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Penetration of Colonic Mucosa by Asbestos Particles

An Electron Microscopic Study in Rats Fed Asbestos Dust*

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A recent report by Seilkoff, Churg, and Hammond¹ concerning asbestosis and mesothelioma also called attention to the relationship between asbestos and neoplasia in the gastrointestinal tract. They noted 3 times the expected incidence of carcinoma of the gastrointestinal tract in asbestos workers. It seemed to us that a reasonable amount of aerosol asbestos must be ingested directly or indirectly as inhaled particles carried on the respiratory mucous blanket to the pharynx.² Acting on this assumption we fed a synthetic diet containing 6 per cent chrysotile asbestos dust to white Wistar rats and examined their colonic mucosas by electron microscopy.

MATERIALS AND METHODS

To study the long term effect of ingested asbestos we gave 60 white Wistar female rats a synthetic diet consisting of 20 per cent casein, 65.8 per cent sucrose, 4 per cent salt mix, 6 per cent asbestos, 2 per cent vitamins, 2 per cent corn oil, and 0.2 per cent choline chloride. In addition, 12 rats were maintained on an identical diet with Alphacel (Nutritional Biochemicals Corp., Cleveland, Ohio) replacing the asbestos. Two rats were killed after 3 months and sections of their colons were examined with the electron microscope. One additional rat was killed 3 days after asbestos had been eliminated from the diet. The colon, mesenteric nodes, and spleen were prepared for electron microscopy.

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Tissues were fixed in 1 per cent osmium with Palade's buffer and in lithium permanganate followed by dehydration in alcohols containing uranyl acetate and lead subacetate. The material was embedded in 50 per cent Araldite and 50 per cent Epon. Sections were cut with a diamond knife on a Porter-Blum ultramicrotome, stained with lead citrate mounted on uncoated grids, and viewed with an RCA EMU-3F electron microscope. In addition, suspensions of intact crystals were placed on coated grids and examined. For comparison, agar blocks containing asbestos fibers were processed in the same manner as tissue.

RESULTS

Scanning for asbestos particles was facilitated by using the sections fixed in lithium permanganate which, because of relatively bland appearance of ribonucleic acid and deoxyribonucleic acid granules, made location of asbestos fibers somewhat easier. Occasional asbestos particles were found near the luminal surface of the cells, and by scanning of many sections chrysotile crystals were detected in intercellular and interstitial locations. Although the direction of movement could not be determined, migration from the lumen inward seemed most likely. The particles usually measured from 0.5 to 1 μ in length and had the typical chrysotile structure of two concentric tubes.^{1,2,6} Crystals in the intestinal lumen were coated with a thin homogeneous layer of material that could also be seen in control suspensions prepared in agar blocks and thinly sectioned (Figs. 1 to 3). In most instances entrance of the particles seemed to be through the goblet cells, as fibers were seen in the mucous droplets. Some appeared to be penetrating the cell membrane at the base of the cell,

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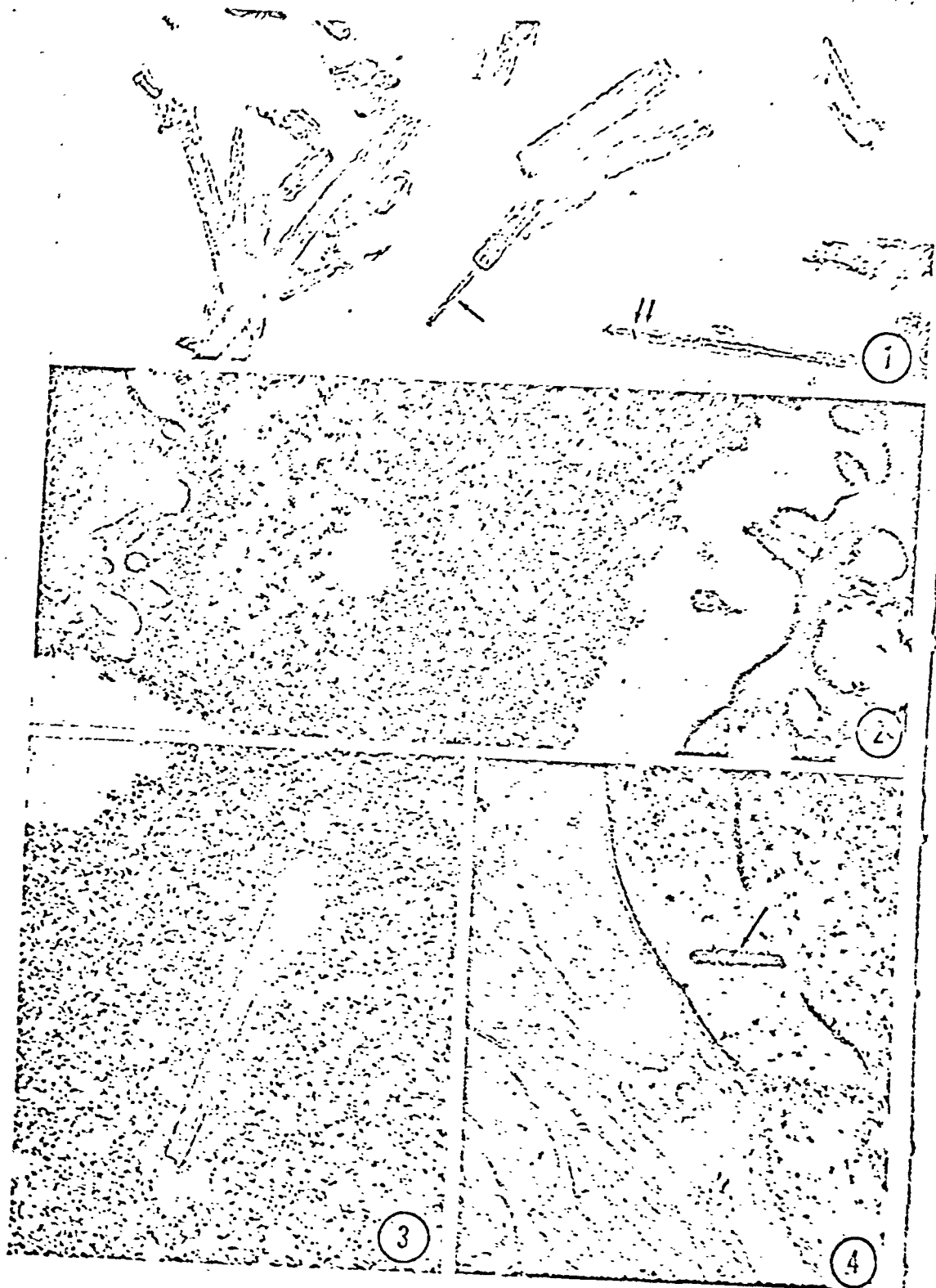
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perhaps representing a phase of migration (Figs. 4 and 5). Similar fibers were found in the cytoplasm of the cell with no apparent isolating membrane (Fig. 6). Asbestos was also found in the lamina propria in both interstitial substance and smooth muscle cells (Figs. 7 and 8). Sections of the regional lymph nodes and spleen were examined with the thought that the particles might be carried by lymphatics. However, no fibers were found in these areas. At this time no alterations in the epithelial cells could be found that were inconsistent with normal mucosa. Sections which had been fixed in osmium were also examined and particles identified.

