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Comparison of the results of asbestos fibre dust counts in lung tissue obtained by analytical electron microscopy and light microscopy

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SUMMARY The efficiency of the light microscope with that of the electron microscope in detecting asbestos fibres in human lung tissue was computed. Necropsy material from 55 patients who had died from asbestos related diseases was analysed independently by phase contrast microscopy and electron microscopy. As expected the number of fibres identified using electron microscopy was higher than that identified by light microscopy. By adjusting the electron microscope fibre counts to allow for the limited resolving power of the light microscope, however, a significant correlation of the number of fibres identified using the two methods was obtained. The best correlation was found with specimens containing crocidolite (correlation coefficient 0·79) and amosite (correlation coefficient 0·74), while chrysotile gave a much lower correlation (correlation coefficient 0·15). The cumulated fibre diameter distribution obtained using the electron microscope suggests that the light microscope is able to visualise only 5% of crocidolite, 26·5% of amosite, and 0·14% of chrysotile present in lung tissue. Therefore, although it is possible, using the electron microscope, to predict the asbestos fibre count that would be obtained by light microscopy, the reverse prediction cannot be made: it is impossible to determine the proportion of the various asbestos mineral types using the light microscope.

In recent years an important aspect in the study of diseases induced by asbestos has been the type of fibre and the amount of exposure that may result in pathological changes.[1] Considerable progress has been made in this field since the development of techniques that enable single asbestos fibres in human tissues to be identified using electron microscopy and electron microprobe analysis.[2-5]

Light microscope studies have been made on lung tissue to assess secular trends in ferruginous body concentrations over a period of time with the aim of establishing the proportion of people in different cities and towns in whom ferruginous bodies can be found.[6] Other studies have been performed to determine whether there is a relation between the concentrations of fibrous particles and ferruginous bodies detected in the lungs and different diseases.[7-9] These studies, using the light microscope, were all performed assuming that the ferruginous bodies observed were all formed on asbestos mineral fibres and that the uncoated fibres observed were also asbestos, although it is well known that they cannot be identified using an optical microscope.

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The electron microscope and microprobe techniques provide the most accurate and detailed information on the identification of asbestos mineral and other fibres, but this equipment is very expensive and is only available in a limited number of research centres. This investigation was undertaken to test the efficiency of the light microscope in detecting asbestos particles in lung tissue, and in particular, to compare counts obtained by light microscopy with those obtained by analytical electron microscopy to determine whether or not there was any relation between the observations obtained by the two techniques.

The samples of lung tissue examined were taken from necropsy material from patients who had mainly died from asbestos related disease, the remainder was taken from patients who had died from cardiovascular disease or bronchial carcinoma. The objective in the selection was to obtain a set of tissue samples with as wide a range of asbestos fibre contents as possible. The specimens of tissue used in the optical microscopic investigation were all taken from the lower lobes of the lung and were prepared and examined in the department of pathology, City Hospital, Nottingham by one of the authors. The specimens of tissue used in the electron microscopic studies

were taken from the upper and lower lobes of the lung, and the composite sample was prepared and examined in the department of mineral exploitation by the other author.

In some cases the number of asbestos fibres in the lung can vary by as much as 50% using electron microscopic counts. It is not known, however, whether this variation is dependent upon the type or severity of exposure to asbestos. Normally, whole lungs are not available for quantitative determinations of asbestos content. However, as the differences between light and electron microscopic counts were expected to be much larger than the possible fibre concentration differences throughout the lung, it was not considered necessary to examine specimens from the same area by both techniques.

Necropsy material from 55 patients was examined, although 59 sets of results were recorded as four specimens were re-examined by optical microscopy for a second evaluation of fibre number concentration. (Table 2, specimen numbers 23/40, 26/41, 27/42, and 36/43).

Necropsy material, although originating from the same patients, was therefore examined by independent laboratories using different specimen preparation techniques and microscopic equipment.

