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# A histochemical study of the asbestos body coating

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**Governa, M., and Rosanda, C. (1972).** *Brit. J. industr. Med.*, **29**, 154-159. **A histochemical study of the asbestos body coating.** Asbestos bodies after iron extraction were tested by histochemical methods for mucopolysaccharides. The results of the reactions suggest that acid mucopolysaccharides are present in the coating of most bodies. During the formation of the bodies acid mucopolysaccharides might act as a matrix for iron deposition on the coating.

Asbestos bodies are formed in the lung of man and certain other animals when asbestos fibres are inhaled. They consist of asbestos fibres coated with a thick layer of hyaline material.

According to some authors (McDonald, 1927; Gardner and Cummings, 1931; Beintker, 1931; Timmermans, 1931; Koppenhöfer, 1935), this coating might include silicates or silicic acid. Recently, Rath (1964) has even suggested that these bodies are formed by the diffusion of dissolved substances from the ends of the chrysotile fibre tube. It is, however, now generally accepted (Suzuki and Churg, 1969) that the coating consists of protein, iron, and probably other material. There is some evidence for this (Beger, 1933; Sundius and Bygdén, 1937; Beattie, 1961; Davis, 1964a, b; Blount, Holt, and Leach, 1966), but the exact chemical nature of the asbestos body capsule is still the subject of study.

Recently, Davis (1970) published the results of an electron microscopic study of asbestos dust, suggesting its role as a nucleation centre in the calcification of old fibrous tissue lesions. The first stage of the calcification process involves the coating of the fibre with a thick layer of material that appears to be acid mucopolysaccharide; in a second stage, calcium in the form of apatite crystals is deposited.

This finding may indicate that acid mucopoly-

saccharides are involved in the initial stages of the production of asbestos bodies. Colloidal iron is known (Davis, 1970) to have an affinity for these polysaccharides. It would appear that asbestos fibres are first coated with acid mucopolysaccharides, and the latter then become impregnated with colloidal iron.

To verify this possibility we supposed that the reactivity of acid mucopolysaccharides—if they are present in the coating—might be masked by the presence of iron in the coating itself. We therefore treated the asbestos bodies in order to extract iron from them.

## Materials and methods

Specimens of lung from seven human cases of asbestosis which had been fixed in formol-saline were studied. They were processed through ethanol and benzene and embedded in paraffin. Representative serial 12  $\mu$ m sections were cut from each block and parts were processed for iron extraction from asbestos bodies.

Extraction of iron was carried out by means of a bath in 5% oxalic acid for about 14 hours. Five minutes' exposure to 1% sodium dithionite in 0.1 M acetate buffer, pH 4.5 (Lillie, 1965), also gave good results. More than 90% of the asbestos bodies showed a negative Prussian blue reaction with either of the two processes.

Sections processed with oxalic acid or with sodium dithionite, as well as non-processed sections, were stained with Perl's and Turnbull's stains, periodic acid-Schiff (PAS) reaction, and by toluidine blue at pH 3, 2.5, and 2, according to standard methods.

Sections processed with oxalic acid or sodium dithionite were also stained with toluidine blue after treatment with testicular hyaluronidase (Jalovis, 15 units/ml, in Na-K phosphates buffer 0.1 M at pH 6.0 for 48 hours at 37°C).

Toluidine blue was also used on sections processed through oxalic acid or sodium dithionite and then submitted to methylation (0.1 N hydrochloric acid in absolute methanol for 2 hours at 60°C) followed or not by saponification (1% potassium hydroxide in 70% ethanol for 20 minutes at room temperature).

Other sections treated with oxalic acid were stained with 0.2% azur A, pH 0.5 to 5, and with fresh 1% alcian blue 8GX at pH 1.0 and 2.5; sections were also treated with fresh solutions of 0.05% alcian blue 8GX in 3% acetic acid, pH 2.4, adding respectively, 0.1 M, 0.2 M, 0.5 M, 0.6 M, 0.8 M, and 1 M MgCl<sub>2</sub>.

