

Observations on the Distribution of Asbestos Fibers in Human Lungs¹

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In an attempt to begin evaluating the distribution of mineral fibers within lung, multiple small adjacent samples were selected from peripheral portions of three normal and six asbestotic lungs, and analyzed for asbestos and nonasbestos fiber content. For both normal and fibrotic lungs there was marked variation in fiber concentration from site to site: the mean in-case ratio of maximum to minimum fiber concentration was 7.5 for amosite and crocidolite, 7.0 for tremolite, 3.8 for chrysotile, 4.0 for nonasbestos fibers, and 5.4 for asbestos bodies. In order to test the precision of the membrane filter method of quantitating particulates, multiple replicate samples of reference mineral standards were examined in a similar fashion: for these standard samples the ratio of maximum to minimum particle concentration varied from only 1.3 to 1.5. It is concluded that: (1) the membrane filter preparation technique used here for counting fibers provides reproducible results, and (2) there is marked variation in asbestos and nonasbestos fiber concentration from area to area within lung tissue. The latter finding implies that analysis of small subpleural lung biopsies may produce misleading results, and that larger pieces or several pieces should be analyzed when attempting to define total asbestos fiber content of the lung.

INTRODUCTION

It is well established that asbestos and nonasbestos mineral particles are present in the lungs of every resident of industrialized countries, and that much larger numbers of asbestos fibers are present in those with occupational exposure to the dust (Churg, 1982). However, little is known about the distribution of any type of mineral particle within lung. Animal experiments have indicated that the initial site of asbestos fiber deposition is in the respiratory bronchioles and alveolar ducts (Brody *et al.*, 1981) and that with time asbestos fibers tend to concentrate under the pleura (Morgan *et al.*, 1977). The few studies in humans which have looked at this problem have concluded that fibers are found in greater numbers near the visceral pleura (Sebastien *et al.*, 1977; Churg and Warnock, 1980); essentially nothing is known about airway deposition.

The differences described above occur over relatively large areas. Little is known about variations in fiber concentration within small areas. It is possible, for example, that the increase in concentration between central and subpleural samples of a given lung is more or less continuous, or equally possible that there are

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distinct concentration jumps from one area to the next. Examination of histologic sections shows quite clearly that there are variations in the distribution of asbestos bodies, and we have previously suggested that in fibrotic lungs there are very marked concentration differences in numbers of bodies in different sites (Churg and Warnock, 1981). In this paper we begin to examine the question of mineral particle, especially asbestos fiber, distribution, by evaluating the concentrations of particles found in adjacent tissue samples.

MATERIALS AND METHODS

This study was based on lung tissue from nine individuals with a history of asbestos exposure, and from whom a long subpleural strip of lung tissue was available. Occupational and smoking histories, and histologic sections were available for all cases. Three cases were chosen in which the lung was grossly and microscopically normal; the remaining six cases were chosen because the lung was markedly fibrotic (asbestotic).

For all cases a subpleural strip of tissue measuring approximately $5 \times 3 \times 1$ cm was cut out. This was then divided into five $3 \times 1 \times 1$ -cm slices, and the slices further cut into three pieces of approximately 1 cc. One of the latter pieces was used for a preparation to count mineral particles by electron microscopy, one was used for a preparation to count asbestos bodies by light microscopy, and one was weighed, dried, and reweighed to determine a wet:dry weight ratio.

Asbestos bodies and mineral fibers were extracted from lung tissue using methods we have previously described (Churg and Warnock, 1980). Briefly, this consisted of dissolving the lung tissue in bleach, and, for the electron microscope preparations, further treating the sediment with hydrogen peroxide. The sediment was then collected on a Millipore filter of $0.45\text{-}\mu\text{m}$ pore size. For light microscopy this filter was cleared and mounted on a glass microscope slide; for electron microscopy, pieces of the filter were cut out and the mineral particles transferred to coated electron microscope grids using the Jaffe washer technique.

Asbestos bodies were counted by examining a known area of the Millipore filter under the light microscope and converting the result to numbers of bodies per gram dry lung. For determination of types and numbers of mineral fibers, 20 squares of each electron microscope grid were scanned at $18,000\times$ and each fiber longer than $0.5\text{ }\mu\text{m}$ was examined for morphology, and by electron diffraction and energy dispersive x-ray spectroscopy to determine mineral identity. Results were converted to values per gram dry lung. For the purposes of this paper, amosite and crocidolite fibers were considered as one group.

