Phagocytosis of Chrysotile Fibers by Pleural Mesothelial Cells in Culture

Marie-Claude Jaurand, Hervé Kaplan, Jacques Thiollet, Marie-Claire Pinchon, Jean-François Bernaudin, and Jean Bignon

Pleural mesothelial cells (PMC) from the parietal pleura of rats were incubated in culture with UICC A chrysotile fibers. The sequence of events in phagocytosis was studied by electron microscopy: phases of attachment, sequestration, and degranulation of lysosomal content into the phagocytic vacuole were observed. This demonstrates that PMC can engage in phagocytosis of chrysotile fibers. (Am J Pathol 94:529–538, 1979)

PHAGOCYTOSIS of particulate matter is generally limited to cells of the reticuloendothelial system; however, some other cells can also ingest particulate matter, especially asbestos, by engulfment, as demonstrated by Suzuki [1] for alveolar epithelial cells. It would be particularly interesting to discover whether pleural mesothelial cells (PMC) can ingest asbestos fibers, since the pleural mesothelium is a target for injury induced by asbestos fibers. The relationship between phagocytosis and carcinogenicity is unknown, but the presence of the fibers in the cells may be a factor in the development of carcinogenesis.

The electron microscopic studies described in this paper demonstrate that PMC in culture can engage in phagocytosis and take in particles such as chrysotile fibers.

Materials and Methods

Isolation and Culture of Mesothelial Cells

Two-month-old Wistar rats were used. Mesothelial cells were isolated from the parietal pleura and cultured according to a technique described previously.[2] Primary cultures were made in Linbro cluster dishes; 15-day-old cultures were used.

Treatment With UICC A Chrysotile Fibers

UICC A chrysotile fibers at a concentration of 100 $\mu g \times ml^{-1}$ were added to the dishes and left in contact with the cultures for 15 minutes, 45 minutes, or 4 hours. The cells were then fixed for electron microscopic studies. Granulometric electron microscopic studies showed that the mean fiber length was $< 4\mu$.

From the Laboratoire de Biopathologie Pulmonaire, Hôpital Henri Mondor, Créteil, France.

Supported by Contract INSERM 78.1.210.5 and by CEE Contract 098-75-1-ENV.F.

Accepted for publication November 2, 1978.

Address reprint requests to M. C. Jaurand, Laboratoire de Biopathologie Pulmonaire, U 139, Hôpital Henri Mondor, 94000 Créteil, France.

0002-9440/79/0308-0529$01.00

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Electron Microscopic Studies

Culture media were replaced by 2.3% glutaraldehyde (TAAB Laboratories) in cacodylate buffer, 0.045 M, pH 7.4, 380 mOsm. After 1 hour, the cells were rinsed with buffer and postfixed with 2.5% osmium tetroxide in cacodylate buffer. After dehydration with ethanol, a thin film of Epon was poured onto the dishes, and they were kept at 37 C. Beem capsules were then placed on the Epon to surround the cells; after polymerization at 60 C, the film was taken off the dishes.

Acid phosphatase staining was used as a lysosomal and phagolysosomal marker; this was carried out by the Gomori method before postfixation.

Conventional staining for ultrastructural studies was with uranyl acetate and lead citrate; for cytoenzymatic studies the sections were left unstained.

Results

Control Cells

Under the light microscope, cultured PMC appeared as monolayers of polyhedric cells in close juxtaposition as described previously.[2]

Ultrastructural studies (Figures 1 and 2) showed that PMC have numerous mitochondria and ribosomes; the rough endoplasmic reticulum was not dilated, but the lumina contained a fairly dense, amorphous material. In some sections, the Golgi apparatus was well defined. A few electron-dense bodies were observed, and some of them were presumably lysosomes. Microfilaments were observed in the cytoplasm and at the periphery of the cells, usually parallel to the junctional system; the cells were strongly attached by junctions.