Also, isolated asbestos bodies were obtained from parts of the same lungs which had been embedded in paraffin. To obtain these, lung specimens fixed in formol-saline were digested with 5% sodium hypochlorite (Gross, de Treville, Cralley, and Davis, 1968). Smears of sediments derived from pulmonary digestion were post-fixed with absolute ethanol. The processes and stainings which have been described as applied to microscopical sections were also carried out on these smears.

Moreover, to assess the validity of the histochemical reactions of mucopolysaccharides which had been used, we stained with PAS, toluidine, and alcian blue some microscopical sections of experimental carrageenin granulomas and asbestotic pulmonary fibrosis containing mucopolysaccharides; part of these sections had been processed through an overnight bath in 5% oxalic acid or given 5 minutes' exposure to 1% sodium dithionite.

Microscopical sections and spread preparations containing asbestos bodies were also processed through oxalic acid for 2, 6, 72, and 90 hours, and other samples were processed through sodium dithionite for 20 and 40 minutes. Parts were stained with Perl's method and parts with toluidine blue.

## Results

### Asbestos bodies not processed for iron extraction

These were Perl's positive, unstained with Turnbull and PAS. After treatment with toluidine blue some bodies were unstained while others were blue (Fig. 1); no body was metachromatic. The same results were obtained with both histological sections and smears.

### Asbestos bodies processed for iron extraction

These were Perl's, Turnbull, and PAS negative. About one-third of asbestos bodies pretreated with oxalic acid stained weakly with alcian blue at pH 1.0 as well as with azur A at pH 0.5 and 1.0. About two-thirds stained with alcian blue at pH 2.5.

About 30% of the bodies treated with toluidine

blue at pH 2.0-3.0 appeared to be unstained, while 70% showed a metachromatic staining of reddish-violet colour (Fig. 2).

About two-thirds of the metachromatic asbestos bodies stained weakly; one-third was more intensely stained.

The coating of some bodies was uniformly stained, while in others the club-like protuberances at their ends stained more than the central part, or vice versa. The metachromasia was readily removed by methylation and could be restored by saponification (Fig. 3).

After treatment with testicular hyaluronidase, many bodies retained their coating, although they lost the ability to stain with toluidine blue. In these samples there were also many fibres apparently without a coating.

About four-fifths of the asbestos bodies pretreated with oxalic acid were positive to alcian blue staining at concentrations of 0.1 M, 0.2 M, and 0.5 M MgCl<sub>2</sub>, while they were negative at concentrations of 0.6 M and 0.8 M. Some bodies stained weakly at a concentration of 1 M MgCl<sub>2</sub>.

The same results were obtained with both histological sections and smears.

## Discussion

Before attempting to interpret our findings we have to consider the techniques used in order to determine whether the stainings, seen under the microscope, were the result of histochemical reactions or whether they could be due to artefacts.

The asbestos bodies studied came either from conventional sections of formol-fixed human lung tissue or from spread preparations of the sediment after digestion of the same lung tissue with sodium hypochlorite solutions. The latter preparations had been subjected to the oxidizing action of hypochlorite and it might be expected that some oxidation of the components, or perhaps even a single component, of the coating of the asbestos body had taken place.

It is possible that hypochlorite treatment alters the histochemical staining reactivity of the coating of asbestos bodies.

A direct control could not be obtained as it was not possible to get spread preparations of asbestos bodies from the lung without hypochlorite treatment, and, on the other hand, treatment of the tissue section with hypochlorite would, of course, dissolve it. However, it is unlikely that hypochlorite treatment influenced our findings as the results obtained in the histological sections untreated with hypochlorite were the same as those obtained in the spread preparations from lung treated with hypochlorite.

