The proper ratio of microscopic to submicroscopic particles yielded a representative sum of measured particles which was then entered into an IBM System 370 computer. From the density and the sum of the calculated volumes, the weight of the counted samples could be obtained and the distribution of particles per microgram of the sample estimated. For convenience, the numbers of particles per microgram were grouped into 34 dimensional ranges as indicated in Table 2. Table 2 illustrates the tabulation of six of the experiments with different samples of glass. By simple inspection the six examples show an apparent relationship between tumor probability and particle distribution that has held for all of the fibers tested thus far. The examples suggest that particles in relatively thin (diameter <0.25 μm) and long (length >8 μm) dimensional categories are associated with the higher tumor probabilities.

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Table 1. Cumulative list of experiments arranged by percent probability of pleural sarcoma.

		Percent
1.	DIHYDROXY SODIUM ALUMINUM CARBONATE V.	100
2.	POTASSIUM OCTATITANATE I	100
3.	POTASSIUM OCTATITANATE II.	100
4.	SILICON CARBIDE GTC #1	100
5.	DIHYDROXY SODIUM ALUMINUM CARBONATE I.	95
6.	BOROSILICATE GLASS (M0L)	85
7.	BOROSILICATE GLASS (M6D)	77
8.	BOROSILICATE GLASS + BINDER (KL)	74
9.	BOROSILICATE GLASS (M6L)	72
10.	ALUMINUM OXIDE -HC	70
11.	BOROSILICATE GLASS + BINDER (KW)	69
12.	DIHYDROXY SODIUM ALUMINUM CARBONATE VII.	68
13.	DIHYDROXY SODIUM ALUMINUM CARBONATE IV	66
14.	DIHYDROXY SODIUM ALUMINUM CARBONATE III.	66
15.	BOROSILICATE GLASS (M6W)	64
16.	ALUMINUM OXIDE #3.	44
17.	ALUMINUM OXIDE #4α	41
18.	ALUMINUM NITRIDE + OXIDE #6α	28
19.	ALUMINUM OXIDE #2.	22
20.	BOROSILICATE GLASS + BINDER (KCP).	21
21.	BOROSILICATE GLASS - BINDER (KUP).	19
22.	BOROSILICATE GLASS (M8L)	14
23.	ALUMINUM OXIDE #4.	13
24.	DIHYDROXY SODIUM ALUMINUM CARBONATE VI	13
25.	DIHYDROXY SODIUM ALUMINUM CARBONATE II	12
26.	BOROSILICATE GLASS (M0S)	8
27.	BOROSILICATE GLASS + BINDER (K2P).	8
28.	MINERAL WOOL (Hi-Ca, Mg)(O2P).	7
29.	BOROSILICATE GLASS + BINDER (KFP).	6
30.	BOROSILICATE GLASS + BINDER (Y2P).	6
31.	HIGH CA-NA (P2P)	6
32.	BOROSILICATE GLASS (M8S)	5
33.	ALUMINUM OXIDE #5.	5
34.	ALUMINUM OXIDE-LC (non-fibrous).	3
35.	BOROSILICATE GLASS (YW) (vehicle) (n=270).	2
36.	BOROSILICATE GLASS (M6S)	0
37.	NICKEL TITANATE.	0

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Table 2. Six example experiments illustrating fiber distribution into 34 dimensional categories by common log of the number of particles per microgram in each size category.

MOL
85.3%

Diameter µm	.01-1	>1-4	>4.8	>8-64	>64
>0.8					
>4.0-8.0					
>2.5-4.0					
>1.5-2.5					
>.50-1.5		2.23	2.53	3.23	3.08
>.25-.50		3.08		3.95	
>.10-.25		2.93	3.35	4.53	
>.05-.10			3.93	4.79	
.01-.05			3.46	4.65	

KI
73.9%

Length µm	.01-1	>1.4	>4.8	>8-64	>64
					0.67
			0.67	1.52	0.97
		1.45	0.67	2.03	2.19
		2.95	2.40	3.42	2.74
	2.55	3.16		3.63	
	3.03	3.16	3.33	3.03	
	2.85	4.09	3.76	3.25	
	3.03	3.73	3.03	3.03	

KCP
21.5%

Diameter µm	.01-1	>1-4	>4.8	>8-64	>64
>8.0				0.97	
>4.0-8.0			1.81	2.01	1.81
>2.5-4.0		1.44	2.05	1.81	
>1.5-2.5		2.05	2.17	2.31	0.97
>.50-1.5	3.00	3.59	3.17	2.85	2.01
>.25-.50	3.24	3.38	3.10	2.55	
>.10-.25	3.24	3.28	3.10	2.50	
>.05-.10	2.50	2.90			
.01-.05	2.20	2.98	2.67		