Figure 3 shows that when the cells were cut perpendicularly to the dish, they looked ultrastructurally like those of the parietal mesothelium *in situ*, ie, with a few microvilli and large nuclei swelling the central part of the cell.

Cytochemical studies showed that in resting cells high concentrations of acid phosphatases were located in membrane-bound structures (Figure 4). The cells generally showed a few primary lysosomes containing acid phosphatases.

Cells Incubated With Chrysotile Fibers

In some cells after 15 minutes of exposure to chrysotile, the fibers were seen to be in contact with cell membranes or within invaginations, consistent with an engulfment of the fibers by the cells (Figure 5). The lysosomes were unchanged (Figure 6) and were distant from the phagocytosed fibers.

After 45 minutes, there were more fibers in the cells and they occurred inside membrane-bound structures (Figure 7). The acid phosphatase reac-

tion inside the phagosome was generally negative; however, in some of them, there was a dense, granular, positive reaction, indicating the presence of phagolysosomes (Figure 8).

By 4 hours after uptake of chrysotile, many fibers were seen inside the cytoplasm, apparently inside phagocytic vacuoles (Figure 9). Acid phosphatase staining very close to the fibers was intensely positive (Figure 10). Primary lysosomes were rarely observed at this time. Elsewhere, cytoplasmic features and organelles were well preserved.

Discussion

The morphologic and cytochemical studies carried out in these experiments with mesothelial cells in culture demonstrated that these cells can engage in phagocytosis of asbestos. Some papers have already been published on the phagocytosis of asbestos by mesothelial cells in organ culture. Rajan et al [3] showed by light microscopy the presence of crocidolite fibers in cells of parietal pleura cultured with crocidolite. According to Allison,[4] mesothelial cells are highly phagocytic; however, phagocytosis was not demonstrated in his study. The phagocytosis of asbestos by pure mesothelial cells in culture has not hitherto been investigated.

For phagocytosis to occur, a number of requirements must be met. Four phases occur in the process of phagocytosis [5]: chemotaxis (for migratory cells), contact and attachment of the particle to the cell membrane, ingestion and sequestration of the particle in the cell, and digestion (as observed in cells that kill bacterial species). The latter phase occurs after discharge of the lysosomal granule content into the phagocytic vacuole.

The experiments described in this paper have demonstrated the three latter phases; under *in vitro* conditions and with closely bound cells, chemotaxis could not be observed. Chrysotile fibers were, however, often seen in contact with the plasma membrane and then ingested and segregated into membrane-bound vesicles; it is in this way that they are transported into the cytoplasm. Subsequently, there is fusion of lysosomal membranes with phagosomes, followed by release of the lysosomal granules into the phagocytic vacuole. Four hours after incubation with chrysotile, the ultrastructure of the cells was still well preserved.

It was thus demonstrated that the various phases of phagocytosis occur when PMC are cultured with chrysotile fibers and that the process of phagocytosis is similar to that of other particles. These results are of interest in the study of the pathogenesis of asbestos; the degranulation phase is of particular importance because it may be a preliminary step in the cytolytic and carcinogenic action of asbestos fibers.

