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ASBESTOS: MECHANISMS OF TOXICITY AND CARCINOGENICITY IN THE RESPIRATORY TRACT

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INTRODUCTION

"Asbestos" is a generic name for a group of minerals well-known for their adverse effects on the respiratory system. After prolonged periods of inhalation, asbestos produced fibrosis of the lung (i.e. asbestosis) and two types of malignancies. Mesothelioma, a tumor of the serosal cells lining the pleural and peritoneal cavities, is an extremely rare cancer in the general population but can account for as many as one in thirty of the malignancies found in asbestos workers (1). The second type of cancer produced by asbestos is bronchogenic carcinoma, a tumor of the epithelial cells lining the upper airways. This cancer occurs with a high incidence in both asbestos workers and smokers in the general population (2).

This review considers new experimental approaches, developed mainly in the past decade, that have been aimed at elucidating the mechanisms of asbestos toxicity. First, we review the physicochemical properties of asbestos that relate to biological activity. Subsequently, we discuss the various

asbestos-induced cellular interactions and responses that might predispose target cells to both injury and neoplastic transformation. Recent monographs related to this topic have appeared (3, 4) and references to the earlier literature can be found in the comprehensive review by Harington et al (5).

PHYSICO-CHEMICAL PROPERTIES OF ASBESTOS

Asbestos is a group of hydrated silicates that have a "fibrous" morphology (defined as a $>$ 3:1 length to width ratio). Two main subgroups are recognized: serpentine and amphibole. Chrysotile ($3MgO \cdot 2SiO_2 \cdot 2H_2O$), a serpentine asbestos that accounts for approximately 95% of the asbestos used worldwide, is a pliable, curly fiber made up of bundles of smaller fibrils (Figure 1*a*). These subunits are comprised of either multi- or single layers of silica and brucite (MgO) that form concentric, scroll-like tubes (Figure 2). In neutral aqueous media, the brucite becomes ionized and gives the fiber a positive charge. In contrast, the amphiboles are a family of straight, rodlike fibers that are either neutral or slightly negative in charge at a neutral pH. The different members of the amphibole family are distin-

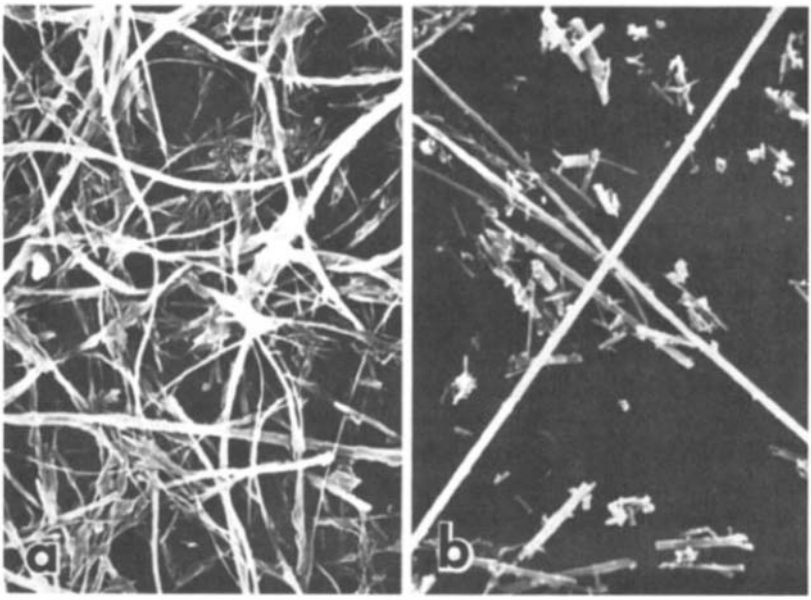


Figure 1 Scanning electron micrographs of International Union Against Cancer (U.I.C.C.) reference samples of Canadian chrysotile (*a*) and Rhodesian crocidolite (*b*) asbestos. Note the different structural features and heterogeneity of fibers both in length and diameter. Photomicrographs were furnished by Mr. Craig Woodworth, Department of Pathology, University of Vermont College of Medicine.

guished from each other by the hydrated cations between the silica layers (Figure 1*b*). Crocidolite ($Na_2O \cdot Fe_2O_3 \cdot 3FeO \cdot 8SiO_2 \cdot H_2O$), the principal amphibole of economic and health significance, is made up of parallel chains of linked, tetrahydral groups having a Si_4O_{11} composition along the fiber axis. Both types of asbestos are heat-resistant and possess great tensile strength. These desirable properties account for the many industrial uses of asbestos.