M8L
14.3%

Length µm	.01-1	>1.4	>4.8	>8-64	>64
					0.17
			0.77	1.73	2.02
		1.49	1.12	2.11	2.27
		2.60	1.45	2.35	2.42
		1.85		2.38	
				1.85	
				1.85	

MOS
8.3%

Diameter µm	.01-1	>1-4	>4.8	>8-64	>64
>8.0					
>4.0-8.0			2.46	2.72	
>2.5-4.0		2.97	2.99	1.76	
>1.5-2.5		3.43	2.37	1.17	
>.50-1.5	3.88	3.91	2.76	2.46	
>.25-.50	4.34	4.02	3.69	3.37	
>.10-.25	4.43	3.88			
>.05-.10	5.90	4.19			
.01-.05	6.77	4.63			

YW
2.8%

Length µm	.01-1	>1.4	>4.8	>8-64	>64
				0.89	0.34
				0.92	0.40
				0.80	0.30
				T.00	0.11
				T.10	0.41

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≤0.25 μm) and long (length >8 μm) dimensional categories are associated with the higher tumor probabilities.

Statistical regression techniques afford a method of analysis that can use a variety of explanatory variables to determine the best correlations between tumor probability and size distribution. The logit transformation [9] was applied to the estimated tumor probabilities (p) according to the formula:

$$\text{logit} = \log \left(\frac{p}{1-p}\right)$$

Then, linear regression methods which find the best fitting function of the form

$$\text{logit} = a + b_1 x_1 + \ldots . b_k x_k$$

were used to compare the common logarithm of the number of particles per microgram in various size categories to the probability of pleural sarcoma. After analyzing various dimensional ranges that might have narrowed the optimum tumor inducing size range, it was determined that the best fit was with the dimensions ≤0.25 μm x >8 μm. The estimated regression equation was:

$$\text{logit} = \log \left(\frac{p}{1-p}\right) = -1.31 + .424x$$

with a correlation coefficient of 0.9. The regression curve for this dimensional range is illustrated in figure 1. Figure 1 also indicates clearly that none of the seven different types of fibers show consistently greater deviations from the curve than any other, and that the curve's steepest slope is between 3-4 log particles per microgram. There was no correlation with particles less than 8 μm in length, but relatively good correlations were also noted with numbers of fibers >8 μm in length and up to 1.5 μm in diameter (correlation coefficient 0.52 to 0.74). Figure 2 illustrates the 34 parameters used for carcinogenicity correlation and those categories in which relatively good correlation was obtained. It should be remembered that absence of correlation does not preclude a low level of tumor response outside these ranges.

Histologic observations suggest the reason for the difference in response to fine, long fibers and those fibers that are either very short or very thick. The lesions in those experiments with a low probability of pleural sarcoma were highly cellular, being composed primarily of fibroblast-laden vascular granulation tissue with a relatively low collagen content and an abundance of macrophages. In lesions from low tumor probability groups in which virtually all fibers were less than 10 μm in length, the fibers seemed completely contained within macrophages. On the other hand, in those lesions from low tumor probability groups in which the fibers were virtually all of large diameter, the fibers seemed sequestered from adjacent tissue by both macrophages and multinucleated giant cells that closely invested the fiber surface. In contrast, the high tumor probability lesions were relatively acellular, with an abundance of collagen at the site of implantation and the fine long fibers lay free in the interstitial tissues unaffected by phagocytes. In ancillary experiments with non-fibrous particles that stimulate collagen such as talc, silica, or carrageenin, collagen deposition equal to that of the high tumor probability lesion has been observed without the subsequent development of tumors. Therefore it seems evident that collagen itself is not the critical factor in carcinogenesis. However, the fact that the fine, long fibers were unaffected by phagocytic activity in the high tumor probability groups suggests that fibers that are fine and long may be more carcinogenic than others simply because they are uncompromised by phagocytic activity.

