Structure and composition of pleural plaques

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The study described below is of human pleural plaques taken from patients occupationally exposed to asbestos dusts; for purposes of comparison plaques taken from patients with calcifications of completely different etiology, such as tuberculosis or emphysema, were studied.

MACROSCOPIC APPEARANCE

The specimens studied were taken preferably from the parietal pleura and had the well-known macroscopic appearance of pearly-white plaques, firm to the touch, a few millimetres thick with an area ranging from a few square centimetres to about 50 cm^2. In most cases the free side of the plaque had a mammillated surface.

Seen in section both the plaque and the mammillae have a laminated texture which can clearly be seen under the magnifying glass. Examination in section also reveals possible calcifications. These almost always appear in the median plane of the plaque and in the centre of the mammillae. If the calcification is already compact, it can be clearly differentiated from the neighbouring parts of the plaque and can be cut away from them. The calcified zones may not be clearly visible in fresh tissue, but if they are kept in formalin for some time they can be distinguished by a markedly yellow colour.

HISTOLOGY

Pleural plaques are made up of fibrohyaline connective tissue, practically free of cells, made up essentially of thick bundles of collagen fibres separated by optically empty pseudolacunae. They are bounded at their lower edge by a thin network of elastic fibres which is stained by orcein and which itself lies on subjacent adipose tissue. The surface, which is bounded by endothelium, is frequently covered with nodules in which collagen fibres are arranged concentrically, like onion rings.

There are only a few cellular elements, mainly fibroblasts, inside the plaque itself. Inflammatory type cells are never encountered. On the other hand, in the peripheral zone at the edge of the plaque, small agglomerations of lymphoplasmocytes or histiocytes or of mast cells can be seen in the vicinity of small vessels.

The calcifications are usually situated in the collagenous zone; and in section, after staining with von Kossa stain or following micro-incineration, they show up as flattened nodules or thin laminae of varying area, or as more diffuse deposits. They are preponderant in the central or deeper zone of the plaque, and they are separated from the endothelium by a relatively thick layer of non-calcified fibrohyaline tissue.

ULTRASTRUCTURE

The fibrous and calcified zones of the plaques will be considered separately.

Fibrous zone

Whatever the size of this zone in relation to the pleural plaque as a whole, a certain number of common ultrastructural characteristics are found in all the cases examined, with only a few variations.

Electron microscopy first of all confirms the structures that can be seen under the light microscope, i.e., bundles of collagen fibres embedded in a hyaline matrix (Fig. 1). These fibres usually show the normal periodicity of collagen (64 nm); on the other hand, some of them have a markedly larger diameter

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than the general average (200 nm as compared to 30–70 nm) (Fig. 2). Also, some cell debris is encountered embedded between the collagen layers.

In addition to these organic components, numerous electron-dense particles can be seen scattered in the fibrohyaline tissue (Figs. 3 & 4). Some of these particles are solid or laminated rounded bodies with a diameter between 0.1 and 0.5 μm. Others, also compact and of similar dimensions, have a marked fibrous external structure which consists of very fine needles with diameters of the order of 7.5 nm. Finally, other types of markedly differentiated dense particles are found, some of them in the form of spheroid or ovoid plaques of fragmented lamellated appearance with a diameter of the order of 1 μm, and others as bundles of acicular crystals of very varied dimensions.

These different types of electron-dense bodies represent incipient calcifications. They are soluble in acids, and most of them produce an electron diffraction pattern corresponding to that of whitlockite in the case of the compact or lamellar ovoid forms and of apatite in the case of acicular crystals.

Examination under high magnification after lead staining shows dark granular patches with incipient crystallisations or impregnating crystalline masses in the course of growth.

It should be noted finally that the nascent calcifications, particularly those of the whitlockite type, are situated mostly in zones containing cell debris. However, early calcification of the apatite type can also be seen along the length of the collagen fibres.

An important point to note is the similarity between these structures and those encountered in the same subjects in the thickened visceral pleura, where fibrohyaline tissue enclosing cell debris and rounded electron-dense bodies made up essentially of whitlockite can also be observed.

Calcified zone

This zone is characterised by an invasion of the fibrohyaline tissue by acicular apatite crystals, among which whitlockite nodules persist (Fig. 5). The crystals apparently grow along the length of the collagen fibres whose underlying periodicity is still visible in many places.

