

Analysis of ferruginous bodies in bronchoalveolar lavage from foundry workers

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Abstract

Classical ferruginous bodies in tissue samples are considered to be markers of past exposure to asbestos. Recent studies have shown that the presence of ferruginous bodies in bronchoalveolar lavage (BAL) fluid correlates with past exposure to asbestos and offers a more sensitive reference than occupational history. Lavage samples from five subjects who had worked in foundries were evaluated by light microscopy for the presence of ferruginous bodies and by transmission electron microscopy for both characterisation of the uncoated fibre burden and analysis of the cores of the ferruginous bodies. All samples at lower magnification (light microscopy (200×)) contained ferruginous bodies that were externally consistent with asbestos bodies. At higher magnification (400×), a separate population from this group could be identified by the presence of a thin black ribbon. Transmission electron microscopy of the core materials of ferruginous bodies and comparable uncoated particulates supported the reliability of higher magnification light microscopy for distinguishing most of those non-asbestos cores; however, a population of transparent non-asbestos cored ferruginous bodies were also shown to exist.

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The usefulness of bronchoalveolar lavage (BAL) in supporting the diagnosis of certain occupational pulmonary diseases has been discussed in several reviews.^{1,2} Studies have shown that this technique is useful in confirming past occupational exposure to asbestos through the presence of ferruginous bodies in the BAL fluid.³⁻⁵ De Vuyst and colleagues have further shown a correlation between the concentration of asbestos bodies in BAL fluid and fibre burden in the lung.⁶ Two other reports used analytical electron microscopy to confirm that the cores of ferruginous bodies in BAL fluids were asbestos fibres.^{7,8} All of these studies included at least one group of subjects having known or suspected occupational exposure to asbestos. The use of light microscopy for defining the number of ferruginous bodies and the assumption that these are asbestos bodies are supported by the arguments: (1) occupational exposure to asbestos is a known stimulus for ferruginous body formation; (2) Churg and Green have stated that "non-asbestos-containing bodies are easily separated from true asbestos bodies with a light microscope."⁹ The criteria used by Churg and Green⁹ and De Vuyst *et al*⁶ for equating ferruginous bodies with asbestos bodies include being formed on a clear, often straight, core, and having a regular or segmented, yellow to brown ferroprotein coating.

In further support of this conclusion, asbestos has been shown by analytical electron microscopy to comprise the core material in samples from the general population.^{10,11} Gross *et al*,¹² however, have reported that transparent non-asbestos fibres are capable of producing ferruginous bodies in experimental animal models. Appreciable numbers of such uncoated non-asbestos fibres^{13,14} have been reported in human lungs and thus offer the potential for various types of cores.

The foundry workers in the present report had been exposed to a mixed dust environment that has been characterised as potentially containing fibrous (asbestos and non-asbestos) and non-fibrous dusts.¹⁵⁻¹⁷ The BAL fluids from five subjects with such exposures were referred for laboratory assessment for the presence of ferruginous bodies and characterisation of the uncoated fibre burden.

Patients and methods

Bronchoalveolar lavage fluid was collected as a part of a diagnostic evaluation from five men who had a history of working from 20 to 31 years in foundries and iron reclamation plants. All were from non-urban areas of east Texas and their ages ranged from 60 to 77 (average 66) years. The group contained two non-smokers and three former smokers.

Fibreoptic bronchoscopy was used to collect samples. A total of 100 ml saline was injected into selected areas (right middle lobe or lingula) of the lung in 20 ml aliquots. The recovered fluid ranged from 19 ml to 83 ml (average 47 ml). A small aliquot (< 0.1 ml) was taken for determination of white cell and differential cell counts. The percentage of phagocytes was obtained by combining the macrophage, neutrophil, and eosinophil percentages. This percentage of phagocytes, which ranged from 84% to 99% (average 89.4%), was then multiplied by the white cell count. The remaining BAL fluid was centrifuged at 700 g for 10 minutes to concentrate the material. The pellet was resuspended in a known volume (2 to 10 ml) of 3% phosphate buffered glutaraldehyde fixative, (pH 7.3, 0.1 M). The white cell count for the fixed BAL fluid ranged from 270 000 to 1 050 000 (average 667 800) white cells/ml and the phagocytes ranged from 226 800 to 1 039 500 (average 613 794) phagocytes/ml.