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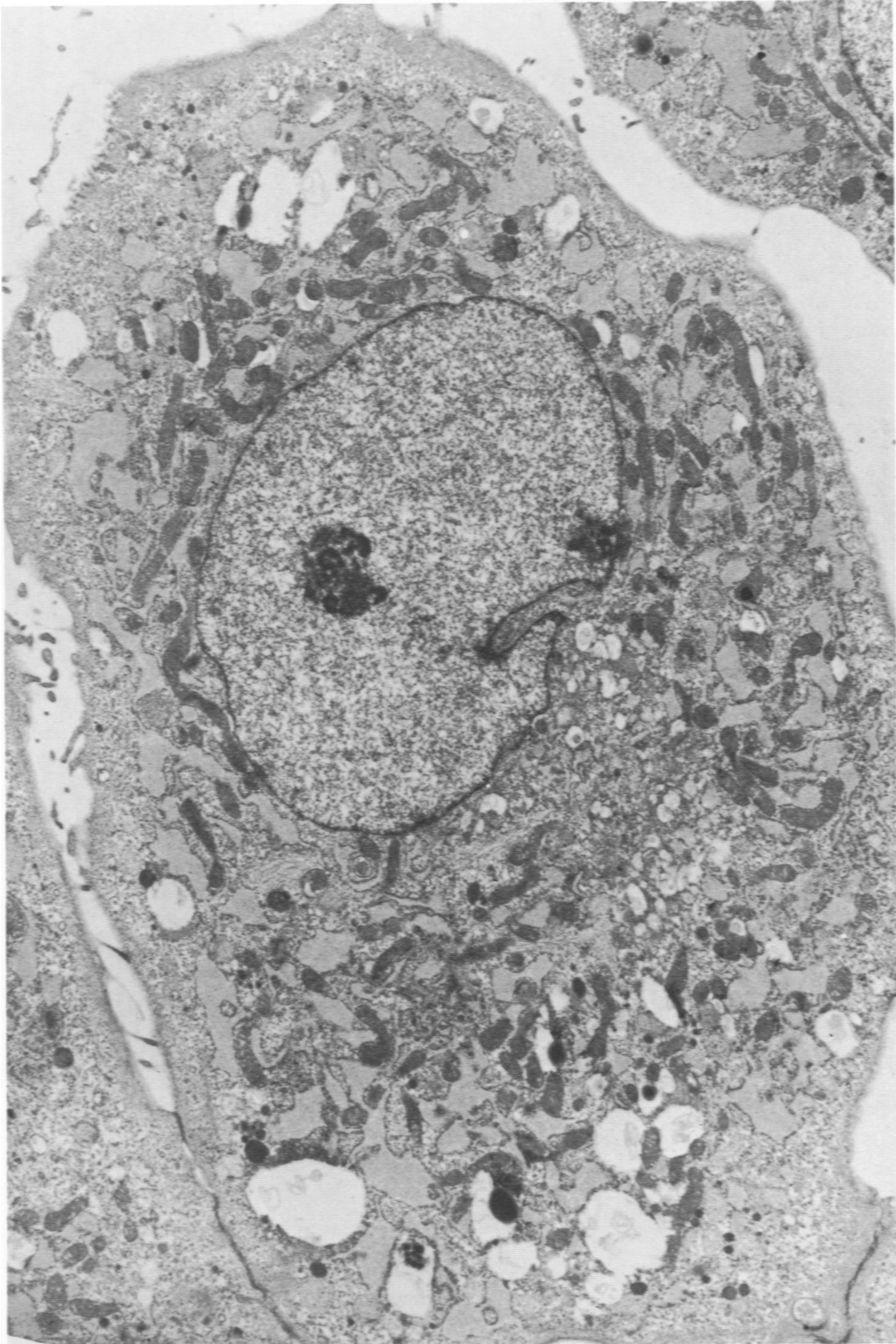

Figure 1—Electron micrograph of control pleural mesothelial cell (PMC) in culture. The section was taken parallel to the bottom of the dish. (Uranyl acid and lead citrate, × 7300)

