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THE MECHANISM OF PRODUCTION OF ASBESTOS BODIES FROM ANTHOPHYLLITE FIBRES

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PLATES CCXVII–CCXXIII

ASBESTOS bodies were first observed by Fahr and Feigel (1914) in the lungs of a worker who had inhaled asbestos. They called them "crystals" and believed that the core was asbestos and that the coating was derived from haemoglobin. Beger (1933) described the structures as being golden yellow, sometimes having the shape of a dumb-bell but more often in the form of a string of irregular discs, the "string of beads" form. At the ends there are often club-like protuberances. A fibre or modified fibre may sometimes be seen running down the middle of the structure. The coating is a gel.

Since Fahr and Feigel published their description, there have been many suggestions as to the chemical nature and origin of asbestos bodies. Sundius and Bygdén (1937–38) gave a bibliography in which the views of various workers were summarised. Typical of the view that the coating of the asbestos body is derived from blood or tissue fluid was that of Cooke (1929), who believed that inhaled asbestos fibres act on the bronchioles and alveoli mechanically, producing small effusions of blood and serum from which protein is adsorbed on to the fibres. Lynch and Smith (1930) and Gloyne (1932) also believed that asbestos bodies are derived from blood. Simson (1928) thought that the coating was deposited by phagocytic cells. An opposite view was held by, for example, McDonald (1927), Gardner and Cummings (1931), Beintker (1931), Timmermans (1931) and Koppenhöfer (1935), who implied that asbestos bodies are asbestos fibres coated with silicates or silicic acid gel, and Beger who thought that the coating consisted of iron oxide and albumin.

Recently Rath (1964) has suggested that asbestos bodies are formed by the diffusion of dissolved substances from the ends of the chrysotile fibre tube. As chrysotile is the only variety of asbestos that has this unique tubular lattice, the explanation could not apply to asbestos bodies formed from amosite, crocidolite or anthophyllite. Beattie (1961) using paper chromatography demonstrated proline and hydroxyproline in a hydrolysate of an asbestos body and suggested that the capsule might be collagen. The amino acid composition of the protein of asbestos bodies is markedly different from that of collagen (Blount, Holt and Leach, 1966), but the hydroxyproline value suggests that 10 per cent. of the total protein could be collagen. Davis (1964) published electron micrographs of sections of asbestos bodies that appeared to show that much of the asbestos body coating consisted of the iron-protein complex, ferritin.

A few weeks after guinea-pigs have inhaled asbestos, the lungs contain many asbestos bodies and also many apparently unchanged fibres. On a first examination, there appear to be no structures representing the intermediate stages in the formation of asbestos bodies. It seemed possible that the transition from fibre to asbestos body might be very rapid, so that in a section of lung in which fibres and asbestos bodies were abundant there might still be few structures in the transition state. On this assumption we examined some hundreds of asbestos fibres in histological sections prepared from the lungs of guinea-pigs that had inhaled asbestos dust, and photographed the few that appeared to show an intermediate stage in the

formation of asbestos bodies. From these the sequence of events in the formation of asbestos bodies was deduced. The sections examined were from animals on which a pathological report has been published (Holt, Mills and Young, 1966) and details of the dusting apparatus used and exposure times are to be found in that report. Anthophyllite was seen to produce the largest and most characteristic asbestos bodies.

We found it preferable to use mainly phase-contrast microscopy for the examination of asbestos bodies, and to examine unstained sections or sections stained by the periodic acid-Schiff method or with Perls' stain only. Phase-contrast microscopy revealed more fibres; many fibres that would have been invisible if viewed in a stained section by direct microscopy were clearly visible by phase contrast.

Dark-ground illumination reveals a large number of asbestos fibres grouped mainly around air vessels. The shorter fibres, up to about 5μ long, are almost all intracellular (fig. 1). Sometimes a much longer fibre—even a fibre 100μ long—appears to be completely enveloped in a cell. Long fibres are sometimes coated, sometimes apparently unchanged. In some cases the coating is so thin as to be invisible unstained, but it can be stained for iron by Perls' method. A few very long fibres, some even longer than 100 μ, are embedded in tissue (fig. 2). In most cases only when a cell has made contact with the end of a fibre does the fibre penetrate the cell wall; cells adjacent to the middle of a long fibre do not appear to be enveloping it (fig. 3). There are many types of coated fibres in the lungs of these guinea-pigs.