The possibility of artifact was strongly considered. Possibly the asbestos crystals could have been forced into the cell during the sectioning, or fibers could have floated onto it from the processing solutions. We attempted to minimize this hazard by examining a rat after elimination of asbestos from the diet until the feces were free of the fiber, hoping that surface fibers would not be present and carried into the cells by cutting. Even in this animal, asbestos crystals were present intracellularly, although in lesser numbers. That the observed intracellular crystals were contaminants from freely floating asbestos is also unlikely from the morphologic evidence. Each of the fibers not only appeared to be within the tissue but most had been thinly sectioned. With electron microscopy the difference in appearance between intact and sectioned crystals is easily apparent. Thus, we believe that the fibers were present in the cells at the time of sectioning.

DISCUSSION

The apparent ability of these fibers to migrate through the colonic mucosa is

significant. First, this may be a partial explanation for a suspected relationship between asbestos and carcinoma of the gastrointestinal tract. Second, penetration of cells provides another means for aspirated fibers to reach the pleura and thus produce mesothelioma, another tumor in which asbestos has been incriminated.⁷⁻⁹

The fact that particles seen in this study were apparently "free" in the cytoplasm and not in vacuoles is not surprising in view of other published results. Dourmashkin and Dougherty⁴ described asbestos particles present in cytoplasm of cells in tissue cultures as free and in vacuoles, and when examining asbestos in macrophages Davis³ also found particles in both locations. In another study, our observations on the intracellular reaction to chrysotile asbestos placed in the pleural space of rats also confirm this finding.¹⁰ Thus, actual incorporation of orally administered asbestos particles into gastrointestinal mucosa can provide contact between the cell and a possible carcinogenic particle.

SUMMARY

In rats fed chrysotile asbestos electron microscopy revealed asbestos crystals in many sites in the colonic epithelium and lamina propria. These particles appeared to have migrated through the mucus of the goblet cells into the cell itself and hence to the lamina propria. This observation may have significance in relation to carcinogenesis in the gastrointestinal tract.

Acknowledgment. The asbestos was kindly supplied by the National Gypsum Company through the National Asbestos Mines, Ltd., Quebec, Canada.

FIG. 1. Agar-embedded crystals, thinly sectioned. Note structure of concentric tubes at point where outer cylinder is absent (arrow). Also seen is the varied appearance where the section passed through different planes of the crystal (double arrows). X72,200.

FIG. 2. Chrysotile fiber in lumen with epithelial cells on either side. X31,100.

FIG. 3. Higher magnification of crystal in Figure 2 showing the characteristic structure. X53,700.

FIG. 4. Crystal in goblet cell (arrow). At this point the particle is intracellular but is in a mucous droplet. X29,300.



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FIG. 5. Fiber at the border of mucous droplet and cytoplasm. X46,800.

FIG. 6. Intracytoplasmic asbestos apparently "free" within the cytoplasm. X37,100.

FIG. 7. Particle (arrow) in interstitial space beneath an epithelial cell and above a smooth muscle cell. X37,100.

FIG. 8. Cluster of chrysotile fibers appearing to penetrate a smooth muscle cell in the lamina propria. Note variation in density of the longer crystal as the plane of section passed obliquely through it. X44,000.