Phosphotungstic acid or hydrochloric acid dissolve the apatite and allow the collagen fibres to be seen (Fig. 6).

Lacunae may persist among the agglomerations of apatite crystals; in these a fairly loose-woven fibrohyaline tissue can be seen which is found with lead staining to consist of a fine reticulation. These lacunae also contain isolated apatite crystals.

Comparison of pleural plaques and pulmonary calcifications of different etiologies

As was stated above, there is a marked similarity between the calcifications found in the fibrous zone of a parietal pleural plaque and those seen in the thickened visceral pleura of the same individual.

To determine whether this similarity applied to other types of calcified lung tissue, we examined three specimens from pathological calcifications of totally different origins, i.e.,

(i) a fibrohyaline plaque from a subject whose occupation apparently involved no exposure to asbestos dust (a search for asbestos fibres, made on the basis of the method described later, proved negative);
(ii) a gas cyst; and
(iii) a tuberculous caseification.

In all three cases electron-microscopy revealed the presence of electron-dense rounded bodies against a background of cell debris and of collagen or elastic fibres, according to the type of lesion. The spheroid calcifications sometimes had a laminated appearance. When oriented crystals were present, these needles grew among the structured elements of the fibrous weft, the collagen or the elastic fibres.

Altogether these calcifications showed a marked similarity to those of pleural plaques.

COMPOSITION OF THE PLEURAL PLAQUES

Fragments of pleural plaques were analysed for their contents of collagen, phosphate, calcium, sulphur and hexosamines. Measurements were made separately in the fibrous zone and in the calcified zone.

Collagen

Specimens were first decalcified, and the collagen level was determined after acid hydrolysis by measurement of hydroxyproline. Table 1 shows the collagen values in the decalcified tissue and, for purposes of comparison, the concentrations in a fibrohyaline plaque from a subject not exposed to asbestos dust.

There appears to be no significant difference between the collagen levels in the two zones of the calcified pleural plaques. Moreover, the proportion

Fig. 1.