Material and methods

The light microscope counts of fibre concentrations were made using the method of Ashcroft and Hepplestone:[10] this entails the potassium hydroxide digestion of lung tissue. The fibres were counted using a modified Fuchs Rosenthal counting chamber and viewed with a phase contrast microscope. Both naked fibres and ferruginous bodies were counted, even though sometimes a fibre could not be discerned in association with the body. The optical count was calculated and expressed as the number of fibres/g of dried lung tissue. The procedure used in the preparation of the tissue specimens consisted of first dissecting two cubes of lung tissue 1–1·5 cm^3 from each specimen, avoiding the pleura, large bronchi, and blood vessels. Both cubes were weighed and one of the cubes was dried for 24 hours in an oven at 60°C. The other cube of tissue digested in 40% potassium hydroxide at 100°C for two hours. The resulting residue was then concentrated by centrifugation and the supernatant removed leaving 0·5–1·0 ml of solution above the deposit in the centrifuge tube. The residue was then resuspended in filtered distilled water and the resulting suspension used to fill a modified Fuchs Rosenthal counting chamber. Using an optical microscope in a phase contrast mode the fibres in the chambers were counted until three consistent readings were obtained. Using the wet weight of both cubes of lung and the weight of the cube that had been dried, the weight of the cube digested was calculated. The fibre concentration of the tissue on a dry weight basis was then computed from the number of fibres counted per unit volume of the residue suspension placed in the counting chamber.

The electron microscopic counts of fibre concentrations were performed using a procedure outlined by Pooley and Clark.[5] The specimens of lung tissue were prepared by initial potassium hydroxide digestion of tissue that had been dried to a constant weight at 80°C. The dust extracted from the tissue samples was filtered onto 0·2 μm nuclepore filters, which were then carbon coated when dry. The dust from a specific weight of tissue was thus deposited on a known area of filter. Sections of the filter, bearing the extracted dust embedded in a carbon film, were then prepared for electron microscopy by dissolving away the nuclepore filter using chloroform, leaving the carbon film. The dust deposit was then mounted on gold electron microscopic support grids. The electron microscope filter preparations were examined at a magnification of 20 000 x and scanned to detect fibres in a regular manner. Each fibre was analysed using an energy dispersive x-ray analysis system and identified. Detecting and analysing fibres in this way established the number of each fibre type present. The dimensions of each fibre were also noted so that size distribution for each type could be obtained: both the diameter and length of each fibre were recorded. The number of fibres/unit area of the electron microscope preparation was determined either directly from the microscope by counting fibres in a given area, or from the subsequent examination of electron micrographs of random areas of the preparations. These fibre counts were then used to obtain the fibre concentration on the original filter specimen, which contained the dust extracted from a known quantity of tissue: in this way fibre concentrations/g of dried tissue were calculated.

The total mineral fibre counts obtained from the analytical electron microscope were therefore reduced to obtain the number of asbestos mineral fibres and also non-asbestos fibres present/unit of tissue.

Results

Tables 1, 2, and 3 list the results of total electron microscopic mineral fibre counts and the abstracted total asbestos fibre counts for each of the specimens together with the corresponding optical microscopic counts. Table 1 shows the results from those specimens, in which the predominant asbestos mineral detected by electron microscopy in the lung was crocidolite; Table 2 shows the results from those lungs in which amosite was the predominant asbestos mineral. Table 3 shows the results from those lungs in which chrysotile was the principal asbestos mineral detected.

Table 1 *Asbestos and other fibrous particle counts obtained from lung tissue specimens containing crocidolite as predominant asbestos mineral**

Specimen No	*Optical microscope counts* $\times 10^6$	*Electron microscopic adjusted counts* $\times 10^6$	*Total electron microscopic asbestos fibre counts* $\times 10^6$	*Total electron microscopic mineral fibre counts* $\times 10^6$
1	0·700	0·230	7·63	10·21
2	0·900	6·130	38·93	116·80
3	4·600	1·580	22·11	22·30
4	1·600	0·140	1·04	2·50
5	1·100	6·540	108·90	116·62
6	1·790	1·630	12·82	23·10
7	0·710	0·310	4·41	6·40
8	0·700	0·670	8·65	21·10
9	0·500	0·260	2·38	3·50
10	0·300	0·800	9·17	12·90
11	3·300	7·230	149·56	180·10
12	25·600	60·690	1213·70	1213·70
13	5·700	6·860	83·90	83·90
14	111·800	35·600	225·80	277·60
15	0·900	2·070	64·32	68·80
16	0·800	6·300	118·66	176·12
17	5·000	2·700	54·89	74·10
18	19·900	16·100	242·00	271·64
19	32·300	106·650	2133·00	2133·00
20	13·600	49·100	646·25	717·40
21	1·000	0·510	10·24	853·10

*Fibre numbers expressed as millions/g of dried tissue.