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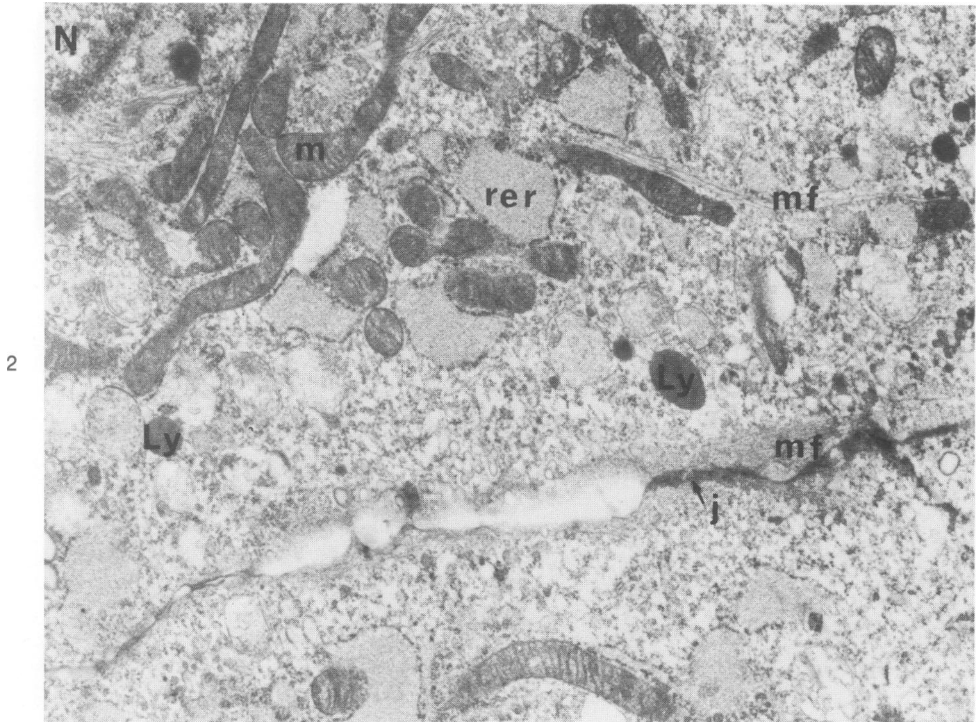

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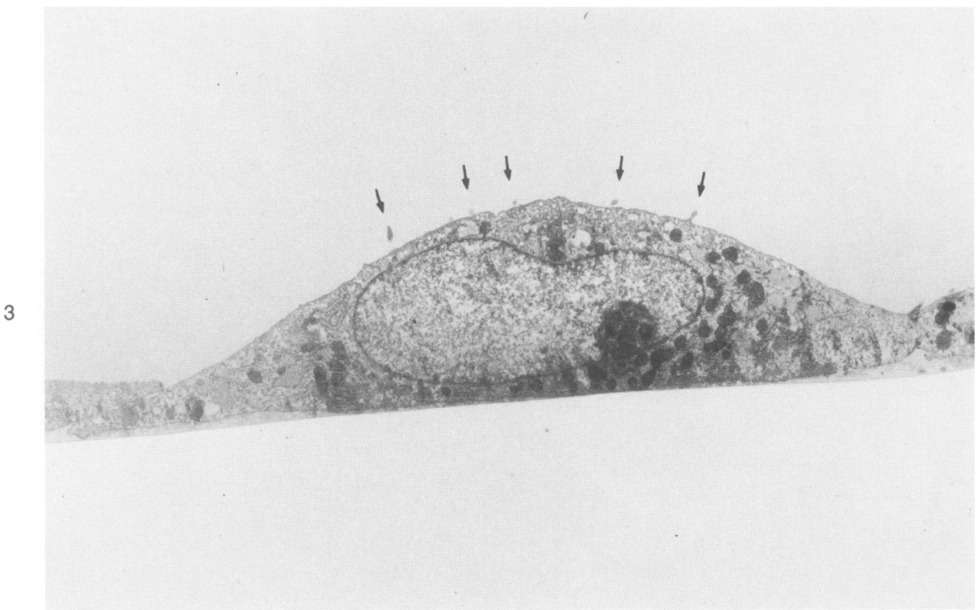
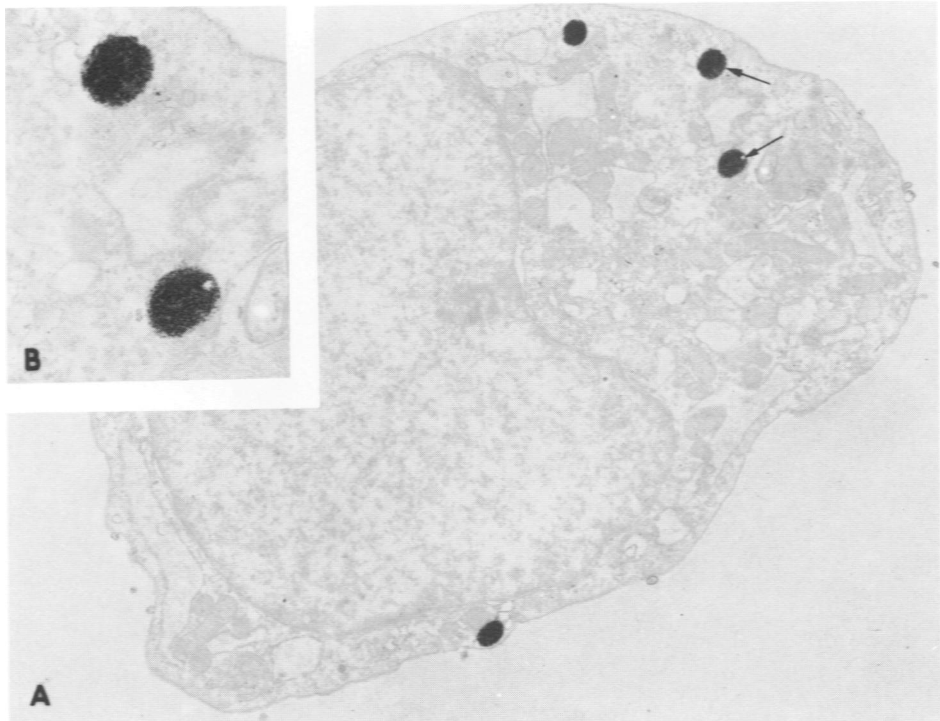


