

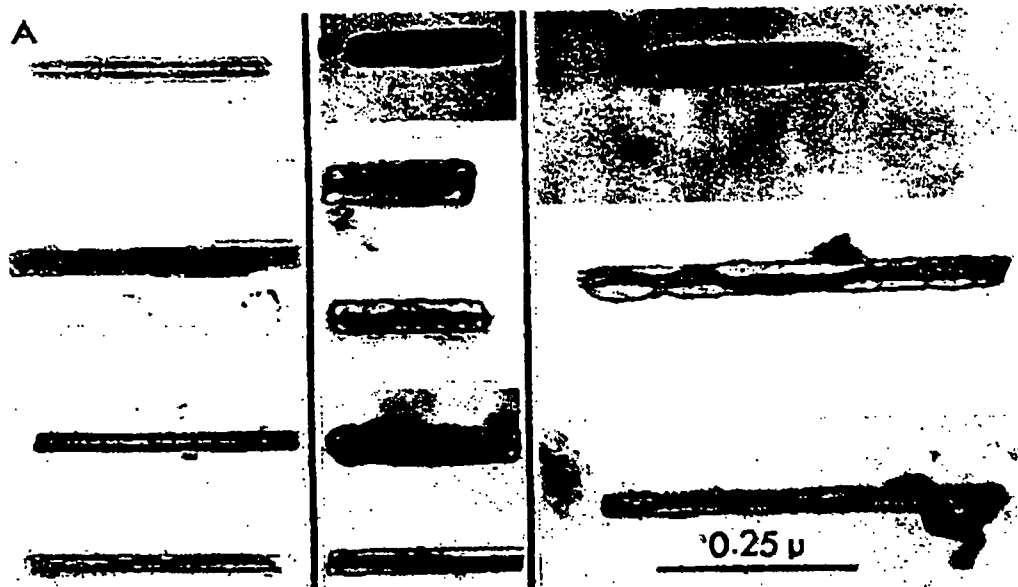


Fig 2.—Chrysotile morphology range in recovered mineral dusts. Rare well-formed fibrils with undeformed capillaries and thick, electron-dense walls without amorphous coatings, (A); more often, electron-dense wall encapsulated in amorphous coating (B); very often, fibrils with deformed internal capillaries and thin crystalline walls encapsulated in thick amorphous covering (C).

findings with those by electron microscopy was made. Study was further undertaken to determine whether the fibers found were or were not chrysotile asbestos.

Separation Techniques and Preparation.—From each of the 28 frozen lungs, 1 cc of tissue was cut and placed in a thick-walled centrifuge tube. A 40% KOH solution was added to the tube until the solution entirely covered the lung specimen (3 ml required). The centrifuge tubes were then placed in a hot water bath, and the water heated to boiling. Digestion was carried out for one hour starting from the time the water began to boil. After one hour very little lung residue remained in most instances. However, in some it was necessary to continue the digestion for an additional hour. It was found that this was sufficient to complete the digestion. The tubes were centrifuged at 15,000 to 17,000 rpm for approximately 30 minutes (head design indicates $F > 30,000 g$). The supernatant was next decanted, and the residue washed with distilled water. Washing, centrifugation, and decantation were repeated 3 times until the residue was free of KOH. Examination of the supernatant with a polarized light microscope indicated that no optically visible asbestos bodies or fibers were present in the supernatant after any of the spinning periods.

The residues were examined by polarized light microscopy. The mounting medium used in all cases was a highly viscous liquid which minimized particle migration²⁹; scanning was commonly done under 250X, 400X, and 500X magnification. High-magnification examination was occasionally undertaken at 1,000X. Each of the 28 cases examined microscopically showed some residual undigested organic materials present, despite the apparently "complete" digestion.

Tissue extracts were also prepared for study by electron microscopy. Small splits (approximately 1 mg) of the washed residues from the 28 specimens were pipetted into 28 smaller test tubes. Each of the latter 28 test tubes was filled with 2 ml of distilled water. The residue and medium were agitated (for dispersal) for 30 seconds. Small proportions of the dispersant residues were removed, and one drop pipetted onto a polyvinyl methylal (Formvar) coated 200-mesh copper electron microscope (EM) locator grid. The drop of water was allowed to remain quietly on top of the grid for 15 minutes to allow the settling of solid materials. At the end of that time, the liquid drop was drawn off with wetted filter paper.

The amount of material that actually settled out in this time onto the polyvinyl methylal grid was invisible to the unaided