The chemistry of asbestos is complex. A number of trace metals, including Ni, Fe, Sb, Cr, and Co, are associated with native chrysotile and crocidolite. These elements can be leached from the fibers after treatment with inorganic and organic acids. Crocidolite is acid-stable whereas chrysotile is more acid-labile. Both fibers can undergo chemical alteration in the lung. For example, chrysotile loses magnesium after intrapleural inoculation into animals (6), inhalation by humans (7, 8), or phagocytosis by rabbit alveolar macrophages in vitro (8). These processes could account for the faster dissolution and disappearance of chrysotile from the lung in comparison to amphiboles, which remain for a longer time period (9). Both types of fibers appear to be coated in vivo with pulmonary macromolecules such as lung surfactant.

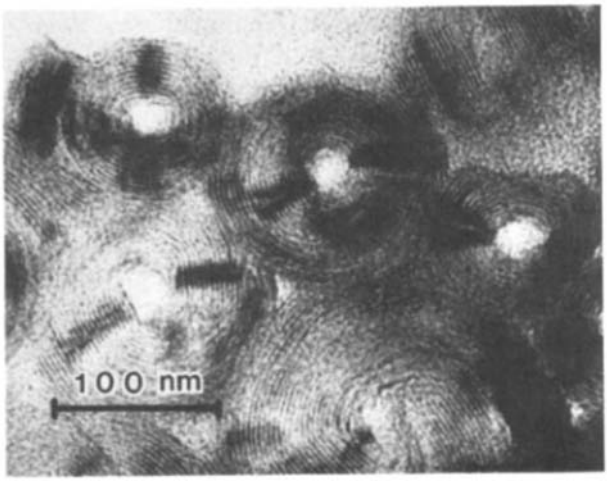


Figure 2 The scroll-like configuration of chrysotile asbestos is illustrated by transmission electron microscopy at high resolution. This chrysotile is from the Thetford Mines in Quebec, Canada and shows an interesting morphology in that multiple rather than single sheets are rolled up into the classical scroll. The photomicrograph was provided by Mr. James S. Webber and Ms. Inga S. Green, Division of Laboratories and Research, New York State Department of Health.

TOXIC EFFECTS OF ASBESTOS

The occurrence of mesothelioma in man generally has been associated with exposures to crocidolite. However, experiments by Wagner (10–12) and Stanton (13–16) have shown that a variety of fine fibers including crocidolite, chrysotile, borosilicate glass, mineral wool, and silicon carbide produces mesothelioma when injected into the pleural cavities of rats. The incidence of tumor indication was associated with fibers $> 8\ \mu m$ in length and $< 1.5 \mu m$ in diameter, whereas pulverized materials were less active. These results were interpreted to mean that physical properties (e.g. fiber size) were more important determinants of activity than the chemical properties of the fibers.

The potentiating effects of exposure to asbestos and cigarette smoking on the development of bronchogenic carcinoma have been documented extensively and verified by both epidemiologic (17–21) and experimental studies (22–31). Cigarette smokers have an approximately 10-fold higher incidence of bronchogenic carcinoma than nonsmokers in the general population (32). However, asbestos workers who smoke have a 6- to 9-fold higher incidence of lung cancer than these individuals. In contrast, nonsmokers occupationally exposed to asbestos have a substantially smaller risk (i.e. 2.5- to 7-fold less than smokers in the general population). Crocidolite is implicated more than other types of asbestos in the development of bronchogenic carcinoma in man (33).