Samples for analysis were prepared with the modified bleach digestion procedure of Williams *et al.*¹⁸ All reagents and distilled water were pre-filtered (0.2 μ m polycarbonate filter, Nuclepore). Reagent blanks were prepared to determine background contamination. Digested BAL fluid material was collected on either 0.2 μ m polycarbonate (Nuclepore) or 0.22 μ m mixed cellulose ester (Millipore) filters. The amount of glutaraldehyde fixed digested material collected on the polycarbonate filters ranged from 1.0 to 2.5 (average 1.5) ml and from 340 000 to 1 039 500 (average 791 394) phagocytes, whereas the amount filtered for the mixed cellulose ester filters ranged from 0.5 to 3.0 (average 1.55) ml and from 319 200 to 1 479 000 (average 740 833) phagocytes. A ratio of 1 ml of glutaraldehyde fixed BAL fluid to 8–10 ml sodium hypochlorite bleach (9.2% Wright's laundry bleach) was used for the digestions. The filters were treated sequentially with 4% potassium permanganate, 8% oxalic acid, 9.2% bleach, and 8% oxalic acid.¹⁸ Each addition was followed by a water rinse. Filters were air dried, mounted on glass slides, and stored in Petri dishes.

LIGHT MICROSCOPY

One quarter of each mixed cellulose ester filter was collapsed in acetone vapour, placed under a cover

slip, and reviewed at 200 \times to 400 \times with an AO MicroStar microscope. All ferruginous bodies were counted and categorised based on external morphology and, where possible, on appearance of the core material.

The general term ferruginous body, denotes a golden brown ferrocoated structure^{11 12 19} and in the initial scan of the filter, various forms of ferruginous bodies were seen in each subject.

To differentiate the separate forms, a classification system of four categories was used. The first category (type A) consisted of elongated reddish or golden brown structures with beaded or segmented morphology and contained a transparent fibrous core. An additional group of similar structures was totally coated. Both of these beaded forms were morphologically identical to classical asbestos bodies as defined by light microscopy.^{9 11 19 20}

The second category (type B) was identical to the type A structures in external morphology, but at 400 \times magnification, a thin black filament was evident as the central core.

The type C ferruginous bodies were also formed on a black core. This core was rectangular or rod like in appearance rather than a thin central filament. The external morphology was consistent with the beaded form of the type A and B ferruginous bodies.

The final form (type D) was covered with a ferruginous coating and was clearly not formed on a fibrous or elongated core, but rather on black or grey particles of various shapes.

All the types of ferruginous bodies had a golden or reddish brown coat. A summary of the ferruginous body classification by light microscopy is: type A—clear core (classic ferruginous body); type B—Thin black filament core (seen at 400 \times); type C—Black, thick with elongated core; type D—Odd shaped ferruginous body with gray or black core.

ELECTRON MICROSCOPY

Each polycarbonate filter was carbon coated at 4×10^{-6} Torr in a Denton 502A vacuum evaporator for transmission electron microscopy and sections were mounted in 100/200 mesh folding copper grids. The filter matrix was dissolved in chloroform with a modified Jaffe-wick procedure, thereby entrapping fibres and ferruginous bodies in the remaining carbon film. For scanning electron microscopy, filter sections were mounted on carbon studs with colloidal graphite. The sections were coated with gold in an Emtech K550 for four minutes at 7.5×10^{-3} Torr argon. Transmission and scanning electron microscopy preparations were evaluated in a JEOL 1200EX interfaced to a TN-5500 Series II x ray energy dispersive analyser. x Ray energy dispersive spectrometry (XEDS) is capable of digital beam x ray mapping of the-