Fig. 2

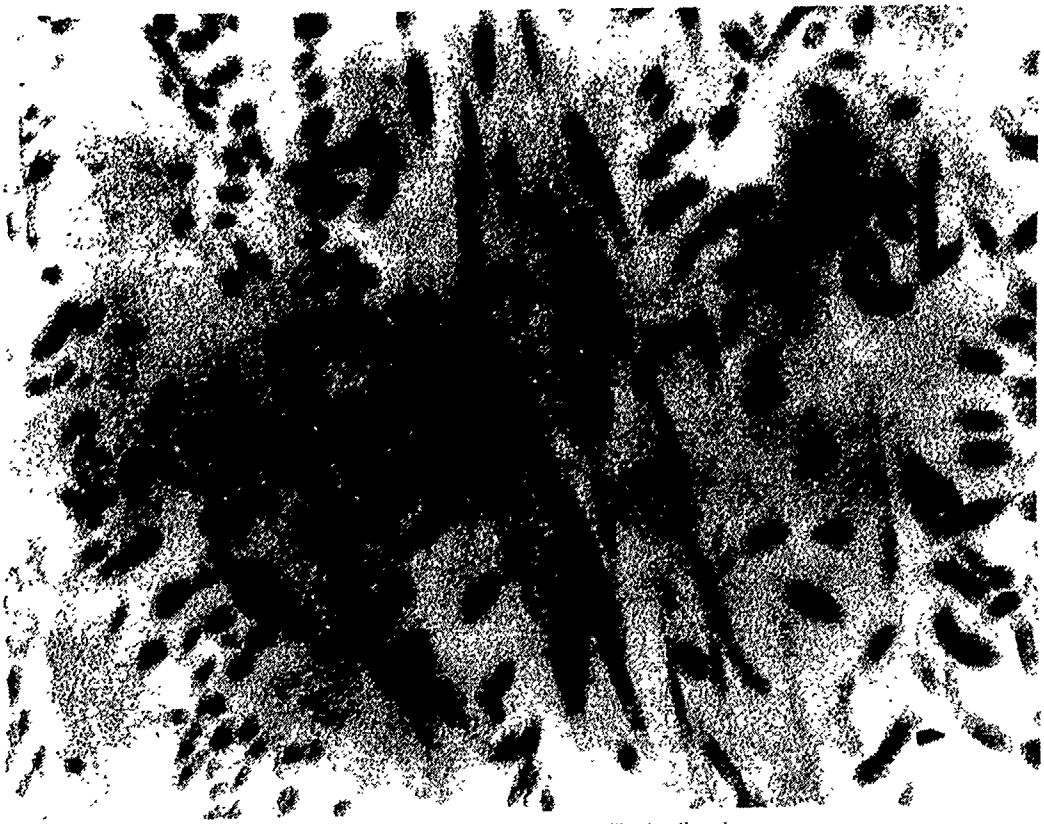

Fig. 1. Parietal pleural plaque—fibrous zone—collagen fibres in the fibrohyaline tissue.

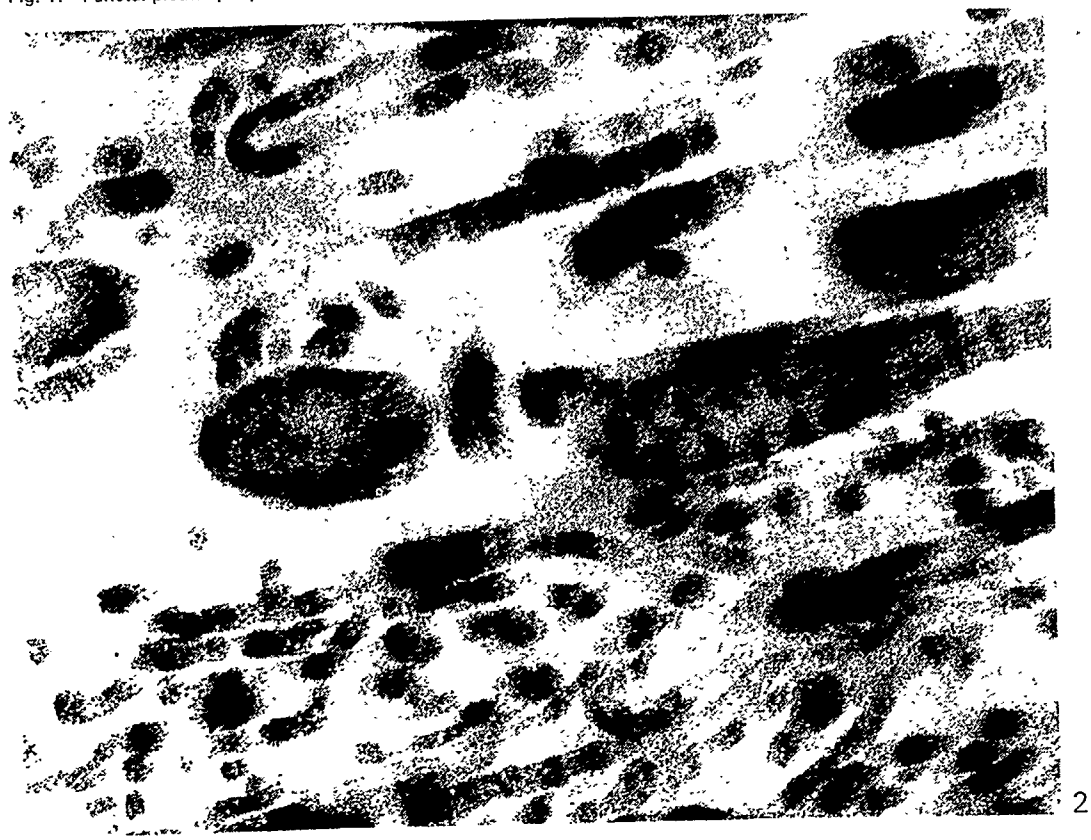

Fig. 2. Parietal pleural plaque—fibrous zone—collagen fibres with large diameter.

