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Phagocytic Activity of the Alveolar Epithelial Cells in Pulmonary Asbestosis

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In the course of pulmonary asbestosis in hamsters, alveolar epithelial cells can transform into large phagocytic cells which ingest asbestos fibers and produce asbestos bodies. In the process, the epithelial cells frequently lose their original Type I or Type II cell structure and change into a form intermediate between the epithelial cell and the alveolar macrophage. This transformation may be the result of continuous stimulation of the cells by repeated phagocytosis of asbestos, followed by rapid death of the host cell, release of the fibers and rephagocytosis by a new cell. It is suggested that some of the large macrophages in the alveolar spaces originate from the alveolar epithelial cells via the intermediate form (Am J Pathol 69:373–388, 1972).

PARTICULATE MATTER of exogenous or endogenous origin is avidly phagocytosed by alveolar macrophages of both free and fixed types, and also by interstitial macrophages.* The phagocytic ability of the alveolar epithelial cells is less well known. The behavior of the alveolar macrophages in pulmonary asbestosis has been studied by us and by others.[1–6] We have also briefly reported on the phagocytosis

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* We use the term "alveolar macrophage" as defined by electron microscopy:[8,9,11,14,15] a macrophage lying in the alveolar space (free macrophage), or attached to an alveolar epithelial cell of Type I but not connected by junctional complexes (fixed macrophage). "Interstitial macrophage" is a similar phagocytic cell lying in the connective tissue of the alveolar septum.[16]

of asbestos fibers by the epithelial cells [5] and wish now to describe this process in detail and to consider its implications.

Materials and Methods

Light and electron microscopic observations were made in 24 male Syrian hamsters which were sacrificed 1, 2, 3, 4, 5, 6, 7, 12 and 16 days and 6, 12 and 24 months after intratracheal instillation of 1 mg of chrysotile asbestos suspended in 0.1 ml of normal saline. Three additional hamsters were used as controls. Single (1% OsO_4), double (2% glutaraldehyde followed by 1% OsO_4) and triple (2% glutaraldehyde, 2.5% potassium bichromate and 1% OsO_4) fixation were used for electron microscopy. Details of preparation technics and fixation were reported previously.[5]

For light microscopy, 4-μ sections from paraffin blocks were stained with hematoxylin and eosin, Mallory's trichrome, periodic acid-Schiff and Gomori's silver reticulum stain.

Results

The normal epithelial lining of hamster alveoli is quite similar in structure to that of other mammalian species.[8–13] The body of Type I cells (Figure 1) is small, but its extensions cover a large area with an extremely tenuous layer of cytoplasm constituting part of the "gas-exchange barrier." The cell organelles are rather poorly developed, and the mitochondria are much smaller than those of Type II cell. The lateral plasma membranes form "terminal bars" or tight junctions with those of adjacent cells. The free surface facing the alveolar lumen is devoid of microvilli. The nucleus often shows indentations. Attached to the attenuated stretches of the cytoplasm are fixed alveolar macrophages. The macrophage and the epithelial cell are separated by a narrow gap, only several hundred Ångstroms wide, but show no intercellular junctions.

The Type II cell is cuboid in shape and quite large (Figure 2). Short microvilli (less than 0.5 μ in length) usually arise from the free surface. Cell organelles such as mitochondria, the Golgi complex, endoplasmic reticulum, multivesicular bodies and free ribosomes are well developed. A characteristic feature of the cell is the osmiophilic lamellar bodies (Figure 2). These are considered by some to be the source of pulmonary surfactant,[17–21] though there is no general agreement on this point.[22] In tissue fixed in glutaraldehyde, microtubules can be demonstrated in both Type I and Type II cells.

As we have previously reported,[5,6] most of the asbestos fibers are phagocytosed by the free and fixed alveolar macrophages, interstitial macrophages and polymorphonuclear leukocytes. However, some of the fibers are also found in the cytoplasm of alveolar epithelial cells

(Figures 3A, B, 4A and B). In both the macrophages and the epithelial cells, the fibers are usually observed in the phagosomes of various shapes (Figures 3B and 4B). The phagocytic epithelial cells are often structurally altered and sometimes detached from the basement membrane (Figure 5). The attenuated cytoplasm of the epithelial cell may have microvilli on the cell surface, thus combining features of Type I and Type II cells (Figure 4A), or the cell may resemble a fixed alveolar macrophage but be in direct contact with the basement membrane and have attenuated cell cytoplasm identical with that of the Type I cell (Figure 6A).

The process of phagocytosis by the macrophage consists of several stages: formation of pseudopods, attachment of fibers to the plasma membrane, formation of a recess containing the fibers, and pinching off of the recess to form a phagosomal vacuole. Some of these steps can be observed in epithelial cells. In Figure 7 fibers are attached to the plasma membrane of a Type II cell. It is interesting that a similar response by the cell to fibrin is observed (Figure 8), though this is more common in the alveolar macrophage. Frequently the asbestos fibers in the epithelial phagosomes undergo partial dissolution or digestion identical with that observed in the macrophages. This dissolution is manifested by the thinning of the walls of the tubular fibrils. Normally the width of the wall is approximately 100 Å and is equal to the width of the lumen. The walls of the intraphagosomic fibrils frequently measure less than 50 Å (Figures 4B, 9B and 11B). Details of the digestion process in the macrophages have been described elsewhere.[5,6,25] The incidence of epithelial cells that contain asbestos increased with the duration of the experiment. These cells have well-developed organelles such as mitochondria, endoplasmic reticulum, Golgi complex and lysosomes, but often lack osmiophilic lamellar bodies. In addition to asbestos fibers and, later, hemosiderin granules (Figures 9A–D, 10B and 12), asbestos bodies may be present in the cytoplasm (Figure 12). Hemosiderin has been suggested as the source of the iron-protein coat of the asbestos body. The consecutive steps in the development of asbestos bodies from asbestos fiber in the phagocytic epithelium are very similar to those occurring in the macrophages (Figures 9B, 9D, 10B, 11B and 12). Some epithelial cells are transformed into multinucleated giant cells (Figures 9, 11).