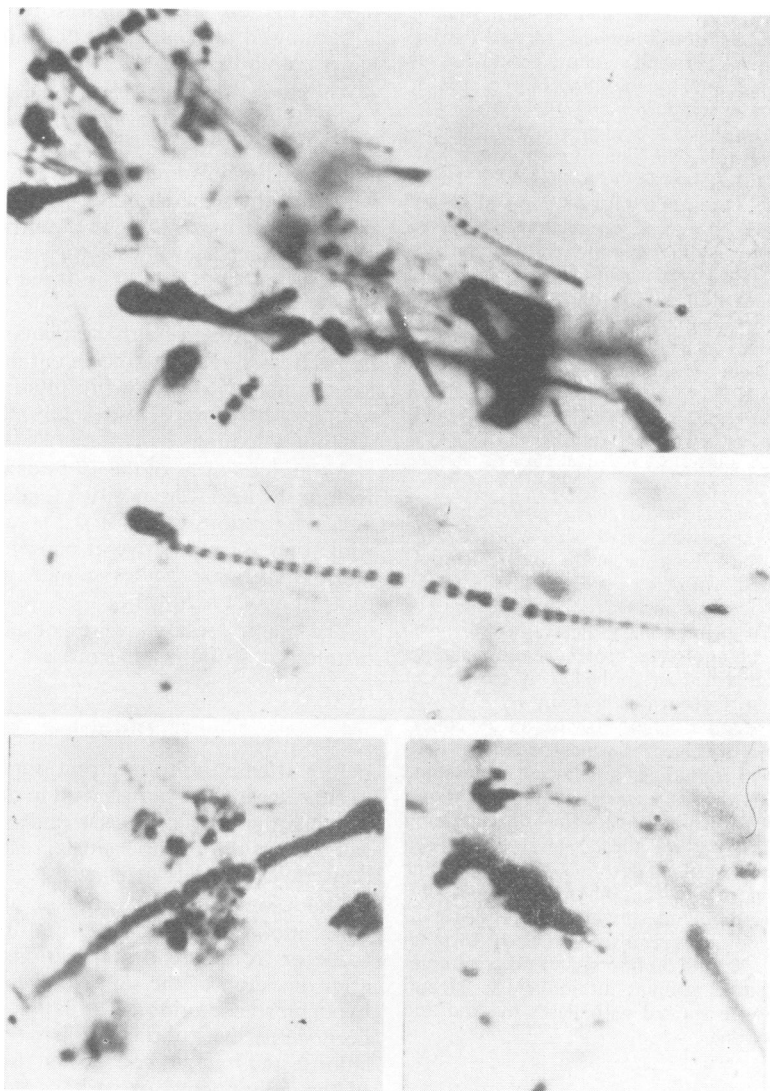


FIG. 1. Asbestos bodies. Samples *untreated* with iron extraction solutions. Toluidine blue at pH 3. All these bodies showed an orthochromatic staining of intense blue colour. Original magnification  $\times 1\ 250$ .

Parts of the asbestos bodies treated and untreated with hypochlorite were processed through oxalic acid or dithionite to remove iron from their coating. After this process they were Perl's negative.

Some histochemical methods for mucopolysaccharides were applied to Perl's negative asbestos bodies as well as to Perl's positive asbestos bodies.

Asbestos bodies from which the iron content had not been removed showed no positive reactions; whereas the majority of the asbestos bodies from

which the iron content had been removed appeared positive to alcian blue at pH 2.5; moreover, they showed a metachromatic reaction to toluidine blue at pH 2.0-3.0. About one-third of the bodies were also weakly positive to alcian blue at pH 1.0 and to azur A at pH 0.5 and 1.0.

