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# ALIGNMENT OF RESPIRABLE ASBESTOS FIBRES BY MAGNETIC FIELDS

V. TIMBRELL

Medical Research Council Pneumoconiosis Unit,
Llandough Hospital, Penarth, South Glamorgan

**Abstract**—Alignment of respirable asbestos fibres by magnetic fields has many advantages in investigations of biological effects of asbestos. Their alignment in thin films or on membrane filters facilitates determination of diameter and length distributions using light and electron microscopes. Fibres aligned in thin film or in liquid suspension produce distinctive light-scattering patterns when illuminated by a laser beam. Photoelectric measurement of the light intensity distribution aids identification of the geographical source of the asbestos; it also permits estimation of fibres in air samples and in dust recovered from human and animal lungs and provides indices of fibre size.

## INTRODUCTION

ASBESTOS fibres of respirable size can be aligned by magnetic fields when in air or liquid suspension (TIMBRELL, 1972a).

This finding has numerous applications in biophysical studies on pulmonary disease associated with exposure to asbestos dust, and its usefulness is enhanced by a number of features. (a) Alignment can be achieved with ordinary magnets. (b) Whereas respirable fibres (fibres less than 3·5μm in diameter and less than 200μm in length (TIMBRELL, 1965)) generally will not align when in contact with solid surfaces, their slow rates of sedimentation when suspended in air or liquid give ample time for alignment before deposition takes place. (c) Of a large number of samples examined, including all the important commercial types, none has failed to align. This apparently universal response is associated with the regular occurrence in asbestos of magnetic elements, especially iron, in which magnetism can be strongly induced, and is not dependent on palaeomagnetism. (d) It is not unusual to find that a large fibre will align with its axis in the direction of the field, and yet, if this fibre is divided into fibres of respirable size, some or all of the latter will show an alignment differing from that of the parent fibre. (e) Durable specimens of aligned fibres can be prepared (Fig. 1).

## ALIGNMENT MODES

Respirable fibres in samples from various asbestos mining areas exhibit differing modes of alignment in magnetic fields. Some samples provide fibres which align *parallel* (P-type) to the direction of the applied field ; some samples, fibres which align *normal* (N-type) to the field; and other samples, a mixture of P-fibres and N-fibres behaving according to type.

These two types of alignment mode are the only ones so far observed in fibres of natural asbestos. The P-fibres all align parallel to the field and hence parallel to one

another (Fig. 2a). The N-fibres align in planes normal to the direction of the field (Fig. 2b), but within these planes there is no magnetically-imposed preferred orientation.

With regard to the mechanism of these phenomena, briefly the present evidence is: (a) all the bulk samples of asbestos fibres examined (at room temperature) have proved to be paramagnetic or dilute ferromagnetic, both before and after thorough magnetic cleaning to remove attached particles of magnetite and other ferromagnetic materials; (b) the P-fibres and N-fibres in a sample have the same chemical composition; (c) fibres of some asbestos minerals, chrysotile for instance, become dipoles which align parallel to the field because of magnetite particles in the fibre; (d) in amphiboles the alignment mode of P-fibres stems from the presence of paramagnetism in the direction of the fibre axis and that of N-fibres from paramagnetism normal to the axis. There are however some types of fibre, certain carbon fibres for instance, which behave like P-fibres by virtue of the presence of diamagnetism normal to the fibre axis (TIMBRELL, 1972b).

Some synthetic fluoramphiboles show an alignment mode differing from those of natural asbestos. These comprise fibres which align with their axes *transverse* (T-type) at a definite acute angle to the direction of the field. The orientation of these fibres (but not their spatial distribution) can be represented by lines on the surfaces of two identical cones with all the lines passing through their common apex (Fig. 2c). This behaviour stems from the presence of paramagnetism inclined to the fibre axis.

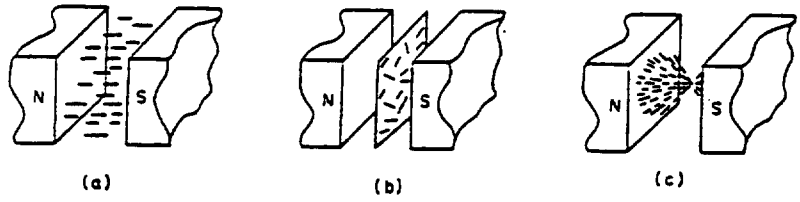


FIG. 2. Alignment modes in air or liquid suspensions: (a) P-fibres; (b) N-fibres; (c) T-fibres.

If a natural alignment mode of fibres in an asbestos sample is inconvenient for some applications it may be possible to alter it. For instance, heating UICC amosite (Transvaal, S. Africa), which has both P-fibres and N-fibres, to 600°C converts the N-fibres to P-fibres. Moreover it may be feasible to convert N-fibres to P-fibres by evaporating onto them a thin layer of iron.