Table 1 Ligand microscopy data

Smoking history	Phagocytes/ferruginous body				Limit of detection	Ferruginous bodies/ml				Limit of detection
	Type A	Type B	Type C	Type D		Type A	Type B	Type C	Type D	
Patient 1 former smoker	3325	1628	3469	927	>79 800	96	196	92	344	<4
Patient 2 former smoker	660	192	168	78	>194 906	1573	5408	6181	13 242	<5
Patient 3 non-smoker	3013	111 483	1570	218	>111 483	296	8	568	4080	<8
Patient 4 former smoker	2501	14 175	5154	4361	>170 100	90	16	44	52	<1
Patient 5 non-smoker	17 607	61 625	8803	370	>369 750	34	10	67	1600	<2

Type A = classic clear core; Type B = thin black filament; Type C = thick black elongated; Type D = odd shape.

elemental components that have a greater atomic number than neon. This technique was applied in assessing the core of the totally coated ferruginous bodies.

Two grids from each patient were scanned at 10 000 \times and 20 000 \times for the uncoated fibre burden. All uncoated fibres with substantially parallel sides, a length/width ratio $\geq 3:1$, and a length of $\geq 0.5 \mu\text{m}$ were counted, as well as analysed by both XEDS and selected area diffraction. Ferruginous body cores were analysed when possible.

Fibres and ferruginous bodies within each BAL fluid samples were categorised and reported as (1) fibres or ferruginous bodies/ml of glutaraldehyde fixed BAL fluid and (2) phagocytes/fibre or ferruginous body.

Results

Table 1 shows a summary of the light microscopy data from the study. The light microscopy confirmed the presence of transparent cored ferruginous bodies (type A) consistent with asbestos bodies in all five patients (fig 1).²¹ The percentage

of type A or classical ferruginous bodies comprised 12%–60% of the total ferruginous body burden in the lavage samples from the patients. The type B and C forms of ferruginous bodies were distinguished from the type A by their visible black cores (fig 2). Similar ferruginous bodies have been referred to previously by Churg *et al* as carbon bodies.¹¹ As noted in the definitions within the methods section, there were physical differences in core structure. Those classified as the B form contained a thin filament that was either difficult or impossible to see at lower magnification, whereas the C structures were thick black rods or plates and easily detected at 100 \times magnification. The B and C forms combined accounted for over 65% of the total ferruginous bodies as formed on elongated core material in four of the five patients and 40% in the fifth patient (table 2).

The pleomorphic particulates that formed the cores of the D form consisted of particulates of various colours ranging from pale yellow to grey and black tones. This form of coated material constituted the highest number of ferruginous entities in three of the five patients. None of these were

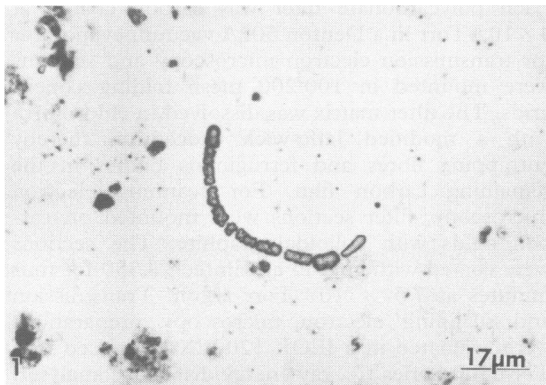


Figure 1 Typical clear cored ferruginous body that was classified as type A. Originally $\times 1200$.

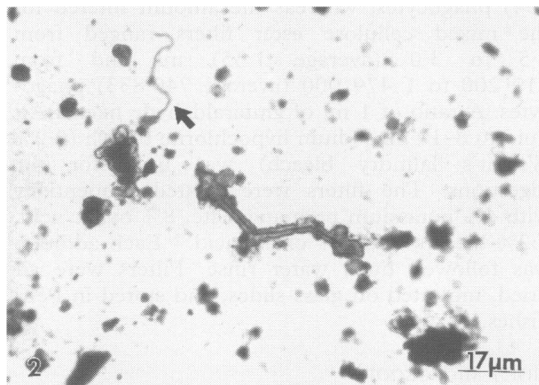


Figure 2 Both type B and type D ferruginous bodies, arrow points to an uncoated black filament. Originally $\times 1200$.

