

Mineralogic Parameters Related to Amosite Asbestos-induced Fibrosis in Humans¹⁻³

PLAINTIFF'S EXHIBIT

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Introduction

Epidemiologic studies as well as analyses of lung asbestos content have suggested that asbestosis (diffuse interstitial fibrosis) is associated with exposure to or retention of very large amounts of asbestos (1-8). Analytic studies quantifying either asbestos bodies or uncoated asbestos fibers have further indicated that on a case by case basis, greater asbestos burdens are associated with pathologically greater degrees of fibrosis (3-8).

These reports have generally considered only the issue of fiber number and evaluated only one sample per case, so that the effects of intrapulmonary fiber distribution and its relationship to local degrees of fibrosis are unknown. The effects of parameters related to fiber size (length, width, aspect ratio, etc.) have mostly been ignored. Because it is generally believed that long fibers are more fibrogenic than short ones (9-12), this question is of interest, but there is disagreement about which parameter may be most useful to measure. Timbrell and colleagues (13) have proposed that one should examine total fiber size parameters, particularly total surface area and mass (i.e., total mass as the sum of the masses of all fibers in a given sample), rather than mean fiber sizes to obtain correlations with fibrosis (see DISCUSSION).

We have previously examined the issues of local fiber burden and size and their relationship to fibrosis in the lungs of a series of chrysotile asbestos miners and millers, and found that the grade of fibrosis correlated positively with fiber concentration but negatively with fiber length (8). In order to determine whether similar effects are seen with amphibole asbestos, in this study we have examined the lungs of workers with heavy exposure to the commercial amphibole amosite and measured the same parameters. In addition, we have compared, on a fiber-for-fiber basis, the fibrogenic effects of chrysotile asbestos and its natural contaminant, tremolite, with amosite.

SUMMARY We have previously shown that in the lungs of a group of chrysotile miners and millers, grade of interstitial fibrosis (asbestosis) is directly proportional to tremolite fiber or chrysotile fiber concentration but is inversely proportional to mean fiber length and length-related parameters. To compare the effects of the commercial amphibole asbestos amosite on parenchymal fibrosis, we histologically graded fibrosis in four different sites in the lungs of 20 shipyard and insulation workers with heavy amosite exposure and measured by analytic electron microscopy fiber concentration and size in corresponding portions of lung tissue. Fibrosis grade was found to be strongly positively correlated with amosite concentration and negatively correlated with mean fiber size parameters, including fiber length, width, surface area, and mass. A comparison of our present results with our data on the chrysotile miners and millers showed that the regression lines of fibrosis grade versus concentration for amosite, chrysotile, and tremolite were statistically different. These findings indicate that amosite concentration, like chrysotile and tremolite concentration, is closely and directly related to fibrosis at the local lung level. Furthermore, these observations again raise the possibility that short fibers may be more important than is commonly believed in the genesis of fibrosis in man. Last, the concentration comparison data indicate that, fiber for fiber, amosite is more fibrogenic than is chrysotile or tremolite, and indirectly suggest that tremolite is more fibrogenic than is chrysotile.

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Methods

Specimen Selection and Preparation

Twenty specimens of autopsy lungs from heavily exposed insulators and/or shipyard workers were selected from a larger group of such specimens. To be selected, we required at least a midsagittal slice of left or right lung so that multiple samples could be obtained. Initial screening of histologic sections was carried out to ensure that the 20 cases represented a range of interstitial fibrosis from none to severe. An initial sample was analyzed to ensure that the patient had been exposed to amosite asbestos.

To examine the relationship between degree of fibrosis and asbestos content, we followed a protocol similar to that used in our previous study of chrysotile miners (8). Four blocks of fixed tissue, approximately 1 × 1 × 0.5 cm, were selected (two upper lobe, two lower lobe; selection random within the lobes). A piece measuring 1 × 1 × 0.2 cm was trimmed off each block, dehydrated in alcohol, and embedded in paraffin. A 5-μm section was cut and stained with hematoxylin and eosin and graded for interstitial fibrosis as described below.