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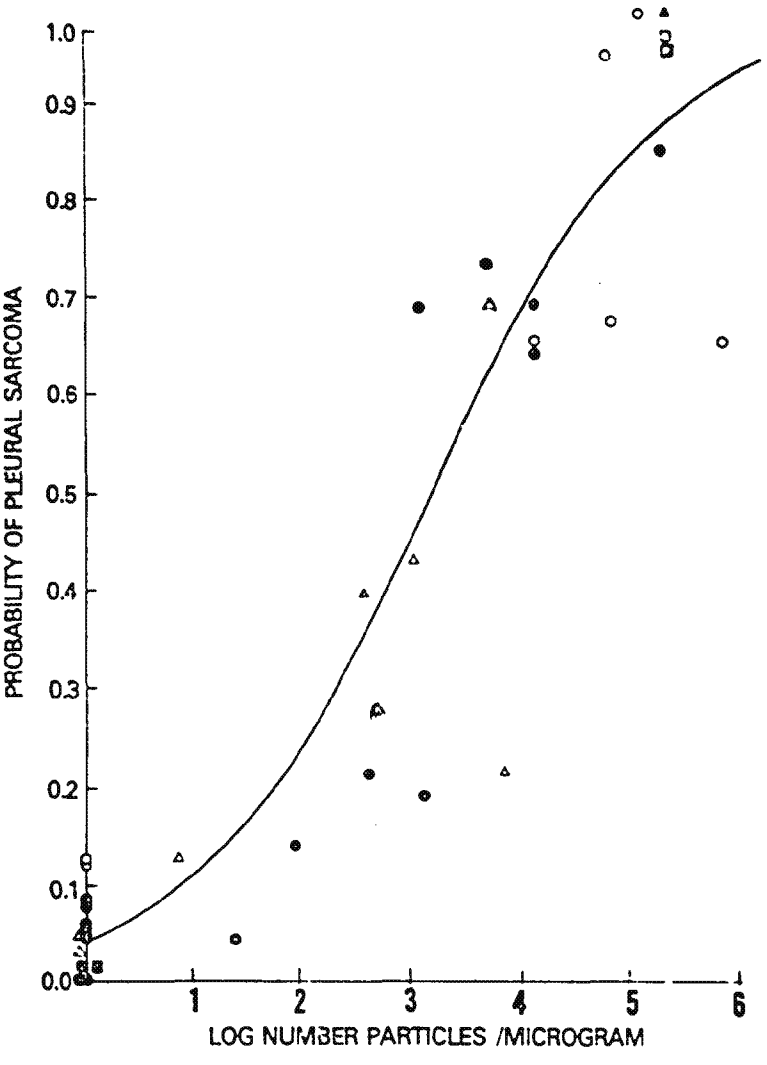


Figure 1. Regression curve relating tumor probability to the common logarithm of the number of particles per microgram with diameters ≤0.25 μm and lengths >8 μm.

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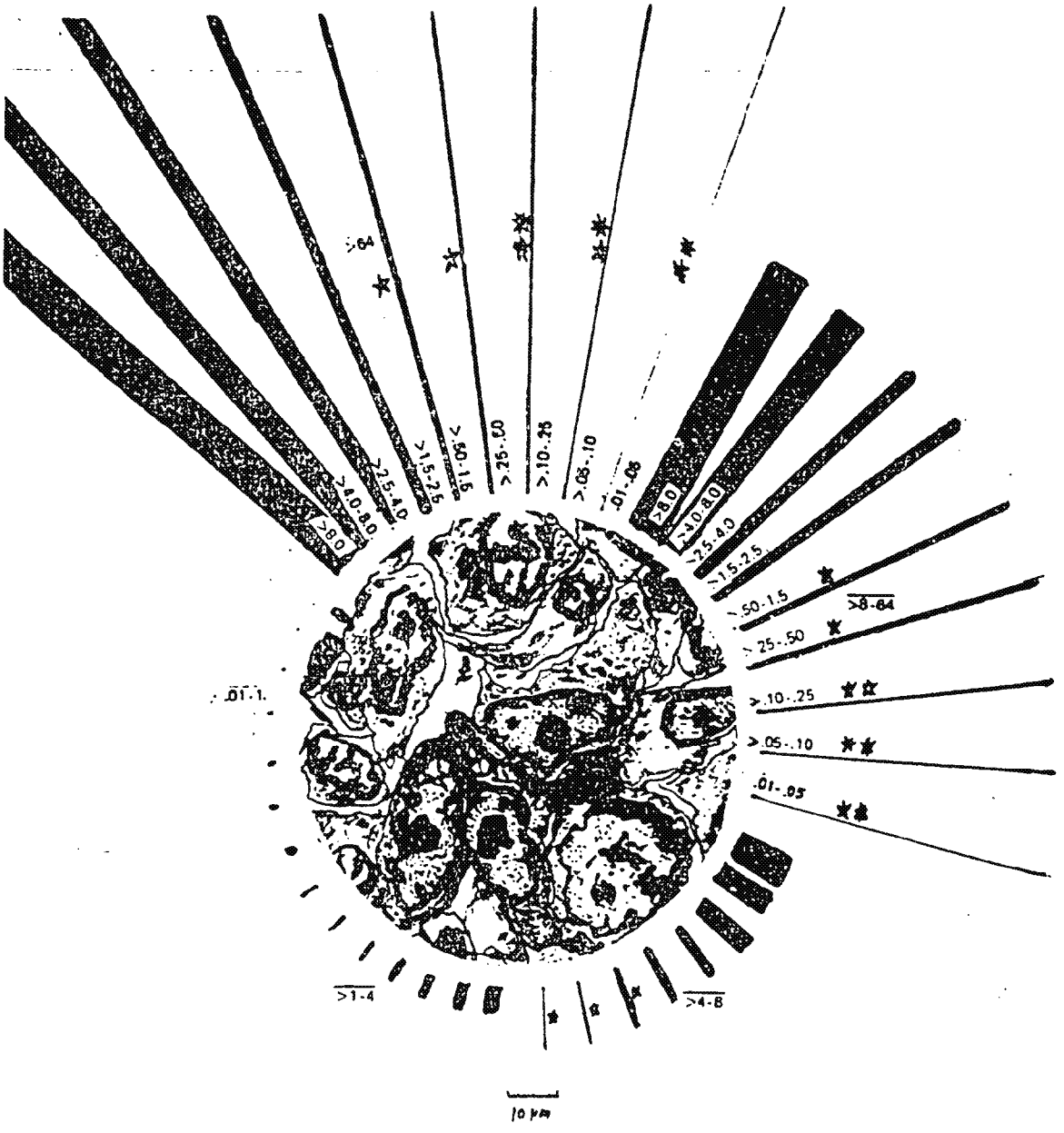