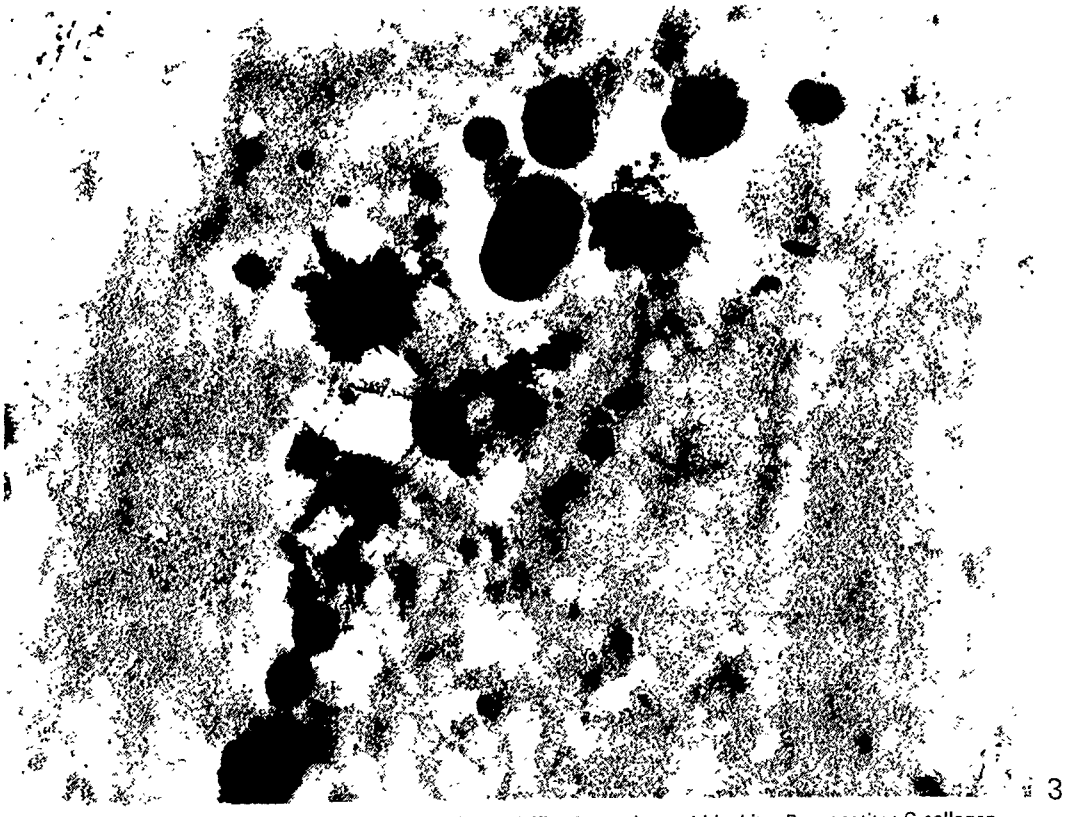

Fig. 3. Parietal pleural plaque—fibrous zone—incipient calcifications—A — whitlockite; B — apatite; C collagen