The similarity of the phagocytic epithelial cell to the macrophage is often striking. However the former can be recognized by its attachment to the basement membrane and particularly by attachment (by means of terminal bars) to the neighboring epithelial cells (Figure

11B). We found no evidence in the literature or in our own material to suggest that macrophages or monocytes are capable of forming terminal bars or tight junctions. This would refute the possibility that the structures designated as phagocytic epithelial cells are actually macrophages lining denuded stretches of the basement membrane. Since the hypertrophic phagocytic epithelial cell is quite similar to the macrophage in both its structure and its ability to transform ingested fibers into asbestos bodies, it may be classified as an intermediate between a normal lining cell and an alveolar macrophage. When such cells desquamate, they can no longer be distinguished from the free alveolar macrophages.

In addition to the two types of phagocytes just mentioned, there is also a third type, the interstitial macrophage which lies in the alveolar septum and is separated from the epithelium by the basement membrane. This cell is quite similar in appearance and function to the other two, including its ability to ingest asbestos fibers, to form asbestos bodies (Figure 13) and to develop into a multinucleated giant cell. It differs from the intermediate epithelial cell by its lack of direct contact with the basement membrane and the absence of tight junctions with neighboring cells such as alveolar septal cells, monocytes, plasma cells and polymorphonuclear leukocytes.

It is noteworthy that all three forms of phagocytic cells may contain uncoated asbestos fibers as late as 2 years after intratracheal instillation. Such fibers are also found in and around degenerating phagocytic cells in the alveolar space (Figure 14) or lying free in the septal connective tissue (Figure 13).

Discussion

Phagocytic activity by the alveolar epithelial cells has been seldom recognized.[7,16,23] Many investigators deny it altogether[2,3,11,14,15,24] or accept it only for Type I cells.[16] Data here presented demonstrate that under suitable conditions the alveolar epithelium is capable of ingesting particulate matter. The number of epithelial cells containing asbestos increases with the length of exposure. It is accompanied by transformation of the cell into a hypertrophic structure with features of both Type I and II cells, as described above. The appearance of this structure is intermediate between that of the epithelial cell and that of the alveolar macrophage, but functionally, the structure has the properties of the macrophage, including the ability to convert asbestos fibers into asbestos bodies and to develop into multinucleated giant cell. The intermediate cell between the epithelial lining cell and the fixed alveolar macrophages may be difficult to recognize. It loses some of the characteristic

features of either altered Type I or II cells and acquires a resemblance to the macrophage. However, its direct contact with the basement membrane and the presence of terminal bars or tight junctions provide evidence of its epithelial origin. We have previously suggested [25] that persistence of uncoated asbestos fibers in the alveoli serves as a potent enhancer of phagocytosis, primarily by the macrophages, but also, as in this instance, by the epithelial cells. After ingestion, the fibers are coated with an iron-protein complex as a protective reaction. However, the cell may die in the process, releasing the uncoated or partly coated fibers, which are then taken up by another phagocyte. Possibly the disintegrating cytoplasm, in addition to the fibers themselves, serves as stimulus for striking mobilization of the macrophages and transformation of the epithelial cells into phagocytes.

Since both Type I and II cells are derived from entodermal epithelium,[10,13,26,27] it may be said that, under certain pathologic conditions such as pulmonary asbestosis, the entoderm may give rise to phagocytes in the alveolar lining. The origin of the alveolar macrophage is less certain. Various cells, such as the alveolar epithelial cell,[28] the capillary endothelium,[29] the monocyte [30] and the mesenchymal cell [11,31] have been suggested as its precursors. Current studies using chromosomal or prealbumin esterase markers,[32,33,34] autoradiography [31,35,36] and tissue culture [38] demonstrate rather convincingly that certain bone marrow cells or, alternatively, monocytes [36,37,39] can give rise to alveolar macrophages. However, it may be premature to exclude other possible sources. There is evidence to suggest that under normal conditions two-thirds of the free alveolar macrophages are derived from bone marrow cells and one-third from local pulmonary cells.[33] We would like to propose that in asbestosis, at least some of the alveolar macrophages are formed by transformation of the alveolar epithelial cells via the intermediate form described above. It is still uncertain whether such transformation occurs only under unusual circumstances, or is, to a lesser or greater degree, a common event of daily life.

The interstitial macrophages increase progressively in size and number after the administration of asbestos. Our material provides no direct clue to their origin: we found no transitions between these cells and the phagocytic epithelial cells, and no evidence for or against derivation from blood and marrow cells [32] or local alveolar septal cells.[31]

