

Comparative Studies on the Cytotoxicity of Amphibole and Serpentine Asbestos

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The chemical and physical properties of serpentine and amphibole asbestos are considered in the context of their interaction with tissue of the tracheobronchial tree and lungs. *In vitro* studies in cultures of several types are evaluated and work with the erythrocyte hemolysis system is reviewed. Although fibers of the two major mineral types differ substantially, it is likely they are modified by secretions and membranes of cells after inhalation to the respiratory tract. Investigations using virgin asbestos might not provide an accurate picture of events *in vitro*.

Asbestos is not one, but a family of, hydrated silicate minerals having a fibrous crystalline structure. The types of asbestos differ mineralogically and, as might be expected, these chemical and physical differences are reflected in the mechanism of interaction of fibers with cells. In this paper, we consider comparatively the characteristics of serpentine and amphibole asbestos and attempt to relate these features to the pathogenetic effects on tissues of the respiratory tract.

Structural and Chemical Properties

Serpentine asbestos derives its name from the pliable, curled, "serpentlike" property of the fiber (Fig. 1). Chrysotile, the only commercially important mineral of this type, comprises over 90% of the asbestos mined in the world today. The members of the amphibole asbestos group are more numerous, but have fewer commercial uses. The amphiboles (crocidolite, amosite, and anthophyllite) exhibit a coarse, fibrous structure (Fig. 2).

The mineralogy of asbestos is complex and incompletely defined. The types vary in major ele-

mental composition, but have a basic Si_xO_x composition. Within the individual classes of asbestos, structural variability is common. Moreover, inorganic and organic chemicals of a variety of types contaminate the mineral. Some of these foreign substances are found in association with the naturally occurring fiber, whereas others are added during milling and industrial processing.

The biological effects of the various asbestos minerals appear to relate to their chemical and crystallographic properties. The fundamental Si_2O_5 subunits of chrysotile are tetrahedra linked to form extensive thin sheets of fixed composition. On the apical oxygens are located hydrated magnesium molecules which account for the highly polar, basic property of the mineral. The layers of this silicate characteristically form concentric cylinders that parallel the fiber axis. In nature, these structures are arranged as loose bundles. This property accounts for the tendency of chrysotile to fragment into subfibrils and segments along both the long and short axes (Fig. 3).

Amphibole asbestos is formed of chains of tetrahedra linked into Si_4O_{11} units. The chains are four tetrahedra wide and are situated parallel to the fiber axis. Cations of various types bind the units, although these minerals cleave along parallel planes and are believed to break down into subunits in tissue (Fig. 4).

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FIGURE 1. Scanning electron micrograph of the serpentine asbestos, chrysotile. Note the variability in length and diameter of individual fibers as well as their twisted and curled "serpentlike" properties.

Asbestos in the Respiratory Tract

The fate of inhaled asbestos in the human respiratory tract is incompletely defined. It is recognized that relatively long fibers either are removed from the inspired air in the upper respiratory tract or eliminated by the mucociliary escalator system of the tracheobronchial tree. The aerodynamic properties of fibers are complex, but studies by Harris et al. (1) indicate that the depth of penetration of a fiber into the lung parenchyma relates to its cross-sectional diameter. Of importance is the fragmentation of fibers that is thought to occur after inhalation.

Because fibers of small size cannot be recognized in tissue by traditional morphological techniques, the distribution and density of these deposits in the lungs is undefined, and their biologic significance unclear.