Table 2 *Asbestos and other fibrous particle counts obtained from lung tissue specimens containing amosite as predominant asbestos mineral**

Specimen No	*Optical microscope counts* $\times 10^6$	*Electron microscopic adjusted counts* $\times 10^6$	*Total electron microscopic asbestos fibre counts* $\times 10^6$	*Total electron microscopic mineral fibre counts* $\times 10^6$
22	0·100	1·530	6·64	25·80
23	7·300	22·750	98·77	99·70
24	10·700	45·500	347·19	347·19
25	0·700	2·400	12·68	15·10
26	6·100	2·070	8·14	8·60
27	4·600	2·490	10·62	10·80
28	1·400	1·490	8·90	12·10
29	1·100	0·450	17·02	18·43
30	5·600	5·200	21·44	1197·44
31	0·100	0·470	3·33	9·38
32	1·300	1·170	2·06	3·00
33	1·200	0·600	2·65	6·91
34	0·800	1·790	6·74	99·10
35	0·600	60·800	374·19	416·64
36	19·100	102·980	657·00	759·80
37	0·700	0·660	18·65	58·90
38	116·700	76·200	368·40	368·40
39	0·300	0·360	1·67	4·90
40	8·160	22·750	98·77	99·70
41	1·780	2·070	8·14	8·60
42	3·740	2·490	10·62	10·80
43	34·600	102·980	657·00	759·80

*Fibre numbers expressed as millions/g of dried tissue.

The results show that the total mineral fibre and also asbestos fibre obtained by electron microscopy were very much higher than those obtained by optical microscopic counting. The reason for this large difference was due to the fact that in the electron microscope the fibres of all sizes can be detected and counted while the optical counts represent only those fibres which can be resolved by the light microscope. The most critical dimension of asbestos fibres in determining whether or not they are observed in the optical microscope is their diameter. If a working resolution of 0·3 μm is assumed for the optical microscope used in this study then all the fibres with diameters below this figure would not be observed and counted.

From the cumulative fibre diameter distributions of the various asbestos mineral fibres obtained from the electron microscopic examination of the tissue samples only 5% of the crocidolite, 26·5% of the amosite, and 0·14% of the chrysotile fibres were visible using

Table 3 *Asbestos and other fibrous particle counts obtained from lung tissue specimens containing chrysotile as predominant asbestos mineral**

Specimen No	Optical microscope counts × 10^6	Electron microscopic adjusted counts × 10^6	Total electron microscopic asbestos fibre counts × 10^6	Total electron microscopic mineral fibre counts × 10^6
44	15·500	0·450	5·1	7·9
45	1·240	2·118	23·15	30·4
46	5·600	1·170	43·49	44·20
47	0·150	0·560	16·31	16·31
48	7·600	0·153	3·93	15·59
49	0·310	0·140	1·08	83·58
50	2·600	0·075	22·00	24·51
51	7·930	0·258	25·63	30·41
52	0·180	0·403	3·85	42·51
53	9·800	0·120	57·59	57·59
54	0·150	0·901	15·14	51·10
55	7·600	0·120	51·94	81·19
56	0·680	2·669	25·89	56·09
57	0·830	0·077	3·24	7·59
58	0·440	0·036	7·06	71·19
59	3·200	0·400	41·31	48·87

*Fibre numbers expressed as millions/g of dried tissue.

an optical microscope. The total electron microscope asbestos fibre counts were therefore adjusted to remove that proportion of the fibre considered to be below the resolution of the optical microscope, and an adjusted electron microscopic asbestos count was obtained. The figures of optical and adjusted electron microscopic counts were fairly compatible (Tables 1 and 2). Table 3 shows that the agreement was not as good for the results as those obtained in Tables 1 and 2. The Figure gives a graphical representation of the optical and adjusted electron microscopic counts, in which the counts from the three Tables are represented by different symbols.

The Figure shows that the largest difference between the optical and electron microscopic counts was obtained as adjusted electron microscopic counts below 0·5 million/g of dried tissue, while the correlation at higher fibre counts was much better. It was those counts obtained from specimens, in which chrysotile was the predominant asbestos mineral, that deviated most from the ideal.