Control studies with sections of carrageenin granulomas and pulmonary fibrosis containing acid mucopolysaccharides were carried out. Positive staining was observed both in the sections not treated

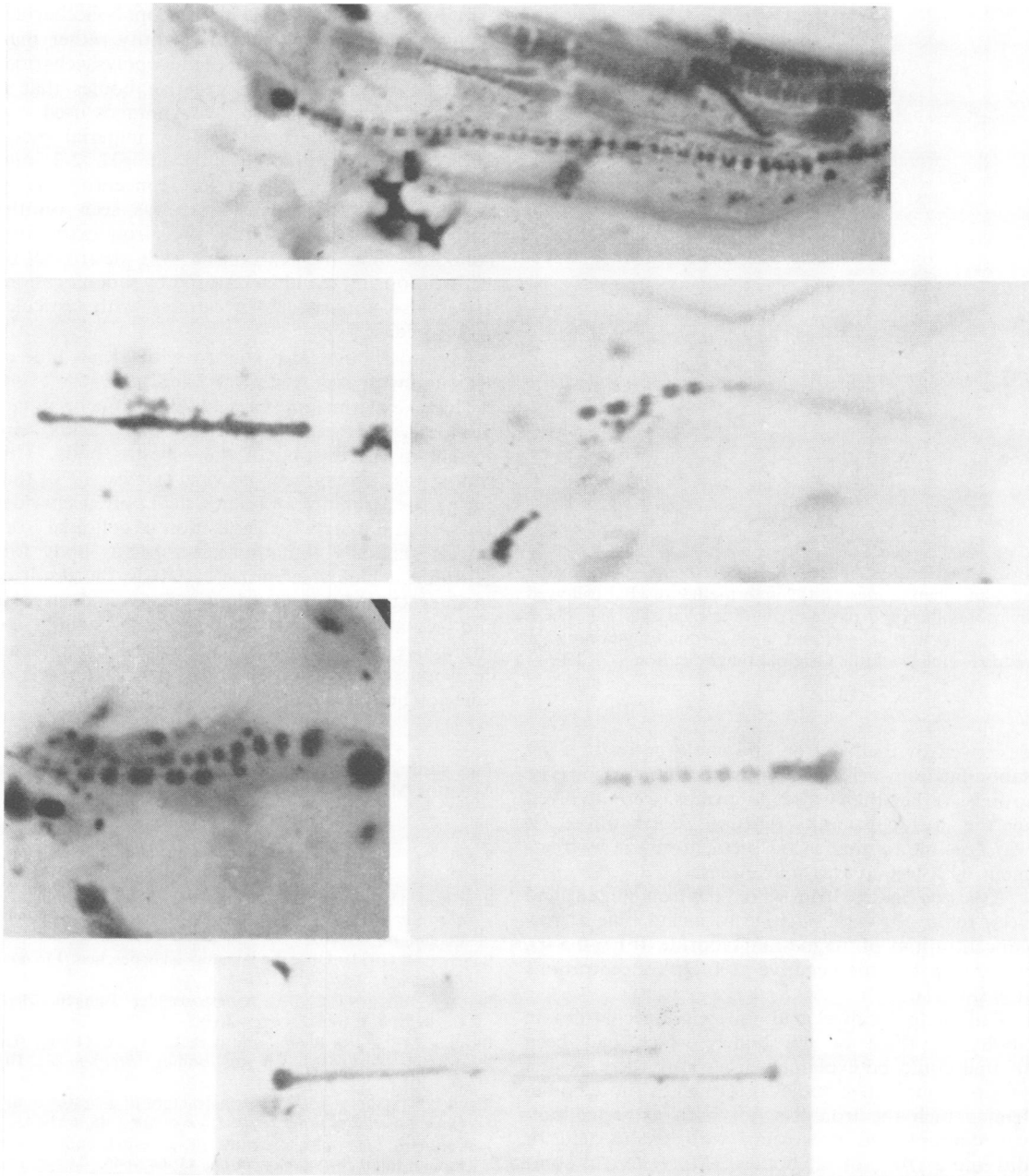


FIG. 2. Asbestos bodies. Samples *treated* with oxalic acid. Toluidine blue at pH 3. All these bodies showed a metachromatic staining of reddish violet colour; some bodies were weakly and others more intensely stained. Original magnification  $\times 1\ 250$ .

and in those treated with oxalic acid or dithionite. These solutions do not seem to affect the staining properties of the connectival mucopolysaccharides tested with PAS, toluidine blue, azur A, and alcian blue.

