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The Relationship between Fibrosis and Cancer in Experimental Animals Exposed to Asbestos and Other Fibers

by John M. G. Davis* and Hilary A. Cowie*

The association between occupational asbestos exposure and the development of both pulmonary fibrosis or asbestosis and pulmonary carcinomas is well documented. It has been suggested that the two pathological conditions are associated with asbestos-related carcinomas developing from areas of asbestosis and not occurring when exposure has been too low to produce this type of pulmonary scarring. Experimental inhalation studies so far published have not been designed to examine this association specifically, but many publications have reported that asbestos samples producing high levels of fibrosis in experimental animals are also very carcinogenic. Samples of asbestos or man-made fibers that produce little fibrosis also produce few tumors. These works are reviewed. In order to examine the association between fibrosis and tumor production in more detail, groups of animals with and without pulmonary tumors and with individual fibrosis measurements were assembled from a number of inhalation studies undertaken over a period of years at this Institute. It was found that animals with pulmonary tumors had almost double the amount of pulmonary fibrosis as animals of similar age that did not. In a few of the animals where tumors were found at an early stage of development, their origin from fibrotic areas could be confirmed, although in most cases where tumor deposits were widespread this was not possible. Experimental confirmation of the site of origin of most pulmonary tumors in asbestos-treated rats would require new studies with rats examined specifically at an age when early tumors would be expected.

While the first cases of asbestosis were recognized within a few years of commencement of the widespread industrial use of asbestos (*1*), even a tentative association between asbestos exposure and cancer was not reported until 1935 (*2*). A further 20 years elapsed before definite evidence that asbestos workers had a significantly raised incidence of lung cancer was published (*3*). The reasons for this delay in hazard recognition may relate to the fact that early factory dust levels were so enormous that many workers developed and died from asbestosis too quickly for tumor production to occur. Paradoxically, it may have been the reduction of exposure levels in the 1930s that allowed many workers to survive long enough to develop cancer.

In Doll's study (*3*), a higher proportion of workers who had developed asbestosis were shown to have lung cancer than those who had not, and this has been confirmed in a number of studies (*4–6*). Subsequently, it has been suggested that asbestos-related pulmonary cancers develop in areas of asbestosis and result from the prior damage and modification to the lung structure. In other words, they are scar cancers (*7*). Evidence in favor of this suggestion is that while most lung cancer occurs in the upper lobes, in asbestos workers cancer of the lower lobes predominates, and it is in the lower lobes that asbestosis is more marked (*8–10*). In addition, it has been reported that a raised proportion of adenocarcinomas occurs in asbestos workers compared to squamous carcinomas, and these are presumed to have developed from Type II pneumocytes in the lung parenchyma, the site where asbestosis occurs. The possibility that asbestos-related lung cancers are scar cancers is extremely important since asbestosis appears to develop only following relatively high asbestos exposures, and it is expected that this condition will have been eliminated by modern factory dust control standards. If asbestos-related lung cancer only occurs with asbestosis, then an increased incidence of these tumors in asbestos workers will also have been eliminated.

Until recently a relationship between pleural fibrosis and mesothelioma production was not considered, particularly since the occurrence of these tumors is less clearly related to dose than is the case with pulmonary cancers. Pleural fibrosis is common in asbestos workers, however, and fibrotic lesions on the parietal pleura frequently become calcified to form placques. Recently Kuschner (*11*) suggested that mesotheliomas may also occur only if there has been pleural fibrosis (perhaps localized) resulting from asbestos exposure.

The association between asbestos exposure, fibrosis,

*Institute of Occupational Medicine, Roxburgh Place, Edinburgh, Scotland.
Address reprint requests to J. M. G. Davis, Institute of Occupational Medicine, Roxburgh Place, Edinburgh, Scotland.