The probable sequence in the formation of these structures can be deduced from the few fibres that appear to be in the transition state. Normally the macrophage is continually producing and withdrawing processes; these processes have been seen with the electron miscroscope in sections of rapidly frozen lung tissue (Davis) and in cell cultures (fig. 4); in tissues killed and fixed by the usual technique the processes are withdrawn and the cells rounded.

When a process of a macrophage makes contact with the end of a fibre, it normally flows along the fibre and, if the fibre is short, the process will engulf it. When the process is withdrawn, the fibre may be taken back into the main body of the cell. If a fibre is too long to be drawn into the cell the process may be withdrawn from it and may then leave behind a small blob of cytoplasm (figs. 5 and 6), which becomes a small sphere on the fibre (fig. 7).

The probability that a long fibre and its attached cell should be entirely in the plane of the microtome knife is remote, but one such structure is shown in fig. 8; the tissue was probably fixed shortly after the structure was formed. In fig. 9 there is a similar structure, but here the cell has disintegrated. The process may become detached from the cell after it has progressed along a considerable part of the fibre, and this leaves a fibre coated along all or part of its length. In fig. 10 a fibre is coated for part of its length and the coating is broken into a series of small tightly packed beads. Later the exterior coat may become smoothed and rounded off at both ends to give the structure shown in fig. 11. The greater part of the cytoplasm of a macrophage may flow around a fibre to which a larger volume of cytoplasm will remain attached when the cell dies. This is shown in fig. 12; the cell nucleus is seen close to the fibre, to which two patches of cytoplasm are attached.

A long fibre might be contained in a giant cell; giant cells are an early feature of lungs of guinea-pigs that have inhaled asbestos fibres (Holt, Mills and Young). If the macrophage has previously ingested very small asbestos fibres these may appear in the coating of the asbestos body; such inclusions have been described by Davis.

The material that is attached to the asbestos fibre will initially have the structure of a denucleated cell; it will be a colloidal solution containing protein enclosed in a cell membrane. Davis has shown that the asbestos body has a mass of iron-containing protein packed around a central asbestos fibre, and Blount, Holt and Leach that, *in vitro*, iron and protein are adsorbed on to asbestos to give a thick

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FIG. 1.—Macrophages containing dust. The shorter asbestos fibres are mostly intracellular. The end of a longer fibre is in contact with a macrophage. Phase contrast. ×720.

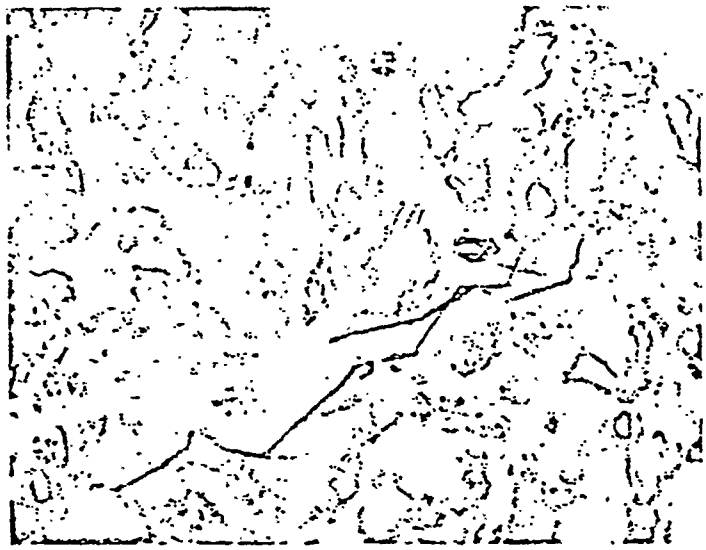

FIG. 2.—Long anthophyllite fibres apparently unchanged in inflammatory lung tissue Phase contrast. ×810.