The toluidine blue metachromasia which appeared on the coating of asbestos bodies after iron extraction seems to be due to the presence of acid mucopolysaccharides, and particularly of hyaluronic acids. In fact the metachromasia was readily

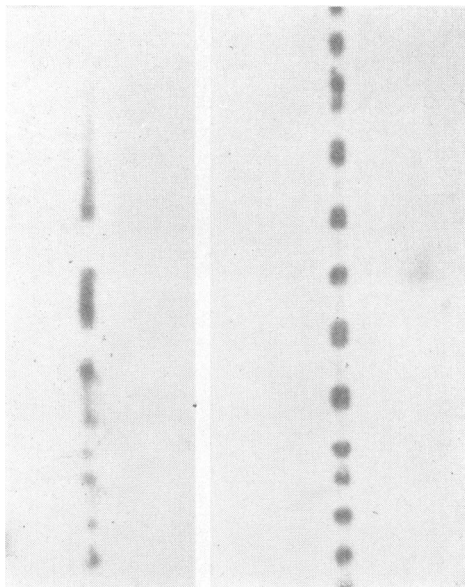


FIG. 3. Asbestos bodies. Samples treated with oxalic acid and then processed through methanol-HCl followed by potassium hydroxide. Toluidine blue at pH 3. These bodies showed a restored metachromatic staining of reddish violet colour. Original magnification  $\times 1\ 250$ .

destroyed by methylation and could be restored by saponification. This suggests that carboxylic groups, rather than sulphate groups, were involved in the metachromatic reaction. Furthermore, it was possible to abolish the metachromasia by treatment with testicular hyaluronidase.

Asbestos bodies, from which the iron content had been removed, were positive to alcian blue at low concentrations of magnesium chloride (0.1 M-0.5 M), while they became negative at high concentrations (0.6 M-0.8 M).

Failure to localize acid mucopolysaccharides in about one-third of the bodies which had been treated could be explained in various ways. A first group of unreactive bodies is formed by those bodies from which the iron has not been extracted notwithstanding the processing with oxalic acid or dithionite (10% of all bodies). Moreover, in some asbestos bodies the hyaluronic acid component of the acid mucopolysaccharides might not be polymerized sufficiently to be metachromatic.

On the other hand, the solutions of oxalic acid and dithionite used did not seem to affect the extent of polymerization of the hyaluronic acids. This view is supported by the results obtained with preparations treated with these solutions for various lengths of time. The percentage of positive bodies did not increase with increasing duration of treatment.

Failure to localize acid mucopolysaccharides might be due to variation in quantity rather than polymerization. The amount of mucopolysaccharides might be so small in the negative bodies that it could not be detected with the techniques used.

Summing up our main finding, material metachromatic with toluidine blue at pH 2-3 and stained with alcian blue at low concentrations of  $MgCl_2$ , but not at high ones, was seen on the majority of asbestos bodies after iron extraction. Toluidine blue metachromasia was destroyed by methylation and could be restored by saponification; it was also abolished by treatment with testicular hyaluronidase.

Asbestos bodies also stained with alcian blue at pH 1 and with azur A at pH 0.5 and 1.0.

These experiments suggest that mucopolysaccharides, and especially hyaluronic acids, are present in the coating of the asbestos body. This finding may strengthen the view (Davis, 1970) that during the formation of bodies, acid polysaccharides may act as a matrix for deposition of colloidal iron.

It is generally thought that asbestos body formation is a biological process, that is, the result of an interaction between asbestos fibres and alveolar and septal cells. Our findings may suggest an alternative possibility, that intercellular components (acid mucopolysaccharides) may play a key role in asbestos body formation.

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