Fibers of both serpentine and amphibole asbestos exhibit complex surface deposits of iron-containing proteins (ferruginous bodies) when observed in lung tissue by light microscopy. These fibers are believed to be chemically inert. It seems unlikely that similar "coats" form on small fiber fragments in tissue, but this remains to be determined. Studies in several laboratories have demonstrated the uptake of both albumin and sialic acid by chrysotile from biologic

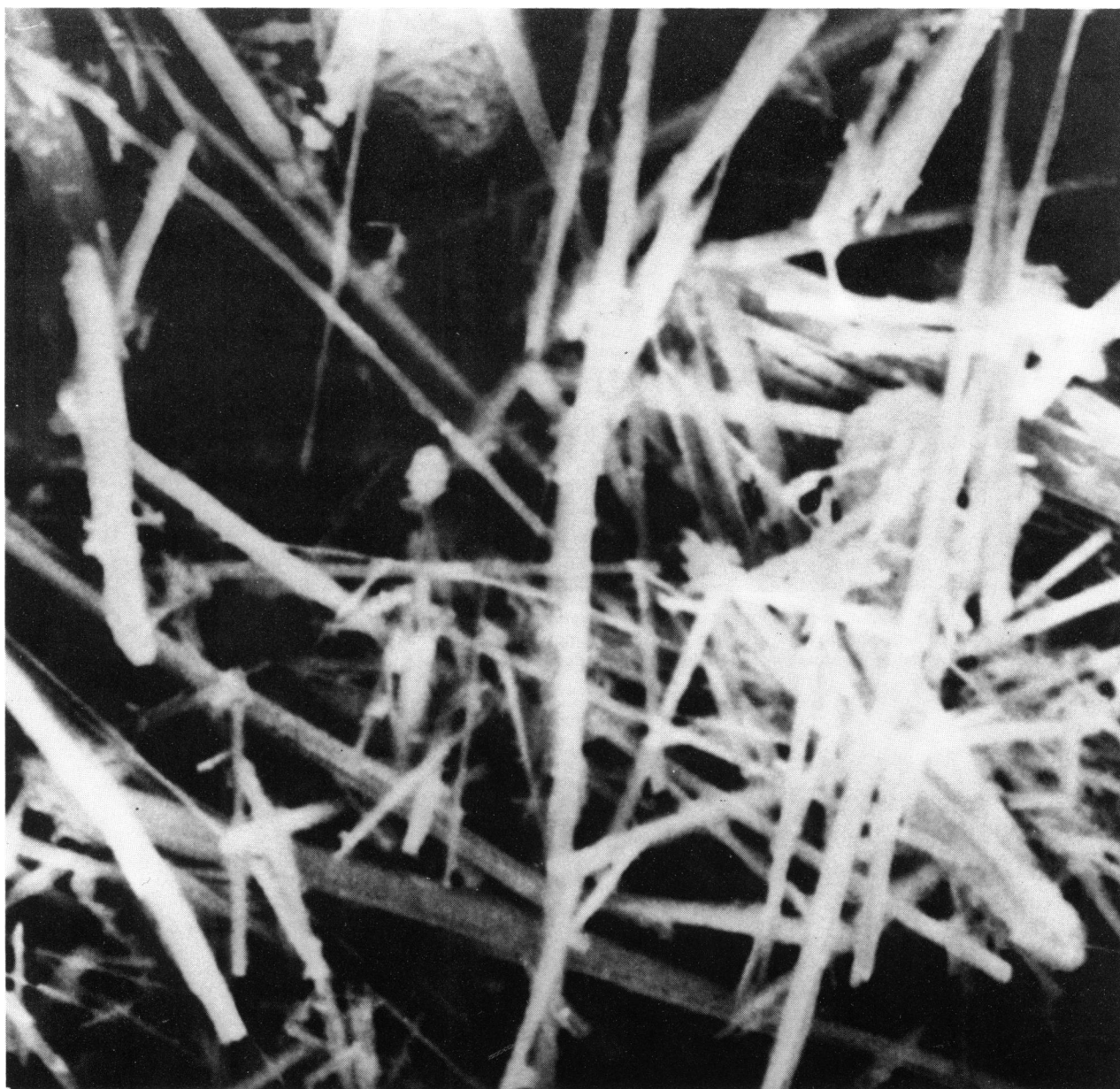


FIGURE 2. Scanning electron micrograph of the amphibole asbestos, crocidolite. As with chrysotile, the fibers vary in length and diameter. However, the amphibole fibers are relatively straight and rigid.

fluids (2-4), and we have shown that the plasma membranes of lysed erythrocytes and epithelial cells adsorb to the particle surface (5). These interactions are attributed to the cationic properties of the fiber. Amphiboles take up dipalmitoyl phosphatidylcholine *in vitro*, and it could be assumed on this basis that adsorption of surfactant to the fiber occurs in the respiratory tract (6). One might speculate that deposits of biologic material on the surface of fibers in the lungs affect the interaction with phagocytic cells.

