A trial of techniques for counting asbestos bodies in tissue

P. D. OLDHAM [1]

variety of different methods is available for mining the number of asbestos bodies in lung , but it is not known if these methods give r results. Further, little is known of the tion in the number of bodies in relation to ee of exposure to asbestos, nor whether in the idual the number of bodies varies in different s of the lung.

n experiment of limited scope was conducted in r to accumulate some numerical information erning these factors. It was intended to cover possible differences between two basically dif- ent methods: (*a*) counting of bodies seen in thin tions under the microscope; and (*b*) counting of dies seen after digestion of a quantity of lung tis- and its redispersion; and to obtain some informa- about the variations of the asbestos body count rom one part to another of the same lung.

Observers have strong preferences concerning the thickness of thin section and the different staining methods. It was decided therefore to let each observer use his own preferred technique. Any conspicuous differences in the resulting counts could be examined to establish whether they were due to variation among the observers or variation among the techniques.

STATISTICAL DESIGN

It was considered that the maximum number of specimens to be counted by each observer should be limited to about 30. In order to cover the four quarters of the lungs, with and without pleura, eight specimens would be needed; consequently four cases were chosen, two with asbestosis, and two without occupational exposure to asbestos but who lived in an area where about a quarter of post-mortem cases had been found to have asbestos bodies in the lungs. From each case the eight blocks were taken in pairs from the right upper, left upper, right lower and left lower zones, one of each pair including some of the pleural surface.

Five sections were cut from each block, three of 6 μm thickness and two of 30 μm. Of the three 6 μm sections, one was unstained, one was stained with haematoxylin and eosin, and the third with Perls's stain. One of the 30 μm sections was stained with Perls's stain and the other was left unstained. The sequence in which each block was cut was carefully randomised to ensure that variation in the profusion of asbestos bodies across the block fell randomly among the five sections and the residue to be used for digestion and redispersion. For example, in case 2, the block including pleural surface from the right lower lobe was cut as follows:

6 μm	Perls
6 μm	unstained
Residue	
30 μm	unstained
6 μm	H & E
30 μm	Perls

On each of the thin sections a square 1½ cm × 1½ cm was marked in ink, unless one dimension was

[1] MRC Pneumoconiosis Unit, Llandough Hospital, Penarth, UK.

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too small, in which case a rectangle of equal area was marked. Observers were required to estimate the total number of bodies within the marked area.

OBSERVERS

Seven observers returned their counts in sufficient time for analysis. Table 1 shows their identifying letters, the type of section they examined, and notes on the technique they adopted. One additional observer who examined 30 μm unstained sections reported so many as "uncountable" that his results have had to be discarded.

Table 1

Observer	Type of section	Method
A	30 μm, unstained	As much section as was necessary to observe 100 bodies was scanned. If this was less than the whole, the number of fields scanned was recorded.
B	30 μm, unstained	When many bodies were seen, a range (e.g., 300–500) was quoted. The upper limit mentioned was 100 bodies; in other cases the report was "innumerable".
C	30 μm, unstained	A sample of fields was scanned, and the position of each body was recorded on a grid (method of C. H. Um). Fibres and pseudo-bodies were recorded separately.
D	30 μm, Perls	When the number of bodies was large, an estimate was given in thousands. The largest estimate given was 4000; in other cases the count was stated as "thousands".
E	6 μm, Perls	The exact number of bodies, fibres, and pseudo-bodies seen was given in every case.
F	6 μm, Perls	The exact number of bodies, fibres and pseudo-bodies seen was given in every case.
G	Residue of block	Digestion of tissue, redispersion of residue, and counting of bodies and fibres in Fuchs–Rosenthal chamber (method of C. Gold).
H	6 μm, H & E	Systematic search of marked area, or until several hundred bodies were seen, when number was scaled up by ratio of searched area to whole.

RESULTS

In order to make comparisons, the raw counts must be adjusted to some standard. How this should be done is debatable, but, as will be seen, the particular method adopted cannot materially have affected the conclusions: observer C expressed his results as the number of bodies per cubic millimetre, and his complex method of estimation made it difficult to express his results in any other form. Accordingly, all the other counts were expressed in these terms, using the assumption that the volume scanned on a 6 μm section was 1.35 mm^3, and that on a 30 μm section, 6.75 mm^3. It was pointed out by several observers that the actual areas marked on the sections were not always 2.25 cm^2, and were sometimes as small as 1.7 cm^2. Thus, errors of 30% or more are introduced by assuming a standard area. However, it will be seen that the range of disagreement among the observers was such as to make this unimportant.

Observer A made counts of 100 bodies in varying numbers of microscope fields; these were adjusted to the standard by making simple geometrical assumptions about the effective area of each field. Those sections said by observers B and D to show an uncountable number of bodies were arbitrarily given a count corresponding to the largest number per mm^3 counted in the series.

That these grossly simplified procedures were nevertheless adequate can be seen from Figure 1. The range of results is such that, here and throughout, logarithms of the counts are used. Figure 1 shows the counts in all 32 sections, one column to each section. The first eight columns relate to case 1, the next eight to case 2, and so on. Within each case the order of the sections is the same:

Right upper zone, with pleura
Right upper zone, without
Right lower zone, with pleura
Right lower zone, without
Left upper zone, with pleura
Left upper zone, without
Left lower zone, with pleura
Left lower zone, without

Zero counts have, arbitrarily, been plotted at 0.01 bodies per cubic millimetre. In case 2, left upper zone without pleura, and in case 3, right upper zone without pleura, the counts of observer F were pseudo-bodies; he specifically recorded no asbestos bodies. Since all the other observers saw asbestos bodies, it seemed best to give the number of objects F saw rather than zeros.