## ALIGNED SPECIMENS FOR MICROSCOPICAL EXAMINATION

The reliable interpretation of results obtained from experimental studies on the biological effects of asbestos demands precise data on the geometric sizes of fibres in the test materials (WAGNER, *et al.*, 1973; TIMBRELL, 1973). This information is obtained through use of the light microscope (LM), the transmission electron microscope (TEM) and, increasingly the scanning electron microscope (SEM) (BECKETT, 1973). The LM allows measurement of the lengths and diameters of visible fibres only. The TEM allows measurement of the diameters of all fibres, but measurement of lengths by this microscope is often not possible because the ends are either outside the boundary field or obscured by the bars of the specimen grid. Consequently, fibre-length and fibre-diameter distributions are usually determined separately using

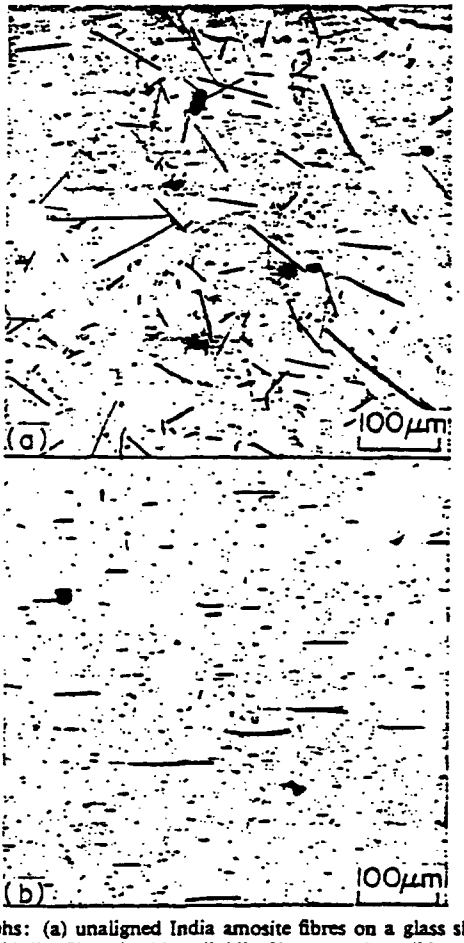


FIG. 1. Light micrographs: (a) unaligned India amosite fibres on a glass slide; (b) aligned UICC crocidolite fibres in thin celloidin film on a glass slide.

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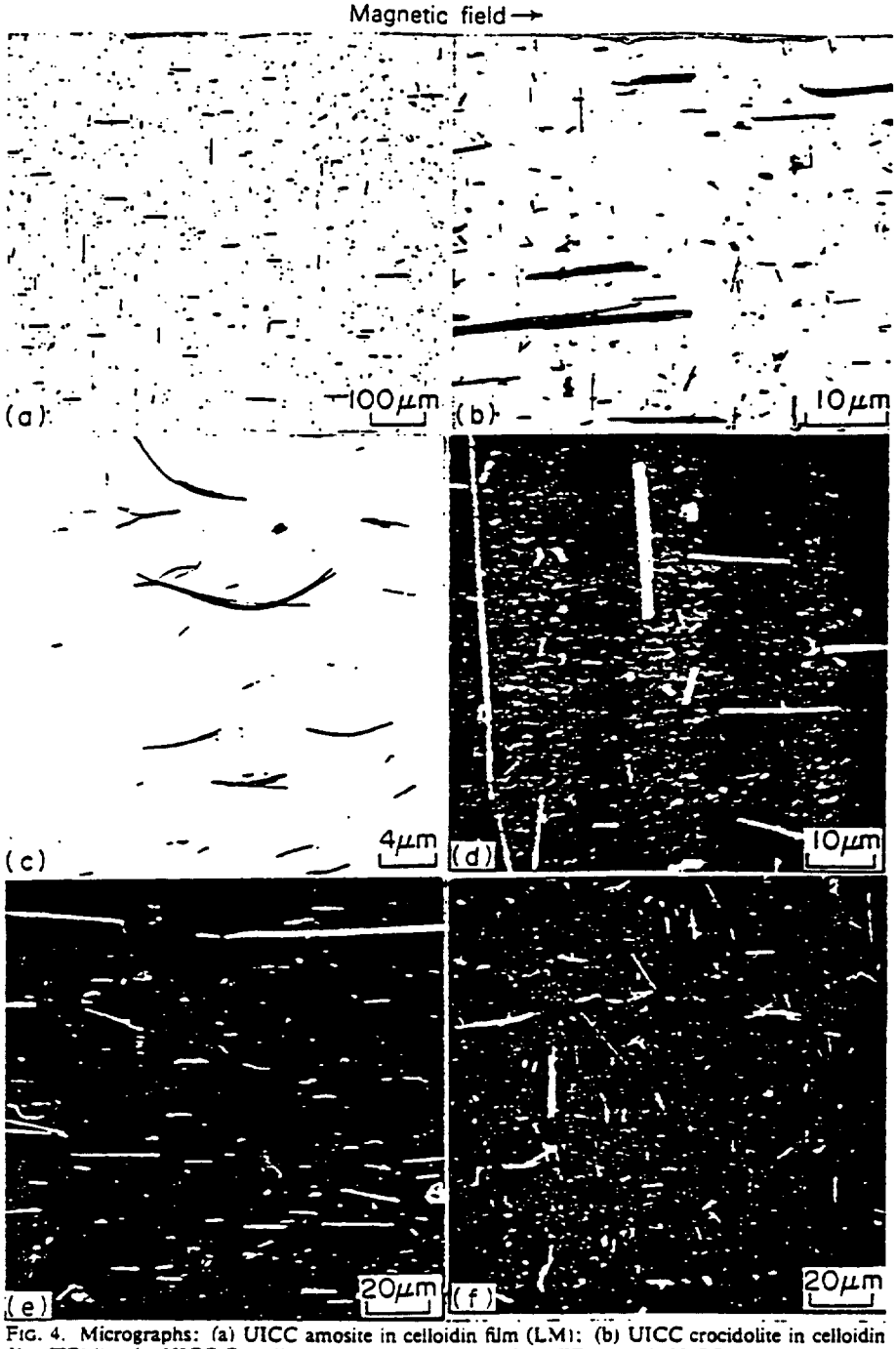


FIG. 4. Micrographs: (a) UICC amosite in celloidin film (LM); (b) UICC crocidolite in celloidin film (TEM); (c) UICC Canadian chrysotile in celloidin film (TEM); (d) UICC amosite deposited on membrane filter from aqueous suspension (SEM); (e) UICC anthophyllite (Casella 113A sample of laboratory aerosol) deposited on membrane filter from aqueous suspension (SEM); (f) Membrane filter sample of laboratory aerosol of UICC amosite (SEM).