The correlation between the logarithms of the counts contained in the Figure is 0·56 ($p < 0·001$) which is significant. A similar correlation of the results from the specimens containing crocidolite, amosite, and chrysotile individually produced correlation coefficients of 0·79, 0·74, and 0·15. The optical results were therefore more accurately predicted from the electron microscope when either crocidolite or amosite was predominant in the tissue specimens. The reason for the larger discrepancy of the results from those cases with mainly chrysotile present is not clear but it may be due to the fact that ferruginous bodies were included in the optical count as representing an asbestos fibre and that such bodies are propor-

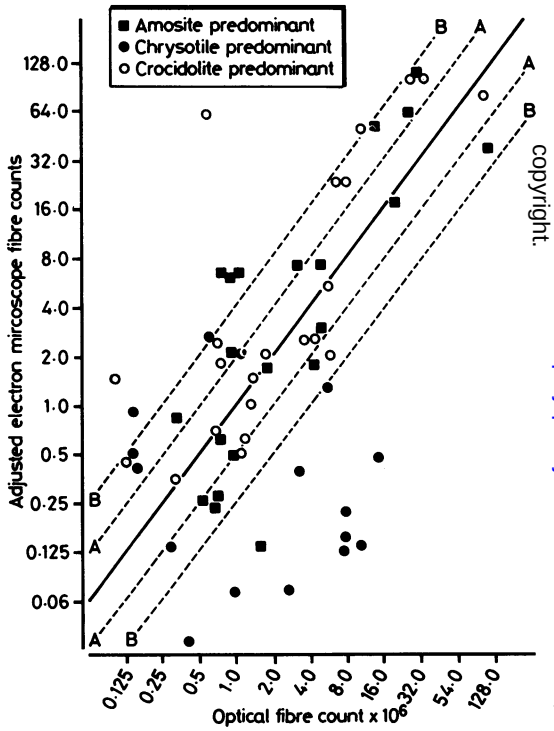

Figure *Solid line represents line on which all points would lie if they were to be perfectly correlated. Pairs of dotted lines labelled A and B either side of the solid line represent limits of position of points if electron and optical counts were to differ by factors of 2 and 4, respectively. About 70% of the results are contained within a range of difference of 4, while 30% are contained within a range of difference of 2.*

Table 4 *Fibres and ferruginous bodies detected (g/dried tissue)*

Specimen No	*Fibres detected* $\times 10^6$	*Ferruginous bodies detected* $\times 10^6$	*Total*
13	4·48	1·22	5·70
19	50·45	8·86	59·31
26	8·51	4·43	12·94
36	28·01	6·38	34·39
50	5·86	3·45	9·31
55	2·61	5·06	7·67

tionally present in much larger numbers in specimens where the fibre count is low. This point is well illustrated by the results contained in Table 4. These were obtained from the optical recount of six of the specimens in which numbers of bodies and fibres observed were assessed independently and their totals noted.

When the optical fibre count was low the number of ferruginous bodies detected was relatively large and even exceeded the number of fibres observed in one case (Table 4). The difference, however, between the fibre and ferruginous body count increased as the number of fibres observed increased. The inclusion of body count with fibres to produce a total optical count of suspected asbestos fibre probably does contribute to the deviation of the optical results from those predicted by electron microscopy.

In the examination of the data obtained by both optical and electron microscopy no attempt has been made to assess the contribution of the non-asbestos mineral fibre to the optical count. It is almost certain, however, that some of the fibres observed using the optical microscope were non-asbestos mineral fibres.

Discussion

It is possible to predict from electron microscopic examination of a lung tissue sample the approximate concentration of fibres that would be assessed from an optical examination of tissue from the same lung. It is impossible, however, to make the reverse prediction, because it is not feasible to determine the proportion of the various asbestos mineral fibre types present in the sample using an optical microscope. The light microscope cannot be used to obtain an accurate assessment of the amount of asbestos fibre in lung tissue. As fibres are not identifiable using light microscopy the presence of non-asbestos fibres may seriously affect the integrity of any count made. Any authors reporting data using the light microscope should, therefore, be careful not to assume that all the fibres they observe are asbestos. The ratio of ferruginous bodies to fibres in tissue samples varies considerably, and the use of such objects in the assessment of an asbestos fibre count in tissue may be very misleading.

Considering the different tissue samples and preparation techniques that were used in this study it is encouraging that the electron microscopic results were able to predict the optical fibre count so well. This is an indication that electron microscopic techniques assess the amount of asbestos mineral fibre in lung tissue fairly accurately.

Although the numbers of asbestos fibres detected in the lung tissue specimens are quoted in millions/g of dried tissue, (Tables 1–3) it must be remembered that because of their size these large fibre numbers represent only very small mass quantities of material. Rough estimates of fibre number and mass for the various types of asbestos show that 1×10^6 fibres of amosite detected by the electron microscope represents in the region of 10^{-6} of material while 1×10^6 fibres of chrysotile, because of its finer size is equivalent to less than 10^{-7} g of material. The mass of 1×10^6 fibres of crocidolite falls roughly midway between the mass figure for amosite and chrysotile respectively.

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