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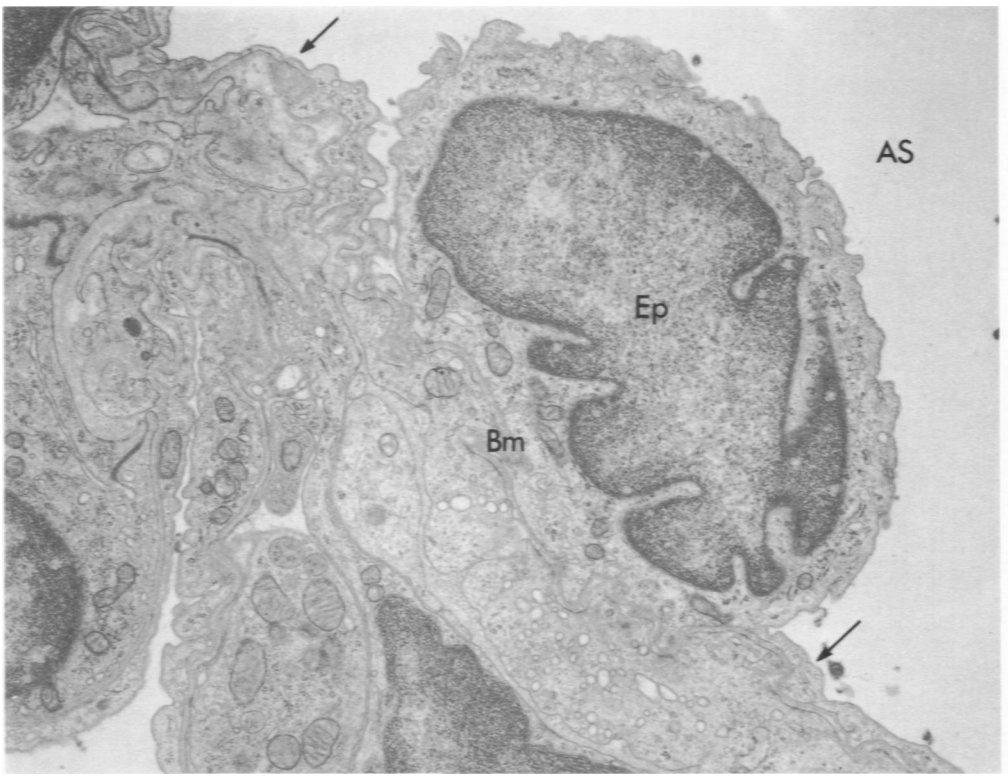
Legends for Figures

Fig 1—A normal Type I alveolar epithelial cell (*Ep*). *Arrows* indicate thin stretches of cytoplasm. AS = alveolar air space, *Bm* = basement membrane (Osmium tetroxide, × 14,000).

Fig 2—A Type II alveolar epithelial cell containing osmiophilic lamellar bodies (*Ep1*). Cell organelles are fairly well developed. The cell surface bears microvilli (*Mv*). *Arrows 1* point to tight junctions and *arrow 2* indicates a desmosome. AS = alveolar air space, *Ep2* = Type I epithelial cells, BM = basement membrane, CL = capillary lumen, Ed = alveolar capillary endothelium (Osmium tetroxide, × 6500).



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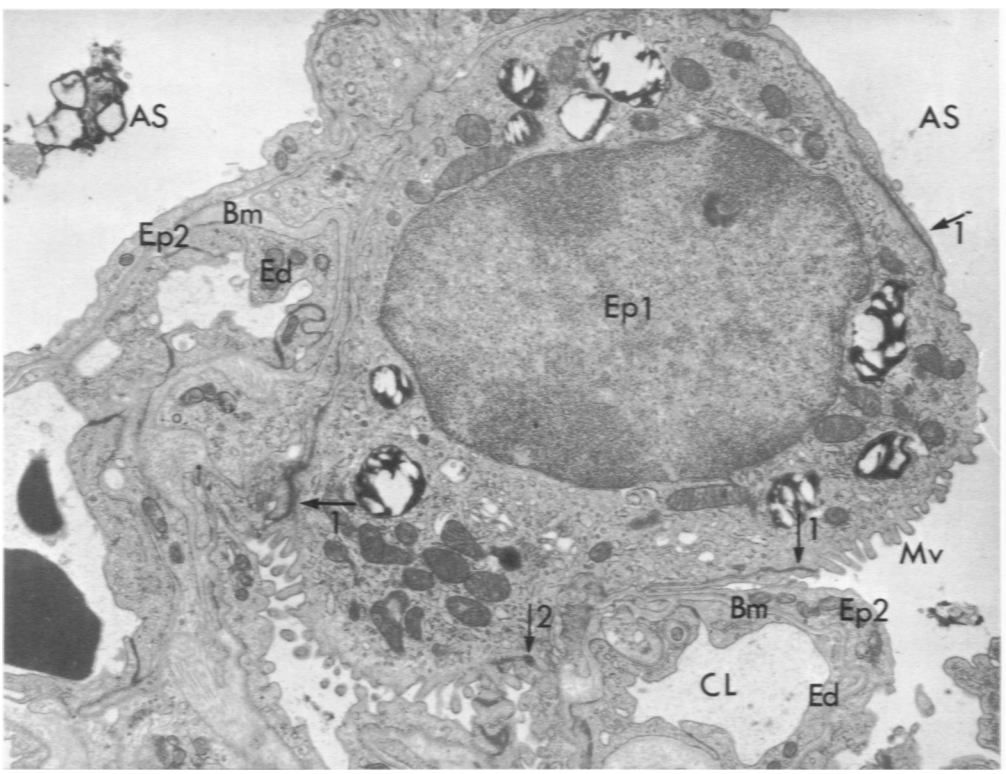

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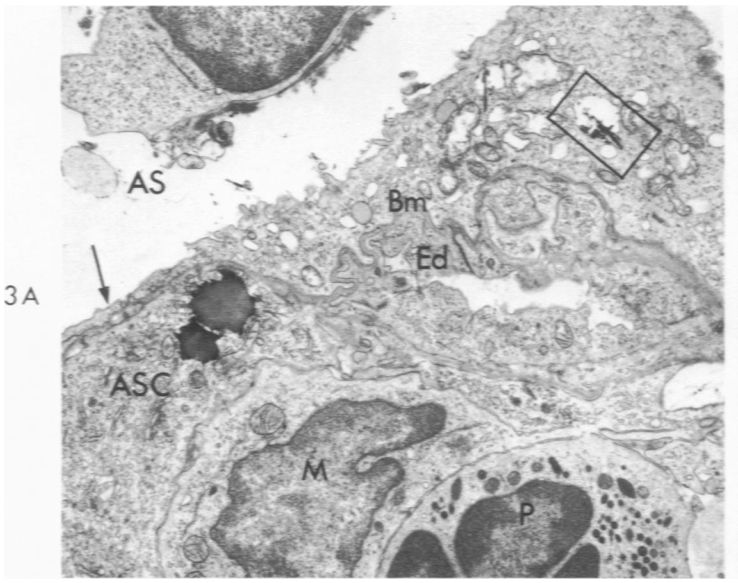
Fig 3A—An alveolar epithelial cell containing asbestos fibers. *Arrow* indicates attenuated cytoplasm. One day after instillation of asbestos. *ASC*=alveolar septal cell, P=phagosome (Osmium tetroxide, × 7400). **B**—A higher power view of the rectangle in Figure 3A. Several fibrils of chrysotile asbestos are seen in a phagosome (× 49,000).