Studies to address this specific question have not been carried out as of yet.

Acids and bases, respectively, affect the surface properties of serpentine and amphibole asbestos *in vitro*, and the capacity of the fiber to interact with the plasma membrane of cells is altered (5, 7). This effect is believed to result from the removal of the hydrate magnesium from the fiber surface. It is reasonable to assume that similar events occur *in vivo*, although at present information on the effects of respiratory

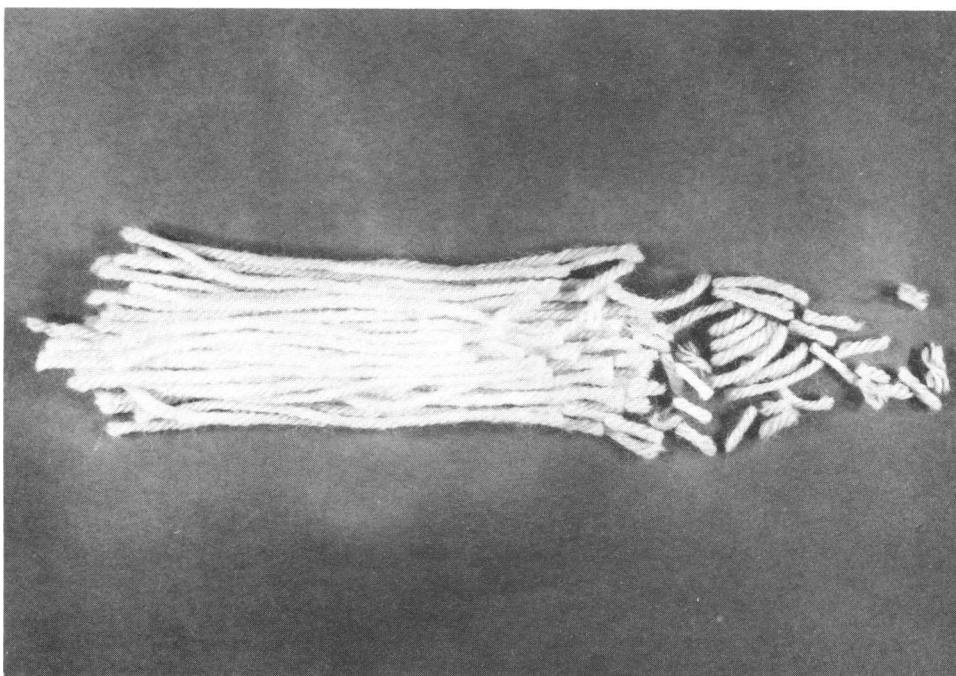


FIGURE 3. Hypothetical representation of the fragmentation of a chrysotile fiber into fibrils along the long and short axes. Chrysotile fibers are comprised of numerous subunits that fragment when exposed to physical forces and biological fluids *in vivo*.



FIGURE 4. Diagrammatic representation of an amphibole asbestos fiber as represented by a stack of 2 × 4 in. pine boards. Fibers of crocidolite also fragment into subunit fibrils when exposed to physical forces and biologic fluids *in vivo*.

tract secretions and fluids on the intrinsic structural and chemical integrity of the fiber is lacking. A decrease in the Mg:Si ratio of chrysotile fibers in pulmonary tissues has been demonstrated (8). This suggests that Mg^{2+} is removed in tissues, as is the case when the fiber is treated with acid *in vitro*.