and tumor production should be amenable to detailed experimental examination, but unfortunately, none of the many published studies reporting the exposure of experimental animals to asbestos or man-made mineral fibers was designed for this purpose. Both the fibrotic and carcinogenic response to dusts have been examined but not in individual animals in a way that allows detailed examination of their association. In early animal inhalation studies where tumor production was found (*12,13*) the presence of pulmonary fibrosis was reported, but it was not quantified for either experimental groups or individual animals. As results from animal inhalation studies multiplied, however, it became obvious that patterns of pulmonary fibrosis found in rats were qualitatively similar to those found in asbestos workers as described in detail by Craighead (*14*). The rat does appear to be a suitable model for the study of the interrelationships of the varying types of asbestos-related disease, and the advanced fibrotic changes in aged rats exposed to chrysotile asbestos were described in detail following examination both by optical microscopy and transmission electron microscopy (*15*). Early asbestotic lesions result from accumulation of dust-containing macrophages and other inflammatory cells in the region of alveolar ducts and respiratory bronchioles that eventually collagenize to produce a micronodular pattern of fibrosis. Pulmonary damage caused by asbestos eventually spreads to the alveolar septa between respiratory bronchioles with the alveolar walls becoming progressively thickened and fibrosed. Even in the early stages of this alveolar interstitial fibrosis, the number of Type II pneumocytes is increased, and as the condition advances, air spaces become lined entirely with cuboidal cells. These are mainly Type II pneumocytes, but some have characteristics of Clara cells. The original alveolar structure may break down, and the remaining air spaces are enlarged with greatly thickened walls forming a pattern very similar to human honeycombing. While in the advanced asbestos lesions the air space lining cells are always rounded, their importance varies. In some cases the main element of disease is the great thickening of air space walls with collagen, while in other areas of lung tissue septal fibrosis may be much less advanced, but hyperplasia of the epithelial lining cells has become so pronounced that a histological pattern that can be described as adenomatosis develops. At this stage epithelial metaplasia has often extended so that some air spaces are lined with ciliated cells similar to bronchial epithelium and in others the lining cells have developed a squamous pattern. In these cases the multilayered squamous epithelium may fill the air space entirely. Both squamous and cuboidal epithelium frequently occur in the same areas of adenomatosis.

When advanced malignant tumors in the rat are discovered, they are often so widespread that it is impossible to determine their site of origin. When adenomas or very early carcinomas are found, however, they are frequently in the center of areas of advanced fibrosis/adenomatosis. That many asbestos-related carcinomas do develop from areas of fibrosis in the rat therefore appears certain. That such tumors only develop when advanced fibrosis is present needs confirmation.

Early studies did not quantify fibrosis, and although later studies have done so, the results have not related to fibrosis and tumor production in more than general terms. Wagner et al. (*16*) reported a very large study in which rats had been exposed to dust clouds of five asbestos types for periods between 1 day and 2 years. Pulmonary fibrosis was estimated on a seven-point scale based on histological patterns of which the first three corresponded roughly to the stages at which fibrosis is limited to the peribronchiolar region and the last four represented progressive stages of air space thickening. With normal lung scored as 1, those lungs showing asbestotic lesions were scored 2 to 8 and the results averaged for the experimental groups. In general, both the scores for asbestosis and the number of pulmonary tumors recorded rose with length of exposure for all five asbestos types, but the correlation was by no means exact, perhaps due to the relatively small experimental groups used in some cases.

Later studies (*17*) used the same experimental protocol to compare three chrysotile samples in rats. All produced pulmonary fibrosis and tumors, but the sample causing the most fibrosis was associated with the highest tumor incidence. Wagner et al. (*18*) again used UICC chrysotile as a control dust in inhalation studies involving glass microfiber, rockwool, and glasswool. All dusts produced some degree of fibrosis, but by far the highest levels were found with the chrysotile, which was the only dust to produce significantly more lung tumors than undusted controls. In a comparison study, McConnell et al. (*19*) used the same chrysotile and glass samples. Again, the chrysotile produced the highest levels of fibrosis and was the only dust to produce tumors. A very similar approach was used by Smith et al. (*20*) to examine the pathogenicity of glass fiber and refractory ceramic fiber in rats and hamsters. On this occasion, UICC crocidolite was used as a control dust. Crocidolite produced by far the highest levels of fibrosis and was the only dust to produce tumors. The same patterns of pulmonary response were reported by Le Bouffant et al. (*21*), who used chrysotile as a control dust for comparisons with glass wool, rockwool, and glass microfiber. Only chrysotile produced tumors. In this study, however, the degree of pulmonary fibrosis was estimated by chemical analysis of collagen content. Muhle et al. (*22*) used a similar experimental protocol with both chrysotile and crocidolite used for comparison with glass fiber. In this case the occurrence of interstitial fibrosis was reported in a similar number of rats from all the experimental groups but the severity was not recorded. No significant production of pulmonary tumors occurred with any of the dusts.