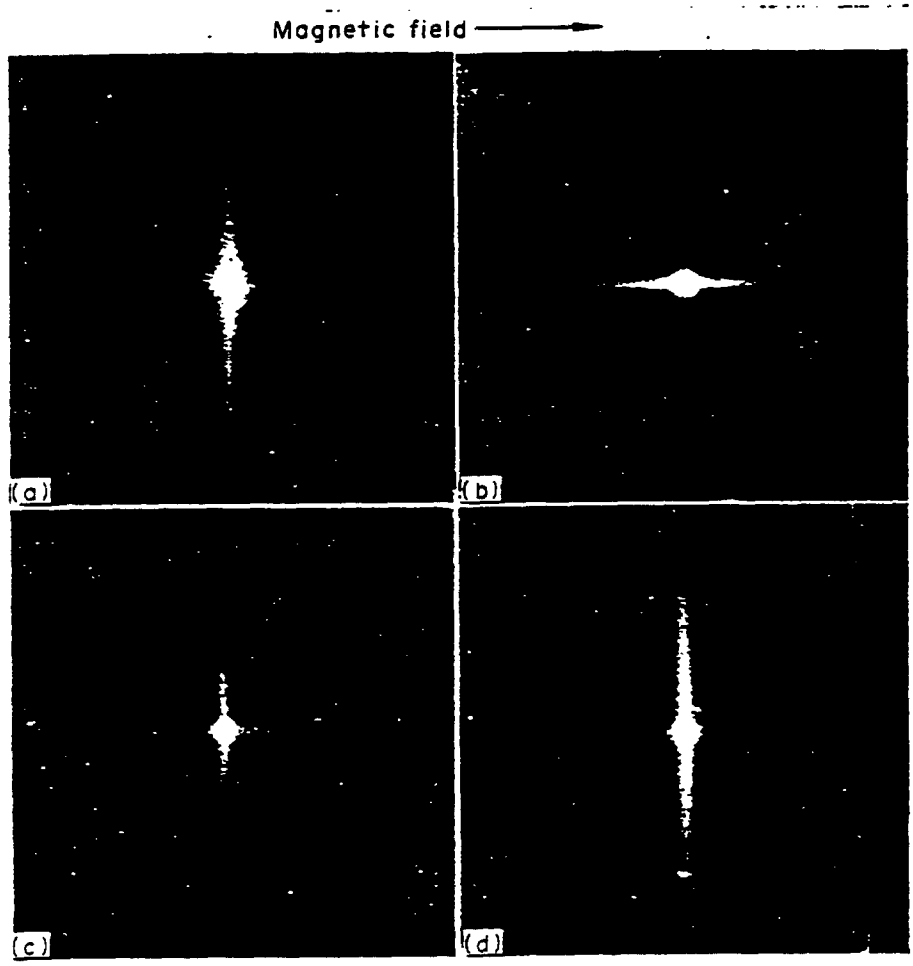


FIG. 7. Light scattering patterns: (a) UICC anthophyllite (Finland); (b) India amosite: (c) UICC amosite (Transvaal, S. Africa); (d) UICC crocidolite (Cape Province, S. Africa).