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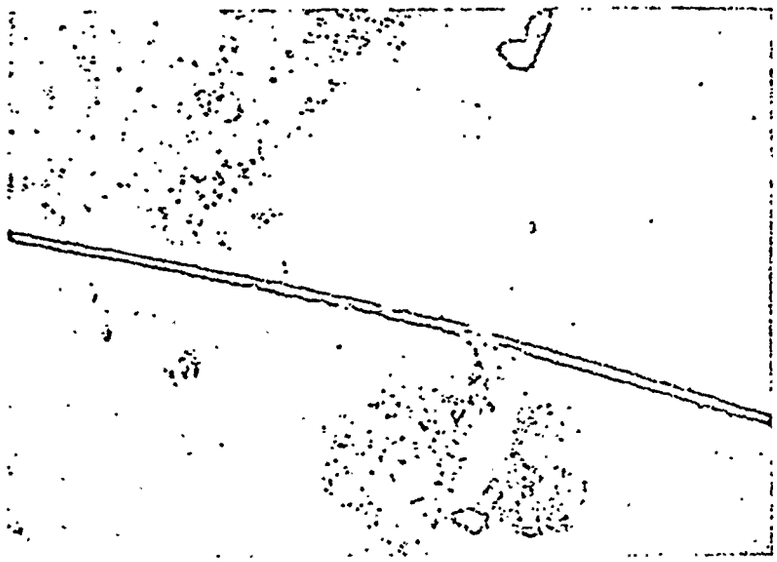

FIG. 3.—Long anthophyllite fibre in lung. Although macrophages are adjacent to the fibre they do not appear to be affecting it except possibly where the fibre is slightly frayed (lower macrophage). Phase contrast. × 675.

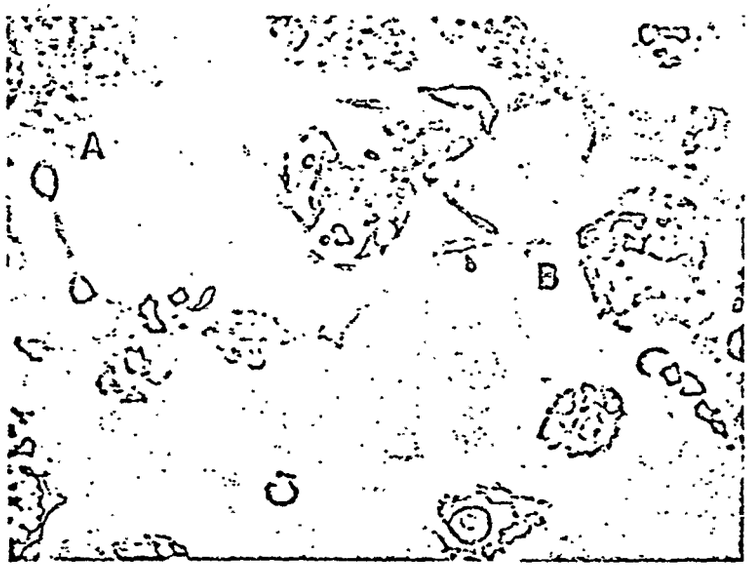

FIG. 4.—Alveolar macrophages of a guinea-pig, showing a long process (AB). Untreated normal cell culture. Phase contrast. ×900.

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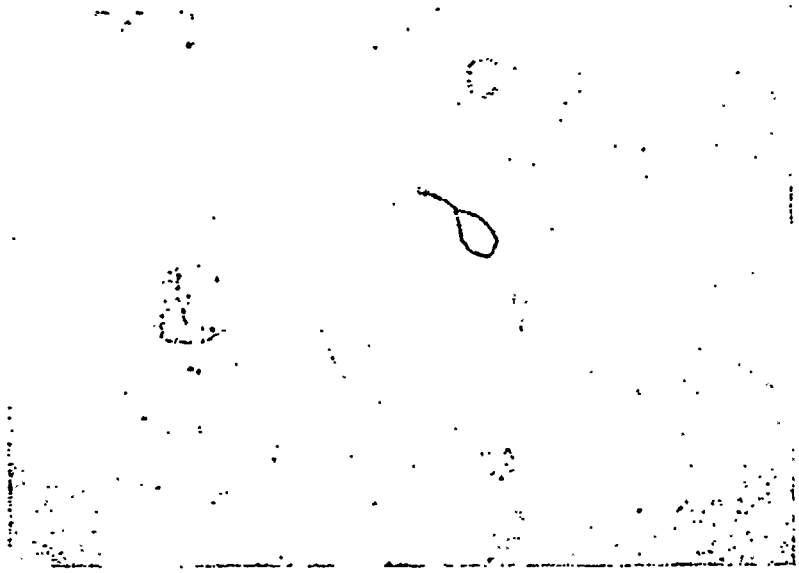

FIG. 5.—Asbestos fibre partly coated by a process. The process has been withdrawn but a blob of cytoplasm remains on the fibre. Phase contrast. 900.