Table 2 Comparison of fibrous cored ferruginous bodies by type

Patient	Type A‡ (%)	Type B + C† (%)	Type B† (%)	Type C† (%)
1	25	75	51	24
2	12	88	41	47
3	34	66	1	65
4	60	40	11	29
5	31	69	9	60

‡Asbestos; †Non-asbestos.

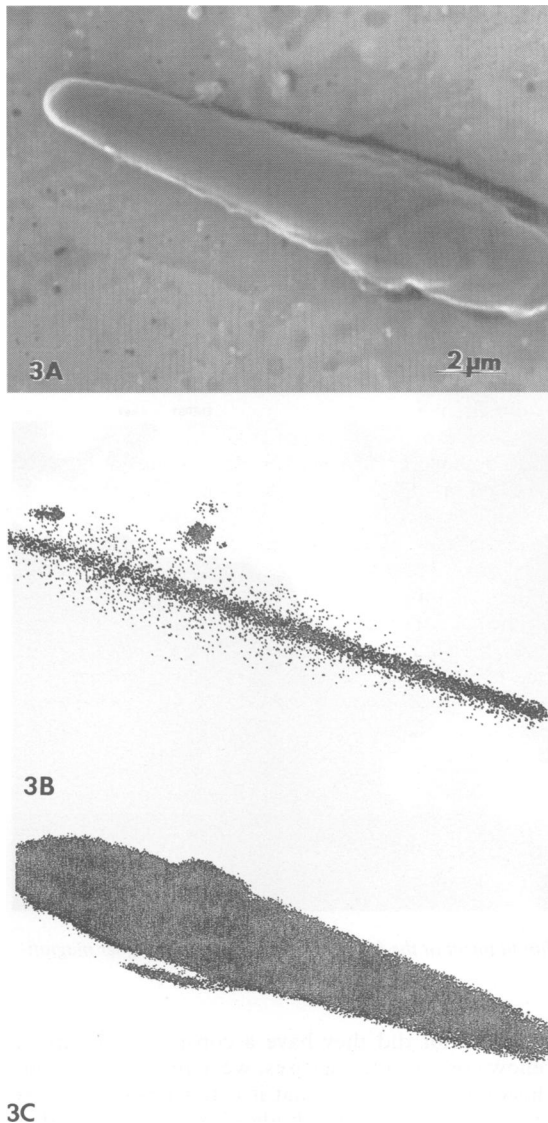


Figure 3(A) Scanning electron micrograph containing a totally coated head region of an asbestos body. Originally $\times 9600$. (B) Elemental x ray map from the asbestos body in (A) indicating that silicon is detectable through the iron coated areas. (C) The iron x ray map of the same field as in (B).

fibrous and therefore would not be confused with the B and C forms. The ferruginous coats of the D form of ferruginous body were often extensive. The coating material often formed terminal blebs that extended from the central region, but the extensions did not seem to contain core material.