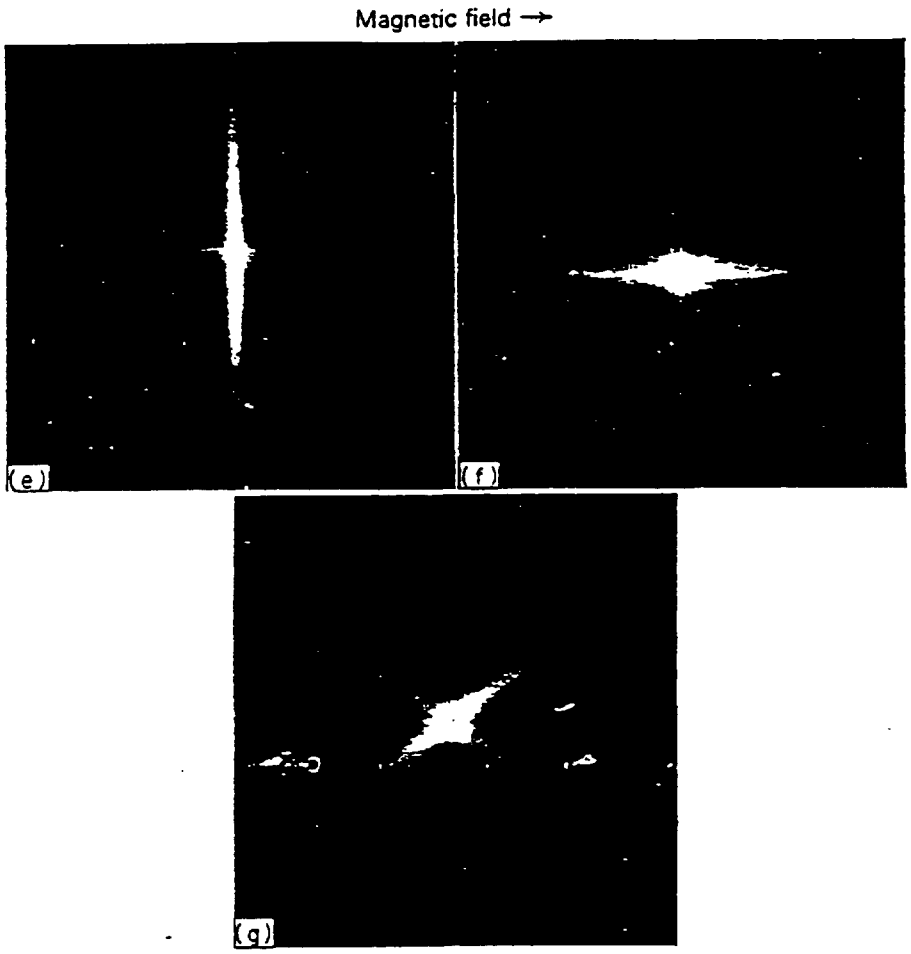


FIG. 7. (e) Uganda tremolite; (f) Zululand tremolite; (g) Synthetic fluoramphibole.

the LM and the TEM respectively. An advantage of the SEM is that the combination of a high resolution (typically 15nm) and a suitable magnification range (typically ×50 to ×20 000) permits the concurrent measurement of lengths and diameters. Aligned specimens can be prepared for these three microscopes.

The alignment of asbestos fibres in a magnetic field is readily demonstrated by applying a drop of an aqueous suspension of the fibres to a glass slide mounted across the pole-pieces of a thin horseshoe magnet which is located on the stage of a light microscope. This is not however a satisfactory method of preparing specimens intended for fibre measurement because : (a) the fibres are moved by convection currents and tend to form aggregates; (b) the magnetic field is too weak to prevent degradation of fibre alignment by the large surface tension forces that occur during the final drying stages; (c) the specimens are not durable. A superior technique is as follows. A low-concentration suspension of the fibres in a 0·5% w/v solution of celloidin in amyl acetate is sonicated to obtain good particle dispersion, and a few drops are then applied to a glass slide located in the air gap of a permanent magnet or electromagnet (Fig. 3a). A strong field (5–10kG) is desirable. This generally requires the air gap to be not wider than 1cm, but standard 3 × 1 in. glass slides or

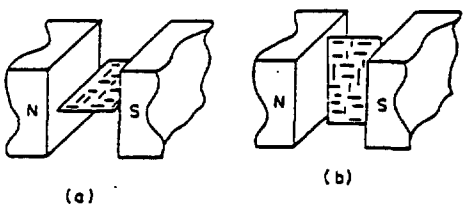


FIG. 3. P-fibres and N-fibres deposited on: (a) horizontal substrate; (b) vertical substrate.

coverslips can still be used by accommodating them in thin horizontal slots milled in the pole-pieces. More slots allow several specimens to be prepared together. After a short drying period (about 15 min) a thin durable celloidin film (about 1cm in diameter) suitable for light microscopy is left on the slide. An advantage of this film is that because of its thinness any N-fibres present do not have the random orientation normal to the magnetic field they show in free suspension (Fig. 2b). The fact that both the P-fibres and the N-fibres are now in one plane is demonstrated (for UICC amosite) in Fig. 4a where the whole lengths of all the fibres are in good focus. The P-fibres are parallel to one another, as also are the N-fibres.

An extra-thin film for examination in the TEM can be produced by using a less viscous solution (0·3% w/v) and by keeping the slide in the field while inclining it so that the liquid drains down and forms an elongated layer. The film is scored with a scalpel and pieces are then floated on water and picked up on specimen grids in the usual way. Figures 4b and 4c show results obtained from UICC crocidolite and UICC Canadian chrysotile respectively.

An aligned specimen for examination in the SEM can be produced by sonicating an aqueous suspension and passing it through a membrane filter located in a magnetic field (Fig. 3a): usually a field of 3kG is adequate. Figure 4d shows a specimen of UICC amosite: here too, N-fibres deposit flat on the substrate.

Dust collected in currently used respirable dust samplers can be examined in the SEM by this method. In the case of a sample from a cyclone or the Casella 113A instrument, a portion is suspended in a suitable liquid which is then filtered through a membrane in a magnetic field. Figure 4e shows a membrane specimen prepared from a Casella 113A sample of a laboratory aerosol of UICC anthophyllite. In the case of a midget impinger sample, if the dust is collected in an organic liquid, a specimen for SEM examination can be prepared using a silver membrane filter.

Airborne asbestos fibres align in a magnetic field with the same alignment modes they show in liquids. Specimens for SEM examination can be obtained by aspirating through a membrane filter. Fitting the filter holder with an inlet tube to protect against air currents promotes good alignment. Here again both P-fibres and N-fibres deposit flat on the substrate. As illustrated by a result obtained from UICC amosite (Fig. 4f), this method has the advantage that unlike the techniques involving suspension in liquids it conserves the state of aggregation of the airborne dust.

Aligned specimens have several advantages in microscopical examinations:

(a) In measurements of size distributions with the SEM or TEM, the minimized fibre-overlapping allows high-concentration specimens to be examined, and hence a high fibre count to be achieved from far fewer electron micrographs than would be necessary using unaligned specimens. Observer fatigue and counting errors are reduced. This is also true in LM work.