Figure 2—Ultrastructure of control PMC in culture. *j*, junctional system; *Ly*, lysosomes; *m*, mitochondria; *mf*, microfilaments; *N*, nucleus; *rer*, rough endoplasmic reticulum. (Uranyl acid and lead citrate, × 15,500) **Figure 3**—Electron micrograph of control PMC in culture showing some microvilli at the apical part of the cell (*arrows*). The section was taken perpendicular to the bottom of the dish. (Uranyl acid and lead citrate, × 5900)



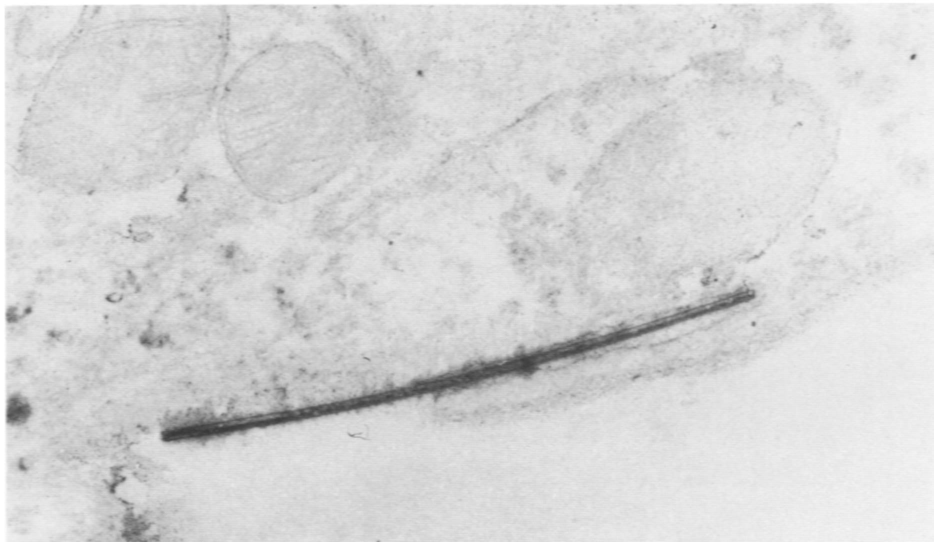


Figure 4—Acid phosphatase staining of a control PMC showing the location of enzymes inside membrane-bound structures. **Inset**—Higher magnification of the two primary lysosomes indicated by *arrows*. (× 9900; **inset**, × 22,900) **Figure 5**—Electron micrograph of PMC incubated with chrysotile for 15 minutes, showing invagination of the cell membrane. (× 50,000)