Long-term inhalation studies have shown that asbestos alone can induce bronchogenic carcinoma in rodents (reviewed in 30). However, few malignancies appear despite protracted regimens of exposure. Thus, comparative dose-response data for different types of fibers are not available. A striking increase in neoplasms can, however, be obtained after intratracheal instillation of chrysotile mixed with benzo(a)pyrene, a carcinogenic polynuclear aromatic hydrocarbon (PAH) found in cigarette smoke (23–26). Similar results are obtained with tracheal organ cultures exposed to crocidolite and PAH after their implantion into syngeneic recipients (27–30). Under these circumstances, asbestos alone does not produce tumors.

CELLULAR MECHANISMS OF ASBESTOS TOXICITY

The epidemiologic data and whole animal studies summarized above provide a framework for further analysis of the cellular mechanisms of asbestos toxicity. A variety of biochemical, morphologic, and in vitro techniques have been utilized to document events that might be associated with asbestos toxicity at the cellular level. Asbestos presents unusual problems to the experimental toxicologist because, unlike most chemicals that are distrib-

uted homogeneously in the biological medium, asbestos is an insoluble mineral with physical characteristics that affect toxicity. Thus, the toxic effects of asbestos tend to be localized and physically limited to the cells it contacts.

Surface Charge and Lysis of Red Blood Cells (RBC)

Red blood cells lyse after exposure to asbestos, and the release of hemoglobin can be quantified spectrophotometrically. Thus, hemolysis has been used as an index of membrane damage by asbestos (34). We have shown that the surface charge on fibers, as measured by the zeta potential, is related directly to the fibers' hemolytic activity (35, 36). When chrysotile fibers are leached with 0.1 M HCl, the zeta potential is decreased concomitantly with its hemolytic activity. In contrast, the hemolytic potential for crocidolite increases as the fibers become more negatively charged after leaching (Figure 3). Incubation of fibers with dipalmitoyl phosphatidycholine (DPPC), the main component of pulmonary surfactant, decreases the zeta potential of both chrysotile and crocidolite with a proportional inhibition of hemo-

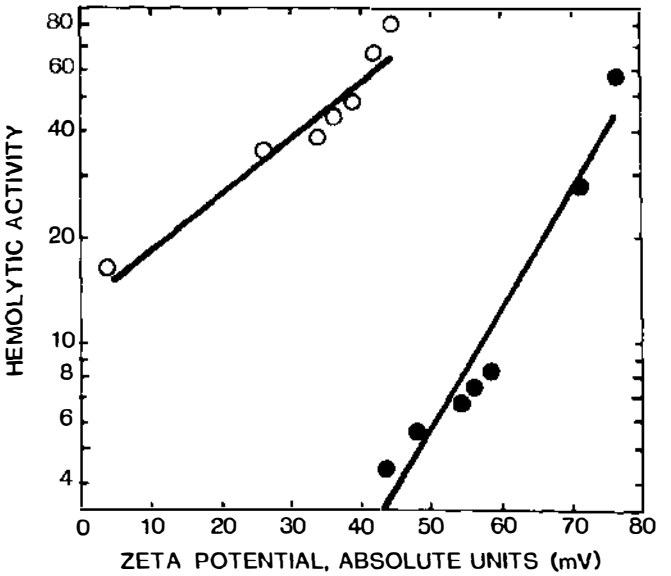


Figure 3 The zeta potential (Z) correlates ($p < .01$) with the hemolytic activity of UICC chrysotile (○) and crocidolite (●). Zeta potentials at pH 7.4 for both types of asbestos are similar in absolute magnitude but opposite in polarity (+44.5 mV for chrysotile and –43.5 mV for crocidolite). After leaching of fibers, Z decreases for chrysotile and increases for crocidolite. Reproduced from (37).

lytic activity (37). These results correlate fiber charge with the capacity of the fibers for membrane damage.