(b) They resolve the problem associated with unaligned specimens, especially acute in TEM examinations, that fibre ends may be outside the boundary of the field or obscured by grid bars. For an examination of P-fibres, the extra-thin film is picked off the water using a grid comprised of parallel bars and positioned with the bars parallel to these fibres. Figure 5 illustrates how a number of successive

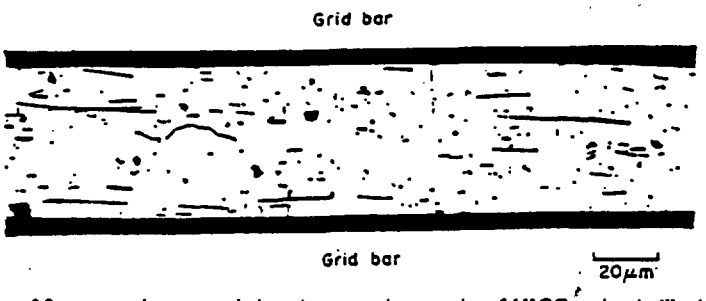


FIG. 5. Series of five successive transmission electron micrographs of UICC anthophyllite in celloidin film.

electron micrographs can then be put together to accommodate extremely long fibres without obscuration. For an examination of N-fibres the film is picked up with these fibres parallel to the bars. Should there be both P-fibres and N-fibres present, separate grids are used for each type. In the LM and SEM, which do not use grids, the solution offered by aligned specimens is always available.

(c) They facilitate fibre counting using automatic microscopes. The current problem of evaluating large numbers of membrane samples in monitoring airborne asbestos dust (ADVISORY COMMITTEE ON ASBESTOS CANCERS, 1973) encourages the development of these rapid, non-subjective methods.

(d) Dust samples collected in industry generally contain several types of particles, for instance silica, glass fibres, asbestos fibres. In microscopic examination of aligned specimens prepared from such samples, the aligned state of the asbestos fibres draws attention to their presence. This greatly reduces the search required prior to use of the electron probe or energy dispersive X-ray analysis to identify the asbestos type.

Aligned specimens also improve X-ray diffraction and some other analytical methods.

## USE OF LIGHT SCATTERING

The technique of fibre alignment promotes the use of light-scattering methods for the rapid determination of the identity and quantity of asbestos fibres in various kinds of specimens. A thin celloidin film as prepared for use in the LM can be examined in the system depicted in Fig. 6, where a horizontal laser beam illuminates

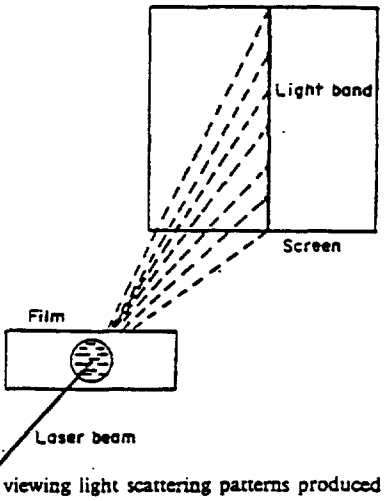


FIG. 6. System for viewing light scattering patterns produced by film specimens.

a vertical glass slide carrying the film and the scattered light is collected on a vertical screen. The film is depicted as containing P-fibres and hence the long axis of the slide was aligned during preparation of the specimen in the direction of the magnetic field. These fibres produce a vertical light band as shown. Fibres aligned in the vertical direction (N-fibres) would show a horizontal light band: the rule is that a light band is always perpendicular to the fibres that produce it. Figure 7 shows results obtained from samples from different sources using this system. UICC anthophyllite (Finland), which the electron micrograph in Fig. 4e shows has only P-fibres, produces a vertical light band which is in effect perpendicular to the original magnetic field. The horizontal light band for India amosite shows the sample contains N-fibres. In sharp contrast, the two light bands produced by UICC amosite (Transvaal) indicates that this amosite contains both P-fibres and N-fibres. This indication is substantiated by LM and SEM examinations (Figs. 4a and 4d). The single vertical light band visible in the pattern given by UICC crocidolite (Cape Province, S. Africa) indicates the presence of P-fibres. Uganda tremolite gives a vertical light band (P-fibres). In

contrast, Zululand tremolite gives a horizontal band (N-fibres). Also shown is the striking result obtained from a synthetic fluoramphibole.

Such a pattern obtained from an unknown sample can suggest the source: for instance whether the fibres could have come from the same Transvaal deposit as the UICC amosite that gave Fig. 7c. More specific evidence can be obtained from measuring the light intensity distribution of the pattern and comparing the result quantitatively with distributions from known sources. Reference samples for use in this method can be prepared directly from the UICC samples (TIMBRELL *et al.*, 1968; TIMBRELL and RENDALL, 1971/72), or from commercial fibre or rock samples after grinding to respirable size using pestle and mortar. Figure 8 depicts a system for use

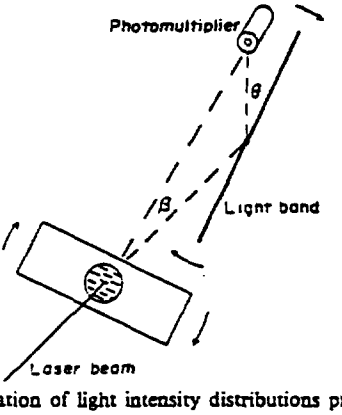


FIG. 8. System for determination of light intensity distributions produced by film specimens.