Fig 4A—Structural alterations of an epithelial cell containing asbestos. Note that the attenuated cytoplasm has microvilli (*Mv*) and a large mitochondrion. Sixteen days after instillation (Glutaraldehyde and osmium tetroxide, × 35,000). **B**—A higher power view of the rectangle in Figure 4A. An elongated phagosome contains a chrysotile fibril 280 Å in width. The electron density of the fibril is decreased (× 55,600).

Fig 5—Partly desquamated epithelial cell showing phagocytic activity. Several phagosomes (*P1–P5*) contain asbestos fibrils. *Unnumbered arrow* indicates an extracellular asbestos fibril. Part of the cell cytoplasm is still attached to the basement membrane (*Bm*). One day after instillation. *AS*=alveolar space (Osmium tetroxide, × 19,300).



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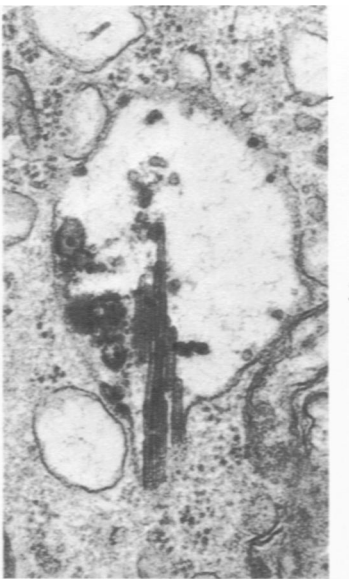

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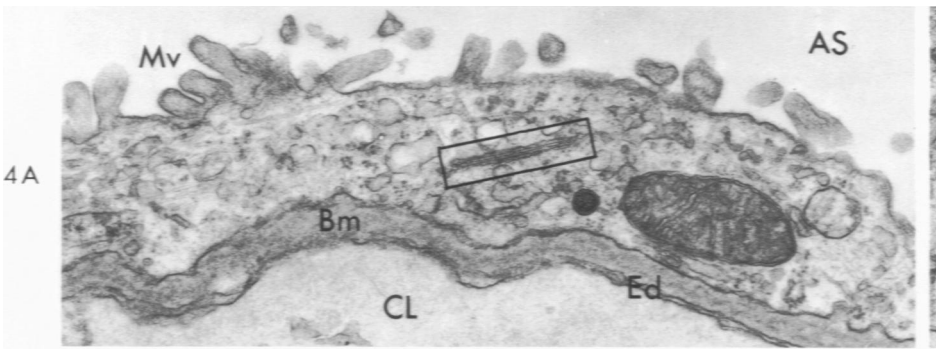

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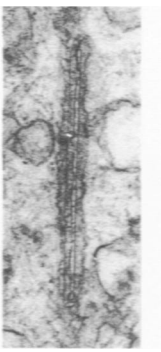

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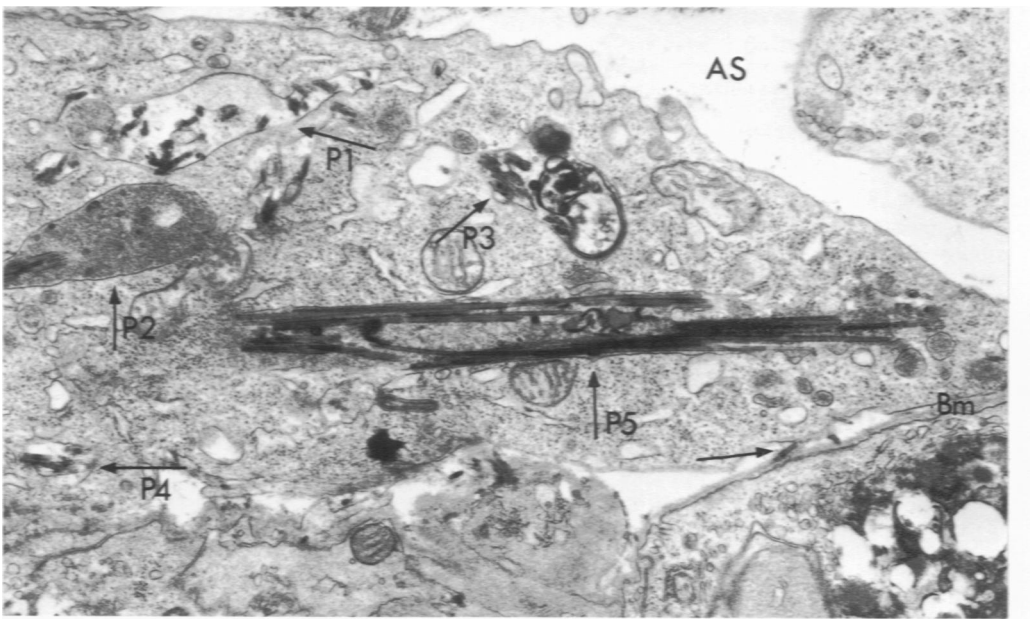