Only two studies so far have reported significant levels of fibrosis and tumor production in the lungs of rats treated with man-made fibers. These were by Davis et al. (*23*), who used ceramic aluminum silicate fiber (details of this study are included in Table 1) and Lee et al. (*24*), who used aramid fiber. In the latter study,

Table 1. The relationship between tumor production and advanced pulmonary fibrosis in rats treated by inhalation with a variety of mineral fibers.

Type of dust	Malignant pulmonary tumors	Benign pulmonary tumors	Mean areas of interstitial fibrosis in old animals, %
UICC chrysotile A + titanium dioxide	22	4	12.9
Tremolite	18	2	14.5
Long amosite + titanium dioxide	18	1	9.5
Long chrysotile	15	8	12.6
Wet dispersed chrysotile (WDC)	14	7	9.6
Milled chrysotile	14	2	8.8
Milled WDC	13	5	12.8
Factory WDC	10	11	12.1
Long amosite	10	4	10.0
UICC chrysotile A	8	7	9.1
Short fiber chrysotile	7	1	2.4
Factory chrysotile	3	8	7.7
Ceramic aluminum silicate	3	1	5.0
Brucite	2	3	2.9
Factory amosite	0	0	8.5
UICC amosite	0	2	2.6
UICC crocidolite	0	1	1.4
Short fiber amosite	0	0	0.1

although the presence of pulmonary fibrosis was recorded, it was not measured.

In Wagner's system rats were scored according to their most severe type of fibrotic lesion regardless of the proportion of lung tissue it occupied. In our laboratory, when inhalation studies began (*25*), we recorded the area of advanced fibrotic lesions although no distinction was made between Wagner's grades 5 and 8. This method of estimating pulmonary fibrosis has now been reported from a number of experiments (*25–27*), and a close overall relationship between mean levels of fibrosis and tumor production in rat lungs has been demonstrated for many asbestos samples and one sample of manmade mineral fiber (ceramic fiber). These results are illustrated in Table 1.

The protocol for these studies included examination of small groups of animals at the end of a 1 year dusting period and 6 months later, at which times almost no tumors or advanced asbestosis were present. Subsequently all animals were allowed to live out their full life span, since an overall estimation of the carcinogenicity of the fiber samples was the major intent. While adventitous deaths occurred in all experiments and increased with time, the majority of animals survived to within 6 months of the end of the study. Experiments were concluded when any experimental group was reduced to 6 in number. Only those animals surviving to within 2 months of the end (25–50%) were used for estimations of asbestotic lesions. For this reason, most tumors were found at an advanced stage of development, and their exact relationship with fibrotic lesions was often impossible to determine. In the aged rats examined in this way, however, a very large range of fibrosis was found, with some animals having only minor fibrotic patches and others having approximately one-third of lung parenchyma occupied by advanced asbestosis. From any one experiment the range for 15 to 20 animals could be between 1 and 35% of lung tissue occupied by advanced fibrotic lesions. With data from animals in a number of different inhalation studies it was considered that sufficient were available for further analysis in which the areas of fibrosis for individual animals with and without pulmonary tumors could be compared. Data from a total of 144 rats were available on computer from 10 studies with chrysotile and amosite asbestos, of which 85 had pulmonary tumors and 59 had not. Twenty-five of the tumors were benign and 60 were malignant.

These data were examined using the statistical technique of analysis of variance to look at the relationship between the areas of fibrosis and tumor presence and type. It was found that the standard deviation of percentage fibrosis was greatest in experiments where the level of fibrosis was greatest and to allow for this, percentage fibrosis was transformed to the log scale before analysis. The 144 rats were examined as one group initially, but by including the relevant interaction terms, the model also examined whether the relationship between tumor presence or type and area of fibrosis differed between individual experiments.

The mean percentage area of fibrosis for animals with and without tumors and with benign and malignant tumors are given for 10 experiments in Table 2, and a summary of results from the analysis of variance studies in Table 3. For the whole group of 144 rats, the percentage of lung occupied by fibrosis in animals with tumors was 12.5, while in those without tumors it was 7.3. This difference is significant ($p < 0.001$). In all but two of the 10 experiments, there was more pulmonary fibrosis in animals with pulmonary tumors, but with the small group sizes in individual experiments these differences were not significant. The figures from these studies, while demonstrating a significant relationship between the extent of pulmonary fibrosis and the development of pulmonary tumors, most probably underestimates the differential between animals that develop tumors and those that do not because of the measurement techniques adopted and because only animals of

Table 2. Mean and standard deviation of percent fibrosis by experiment and tumor presence and size.