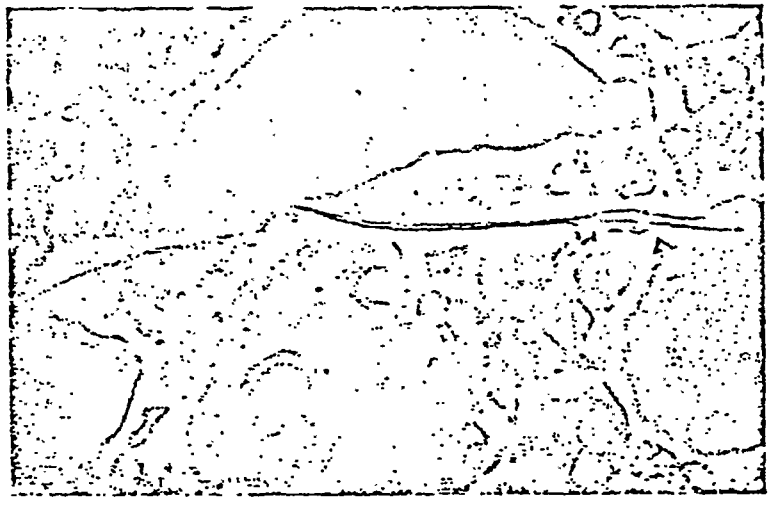

FIG. 6.—A longer fibre with an irregularly shaped blob of cytoplasm (arrowed) attached. Phase contrast. ×765.

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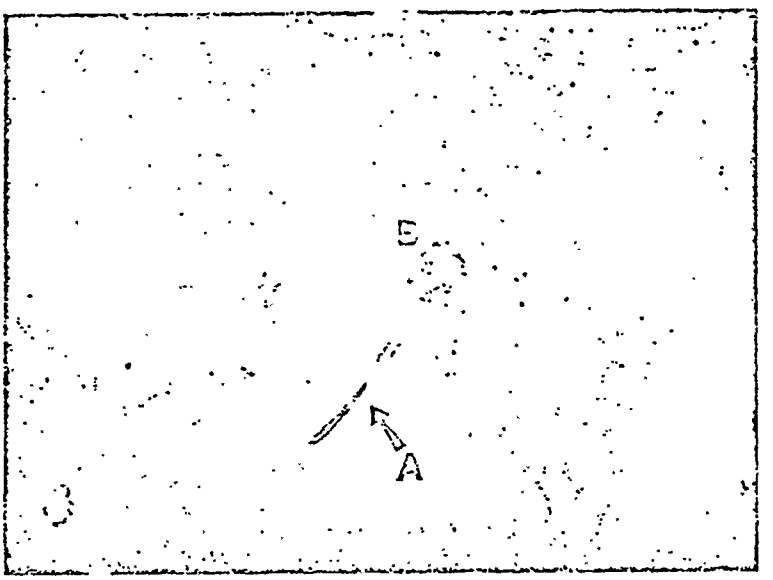

FIG. 7.—A small blob of cytoplasm (A) left on a fibre by the macrophage (B) has become spherical. Phase contrast. 765.

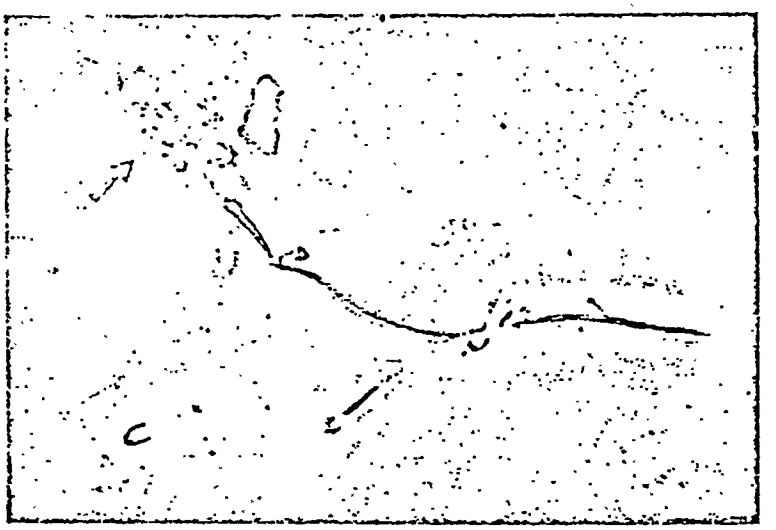

FIG. 8.—Macrophage attached to the end of a long anthophyllite fibre. A process from the cell (arrowed) has completely coated the fibre giving a rounded thickened distal end. Phase contrast. ×810.