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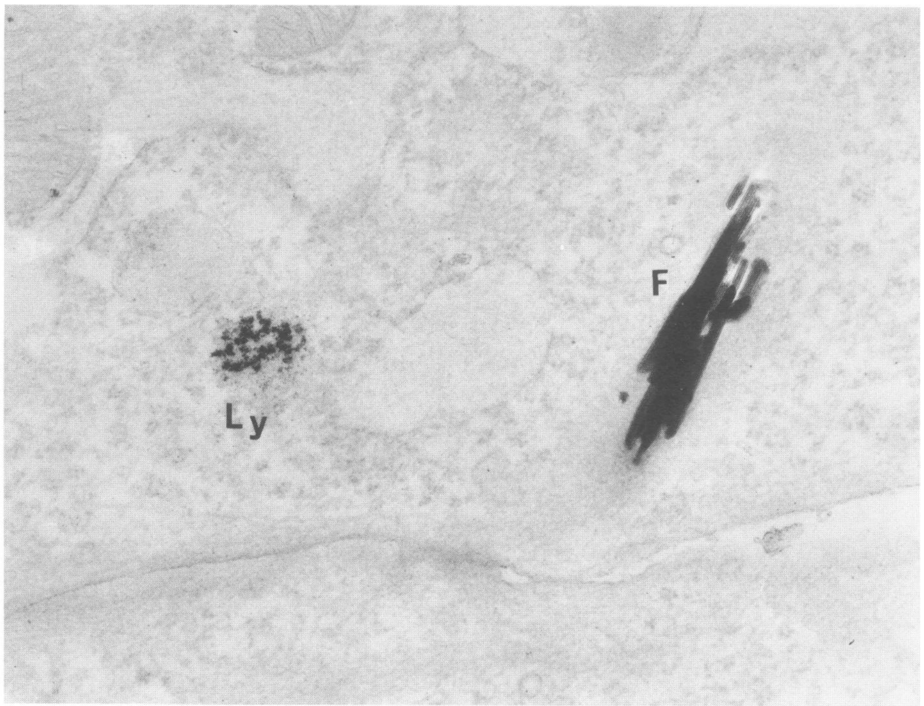