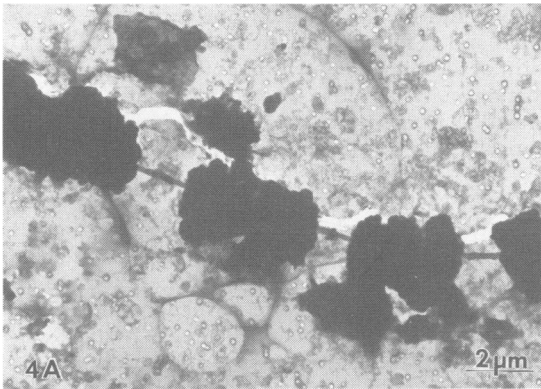
TRANSMISSION ELECTRON MICROSCOPY

Fifty two apparently classical ferruginous bodies (type A by light microscopy) were analysed to determine core composition. Eight were formed on amosite asbestos, two were formed on fibreglass, and six were formed on thin elongated sheet silicates. Thirty seven of these bodies were totally coated and only iron peaks were obtainable by XEDS. This implies that the central fibre was either iron or carbon, or shielded sufficiently by the coating to prevent peak production. Our XEDS analysis suggests that the core material was often of carbon composition (type B filaments), as no energy peaks except iron were produced. This contrasts with the generation of peaks of silicon from totally coated asbestos bodies (figs 3(A), 3(B), and 3(C)).

The exposed cores of sixty type B ferruginous bodies were analysed. The exposed black filament in type B ferruginous bodies (figs 4(A) and 4(B)) did not give peaks by x ray energy analysis and was considered to be an amorphous organic material, as was the central area of the type C form. By electron microscopy, the distinction between the type B and C forms at the light microscopy level was based only on thickness of the core material. The analysis of cores from nine of the C types gave the same information as the analysis of the cores of the type B forms.

The type D ferruginous bodies could not be confused by light or electron microscopy with ferruginous bodies formed on fibrous material. The analysis of the core material of 140 such structures was confirmed to be mixed dust (fig 5). These included carbon material, (no XEDS peaks above neon) silica, and titanium, as well as various alumina silicates and mixed silicates.

Uncoated chrysotile or amosite asbestos fibres were found in the lavage samples from four of the five patients whereas fibreglass fibres were found in material from two patients. Four of the patients were found to have amorphous fibres as determined by selected area diffraction and did not yield detectable elements when analysed by XEDS (fig 6). These fibres were considered to be the equivalent of the cores of the B and C form of ferruginous body and comparable by XEDS to the carbon cores described by Churg and Warnock.¹⁹ Each of the samples also had other uncoated fibres that were found to be silicon, titanium, iron, or various aluminum silicates by XEDS analysis (table 3).



variation in orientation of coating material, many of these are not evident at lower magnification. This may seem like a trivial issue, easily overcome by simply shifting to high dry objective; however, there are numerous studies in which counts of ferruginous bodies are made at $100\times$. As these bodies are externally identical to asbestos bodies, such a count would have likely not differentiated these non-asbestos types. Also, those formed on sheet silicates and fibreglass were similar to asbestos bodies in external appearance. Compounding the problem of differentiating the cores of sheet silicates was the fact that the particles were neither noticeably