To determine the error in electron microscopic counting introduced by the method itself, a series of six replicate samples of UICC amosite (as an example of an amphibole and a fiber) and six samples of cosmetic talc (as an example of a nonfibrous particulate) were created. These were prepared by suspending the reference mineral in water, taking six aliquots of known volume (the equivalent of taking six samples of lung), and processing them through bleach, peroxide, and Millipore filter steps as described above. Twenty squares were then counted for each sample, and the fibers or particles per milliliter calculated as above.

RESULTS

Reference standards. Table I shows the counted values and the mean, standard deviation, range of counts, and ratio of highest to lowest count for the amosite and the talc standards. Excellent reproducibility was seen from sample to sample. The observed values for the amosite standard varied from 237- to 310×10^6 fibers/ml, with a mean of 286×10^6 and standard deviation of 27×10^6 . The ratio of highest to lowest observed value was 1.3. For the talc sample, values ranged from 159 to 238 with a mean and standard deviation of 198 and 37×10^6 particles/ml. The ratio of highest to lowest count was 1.5.

Asbestos-exposed cases. Detailed results for the test cases are shown in Tables 2, 3, and 4. As a measure of the variation between sites we have also calculated the ratio of highest to lowest observed value for each case, and have summarized these ratios in Table 5.

For every type of fiber examined there was considerably more variation from site to site than was present in the replicate samples of standard minerals. In a few cases the observed ratio was nearly as low as that for the reference standard (for example, nonasbestos fibers in case 1059 and case 1125, and amosite and crocidolite fibers in case 1012), but for the rest the ratios were much higher, reaching 16.5 for amosite and crocidolite in case 1054. The mean ratio for amosite and crocidolite over seven cases was 7.5, and for nonasbestos fibers over nine cases, 4.0. Similar variations were seen for chrysotile and tremolite (Table 5). For seven cases, a nonparametric correlation test (Spearman's ρ) was used to determine if the concentration of amphibole asbestos fibers was related to the concentration of nonasbestos fibers; the result was not significant ($\rho = 0.21$, P less than 0.56).

Considerable variation was also found between highest and lowest ratio values of asbestos bodies (Table 4); these ranged from 1.5 to 15, with a mean ratio over seven cases of 5.4. In two cases asbestos bodies were not evaluated because of technical problems in sample preparation. A correlation test for asbestos bodies and fibers of amosite and crocidolite gave a nonsignificant result ($\rho = -0.60$, P less than 0.48).

TABLE I
REPLICATE ANALYSES OF REFERENCE STANDARDS

Sample	Amosite standard	Talc standard
1	286	162
2	275	159
3	299	238
4	308	223
5	237	172
6	310	233
Mean	286	198
SD	27	37
Range	237-310	159-238
Ratio ^a	1.3	1.5

Note. All values $\times 10^6$ fibers/ml.

^a Ratio of highest to lowest observed count.

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TABLE 3
LUNGS WITH ASBESTOSIS

Case: Mineral: Sample	1117 Chrysotile	1117 Tremolite	1117 Nonasbestos fibers	1066 Amosite/ crocidolite	1066 Nonasbestos fibers	1070 Amosite/ crocidolite	1070 Nonasbestos fibers
1	8.0	54.6	14.6	8.0	5.7	8.3	5.5
2	10.3	35.1	13.8	4.7	5.5	6.6	7.0
3	5.8	5.6	4.4	9.4	15.6	5.8	4.1
4	16.9	29.7	26.8	25.8	26.2	14.8	9.8
5	3.7	8.4	4.4	49.8	36.7	1.1	8.0
Mean	9.0	26.7	12.8	19.5	18.0	7.3	6.9
SD	5.0	20.2	9.2	18.8	13.5	5.0	2.2
Range	3.7-16.9	5.6-54.6	4.4-26.8	4.7-49.8	5.5-36.7	1.1-14.8	4.1-9.8
Ratio ^a	4.6	9.8	6.1	10.6	6.7	13.5	2.4

Case: Mineral: Sample	1025 Amosite/ crocidolite	1025 Nonasbestos fibers	1125 Amosite/ crocidolite	1125 Nonasbestos fibers	1054 Amosite/ crocidolite	1054 Nonasbestos fibers
1	25.3	11.9	26.8	13.1	5.2	8.4
2	24.0	15.3	28.8	11.6	1.5	6.8
3	32.9	10.0	26.0	8.6	4.0	15.3
4	54.3	6.8	67.3	15.4	24.8	35.9
5	43.6	3.4	42.9	9.2	5.3	19.7
Mean	36.0	9.5	38.3	11.6	8.2	17.2
SD	12.9	4.6	17.5	2.8	9.4	11.6
Range	24.0-54.3	3.4-15.3	26.0-67.3	8.6-15.4	1.5-24.8	6.8-35.9
Ratio ^a	2.3	4.5	2.6	1.8	16.5	5.3

Note. All values $\times 10^6$ fibers/g dry lung.