Interactions of Asbestos With Cells

It generally is assumed that alveolar macrophages phagocytize the bulk of the small asbestos particles entering the acini of the lungs. Because chrysotile asbestos is cytotoxic for these cells *in vitro* (4), one might conclude that injury to the macrophages also occurs in the respiratory tract. Whether or not this is a correct assumption is a matter for debate. As briefly discussed above, the surface properties of the fiber doubtless are changed by their interaction with respiratory secretions. Thus, laboratory studies using virgin asbestos may not appropriately simulate events occurring *in vivo*. Studies by Brody et al. (9) have demonstrated phagocytosis of chrysotile by macrophages and type I pneumocytes in the lung of rats several days after inhalation exposure. These cells fail to reveal evidence of cytotoxicity when examined ultrastructurally.

Erythrocytes

The interaction of asbestos with mammalian erythrocytes *in vitro* has been examined by a number of investigators in an effort to assess the mechanism of cell injury (4-7, 10). We have pursued the model using scanning electron microscopy to define the structural alterations that occur in cells interfaced with fibers. As can be seen in Figure 5, dramatic configurational changes become evident in the erythrocytes shortly after chrysotile is added to a cell suspension. These studies strongly suggest that hemolysis occurs, at least in part, consequent to physical distortion of the red cell membrane. Injury to the cell also might result from alterations in the intrinsic molecular structure of the bilipid plasma membrane of the cell as has been suggested by Harington (4). Experimental data from our laboratory suggest that both mechanisms play a role in hemolysis (5).

When virgin chrysotile asbestos is mixed with erythrocytes *in vitro*, the cell membrane and the fiber interact intimately and the cell leaks hemoglobin. This fails to occur when fibers are "coated" with either albumin or sialic acid, and after acid digestion. The amphibole asbestos, crocidolite, hemolyzes red blood cells after prolonged periods of exposure *in vitro*. The mechanism is unclear. Configurational

changes in the cells fail to develop and the interaction of fiber with erythrocytes is less intimate. (Fig. 6). Since the fibers lack the strong positive charge of chrysotile, these observations suggest that ionic surface properties or the asbestos fibers are intrinsic to the cellular interactions of asbestos.

Cultured Fibroblasts and Pulmonary Macrophages *in Vitro*

Studies concerned with the toxicity of asbestos for cultured cells have been reported from a number of different laboratories. Although general conclusions can be drawn from a review of the literature, individual reports conflict somewhat with another. It seems likely that the contrasting results obtained by various investigators reflect differences in the cell culture systems employed and subtle variables in experimental protocols. The intrinsic properties of the asbestos particle also are important. It is apparent that results with fibers of different length cannot be compared. Moreover, weight would seem to be a poor criterion upon which to base an assessment of dosage effects inasmuch as the surface properties of the fibers appear to be critical determinants of cellular injury.

The work of several investigators clearly demonstrates the cytotoxicity of chrysotile both for fibroblasts (11-13) and pulmonary macrophages (14, 15). The effect on fibroblasts doubtless is consequent to the surface charge of the particle and its Mg^{2+} content. Amphiboles appear to exhibit variable toxicity for reasons that are not evident. The effect could be attributable to either inorganic or organic surface contaminants, but this is uncertain. The mechanism of macrophage cytotoxicity is more complex, inasmuch as the fibers interact with membranes of both the cell surface and the phagolysosomes. Release of lysosomal enzymes consequent to ingestion of the particle has been amply demonstrated. Since many fibers are too long to be taken up by cells, there might be an accentuated effect on enzyme release due to leakage from the phagosome (exocytosis).