Figure 2. Graphic display of the 34 size categories in scale with phagocytic and mesothelial cells. The starred fibers are those that correlate with pleural sarcoma probability.

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Discussion

A. SUNDARAM: These pleural sarcomas, are they localized? If you leave them for a duration of time do they metastasize?

M. STANTON: Yes, this is real cancer, but they do not metastasize early.

A. LANGER: You allow your animals to live only two years, whereas Wagner allows his rats to live three years. Have you had any control groups run for the duration of the animals' lives? In those animals which you are reporting here, you are reporting frank malignancy? How many of the other animals had hyperplastic lesions?

STANTON: It is difficult to detect precancerous lesions in mesenchymal tissues, so that it really is difficult to say what might be precancerous. We find that after two years plus the twenty weeks the animal has aged by the time we treat them, most rats are in their terminal stages. These rats do not have normal lung capacity and so they do not survive as long as the untreated rat does. By the end of the two years there is only a small percentage of the rats left.

P. KOTIN: This business of assuming that hyperplasia carries with it as a corollary, or is even indicative of subsequent malignant transformation or neoplasia, particularly in mesenchymal tissue, has no substance at all. Now I am speaking as a pathologist. The other thing is, there is a tendency to denigrate experiments which are terminated for the reasons you said, when in fact the confounding findings which arise in the last six to eight months of a rat or any experimental animals, are such as to muddy up the results. I'd like your comment on this. The elegance of the observation occurs before you get into

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ne agonal state, where the exposure of the animal probably has little as anything to do ith what he ultimately gets and dies from.

STANTON: No further comment on that.

WARREN: Our firm recently completed a report for OSHA, "The Economic and Inflation Impact Study for the Effects of a Proposed Standard for Asbestos in Construction," nd in the process of doing this report for OSHA we had to talk to a lot of your firms, niversities - everybody from environmentalists to producers, maybe not some of you in ere personally. This type of meeting is needed. We need people not just talking to each ther, but with each other, and I think you have seen this today. You got one group over ere saying, "Lookout! we are going to put out of business x number of people." Another roup says you have to protect the worker - the worker comes first even at zero exposure. ne only way we are going to resolve this problem, and it is a very sticky problem, is by verybody talking together. So that is just a comment; we found there is not enough alking to each other; even if you don't agree you can still talk. Has anyone looked at ne possibility of using experimental animals other than rats, say a primate? Would this hange results or give better data if we had an animal that would live longer? Has nyone used a rat that has been exposed to cigarette smoke at the same time?

STANTON: Yes, other species have been used. Dr. Bill Smith is here today who has een using hamsters for many years and has some very elegant data with hamsters. We rselves have used mice, and have been successful with mice as well as rats. I don't hink that unless there was an exotic species that we would particularly contribute a reat deal more by using another species. Chickens have been used and various other types birds with some interesting results.

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