Mineralogic Analysis

The remaining portion of each tissue block was dissolved in bleach and the asbestos fibers collected and transferred to coated electron microscope grids as per our published pro-

tol (14). For each sample, approximately 75 sequentially encountered amosite fibers were identified and measured using a Philips 400T electron microscope equipped with a Kevex 7000 energy dispersive X-ray spectrometer. At the time of tissue sampling, an additional piece of tissue was taken and dried to constant weight to allow final expression of results in terms of fibers/g dry lung. Using an algorithm relating weight of lung dissolved and area of grid examined, the concentration of fibers/g dry lung was determined. Further details of the analytic procedure can be found in reference 14. Amosite surface area and mass were calculated using a height-to-width ratio of 2.5 (15) and a density of 3.4. All size, surface, and mass data were expressed as geometric means because fiber sizes are usually log normally distributed. Additionally, the to-

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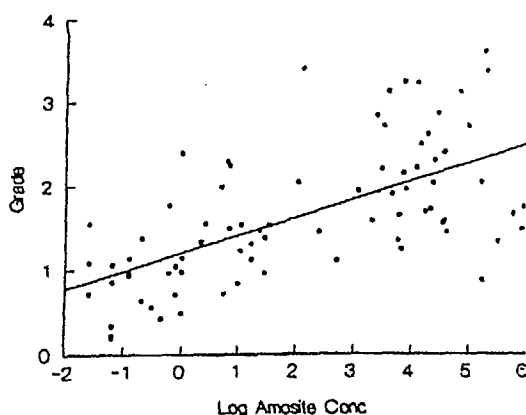


Fig. 1 (left). Grade versus log amosite concentration as fibers/g dry lung $\times 10^4$: $r = 0.62$, $p < 0.001$.

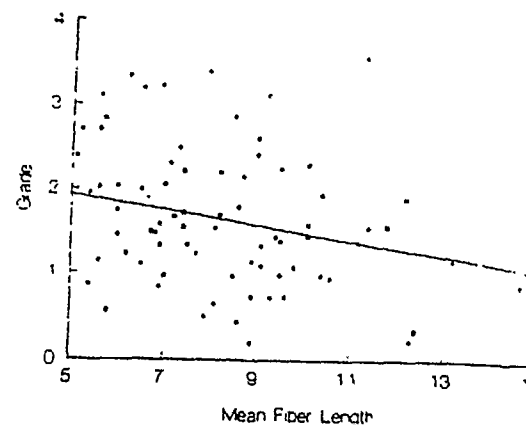


Fig. 2 (right). Grade versus mean fiber length in microns: $r = -0.26$, $p < 0.01$.

tal length, width, and so forth of all fibers in each site were calculated (see below for the significance of these values).

Grading of Interstitial Fibrosis

Fibrosis was graded in exactly the same fashion (16) as used in our previous study of chrysotile miners (8). In brief, each hematoxylin and eosin-stained section was examined in its entirety using a $10\times$ objective, and each microscopic field was assigned a grade of zero (no fibrosis) to 4 (most severe fibrosis). The value for each field was recorded and an average taken over all the fields to provide a value for each site. Some of the cases graded for the study on chrysotile miners (8) were regraded to ensure consistency between that study and the present one.

Statistical Analysis

Using simple regression, the relationship of the various measured parameters to grade of interstitial fibrosis was examined. The SYSTAT system (17) was used for this purpose. Initial inspection showed that the concentration data

could be normalized by a log transformation, and the analyses using concentration were performed on the transformed data. Bonferroni corrections for multiple comparisons were used as appropriate. To evaluate the question of whether amosite, chrysotile, and tremolite produce different amounts of fibrosis on a fiber-for-fiber basis, comparisons were made using the method of Feldman (18) for examining families of lines.

Results

Demographic Data

The mean age of the patients $\pm$ SD was 68 ± 9 yr; mean pack-years of smoking, 29 ± 26 ; mean years of asbestos exposure, 24 ± 13 ; and mean years since first exposure, 44 ± 4 .

Correlations with Concentration Data and Size Data

A strong, positive correlation ($r = 0.62$) was seen between fibrosis grade and amo-

site fiber concentration (figure 1). Significant negative correlations were seen between fibrosis grade and all measured mean size parameters except aspect ratio (figures 2-6). By contrast, a significant positive correlation was seen between fibrosis grade and total fiber length, width, and aspect ratio, but no correlation was found between fibrosis grade and total fiber surface or mass (figures 7-11).