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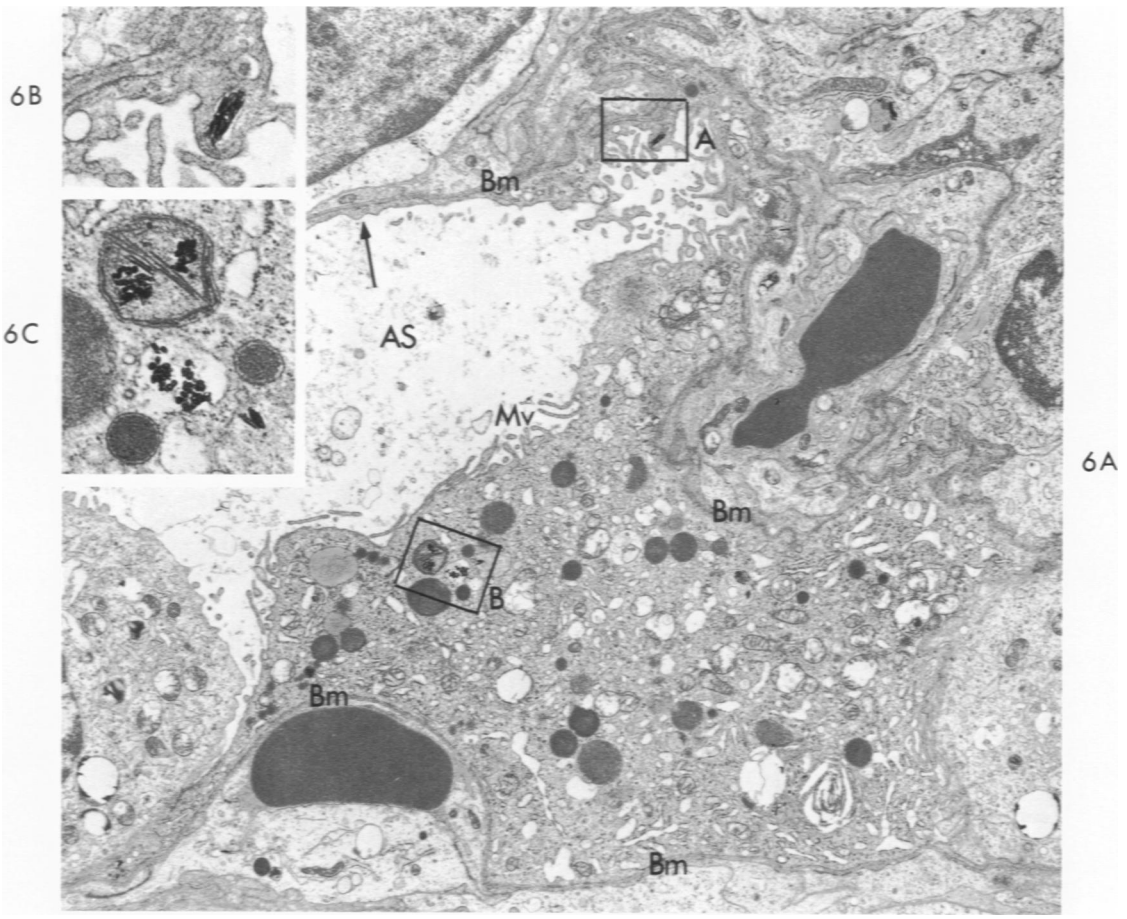
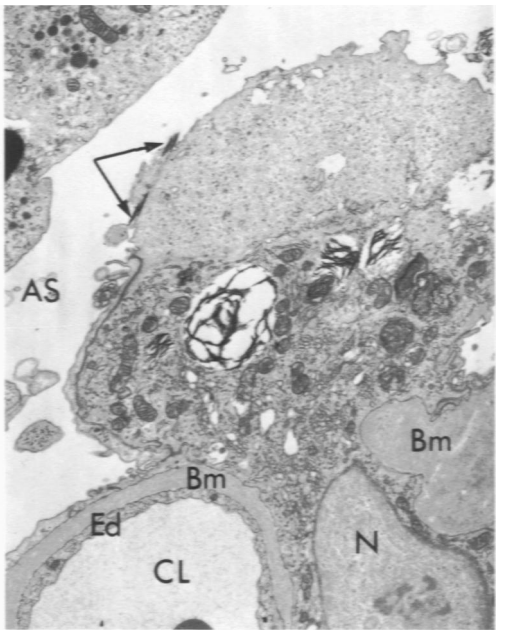


Fig 6A—A cell intermediate between an epithelial cell and a fixed alveolar macrophage. It has well-developed organelles including lysosomal granules but maintains direct contact with the basement membrane (*Bm*) and has attenuated cytoplasm (*arrow*). One week after instillation. *AS*=alveolar space, *Mv*=microvilli (Glutaraldehyde and osmium tetroxide, × 6000). **B and C**—Higher power views of Rectangles A and B, showing asbestos in phagosomes (× 20,400).

Fig 7—Attachment of asbestos fibrils (*arrows*) to the cell surface of a Type II cell. The upper half of the cell cytoplasm is transformed into a pseudopod-like structure. (Similar changes have been observed in alveolar macrophages and are believed to represent the initial step of the phagocytosis). One year after instillation. *N*=nucleus of the cell, *Ed*=endothelial cell, *CL*=alveolar capillary lumen, *AS*=alveolar air space (Glutaraldehyde and osmium tetroxide, × 4500).

Fig 8—Type II aveolar cell in direct contact with fibrin. The cell has lost most of its microvilli. *Arrow* indicates a pseudopod-like part of the cytoplasm. One day after instillation. *F*=fibrin, *AS*=alveolar space, *Bm*=basement membrane (Osmium tetroxide, × 4500).

Fig 9—An intermediate form between an epithelial cell and a fixed alveolar macrophage. Well-developed organelles and microvilli are seen. Six months after instillation (Glutaraldehyde and osmium tetroxide, × 6100). **B**—Enlargement of Rectangle A. Phagosome containing a chrysotile fibril and iron particles (× 22,500). **C**—Enlargement of Rectangle B. Two hemosiderin granules; the one on the right contains numerous iron particles (× 22,000). **D**—A phagosome containing several chrysotile fibrils. Two hemosiderin granules are located closely to the phagosome (× 22,500).