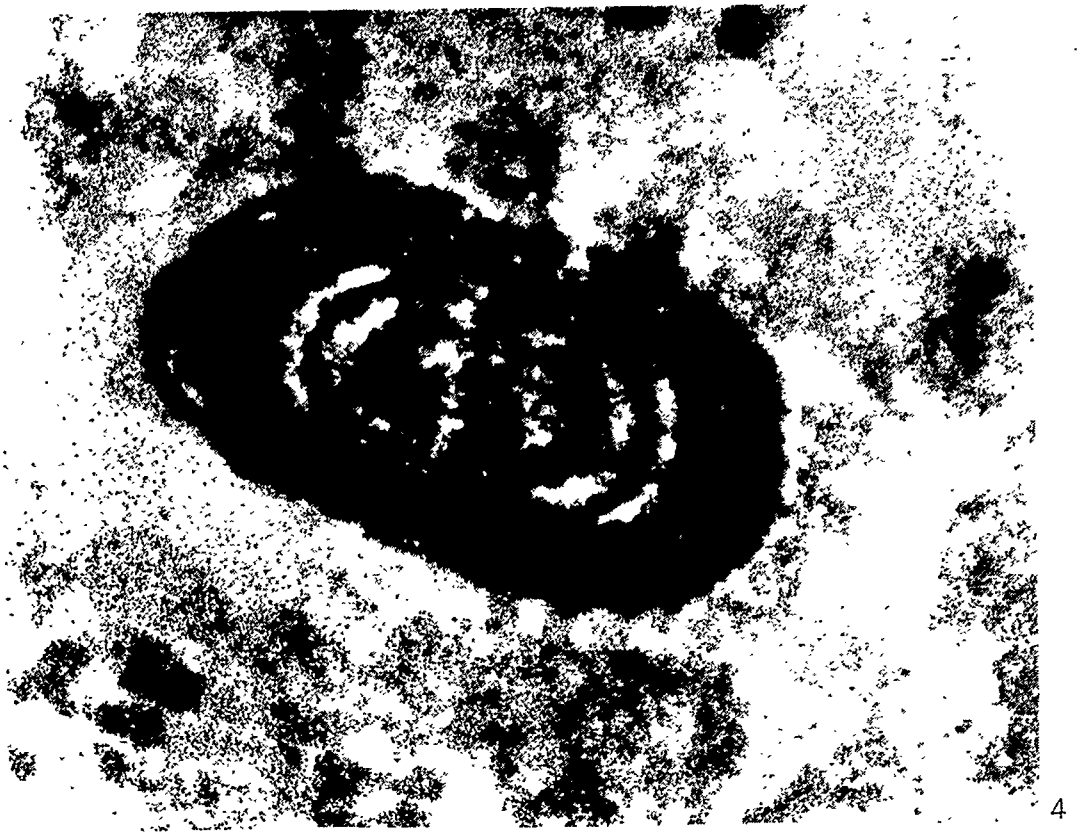

Fig. 4 Parietal pleural plaque—fibrous zone—lamellated structures.

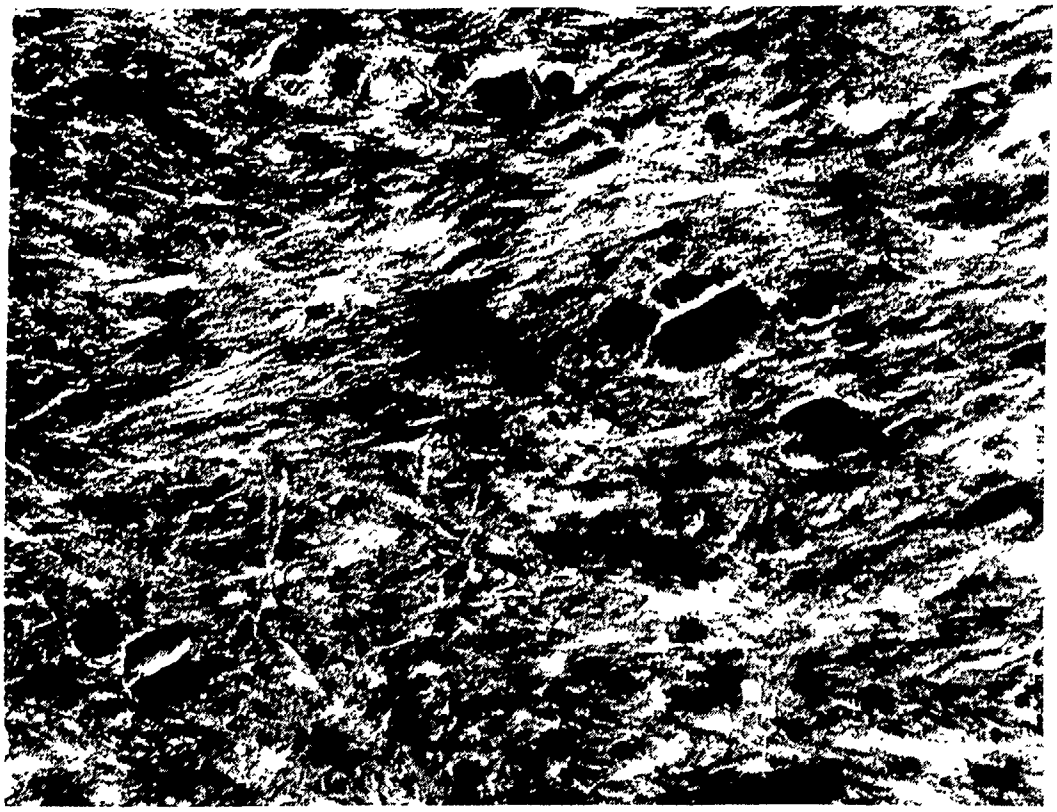

Fig. 5. Parietal pleural plaque—calcified zone—massive calcifications—A -whitlockite; B--apatite; C -periodicity of collagen.