Tracheal Epithelial Cell Monolayer Cultures

Figure 7 summarizes the results of studies carried out in this laboratory using monolayer cultures of epithelial cells derived from the mucosa of the hamster trachea (16). These cells exhibit the differentiated properties of respiratory epithelial cells for they produce extracellular mucins (17), and contain both cilia and mucous vacuoles (16). As can be seen, chrysotile inhibits growth of cells over a range of dosages when the mineral is introduced at the time cells are plated (18). Equivalent amounts of crocidolite have no effect. Thus, observations using differ-

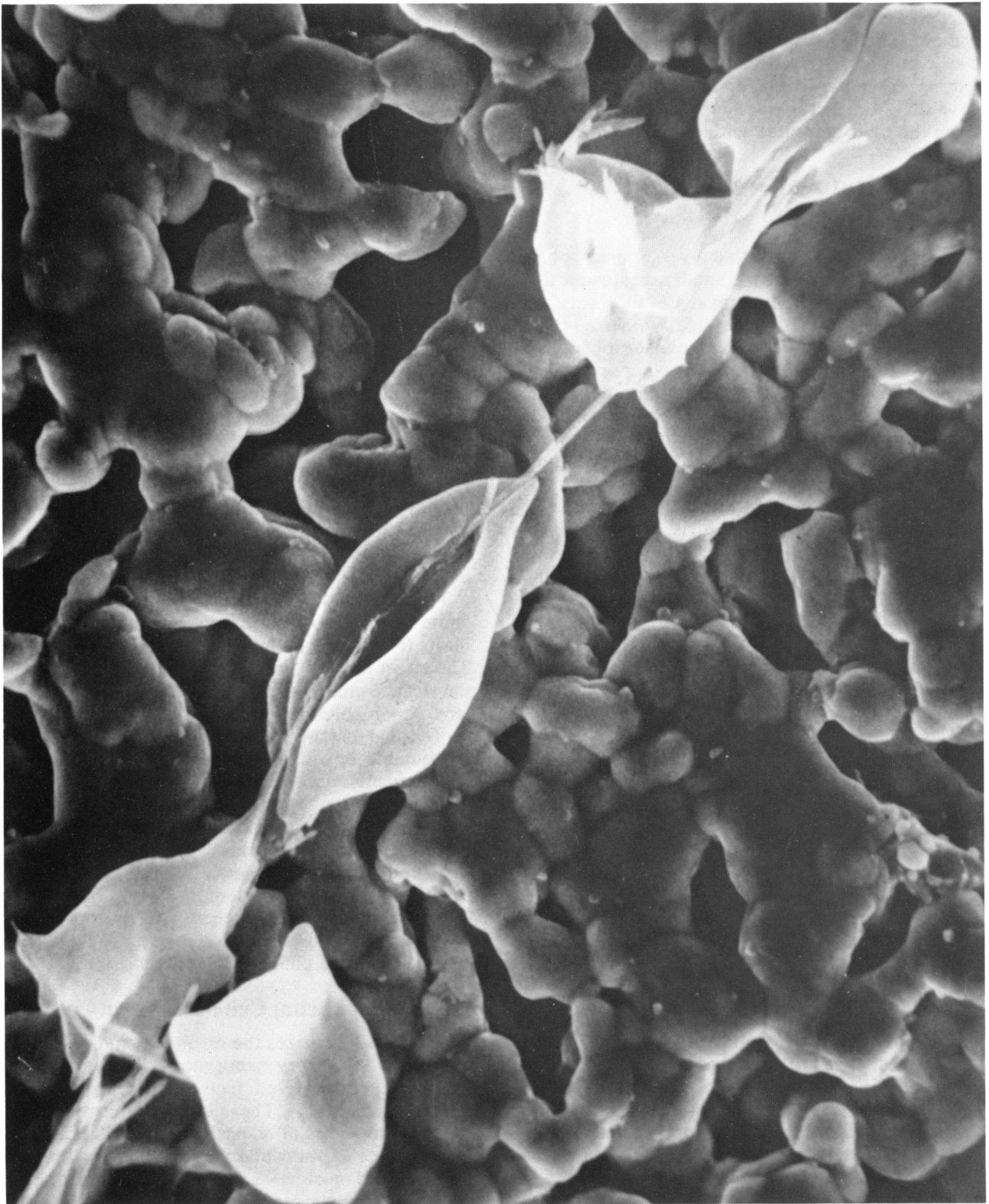


FIGURE 5. Chrysotile fibers interacting with human erythrocytes. The fibers are engulfed by the cells which approximate them. This is believed to be consequent to the positive charge of the chrysotile fiber interacting with the negatively charged polysaccharide and protein components of the plasma membrane of the erythrocyte. Chrysotile is highly cytolytic.