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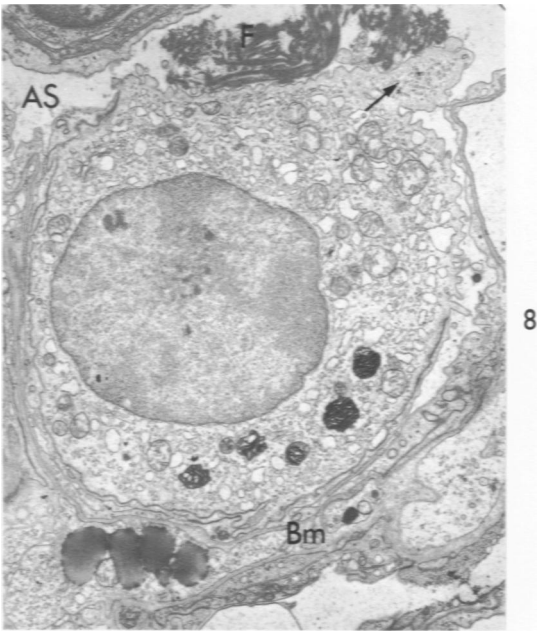

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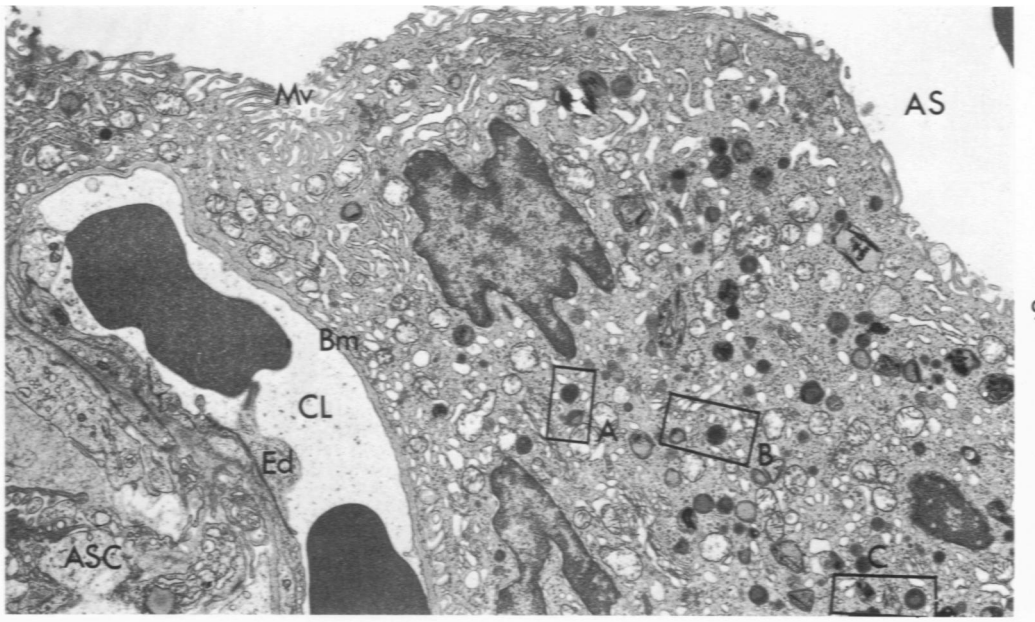

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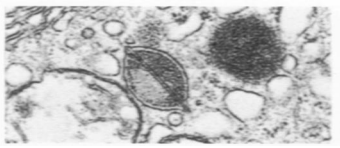