Tumor		Experiment number 1	2	3	4	5	6	7	8	9	10	All
No tumors	Mean	4.1	8.8	8.7	5.1	9.7	5.5	12.7	1.6	14.8	7.4	7.3
	SD	0.7	7.1	5.3	4.0	7.8	6.0	7.9	1.8	11.2	4.8	6.4
	n	4	6	6	9	5	8	5	6	3	7	59
Any tumors	Mean	11.1	16.6	13.9	14.9	13.7	9.7	12.5	3.6	12.3	11.2	12.5
	SD	4.2	11.2	10.8	12.3	7.1	3.2	3.2	2.5	3.2	6.2	8.0
	n	10	9	14	9	8	8	6	4	9	8	85
Malignant	Mean	10.5	14.0	13.5	18.5	13.6	8.5	12.0	3.6	12.1	11.2	11.9
	SD	4.0	7.6	11.4	14.4	7.9	3.3	3.5	2.5	3.4	6.2	7.4
	n	9	7	6	5	4	5	4	4	8	8	60
Benign	Mean	16.3	25.5	14.2	10.4	13.8	11.7	13.6	—	13.7	—	14.2
	SD	—	21.4	11.2	8.7	7.4	2.3	2.9	—	—	—	9.3
	n	1	2	8	4	4	3	2	0	1	0	25

Table 3. Summary analysis of variance table.[a]

Variable	df	SS	MS	*F*-ratio	*p*-Value
Experiment	9	33.26	3.70	4.33	< 0.001
Tumor presence	1	13.61	13.61	15.94	< 0.001
Tumor type (benign or malignant)	1	0.01	0.01	0.01	0.92
Experiment × tumor presence	9	4.25	0.47	0.55	0.84
Experiment × tumor type	7	2.31	0.33	0.39	0.91
Residual	116	99.04	0.85		
Total	143	152.48	1.07		

[a] Dependent variable, log (% fibrosis).

advanced age were examined in detail both for fibrosis and the presence of tumors. It would be expected that a malignant tumor expanding through lung tissue would overgrow and destroy any area of fibrosis/adenomatosis from which it originated. Consequently, if only one major area of fibrosis was present in any lung and the tumor developed in this area, its subsequent destruction could result in a low figure for fibrosis from an animal with an advanced tumor. We adopted the formula of excluding areas of tumor from the overall lung areas measured for fibrosis, but that procedure did not greatly affect the issue in question.

It would be expected that animals dying with early pulmonary tumors might show the best relationship to pulmonary fibrosis, and the 25 rats with benign tumors in the group examined did have more fibrosis (14.2%) than those with malignant tumors (11.9%). This difference was not, however, significant. Many animals without pulmonary tumors had high figures for pulmonary fibrosis/adenomatosis, but this was to be expected, for while it has been suggested that asbestos-related tumors may develop only from areas of advanced asbestosis, tumor production would not be expected to be universal even when very large areas of fibrotic change were present. Of greater importance is the situation where some animals develop pulmonary tumors with low recorded levels of fibrosis. In the series of 85 tumors recorded in this paper, 10 developed in animals with less than 4% of lung area occupied by advanced asbestotic lesions. Histological sections from these cases were reexamined individually, and it was found that they were clearly divided into two categories. Five were advanced tumors that had entirely occupied a single lung lobe but had not spread to others where only relatively small amounts of fibrosis were present. Five were early tumors either benign or showing only the earliest signs of invasiveness and all showed strong evidence of originating from the center of areas of interstitial fibrosis/adenomatosis. The rest of the lung parenchyma contained relatively few areas of this type of pathological change.

The accumulation of data from a number of inhalation studies in rats exposed to asbestos or other mineral fibers thus supports the suggestion that when pulmonary tumors develop in these animals they usually do so from areas of adenomatoid change occurring along with advanced fibrosis. Closer study of this association would require specifically designed studies. The same experimental system of long-term inhalation of fiber by rats would permit a much more detailed examination of this relationship if a different experimental protocol was adopted. Instead of permitting most rats to live out their full life span following the cessation of dust exposure, larger groups of rats would be used and the study terminated 2 years after the start of dust exposure (rats aged 2.75 years). It is known from our accumulated study with a variety of asbestos samples that few pulmonary tumors develop in animals less than 2 years old, but they occur with progressively greater frequency after this time. Examination of a relatively large population of rats during the period of early tumor development should enable the sites of tumor origin to be determined with precision in most cases. If the mater is considered of sufficient importance this study should certainly be undertaken.

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