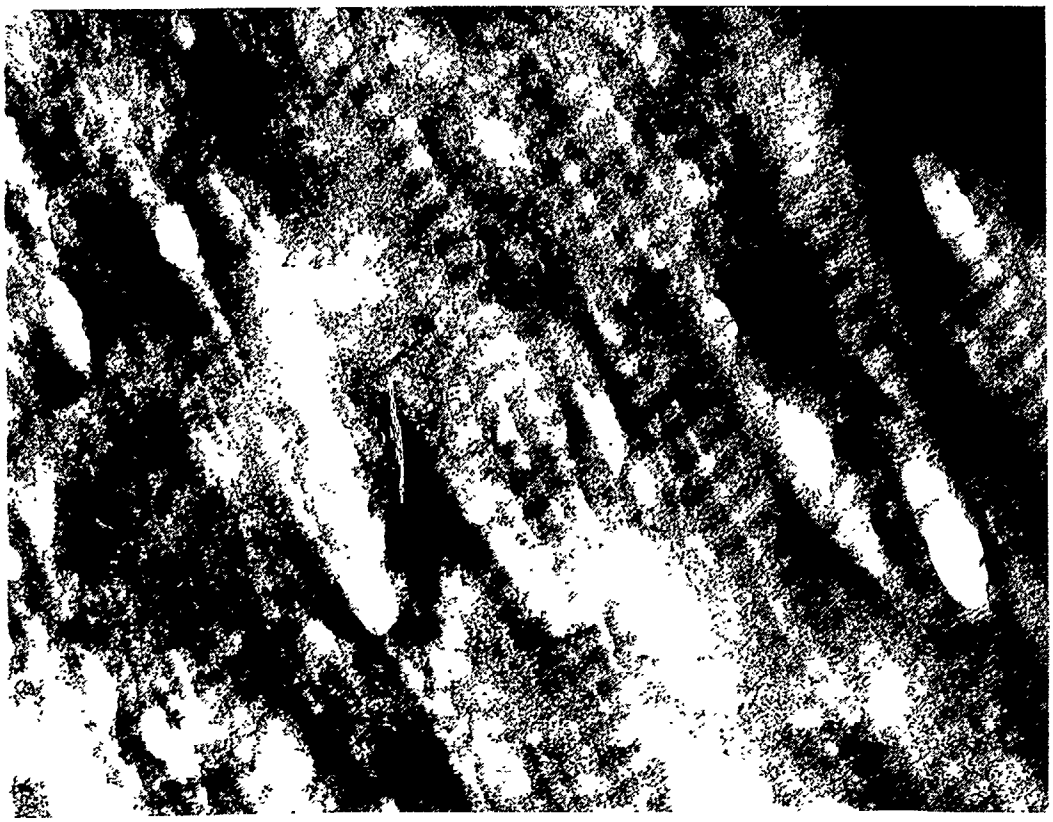

Fig. 6. Parietal pleural plaque—calcified zone—collagen fibres after decalcification by phosphotungstic acid.