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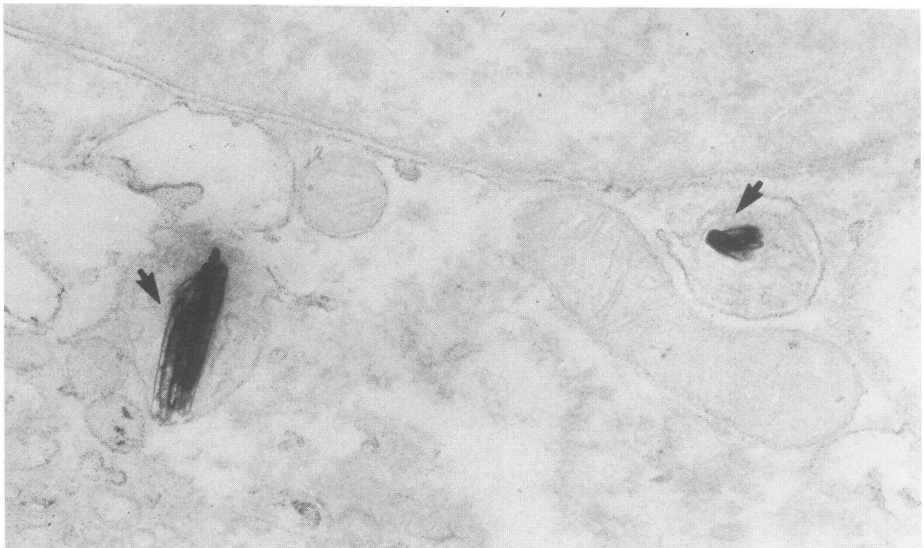
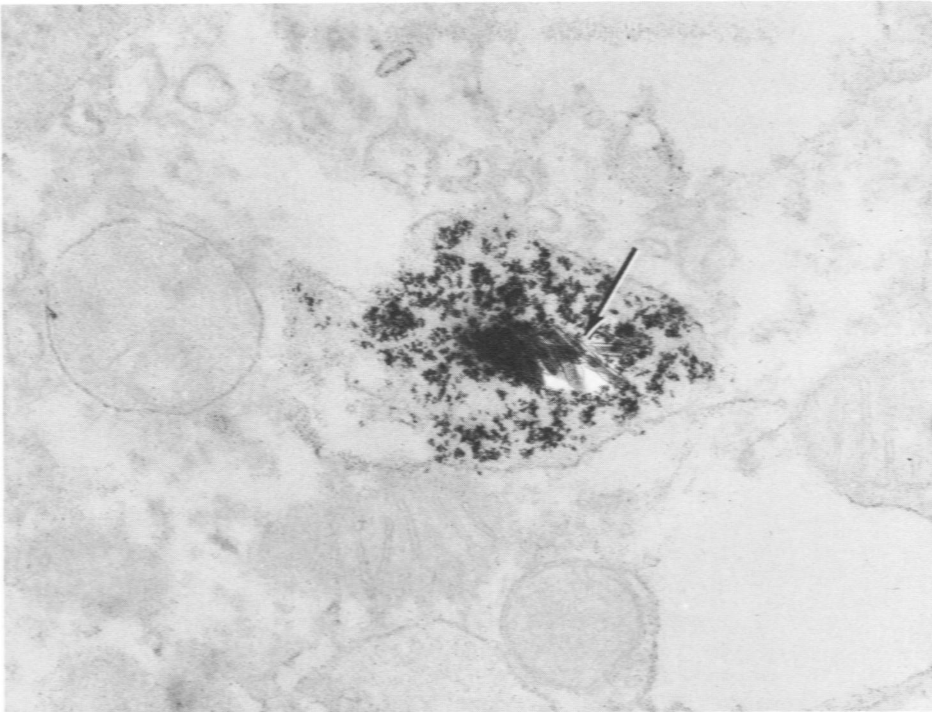


Figure 6—Acid phosphatase staining of a PMC incubated for 15 minutes with chrysotile, showing lysosomes distant from the fibers. *Ly*, lysosomes; *F*, asbestos fiber. (× 34,200) **Figure 7**—Electron micrograph of PMC incubated for 45 minutes with chrysotile fibers. Two vesicles (*arrows*) surrounded by a membrane. (× 50,800)