Comparison of Asbestos Fiber Types

The regression data for fibrosis grade and log fiber concentration for amosite, chrysotile, and tremolite are shown in table 1. The latter data were derived from our previous report (8). Reinspection of the chrysotile data from that report showed that four sites had extremely small fiber concentrations ($< 10,000$

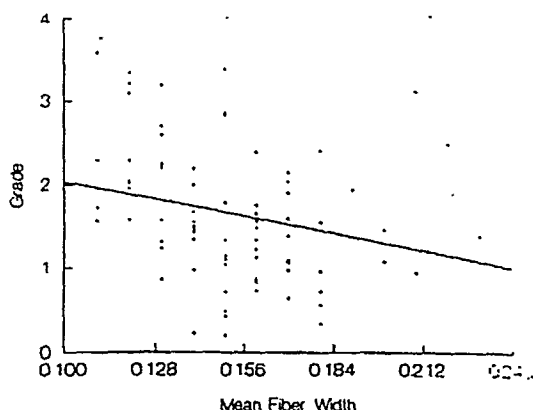


Fig. 3 (left). Grade versus mean fiber width in microns: $r = -0.28$, $p < 0.01$.

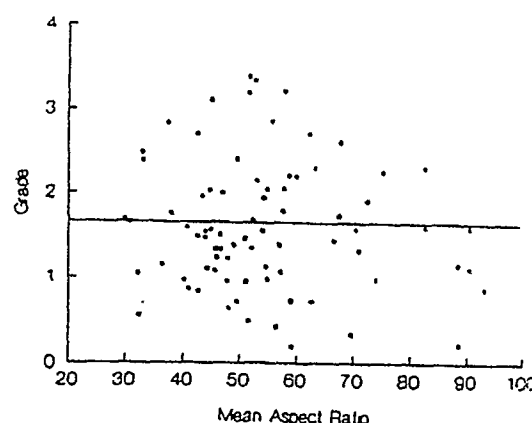


Fig. 4 (right). Grade versus mean fiber aspect ratio: $r = -0.01$, p value is not significant.

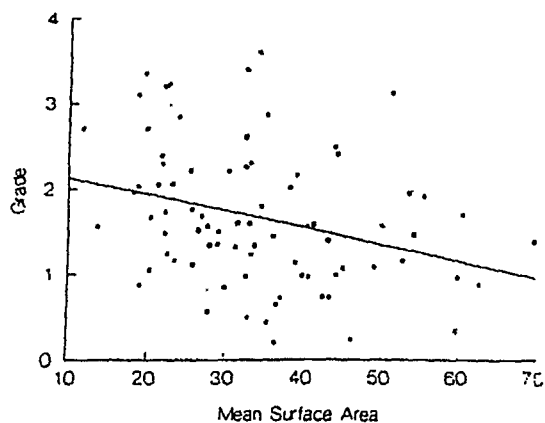


Fig. 5 (left). Grade versus mean fiber surface area in microns squared: $r = -0.32$, $p < 0.003$.

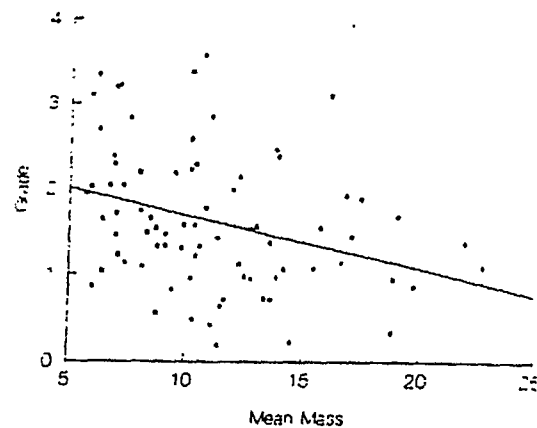


Fig. 6 (right). Grade versus mean fiber mass $\times 10^{-12}$ g: $r = -0.33$, $p < 0.003$.