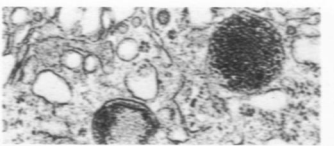

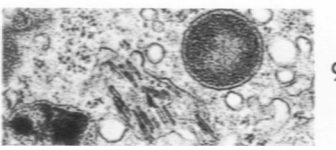

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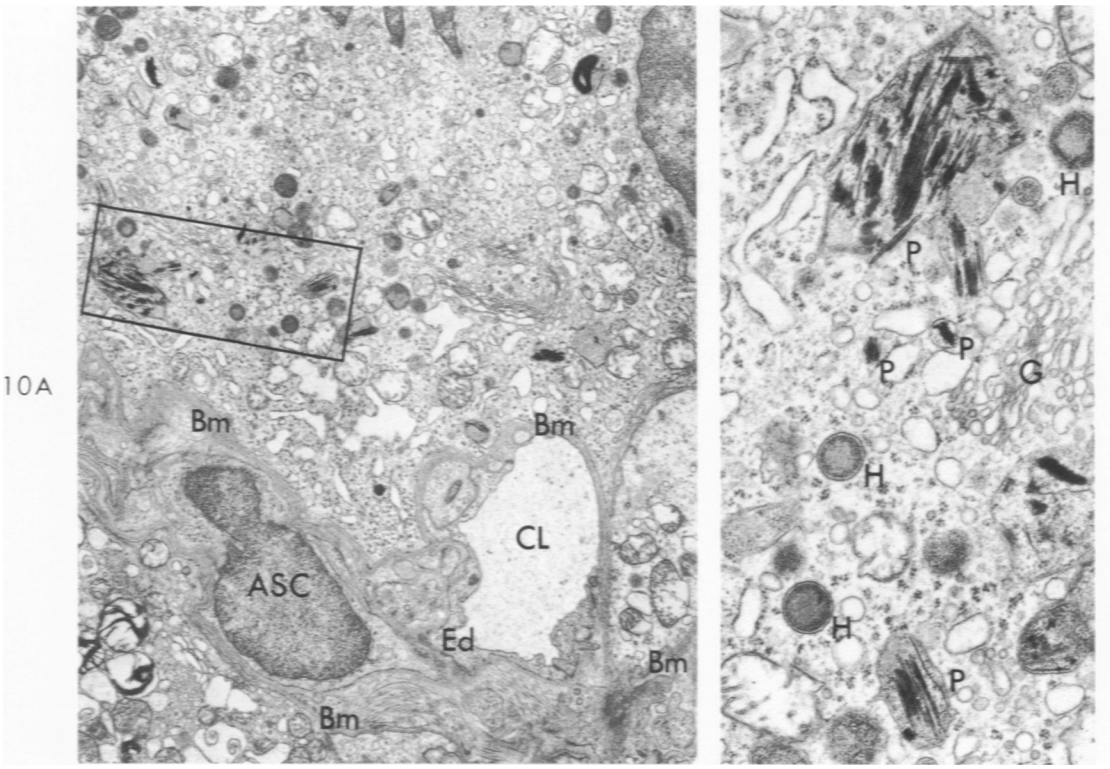
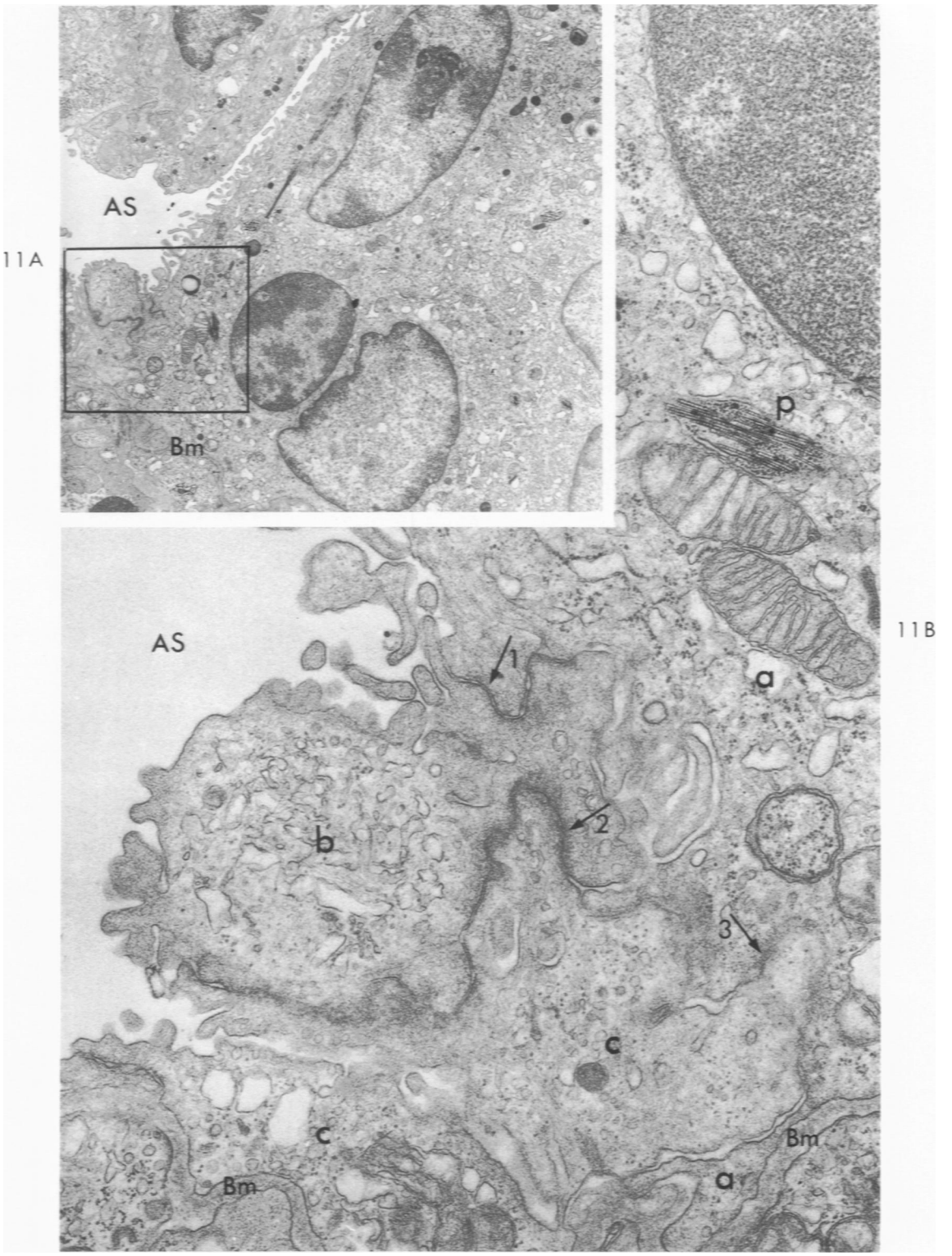


Fig 10A—Another intermediate cell showing well-developed organelles. The basal plasma membrane is in direct contact with the basement membrane. Six months after instillation. *BM*=basement membrane, *ASC*=alveolar septal cell, *Ed*=endothelial cell, *CL*=capillary lumen (Osmium tetroxide, × 6900). **B**—Higher power view of the rectangle in Figure 10A. Golgi complex (*G*), hemosiderin granules (*H*) and phagosomes (*P*) containing asbestos are seen (× 20,400).

Fig 11A—An epithelial multinucleated giant cell. Sixteen days after instillation. *AS*=alveolar air space, *Bm*=basement membrane (Glutaraldehyde and osmium tetroxide, × 53,000). **B**—Enlargement of the rectangle in Figure 11A. *Arrows* indicate terminal bars; *a*, *b* and *c* represent parts of cytoplasm of three different cells. The giant cell (*a*) has two junctions: *arrow 1* (between *a* and *b*) and *arrow 3* (between *a* and *c*). A phagosome containing chrysotile fibrils and iron particles is seen at *P*. *AS*=alveolar space, *Bm*=basement membrane. (× 28,400).