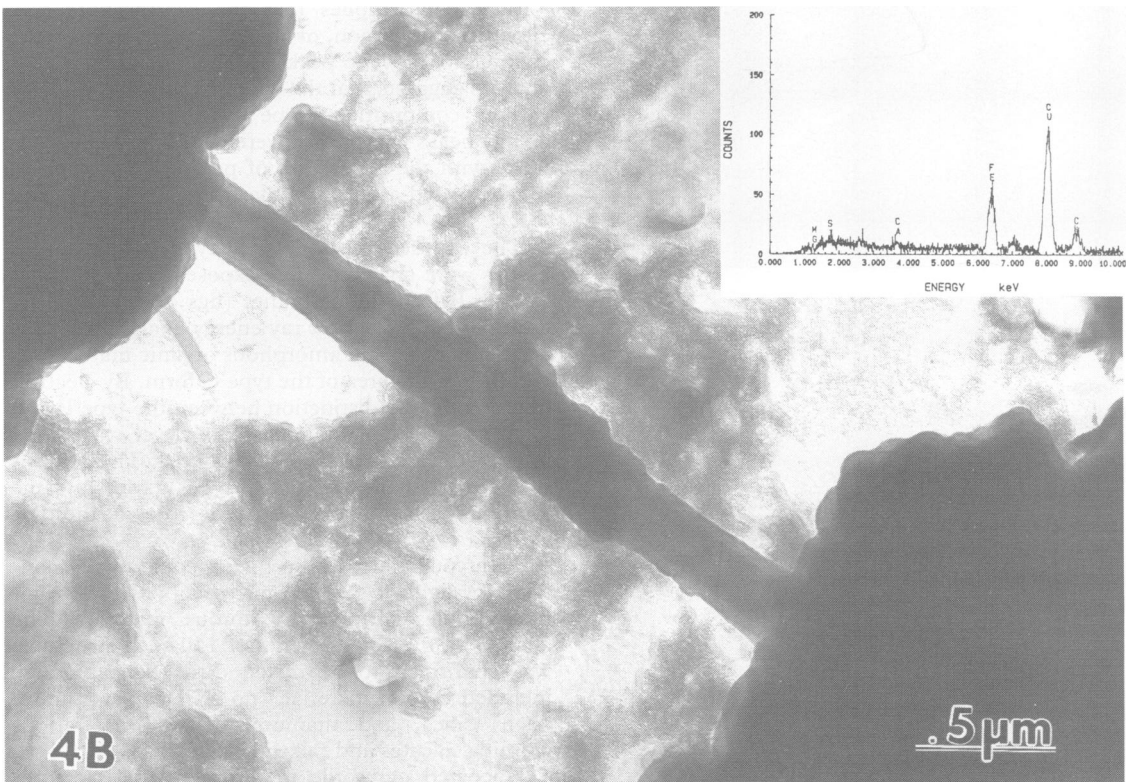


Figure 4(A) Type B ferruginous body. Originally $\times 8600$. (B) Thin filament in the ferruginous body of (A) at higher magnification. Inset shows XEDS of filament. Originally $\times 58\,000$.

Discussion

The BAL material from the foundry workers contained four types of iron coated structures. Our data are generally in agreement with Churg's²¹ conclusion that non-asbestos ferruginous bodies can be detected by light microscopy. We would, however, offer the qualifier that those formed on thin filaments (type B) often require higher magnification ($400\times$) to resolve the core material. Due to the

widened nor did they have a conspicuously bright yellow core.¹⁹ In these cases, we agree with the conclusion of Churg *et al* that it is not possible to distinguish between these bodies and asbestos bodies with the light microscope.¹¹

The isolated C and D forms of ferruginous bodies are readily distinguished from classical ferruginous bodies (type A) by light microscopy analysis of isolated preparations on a cleared filter. These

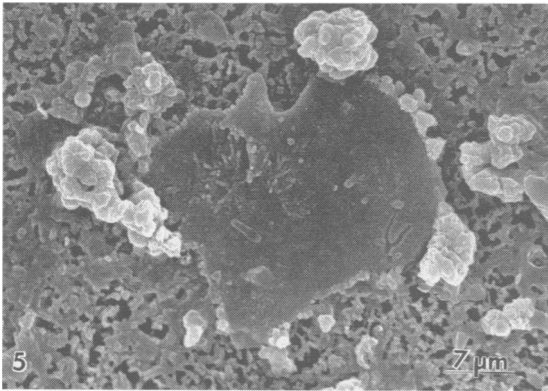


Figure 5 Type D classification illustrates that the ferruginous material was formed on a central plate. No peaks, other than iron, were obtained by XEDS of the material. Originally $\times 2900$.

same structures seen in various orientations in tissue sections may not be as easily interpreted.

The presence of an iron coat in any form may increase the cytotoxic potential of the core particulate. Although the process of coating seems to physically isolate particulates from the tissue environment, Lund *et al* found when comparing single strand breaks in a DNA model that there was a greater reactivity associated with coated asbestos compared with equivalent concentrations of uncoated asbestos (unpublished data).

Our present study of lavage samples from foundry workers confirmed the presence of classical ferruginous bodies as reported in previous studies of tissue samples,^{22 23} and lavage material.²⁴ The data from the present study likewise confirm an exposure to mixed dusts that included both fibrous and non-fibrous forms that were capable of stimulating the formation of ferruginous bodies. Those ferruginous bodies formed on non-asbestos fibres can greatly exceed the numbers of classical asbestos bodies in such exposures.