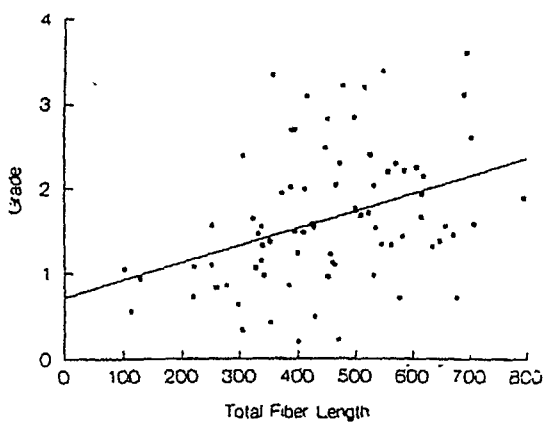


Fig. 7 (left). Grade versus total fiber length in microns: $r = 0.37$, $p < 0.001$.

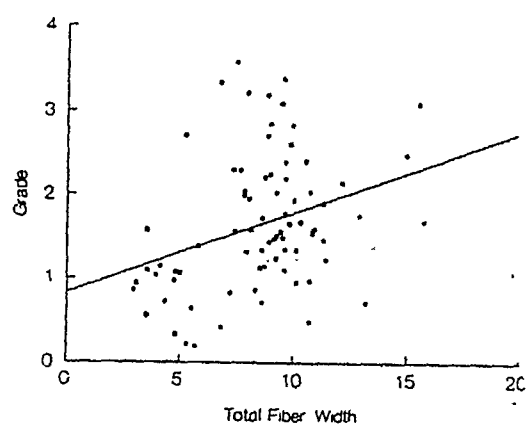


Fig. 8 (right). Grade versus total fiber width in microns: $r = 0.33$, $p < 0.003$.

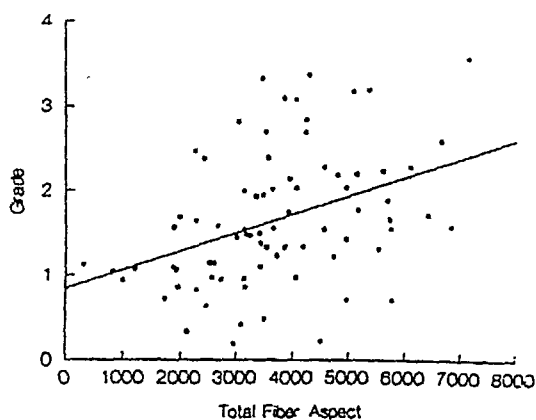


Fig. 9 (left). Grade versus fiber aspect ratio: $r = 0.40$, $p < 0.001$.

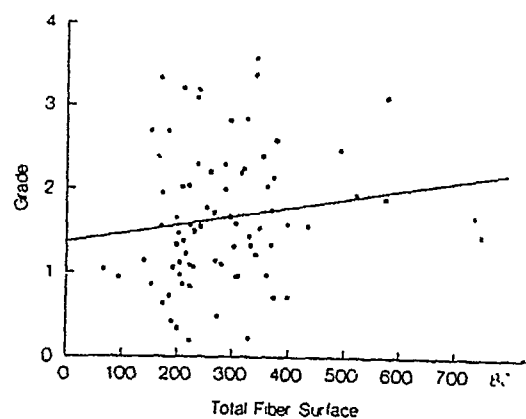


Fig. 10 (right). Grade versus total fiber surface area in microns squared: $r = 0.16$, p value is not significant.

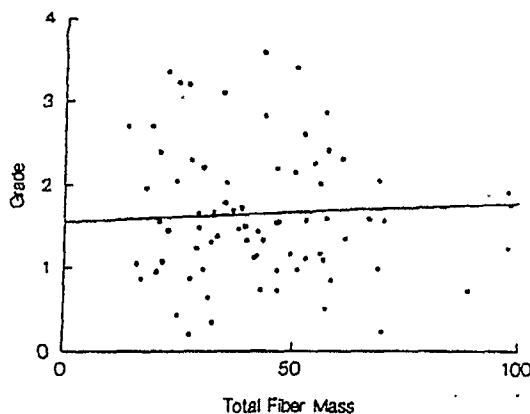


Fig. 11. Grade versus total fiber mass $\times 10^{-13}$ g; $r = 0.07$, p value is not significant.

fibers/g dry lung) but considerably greater than zero fibrosis grades. It was felt in retrospect that these data, which as a group formed outliers from the main mass of points, were unlikely to be correct, and these points were dropped for this analysis. This procedure had the effect of bringing the intercept of the chrysotile regression line through the origin at about the same point as the tremolite and amosite regression lines, a finding which makes biologic sense.