^a Ratio of highest to lowest observed value.

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TABLE 4
ASBESTOS BODIES

Case: Sample	Normal lungs		Fibrotic lungs				
	1059	1012	1117	1066	1070	1025	1125
1	4	44	7.2	320	31	1700	440
2	34	45	5.0	380	45	930	660
3	4	40	3.6	ND	48	1000	200
4	6	32	5.5	390	37	700	410
5	11	3	2.4	260	12	1000	320
Mean	12	33	4.7	340	35	1000	410
SD	13	17	1.8	60	14	380	170
Range	4-34	3-45	2.4-7.2	260-390	12-48	700-1700	200-660
Ratio ^a	8.5	15	3.0	1.5	4.0	2.4	3.3

Note. All values $\times 10^3$ bodies/g dry lung.

^a Ratio of highest to lowest observed value.

DISCUSSION

In the Introduction we have summarized much of the available data on variation of asbestos and other mineral fiber and particle distribution within the lung; it is clear that little information is available on this topic. Questions about asbestos fiber distribution are of particular interest, since asbestos-induced disease tends to have rather distinct localizations: asbestosis is usually worse in the lower sub-pleural zones. Asbestos shows a distinct tendency to produce disease in the pleura: this may appear in the form of effusions, fibrosis, plaques, or mesothelioma (Selikoff and Lee, 1978). Asbestos also causes peribronchial fibrosis (Craighead *et al.*, 1982) as well as bronchogenic carcinoma, lesions probably related to airway deposition of fibers. We have also recently shown that persons exposed to inorganic nonasbestos dusts may develop disease of the small airways, again implying the importance of particle localization (Churg and Wright, 1982).

In this paper we have examined a method used by us and others for quantitating fibers and particles, and have considered the variation in particle distribution over small areas of lung. The data in Table 1 indicate that concentration variations

TABLE 5
MEAN VALUES OF THE IN-CASE RATIOS OF HIGHEST TO LOWEST OBSERVED COUNT

	Number of cases	Mean ratio
Asbestos bodies	7	5.4
Amosite/crocidolite	7	7.5
Tremolite	2	7.0
Chrysotile	2	3.8
Nonasbestos fibers	9	4.0
Standard amosite	6 ^a	1.3
Standard talc	6 ^a	1.5

^a Number of samples.

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introduced by the dissolution, centrifugation, and membrane filter collection technique are satisfactorily small, both for fibers and for nonfibrous particulates. The standard deviations for the asbestos and talc standards are approximately 10 to 20% of the mean values, and the ratio of highest to lowest observed count is 1.3 to 1.5. Values for this ratio of much greater than 1.5 can be presumed, therefore, to reflect variations in the actual concentration in lung samples. It should be noted, however, that these conclusions apply to particulates which do not have a tendency to aggregate tightly; aggregated particulates are likely to produce much greater errors arising from uneven distribution on the electron microscope grid.

As shown in Tables 2-4, most lungs demonstrate marked concentration differences from area to area for both asbestos and nonasbestos fibers, although a small number of lungs demonstrate fairly even distributions. This is also true of the concentration of asbestos bodies. There is a suggestion from the data that there is more variation in fibrotic lungs, but the numbers of cases are too few to be statistically significant. The results of the correlation tests indicate that the different fiber types appear to vary independently in concentration; this is also true of asbestos bodies and fibers.

These findings suggest that when studies are performed using digestion and membrane filtration techniques, as large a piece of lung as possible should be analyzed to minimize local site variation. They also suggest that small peripheral biopsies, i.e., the kind of biopsy which is exactly equivalent in size and location to the samples we have examined, may be somewhat misleading if used to determine particle concentration. Certainly it would seem preferable to analyze at least two different pieces to minimize this type of error, as can be seen by selecting any 2 values for cases in Tables 2 and 3.

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