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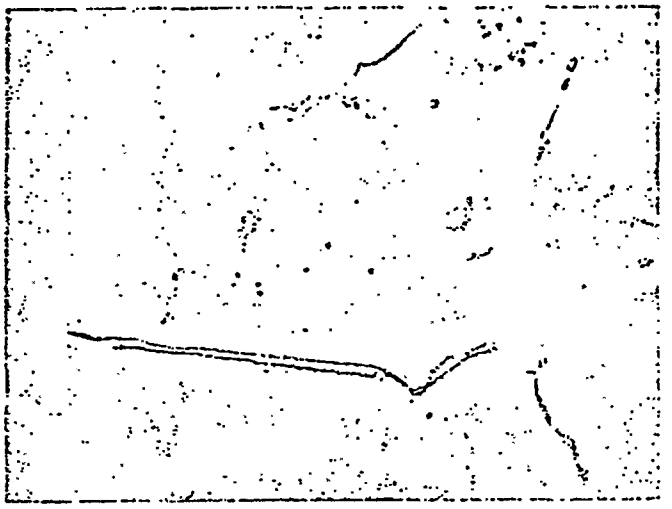

FIG. 9.—A similar fibre to that shown in fig. 8, but here the macrophage has disintegrated. Note the series of granules in the coating. Phase contrast. ×765.

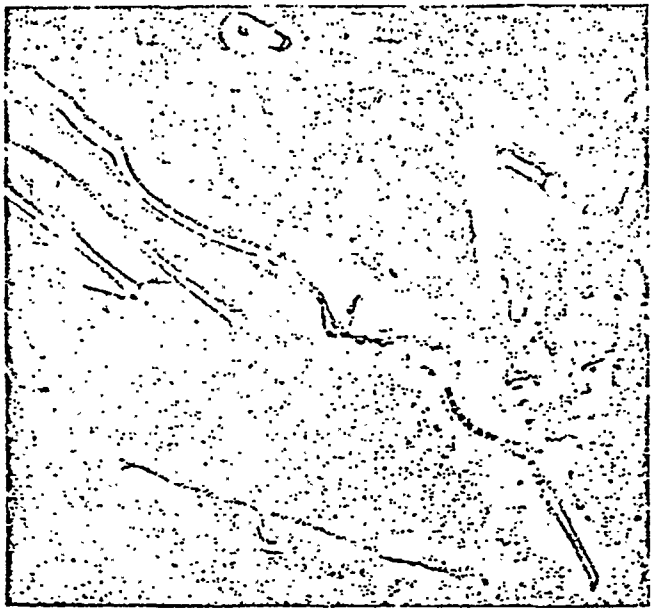

FIG. 10.—A long fibre is partly coated. The coating is closely beaded. Phase contrast. ×765.

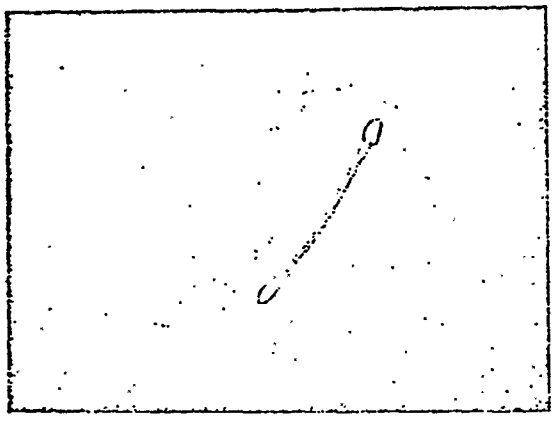

FIG. 11.—Anthophyllite asbestos body with thickened coating at the ends. Phase contrast. ×675.