Analysis of these data by the method of Feldman (18) showed that all three regression lines were significantly different from each other (each $p < 0.01$), i.e., for a given fiber concentration, amosite produced more fibrosis than did chrysotile, which produced more fibrosis than tremolite. A similar analysis of the effect of mean fiber length and fibrosis grade, also shown in table 1, indicated no difference between amosite and chrysotile, but a significant difference existed between tremolite and the other fibers ($p < 0.05$ comparing tremolite to chrysotile or amosite).

Discussion

In this study, we have used the lungs of workers exposed to amosite asbestos to examine the correlation of fibrosis grade

and a variety of mineralogic parameters. We have selected this group of workers because their amosite exposure was, historically and analytically, quite high and, in a very broad sense, reasonably homogeneous, so that the effects of this specific fiber type on fibrosis can be evaluated. However, this study population is not ideal: all of the workers historically had chrysotile exposure, generally at a very high level, as well. As is usually true of remote chrysotile exposure (6-12, 14, 15), most of the chrysotile had disappeared from these lungs by the time of analysis, and there were too few chrysotile fibers left to even attempt to count and correlate with fibrosis grade. To the extent that past chrysotile exposure played a role in producing fibrosis, our amosite results may therefore be in error; however, the comparative data on amosite, tremolite, and chrysotile discussed below suggest that this is not a serious problem.

Using this group of lungs, we have shown that interstitial fibrosis (asbestosis) can be related to the concentration and size of amosite fibers at the local level. The correlation between fibrosis grade and concentration was positive, i.e., the greater the fiber concentration, the greater the fibrosis grade. In contrast, the correlation between fibrosis grade and

mean fiber length was negative, i.e., the longer the fiber, the lower the fibrosis grade. A negative correlation of roughly similar magnitude was found for most of the other parameters related to mean fiber size. Length thus appears to be a suitable representative parameter to measure the effects of mean fiber size on fibrosis.

Several different groups have found a positive correlation between fibrosis grade and fiber concentration at the whole case level by counting asbestos bodies, uncoated fibers by light microscopy, and uncoated fibers by electron microscopy (3-8). In most of these studies, the samples for mineral analysis were not taken from the same areas as were used to grade interstitial fibrosis, and generally only one sample was obtained per case. The present sampling scheme has the advantage of examining multiple areas within the same lung and allows examination of histologic and mineralogic samples that are virtually identical. Because multiple points are sampled in each case, this protocol also permits us to use a newly developed statistical method (18) for examining series of lines and thus to compare fibrosis as a function of concentration and size parameters among the different asbestos minerals.

Using this sampling protocol (8), we previously showed that for chrysotile asbestos and its natural contaminant tremolite, positive correlations were present between fibrosis grade and fiber concentration. The finding of a similar effect in our present study of amosite-exposed workers suggests that for all types of asbestos, the degree of local fibrosis is closely related to the local fiber concentration. What our present and previous studies do not explain, however, is the distinct lower zonal distribution of asbestosis in man; thus far, studies from our laboratory and from other laboratories have failed to find a consistently greater fiber concentration in the lower lung zones (19-22).

Much less information is available concerning the relationship of fibrosis grade and parameters related to fiber size in man. In our study of chrysotile workers (8), we found a negative correlation between mean fiber length and length-related parameters (aspect ratio, surface area, mass) and fibrosis grade, and the same effect is present in the data on amosite-exposed workers. This is a very surprising finding because animal studies consistently indicate that long fibers are more fibrogenic (and more carcinogen-

TABLE 1
REGRESSION EQUATIONS FOR FIBROSIS GRADE AS A FUNCTION OF FIBER CONCENTRATION OR LENGTH AS DETERMINED BY THE METHOD OF FELDMAN*