FIGURE 6. Scanning electron micrograph illustrating the interaction of crocidolite with erythrocytes. The physical relationship contrasts strikingly with chrysotile. Amphibole fibers exhibit a relatively negative surface charge, and therefore would not be expected to alter the erythrocyte plasma membrane after prolonged exposure.

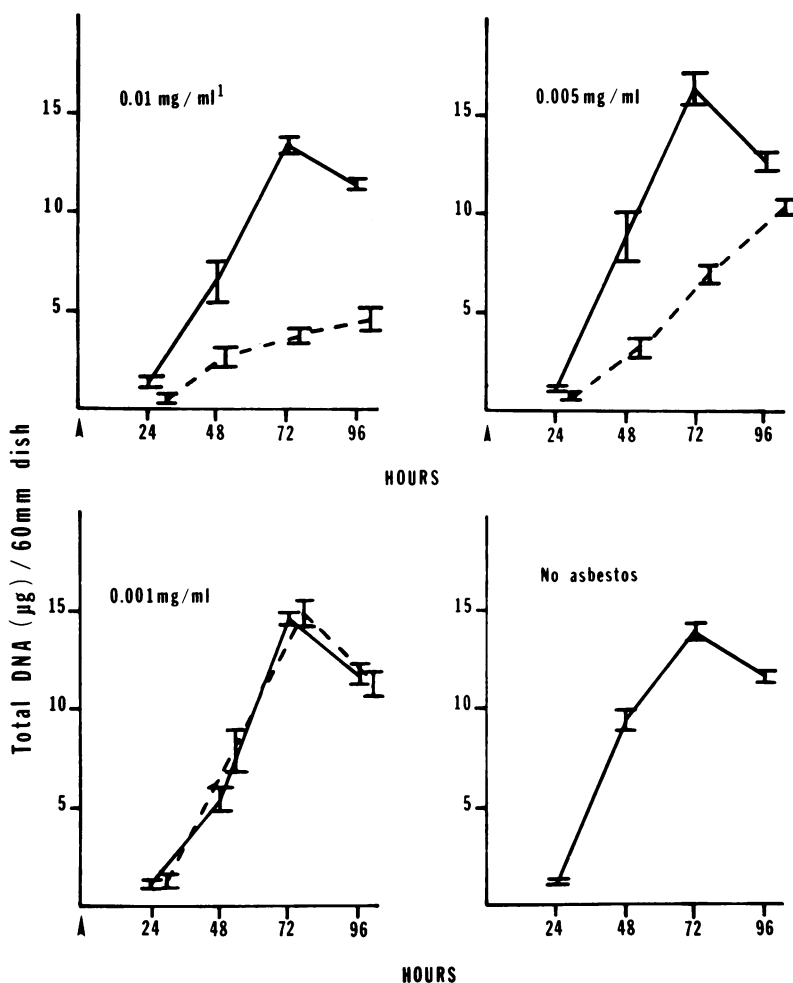


FIGURE 7. Effect of (- -) chrysotile and (—) crocidolite on growth of monolayer cell cultures derived from the hamster tracheal epithelium. The effect of chrysotile is attributed to cytotoxicity.

entiated epithelial cells in monolayers correspond with the results of studies by other investigators using both fibroblasts and macrophages.