with film specimens. The glass slide is arranged to rotate (1 rev/min is technically convenient) with the laser beam as axis, resulting in rotation of the pattern (cf. Fig. 6) in the same direction, $\theta$ being the phase angle. The light scattered at angle $\beta$ to the direction of the laser beam is collected by a photomultiplier fitted with a small aperture, and the amplified signal is applied to a chart recorder. Figure 9 shows chart traces obtained from the samples which produced some of the patterns in Fig. 7. The trace given by UICC anthophyllite (Finland) shows two identical P-peaks, at

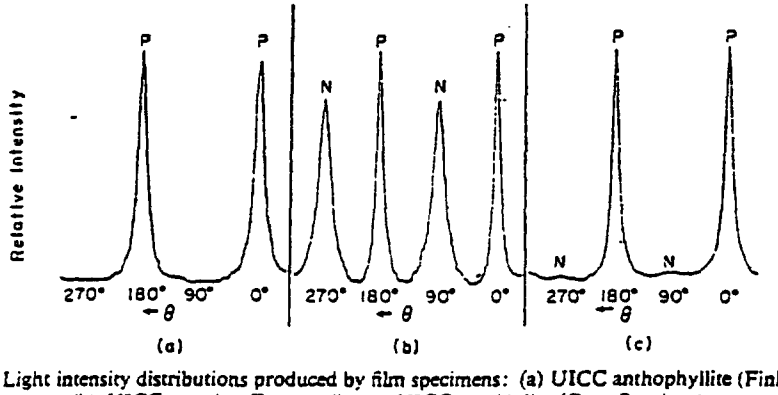


FIG. 9. Light intensity distributions produced by film specimens: (a) UICC anthophyllite (Finland); (b) UICC amosite (Transvaal); (c) UICC crocidolite (Cape Province).

values of $\theta$ of 0° and 180°, produced as the light band due to its P-fibres crossed the aperture twice in the complete revolution. In contrast, the trace for UICC amosite (Transvaal) shows two P-peaks at 0° and 180° due to the light band from its P-fibres and two N-peaks at 90° and 270° due to the light band from its N-fibres. UICC crocidolite, which shows no light band from N-fibres in the pattern (Fig. 7d) but which shows small N-peaks in the trace, illustrates the sensitivity of the system to minute quantities (nanograms or picograms) of fibre. The fact that the N-fibres in this crocidolite are invisible in the LM (Fig. 1b) but are visible in the TEM (Fig. 4b) illustrates the sensitivity to extremely fine fibres.

A similar system, depicted in Fig. 10, is used to examine a liquid suspension of a sample in a spectrophotometer cell. Samples when examined by this system produce

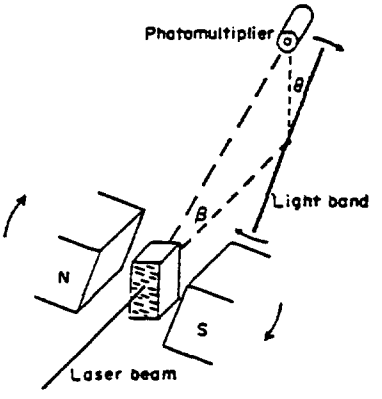


FIG. 10. System for determination of light intensity distributions produced by liquid suspensions.

light patterns similar to those they give in the film system (Fig. 8). Recording of the light intensity distribution is achieved by rotating the magnet with the laser beam as axis, causing each fibre to rotate about its own centre. With a field of 3 kG, a rate of 1 rev/min ensures that all the fibres are virtually in phase with the field.

A comparison of Figs. 9a–c and Figs. 11a–c shows that while given samples produce similar traces in the film and suspension systems there is one important difference exemplified by UICC amosite. For this sample, when compared with the P-peaks, the N-peaks are larger in the film-derived than in the suspension-derived traces, the reason being that in a film the N-fibres occur in a single plane, each N-fibre making its maximum contribution to the intensity of the light band, whereas the same N-fibres in suspension, owing to their random orientation normal to the field (Fig. 2b), make contributions varying from maximum to nil.

The film and the suspension systems provide means for using the relative proportions of P-fibres and N-fibres in a sample, as measured by the sizes (areas) of the peaks, to assist identification of its source. The greater width of peaks in the trace obtained from UICC Canadian chrysotile (Fig. 11d), which is related to the curvature of the fibres (Fig. 4c), illustrates one of the many other features that assist identification. The trace (Fig. 11e) obtained from the fluoramphibole which gave the pattern in Fig. 7g illustrates the novel traces produced by synthetic fibres. In this example the

shape of the trace changes with time elapsed from starting the magnet into rotation, as the fibres drag on one another until they attain an orientation equilibrium.

The film and the suspension systems can be used to estimate asbestos in a dust sample by calibrating with weighed samples of fibres from the identified source. A unique advantage of these methods is that they allow fibres to be distinguished from other particles of respirable size which, either by being non-magnetic or non-fibrous, do not align and hence scatter the laser light in all directions. When, as is usual, a low-concentration specimen is used, these particles merely cause (as illustrated in