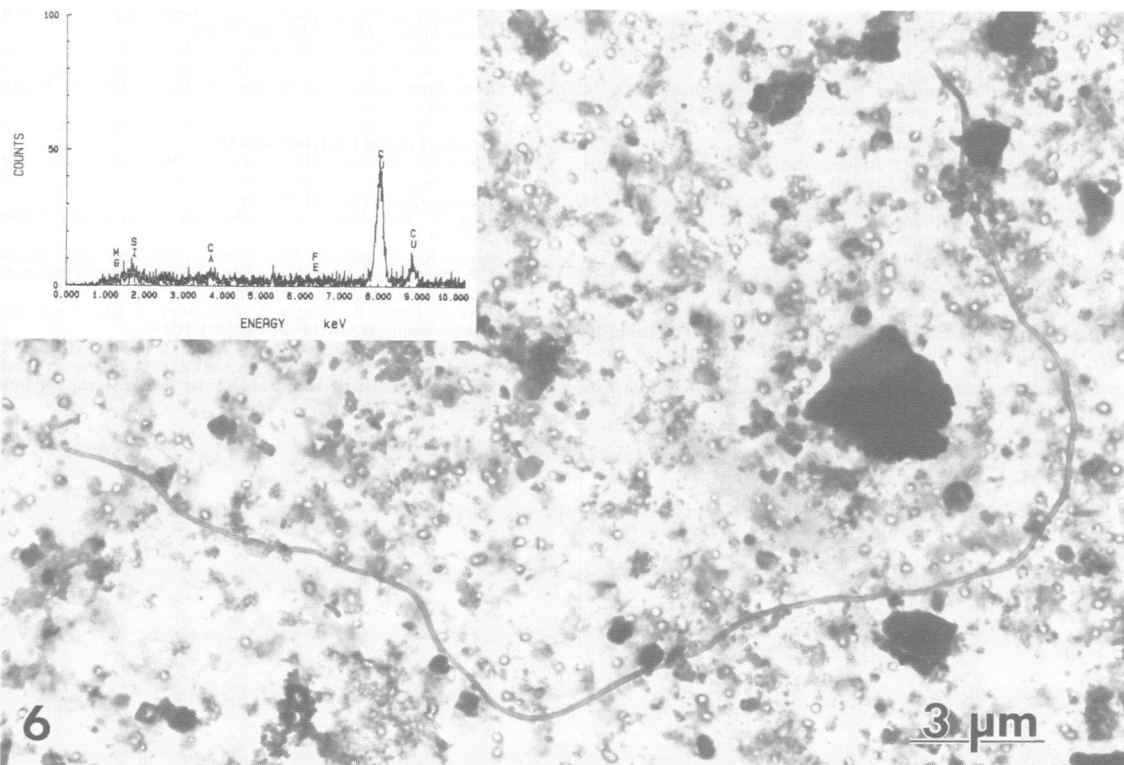


Figure 6 Only a background peak of copper is generated on this XEDS (inset) of an uncoated fibre that is equivalent to the core material in the type B ferruginous body, originally $\times 8000$.

Table 3 Other uncoated fibres, identified by x ray energy dispersive spectrometry

Patient	Fibres/ml					Phagocytes/fibre				
	Chrysotile	Amosite	Glass	Organic	Other*	Chrysotile	Amosite	Glass	Organic	Other*
Patient 1	ND	50	ND	251	377	ND	6352	ND	1270	953
Patient 2	1256	1005	ND	1570	5025	827	1034	ND	690	207
Patient 3	3832	ND	377	5026	6596	3633	ND	2382	178	188
Patient 4	838	ND	1815	ND	964	271	ND	248	ND	519
Patient 5	ND	ND	ND	837	3350	ND	ND	ND	706	176

ND = none detected; *Other = Si, Ti, Fe, Al, AlSi, AlSiCa, AlSiFe, SiFe.

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