It will be seen that the range of counts on each section is more than tenfold, however it is clear

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Fig. 1. Counts by seven observers on each of 32 sections. Each column is one section; successive sets of eight sections come from the same case.

that there is a degree of consistency in the results. No particular technique gives consistently high or low results; the variation seems attributable primarily to observers. Such distinction as there is among the four cases is made equally well by all observers and by all techniques.

There is no consistent difference between the pleural sections and the purely parenchymal ones. Only in case 3 is there a marked difference between the upper and lower parts of the lung: all observers counted more bodies in the four sections from the upper lobes. In case 4 four of the observers found more bodies in the left lung than in the right; three made counts in 6 μm sections, and the fourth was observer A who counted 30 μm unstained sections.

Figure 2 shows the results obtained by observer G, who examined the residue of each block by digestion and redispersion. His results are expressed on a mass basis, as the estimated number of bodies per unit weight of dried lung tissue, and are plotted against the geometric mean counts per mm³ of observers A to F (the results from observer H arrived too late to be included). It will be seen that the correlation is good. The sixteen points at the top right-hand side come from cases 1 and 2; the four samples from the upper lobes of case 3 appear at about 10 mm³; while case 4 and the lower lobes of case 3 provide the remaining points. In three cases where observer G found no asbestos bodies (although he did find fibres) the points appear with downward arrows, indicating that the estimates are less than the 1.4 bodies per mg shown.

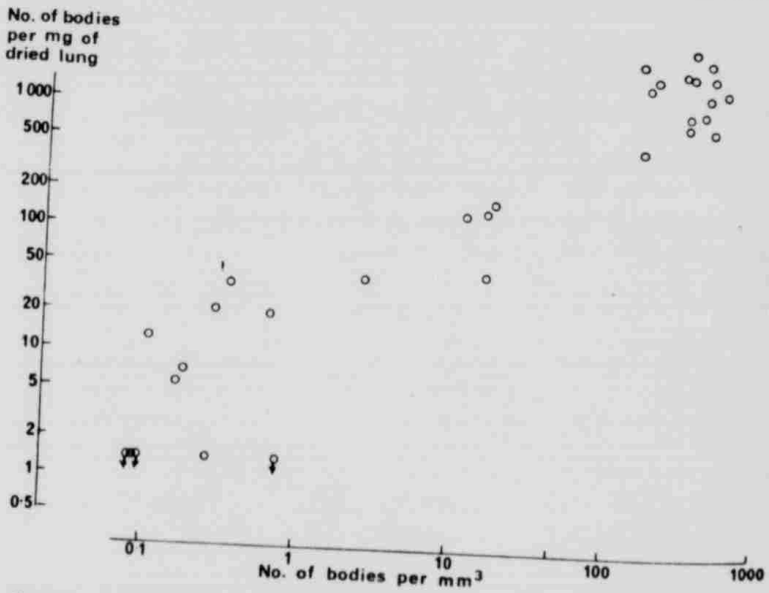


Fig. 2. Correlation between the counts by one observer of redispersed, digested blocks of lung and the average count of thin sections from the same blocks.

DISCUSSION

No detailed numerical analysis of these results is called for. It is evident however that, with all the techniques used, there is a range of uncertainty of about an order of magnitude, and that this should be borne in mind when assessing reported numerical counts of asbestos bodies. At the same time, no one method appears to have a serious bias. Clearly

0 μm sections, stained or unstained, show embar-assingly large numbers in cases of asbestosis; thus t would be advisable to specify an exact procedure o be adopted in such cases. On the other hand, μm sections are more likely to give zero counts in ases where other techniques can show asbestos odies to be present; this may be undesirable in the tudy of diseases suspected to be asbestos-related.

The scrupulous but extremely laborious technique troduced by Um (1971) evades both these diffi-ulties; however the results which it provides are ot so markedly different from those obtained by impler methods as to justify a recommendation for s general adoption.

Gold's technique (Gold, 1968) compares very well n numerical terms with thin-section methods, and t is evident that the digestion of lung tissue with KOH does not cause serious loss of asbestos bodies as had been feared.

It appears that practical considerations of expediency can dictate the choice of method. It is probable that material reductions in the differences between observers could be achieved, with any technique, by a set of specified rules for scanning under the microscope. This should probably involve a rectangular grid, a set procedure to relate this to a marked area on the slide, and conventions for sampling the section when large numbers of objects are seen.

The evidence provided by this study of variation in the profusion of asbestos bodies from one part of the lung to another is extremely limited; but it is at least clear that large differences may occur in some cases.

SUMMARY

Sections were cut from 32 blocks of lung tissue taken rom four subjects. 6 μm and 30 μm sections were stained ither with Perls's stain, or haematoxylin and eosin, or eft unstained. They were scanned by eight different bservers, and the profusion of asbestos bodies seen was ecorded. The residue of each block was digested with KOH, redispersed, and then scanned.

A tenfold variation was found in the counts obtained; this appeared to be more a function of difference in observer than in technique. Nevertheless, the tissue samples from the four cases were distinguishable one from another; some material differences between the upper and lower lobes of one case were seen; and the average count of asbestos bodies in the thin sections correlated well with the count in the redispersed digested residue.

ACKNOWLEDGEMENTS

This work was carried out by the following pathologists of the UICC Committee on Asbestos and Cancer: Dr G. Chiappino; Dr R. Guy; Dr M. Kannerstein; Prof D. Magner; Dr L. Meurman; Prof H. Otto; Dr H. T. Planteydt; Prof L. Santi; Dr J. C. Wagner; Prof I. Webster; co-opted, Dr C. Gold and Dr C. H. Um.

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