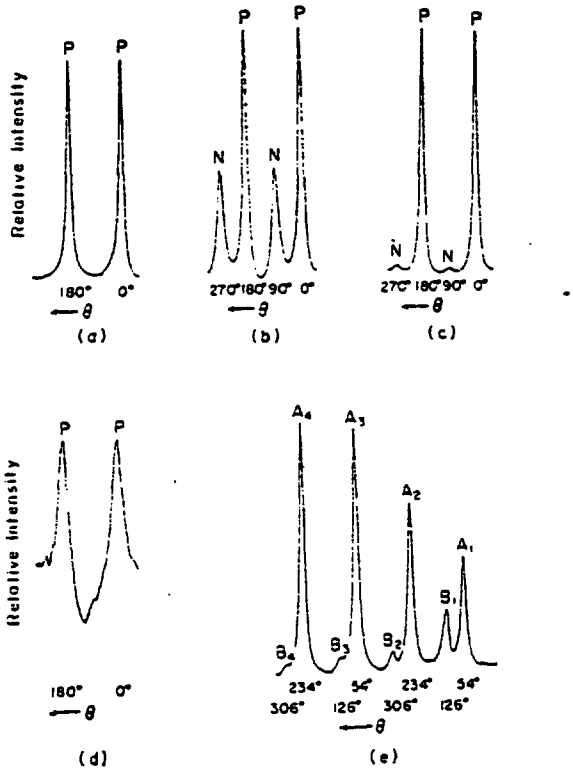


FIG. 11. Light intensity distributions produced by aqueous suspensions : (a) UICC anthophyllite ; (b) UICC amosite; (c) UICC crocidolite; (d) UICC Canadian chrysotile; (e) Synthetic fluoramphibole.

Fig. 12) a raising of the trace with respect to the baseline without altering the sizes (areas) of the peaks. In cases where fibres represent a small proportion of a sample an improved evaluation is obtained by first separating them from the other particles. Magnetic alignment is providing a basis for the development of such separation methods.

For the estimation of asbestos fibres in tissue the organic material is first digested with a potassium hydroxide (GOLD, 1973) or a sodium hypochlorite solution which is then examined in the suspension system (Fig. 10). The identification of the fibre in

the lung which gave the trace in Fig. 13 was confirmed by the subject's occupational history showing a moderate exposure to Transvaal amosite. The fact that the N-fibres in this asbestos tend to be thinner than the P-fibres causes them to penetrate more efficiently into the lungs (TIMBRELL, 1965). The consequence is seen in the greater size of the N-peak in relation to the P-peak in the lung-derived trace than in

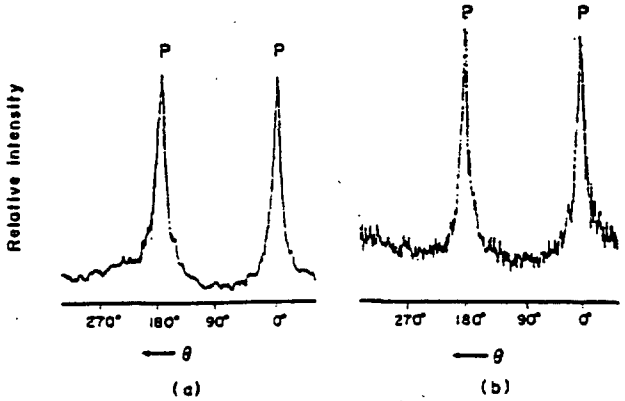


FIG. 12. Effect of adulterants: (a) light intensity distribution produced by a film specimen of UICC anthophyllite; (b) light intensity distribution produced by a similar film specimen but with silica particles added.

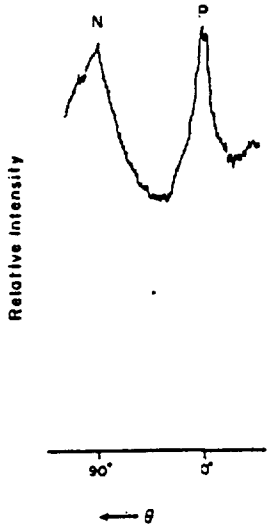


FIG. 13. Light intensity distribution produced by liquid suspension of particles recovered from an asbestos worker's lung.

the sample-derived trace (Fig. 9b). This is an example of use of the system to obtain information on the behaviour of fibres in the respiratory tract.

A durable specimen of a sample can be obtained by adding a thixotropic material to an aqueous suspension in a spectrophotometer cell and keeping the cell in a magnetic field until the suspension gels. The specimen can then be evaluated in the

system depicted in Fig. 8. Such a specimen is also useful for LM examination of the fibres to check the alignment modes when, for instance, one meets an unusual light pattern or chart trace for the first time.

*Indices of fibre size*

Some investigations on asbestos demand such a full description of fibre size that there is no alternative to using light and electron microscopes to determine the diameter and length distributions. The substantial effort that this involves imposes a severe limitation on the number of samples that can be examined, and in our laboratory we are investigating easier ways for obtaining comparable information. However, for many studies, simpler measures of fibre size such as single-number indices are much more useful: in fact, in many cases the study would not be possible without them. The chart traces produced by the suspension system depicted in Fig. 10 provides two such indices. These indices facilitate a wide range of investigations; frequently, when the requirement is to compare fibre size in samples of asbestos from the same source, complete experiments can be carried out using these indices alone. The indices are outlined below.

Referring to Fig. 10, one index involves measurement of the change which occurs in the height of the peaks as the value of $\beta$ is altered. This is readily achieved by using automatic means for moving the photomultiplier at a steady rate while the chart recorder is run at a slow speed. The chart trace thus produced shows a large number of closely-spaced peaks, the heights of which can be measured and plotted against $\beta$, for both P-fibres and N-fibres. Examinations of a wide range of samples have shown that the results consistently take a simple form if: (a) the photomultiplier is moved along a circular arc centred at the spectrophotometer cell, rather than along a vertical straight line for instance ; (b) as depicted in Fig. 10, $\beta$ refers to the light as it emerges from the spectrophotometer cell ; (c) peak height is expressed as a percentage of peak height at some fixed value of $\beta$, say 10°, and plotted on a logarithmic scale. This index may be illustrated using data obtained from aqueous suspensions of progressively decreasing fibre size. Six suspensions of UICC anthophyllite were subjected to centrifugation at $18g$ for various periods, in each instance a standard volume of the suspended phase being withdrawn for examination. Figure 14 shows that each suspension gives a straight line, the index being the gradient of this line. This index thus decreases with decreasing fibre size.