Fiber Type	Fibrosis Grade as a Function of Log Fiber Concentration		Fibrosis Grade as a Function of Geometric Mean Fiber Length	
	Intercept as Fibrosis Grade	Slope as Δ Fibrosis Grade/ Δ Log Concentration	Intercept as Fibrosis Grade	Slope as Δ Fibrosis Grade/ Δ Mean Length
Amosite	1.20	0.21	2.04	-0.17
Chrysotile	0.99	0.17	1.72	-0.22
Tremolite	0.59	0.18	2.53	-1.05

* See reference 16

ic) than are short fibers (9-12). Timbrell (23) has suggested that fibrotic lungs retain more fibers than do nonfibrotic lungs, and it is conceivable that what we are observing is a very local increase in fiber retention in any area in which fibrosis develops. However, to produce a negative correlation with fibrosis grade, preferential retention of short fibers would have to occur. Such an effect might be seen if fibrosis simply impaired clearance of all types of fibers because short fibers generally outnumber long fibers in dust clouds; however, one does have to question if given the variability of the dust clouds inhaled by workers under different conditions, the general person-to-person variability of clearance of any dust and the necessity that fiber clearance impairment be exactly proportional to local degree of fibrosis, whether this effect would be consistent enough to produce the type of negative correlation found here.

The other possibility is that, at least in humans, short fibers are in and of themselves more important generators of fibrosis than, has generally been believed and/or that, once impaired clearance is established for any reason, short fibers become more dangerous than they would otherwise be. The situation in man may also be complicated by other types of exposures; for example, we have shown in guinea pigs that cigarette smoke increases retention of short fibers to a greater degree than retention of long fibers and also changes the way in which specific cells in the lung handle long or short fibers (24, 25), and it is possible that cigarette smoke also changes the retention pattern in humans.

In contrast to the negative correlation of fibrosis grade and mean fiber size parameters, a significant positive correlation was seen between fibrosis grade and total fiber length, width, and aspect ratio, and a positive but nonsignificant correlation was seen for grade and total surface area or mass. Timbrell and colleagues (13) originally proposed measuring this type of parameter because it could be easily determined with a light scattering system and hence was far more economic and faster than using electron microscope methods. Timbrell and colleagues found that surface area measured in this fashion gave consistent predictions of degree of fibrosis with different types of asbestos. We have confirmed the idea that total rather than mean size parameters may provide predictive information in regard to amosite-induced fibrosis, al-

though surface area does not appear to be one of the parameters that is useful, but we were unable to find any correlations of total size parameters and fibrosis when we examined chrysotile and tremolite (8). The reasons for these discrepancies are unclear. It should be appreciated that total fiber parameters in the present study correlate strongly with fiber concentration, so in practice the total fiber size parameters act as a substitute for fiber number.

A question of considerable interest for the understanding of asbestos-related disease is whether all asbestos types are equally fibrogenic. Epidemiologic studies have shed little light on this issue in humans. It is interesting that in two animal inhalation experiments examining the fibrogenicity of long fiber chrysotile or amosite, Davis and coworkers (9, 10) found a roughly equal mean fibrosis grade after approximately 30 months of exposure to both fiber types even though the measured amosite exposure in terms of fibers/ml was only about half the measured chrysotile exposure. This finding implies that amosite is more fibrogenic than is chrysotile.

The present analyses were designed to allow a direct examination of this question in humans. Using the data on chrysotile and tremolite from our previous study, and employing the newly developed statistical method of evaluating families of lines proposed by Feldman (18), we found that on a fiber-for-fiber basis, amosite was more fibrogenic than was either chrysotile or tremolite (table 1). Although the regression data in table 1 suggest at first glance that chrysotile is more fibrogenic than tremolite, this may not actually be true. Extensive animal and human data indicate that chrysotile is rapidly removed from lung (6-12, 14, 15), even in experimental conditions in which it produces marked fibrosis (9, 10), so that the amount of chrysotile that must once have been present in our chrysotile miner lungs, and which is at least in part responsible for fibrosis, was enormously greater than the analytic data imply. Tremolite, by contrast, constitutes only a few percent of the chrysotile ore but, like other amphiboles, is poorly cleared from lung. Thus, our regression data can be interpreted as indicating that the vast amount of (now invisible) chrysotile exposure produced only about as much fibrosis as the several orders of magnitude lower tremolite exposure, i.e., tremolite is actually more fibrogenic than is chrysotile on a fiber-for-fiber basis.

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