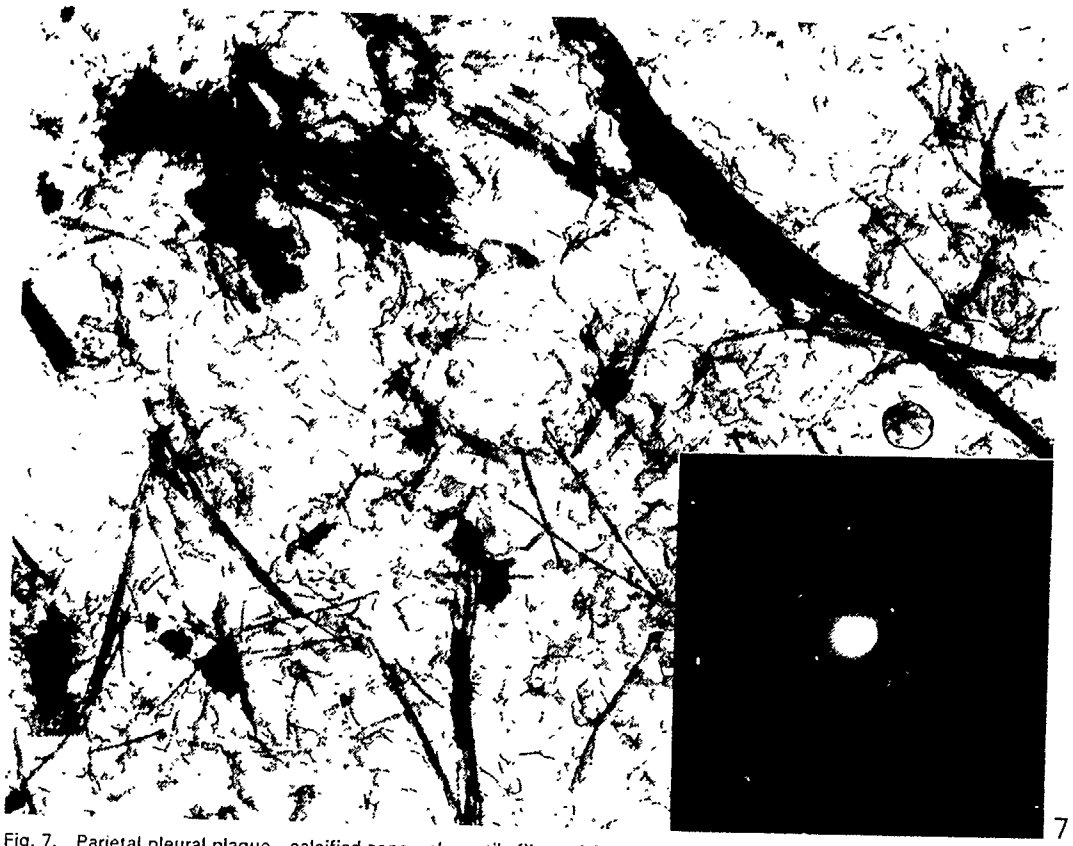

Fig. 7. Parietal pleural plaque—calcified zone—chrysotile fibres visible after incineration and washing with acid.

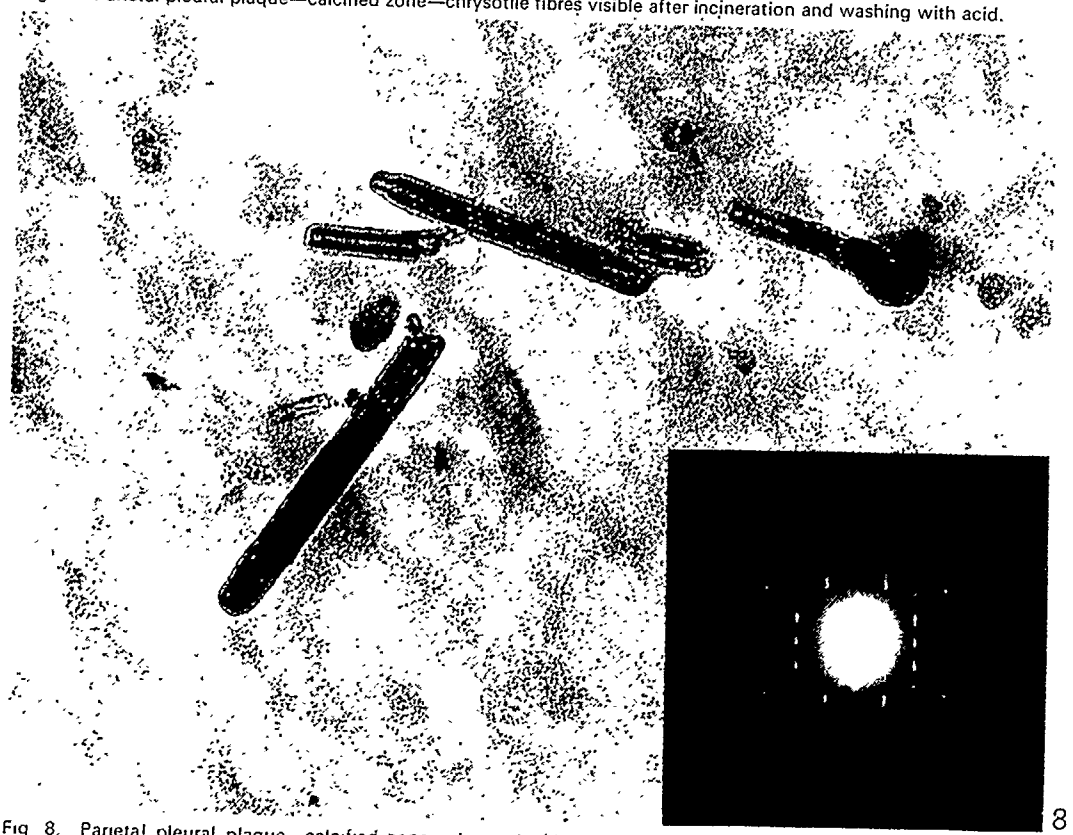

Fig. 8. Parietal pleural plaque—calcified zone—chrysotile fibres visible after treatment with phosphotungstic acid and trypsin.