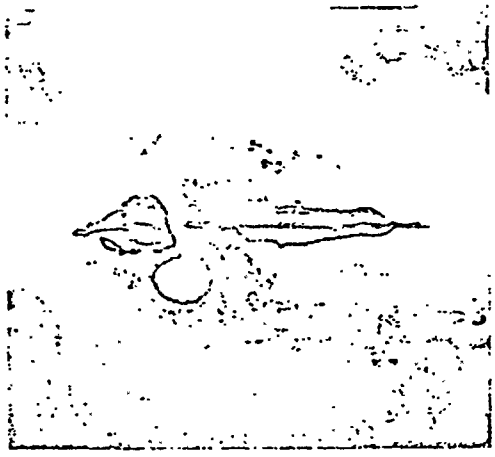

FIG. 12.—A large part of the cytoplasm of a macrophage is attached to an anthophyllite fibre. The nucleus of the disintegrated cell (arrowed) is close to the structure. The cytoplasm at one end is already assuming the pointed form characteristic of some asbestos bodies. Phase contrast. ×675.

FIG. 13.—"String of beads" type of asbestos body. Only part of the anthophyllite fibre, the end of which is indicated by an arrow, is coated. Phase contrast. × 675.

layer. The adsorption of protein from the cytoplasmic fluid will imply the orderly packing of the protein units around the fibre and expression of the water that hydrates the colloidal protein. This water will pass through the membrane; there will be an increase in density and decrease in volume of the contents of the bag of fluid. The shrinkage will produce a corrugation of the membrane. Types seen in these guinea-pigs included some with thickened ends (fig. 11) (referred to in earlier literature as the dumb-bell type), some with a sequence of spheres or flattened spheres (figs. 13 and 14), corresponding to the "string of beads" described in earlier literature, and some with deep corrugations (fig. 15). The structures usually appeared a brilliant iridescent orange colour when viewed by phase-contrast microscopy. Some large asbestos bodies were found to be built around a part of a long, very thin fibre. One such asbestos body is that shown in fig. 16 when a fibre about 80 μ long has an asbestos body 25 μ long with the fibre projecting from one end. The final form of the asbestos body will depend on factors such as the thickness of the fibre and the volume of fluid surrounding it. There are many unexplained features of asbestos bodies—for example, the nature of regularly spaced "granules" along some coated fibres. Moreover, it has not yet been determined at what stage the large amounts of iron enter into the composition of the coating. These features are being studied.

Summary

Histological studies of sections of guinea-pig lungs suggest that asbestos bodies are derived from the cytoplasm of macrophages that have attempted to ingest asbestos fibres. Proteins are probably adsorbed from the cytoplasm on to the asbestos and this leads to a shrinkage of the structure which produces corrugations or divides the structure into beads.

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REFERENCES

Beattie, J.	1961.	*In* Inhaled particles and vapours. ed. by C. N. Davies, *Oxford*, p. 434.
Beger, P. J.	1933.	*Arch. path. Anat.*, 290, 280.
Beintker, E.	1931.	*Arch. Gewerbepath.*, 2, 345.
Blount, M., Holt, P. F., and Leach, A.	1966.	*Biochem. J.*, 101, 204.
Cooke, W. E.	1929.	*Brit. Med. J.*, 2, 578.
Davis, J. M. G.	1964.	*Brit. J. Exp. Path.*, 45, 634.
Fahr, T., and Feigel, F.	1914.	*Dtsch. med. Wschr.*, 40, 1548.
Gardner, L. U., and Cummings, D. E.	1931.	*J. Industr. Hyg.*, 13, 65.
Gloyne, S. R.	1932.	*Lancet*, 1, 1351.
Holt, P. F., Mills, J., and Young, D. K.	1966.	This *Journal*, 92, 185.
Koppenhöfer, G. F.	1935.	*Arch. Gewerbepath. Gewerbehyg.*, 6, 38.
Lynch, K. M., and Smith, W. A.	1930.	*J. Amer. Med. Assoc.*, 95, 659.
McDonald, S.	1927.	*Brit. Med. J.*, 2, 1025.
Rath, R.	1964.	*Beitr. Silikose-Forsch.*, 81, 1.
Simson, F. W.	1928.	*Brit. Med. J.*, 1, 885.
Sundius, N., and Bygdén, A.	1937–38.	*Arch. Gewerbepath. Gewerbehyg.*, 8, 26.
Timmermans, F. D.	1931.	*Zbl. Gewerbehyg.*, 18, 280.

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