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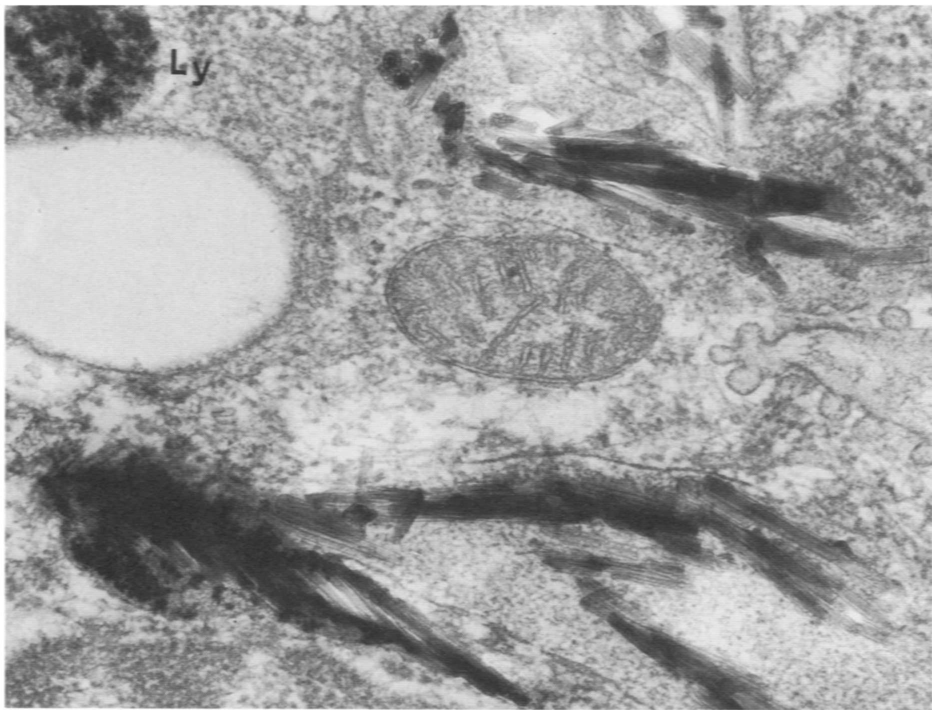

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Figure 8—Electron micrograph of PMC incubated for 45 minutes with chrysotile fibers (*arrow*). The positive acid phosphatase staining is located well inside the phagolysosomes. (× 55,350) **Figure 9**—Electron micrograph of PMC incubated for 4 hours with chrysotile. There are numerous fibers inside phagolysosomes positive to acid phosphatases. *Ly*, lysosome. (Uranyl acid and lead citrate, × 50,600)

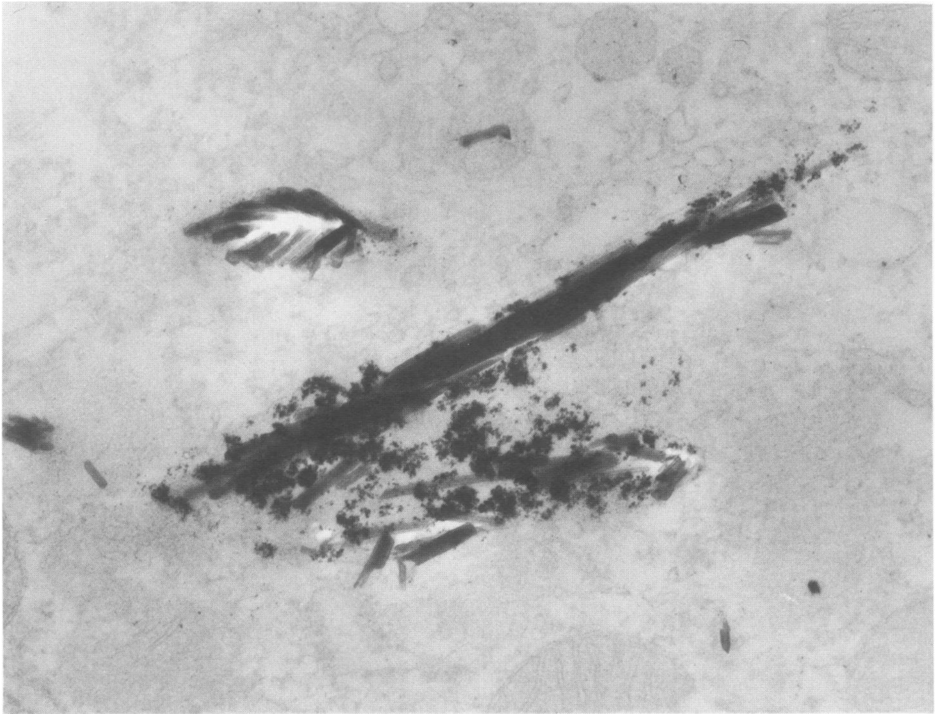

Figure 10—Acid phosphatase staining of PMC incubated for 4 hours with chrysotile. Intense positivity is observed very close to the fibers. (× 45,950)