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studied the fibres were those of chrysotile. In the sections treated with phosphotungstic acid and trypsin the fibres are surrounded by a thin sheath of an unidentified substance.

The results given in Table 4 show that pleural plaques found in persons who have been exposed to asbestos dusts may contain considerable quantities of very fine fibres whose presence can be demonstrated only after calcium deposits and collagen fibres have been eliminated.

Table 4. Results of asbestos fibre counts

Nature of specimen	No of fibres per cm^3 of tissue	
	Case No 1	Case No 2
Lung tissue	10×10^6	1.6×10^6
Pleural plaque		
fibrous zone	6×10^6	3.6×10^6
calcified zone	30×10^6	40×10^6

Diameter of fibres : 25–30 nm
Length of fibres : 0.1–2 μm

DISCUSSION

The organisation of pleural plaques as seen in the light microscope has already been described and calls for no particular comment. On the other hand, infrastructural and biochemical study and electron diffraction techniques have thrown light on a certain number of points:

Calcifications

Microscopic nodular or acicular calcareous structures can be seen scattered throughout the fibrous part of calcified plaques as well as in fibrohyaline plaques. These structures are similar to those which form large areas of mineralisation in the calcified zones, thus indicating that these different tissues are related and essentially differ only in their degree of calcification.

Nascent calcifications are encountered in the fibrous zone mainly in the sectors which contain cell debris, or within the hyaline substance. In the calcified zone, the growth of the crystals is orientated in part by the collagen fibres.

The calcifications are essentially of the whitlockite and apatite type. Significant differences are found, however, in the phosphorus and calcium composition of these minerals, so that a more thorough study would appear to be necessary to clarify this point.

Organic matter

This consists essentially of fibres and of amorphous matter. The collagen fibres are of normal periodicity with a small proportion of wide diameter fibres.

Ultrastructurally, the amorphous matrix shows a microfilamentous organisation similar to that described for the ground substance of collagen tissue. Furthermore, biochemical analysis demonstrates the presence of hexosamines in greater quantities in the calcified zone than in the fibrous zone; and this is confirmed more specifically for the acidic mucopolysaccharides by histochemical methods.

Asbestos fibres

The presence of numerous asbestos fibres in the pleural plaques of persons exposed to asbestos dust constitutes an important finding. In the cases examined the fibres were all of very small size and hence completely invisible under the light microscope; moreover, their detection by electron microscopy requires a suitable technique. It should be noted that they are present in a much higher concentration in the calcified zone than in the fibrous zone.

Histogenesis of the pleural plaques

Data on this are still very incomplete and thus we are unable to sketch the probable process for the formation of pleural plaques.

It may, however, be noted in regard to the conditions under which fibrohyaline plaques appear that they are not the specific result of exposure to asbestos and that they can be observed in other circumstances.

On the other hand, it is interesting to note that the only pleural plaques showing massive calcifications contained large amounts of asbestos fibre, and that the calcified zone contained more abundant fibres than did the surrounding fibrous zones.

At a more basic level, the initiation of these calcifications and their shape seem to be dependent on the substrate on which their nucleus is formed.

To sum up, although the appearance of fibrohyaline tissues and of calcifications are both relatively common phenomena in general pathology, it may be assumed that the occurrence of these two processes together in this particular site suggests a definite relationship with the risk of asbestosis.

SUMMARY

A study has been made of the ultrastructure of fibrohyaline tissue and calcification in pleural plaques. The microscopic calcifications present in the fibrous zone of these plaques are comparable to those in the calcified zone which have the structure of whitlockite and apatite; however, their P:Ca ratio is different from that of those phosphates, particularly in the highly calcified zone. The level of hexosamines, and particularly of acidic mucopolysaccharides, is higher in the calcified zone than in the fibrous zone. Calcified pleural plaques from persons exposed to asbestos dust contain numerous fibres of that mineral.

ACKNOWLEDGEMENTS

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REFERENCE

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