The second index is provided by the chart traces obtained when the suspension-system is operated in the normal mode with $\beta$ fixed. This index is the peak width at half peak-height as illustrated in the insert in Fig. 15. Expressed in terms of $\theta$, it is a measure of the deviation of the fibres from exact alignment with the field as rotational diffusion acts against magnetic torque. The results given in the Figure, also obtained from the UICC anthophyllite suspensions mentioned above, show that this index increases with decreasing fibre size.

The indices can provide valuable information on fibre size in air samples and lung-extracted dusts. It would be convenient if the two indices depended on different parameters and were capable of providing separate evidence on fibre diameter and length. In fact, the indices are affected to some extent by both diameter and length. In many applications however this limitation detracts little from the value of the indices. Usually a correlation exists between diameter and length, the thickest fibres tending to be the

longest; with the result that when sedimentation and impaction act directly in air sampling and inhalation to decrease fibre diameters they also operate indirectly to cause a parallel decrease in the lengths. Many situations in which we are interested involve these deposition mechanisms. In these circumstances the parallel decrease in the parameters can be anticipated and it matters little that the indices may not give separate evidence on diameter and length.

An important use of the indices is in calibrating the suspension system for measurement of the mass of fibre in an air sample or a specimen of lung-extracted dust. Accuracy in such a determination requires that after the series of aqueous

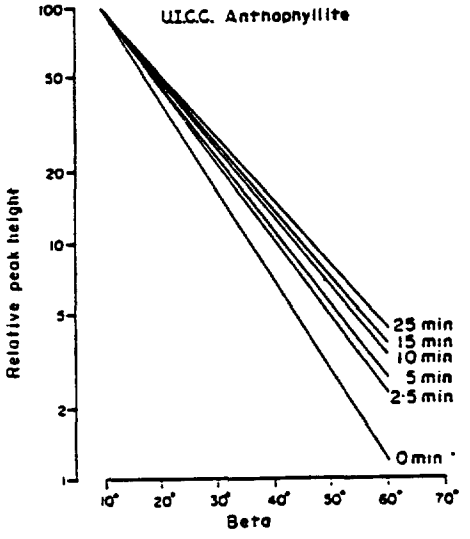


FIG. 14. Peak height expressed as percentage of peak height when β is 10°, plotted against β for various centrifugation times.

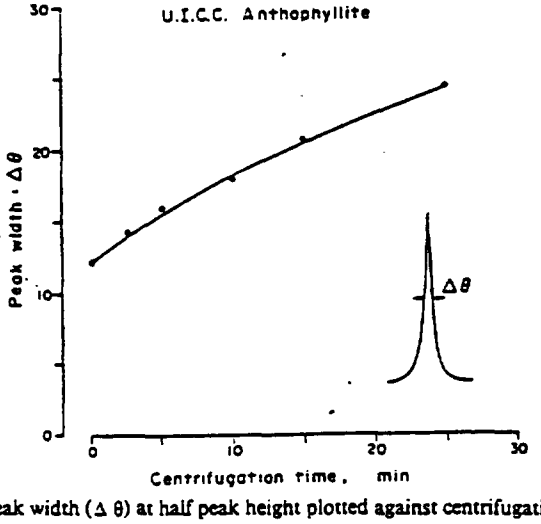


FIG. 15. Peak width (Δ θ) at half peak height plotted against centrifugation time.

suspensions of known mass concentration for use in the calibration have been prepared, the fibre size should be comparable to that in the sample being examined. This means that a method must be provided for measuring fibre size in the sample, as well as a technique for preparing the calibration materials, which permits adjustment of both fibre diameter and length. In relation to these requirements, microscopy is too time consuming for use except as a reference method, while conventional grinding methods do not give separate control of the size parameters. A satisfactory combination is provided by using the two indices in conjunction with centrifugation, centrifugation having an effect on fibre diameter and on length comparable to the influence sedimentation and impaction exert in air sampling and inhalation. That the combination is effective is seen in practice from the way centrifugation brings the two indices simultaneously to the correct values. An excellent match with the indices in the sample is obtained when the asbestos involved is one of the UICC fibres: in this case the calibration material starts with length and diameter distributions comparable to those in lungs (TIMBRELL *et al.*, 1970) and all centrifugation has to achieve is a reduction in maximum diameter and length. Good results are achieved with other asbestos samples by first milling the calibration fibres to a size approximating the UICC specifications and then applying centrifugation. To avoid having to produce a calibration material for each sample, a set of calibration materials with a range of values of the indices is being prepared whenever asbestos from a new source is encountered. This will reduce calibration to selecting data from a bank.

The indices enable large-scale investigations to be carried out which otherwise would not be feasible. They also encourage the effective use of electron microscopy by confining it to detailed examinations of a small number of samples for confirmation. In a current study, designed to establish the spatial distribution of fibre size in a rat lung, we are employing the suspension system to examine hundreds of lung portions. This is a case where separate data on fibre diameters and lengths in each specimen would be valuable. An investigation of methods for obtaining this extra information by comparing the indices has given encouraging results.

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