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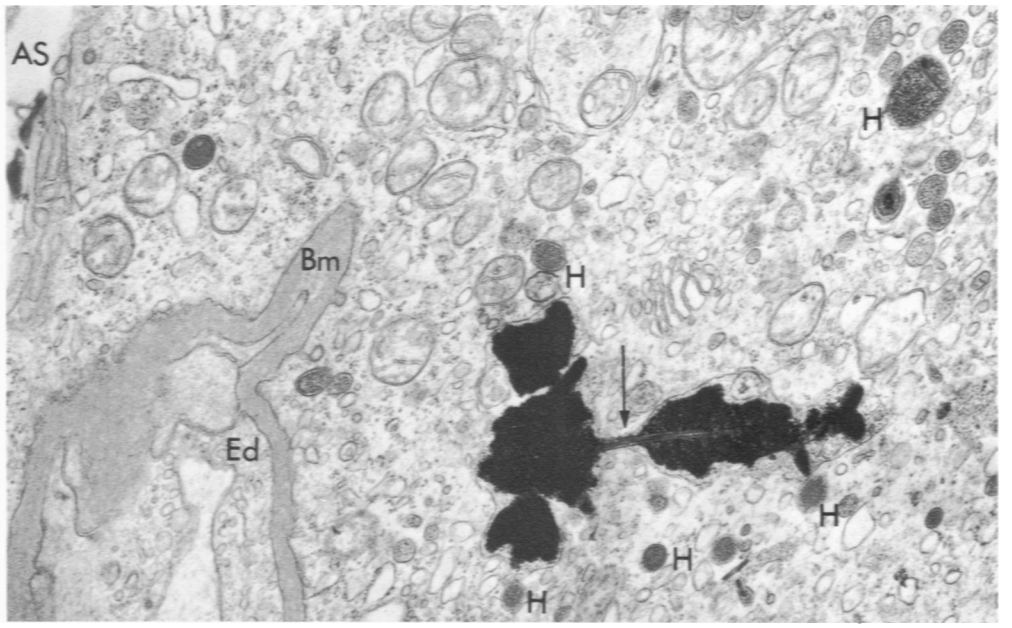

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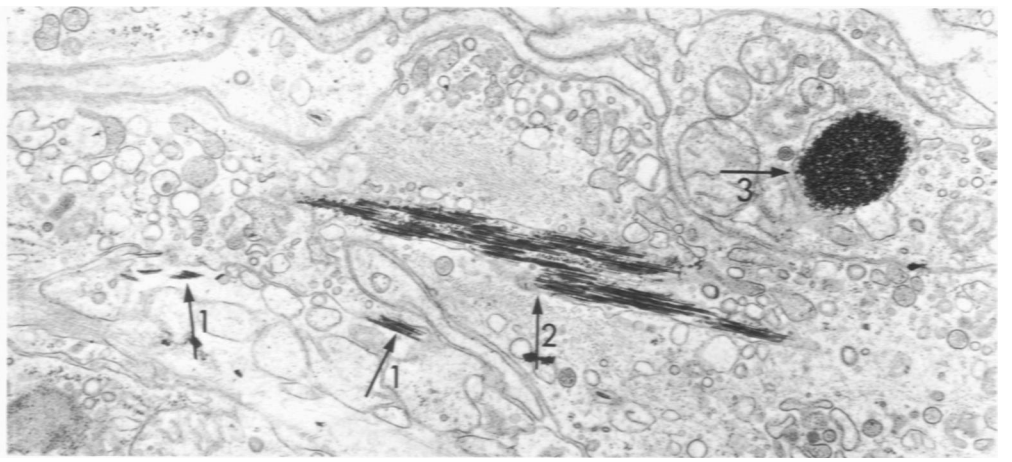

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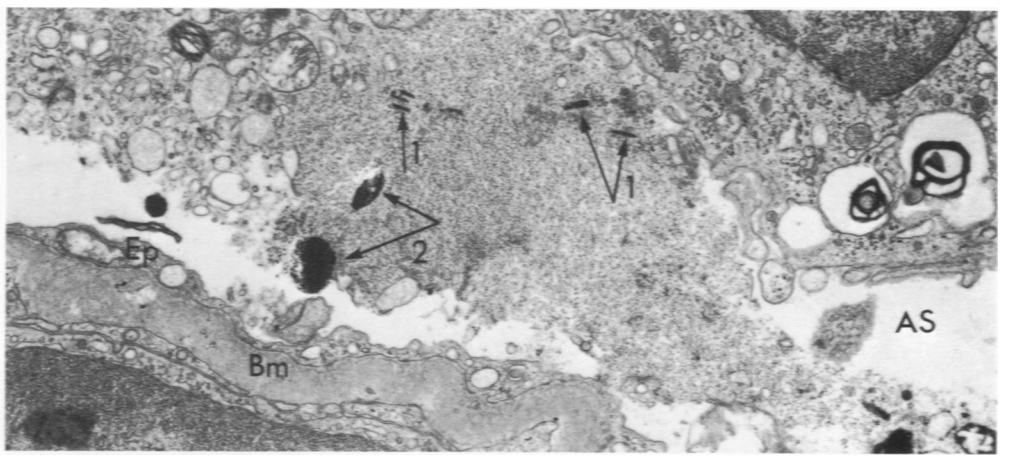

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Fig 12—An asbestos body (*arrow*) of irregular shape in an intermediate cell. Some of the hemosiderin granules (*H*) are intimately associated with the body. One year after instillation. *AS*=alveolar air space, *BM*=basement membrane, *Ed*=endothelium (Osmium tetroxide, × 17,500). **Fig 13**—Two interstitial macrophages containing an immature (*arrow 2*) and a mature asbestos body (*arrow 3*). Uncoated fibrils (*arrows 1*) are seen extracellularly. One year after the instillation (Osmium tetroxide, × 14,000). **Fig 14**—Asbestos fibrils (*arrows 1*) and asbestos bodies (*arrow 2*) apparently in the process of release from a degenerating phagocytic cell. One year after instillation. *AS*=alveolar air space, *Ep*=alveolar epithelium, *Bm*=a markedly thickened basement membrane (osmium tetroxide, × 9200).