Tracheal Epithelial Organ Cultures

In a recent report, Mossman and Craighead described the events that occur when explants of hamster trachea are exposed to crocidolite (19). These studies document the cytotoxicity of the mineral for the mucociliary mucosa. This effect appears to be consequent to a direct interaction of the fibers with these highly differentiated cells. Interestingly enough, basal cells were not affected, and an intact layer was observed adjacent to the basal lamina after the superficial cells had sloughed. These cells phagocytized the asbestos particles and responded by undergoing hyperplasia and metaplasia. When chrysotile was introduced into cultures of trachea,

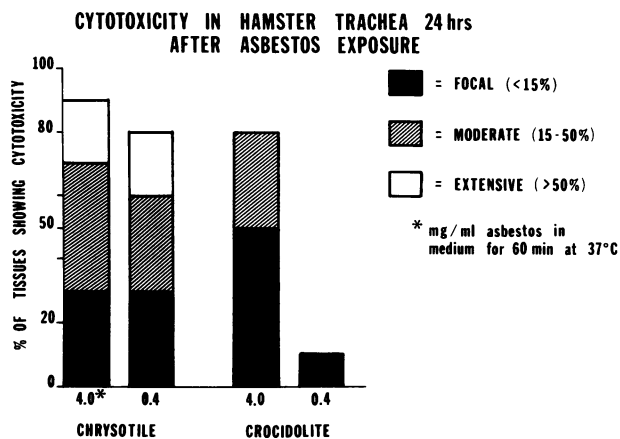


FIGURE 8. Effect of chrysotile and crocidolite on the intact differentiated respiratory mucosa of hamster tracheal organ cultures. Concentrations indicate the amount of asbestos (mg/ml) in the medium.

cytotoxicity was more pronounced. (Fig. 8). As with crocidolite, the basal cells remained intact. Thus, the differentiated epithelium responded to both types of asbestos in a comparable fashion.

Discussion and Summary

Asbestos is a generic term referring to a family of hydrated silicate minerals having a basic fibrous composition. Serpentine and amphibole asbestos differ structurally and in chemical composition. These features are reflected in the biologic effects summarized here.

The cytotoxicity of the serpentine, chrysotile, appears to relate to its magnesium content. Most, if not all, of the effects are believed to be consequent to the strong positive charge this cation introduces on the fiber surface. Chrysotile avidly interacts with the membranes of the cell surface and lysosomes. The mechanism whereby it injures these structures remains to be defined.

In biological systems, chrysotile would appear to interrelate dynamically with its environment. The cytotoxic properties of the mineral are attenuated by a wide variety of naturally occurring anionic substances, and the highly reactive Mg^{2+} ions are leached during residence *in vivo*. Thus, inhaled chrysotile in respiratory tract tissue is not comparable to the virgin mineral used in most *in vitro* model systems. The amphiboles, amosite and crocidolite, are less cytotoxic than chrysotile. These minerals also absorb biologic substances from fluids and tissue *in vivo*. Thus, the surface properties of the amphiboles also change in the respiratory tract.

The cytotoxic effects of the various asbestos types may, in part, be consequent to the inorganic and organic materials that contaminate the basic minerals. This is a hypothetical consideration, however, for conclusive evidence to indicate that these substances injure cells has not been published as of yet. Asbestos triggers the alternate pathway of the complement system. It remains to be determined whether or not complement-mediated injury is important. Moreover, it is not known if the interaction with complement enhances uptake of particles by alveolar macrophages, cells having plasma membrane C_3 receptors.

In our experimental organ culture system, both crocidolite and chrysotile were cytotoxic for the differentiated mucociliary cell layer of the tracheal mucosa. At present it is not clear why the basal cell layer is resistant to the injurious effects of asbestos. However, as discussed above, the fiber surface is modified by its interaction with biologic substances. This may render the particle nontoxic. Whatever the mechanism, the lack of an apparent effect of asbes-

tos on basal cells is important for the injured epithelium regenerates from the basal layer. It is of interest in this regard that hyperplasia and squamous metaplasia develop in tracheal organ cultures as a consequence of their interaction with the amphibole asbestos, crocidolite and amosite.

The experimental work briefly summarized above indicates that the surface properties and configurational forms of both amphibole and serpentine asbestos are altered after inhalation into the respiratory tract. This conclusion should be considered in the design of *in vivo* laboratory experiments to examine the pathogenesis of asbestos-associated diseases.

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