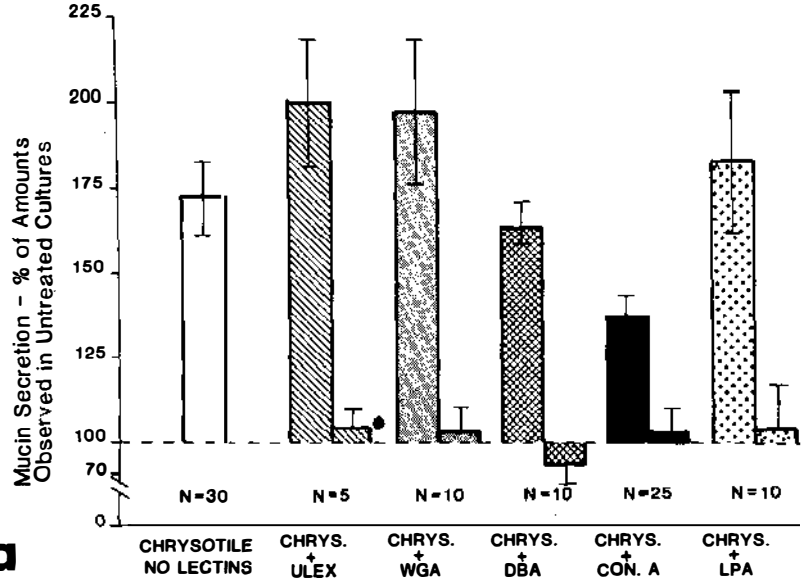
Cell Membrane Receptors for Asbestos

Jaurand et al have shown that chrysotile adsorbs both proteins and phospholipids from RBC membranes (38). These events appear to modify the hemolytic capacity of the fibers (39, 40). Treatment of RBC with neuraminidase, an enzyme removing sialic acid from the cell surface, reduces the hemolytic activity of chrysotile (41), which suggests that asbestos interacts with specific sites on the plasma membrane to induce hemolysis. The external membrane of the RBC contains a variety of unique glycoproteins including glycophorin, its major sialoprotein. Thus, we have used another in vitro model to elucidate membrane receptors that may be associated with asbestos toxicity in the respiratory tract.

Alcian blue-periodic acid Schiff positive mucin-containing granules rapidly appear in mucin cells of rodent airways after in vivo or in vitro exposure to chrysotile (42). This phenomenon (i.e. hypersecretion) can be studied in rodent tracheal organ cultures. Tissues are prelabelled with ^{3}H-glucosamine, a precursor of mucin, and secretion of label into the culture medium before and after exposure to chrysotile can be quantified by scintillation spectrometry. We examined different lectins, i.e. proteins binding to selected carbohydrate residues on the plasma membrane, and enzymes for their ability to block chrysotile-induced secretion of mucin.

Pretreatment of tracheal explants with Concanavalin A, a lectin binding to α-D-mannose and α-D-glucose sites, partially blocked chrysotile-induced secretion of mucin, whereas lectins that blocked residues of α-D-N-acetylgalactosamine, α-D-N-acetylglucosamine, α-L-fucose, and sialic acids were ineffective (Figure 4*a*). The addition of phospholipase A_2

Figure 4 Exposure of tracheal explants to chrysotile asbestos induces enhanced secretion of mucin into the culture medium. (*a*) Hypersecretion caused by asbestos is decreased ($p < .05$) by pre-addition of Concanavalin A, a lectin that binds selectively to α-D-mannose and α-D-glucose residues on the plasma membrane. In contrast, no inhibition is observed with lectins blocking sites of sialic acid (i.e. Limulus polyphemus agglutinin, LPA), N-acetylgalactosamine (i.e. dolichos biflorus agglutinin, DBA), N-acetylglucosamine (wheat gern agglutinin, WGA), and L-fucose (ulex europaeus agglutinin, ULEX). Mucin secretion is expressed as a percentage of increase over control cultures (i.e. those without exposure to asbestos). Bars with asterisks (*) show mucin secretion in cultures exposed to lectins without chrysotile. (*b*) Pretreatment of tracheal explants with carboxypeptidase A and phospholipase A_2 inhibits chrysotile-induced secretion of mucin ($p < .05$) whereas neuraminidase, an enzyme cleaving sialic acids, is without effect. These observations suggest the interaction of chrysotile with both glycoproteins and glycolipids on the cell surface.



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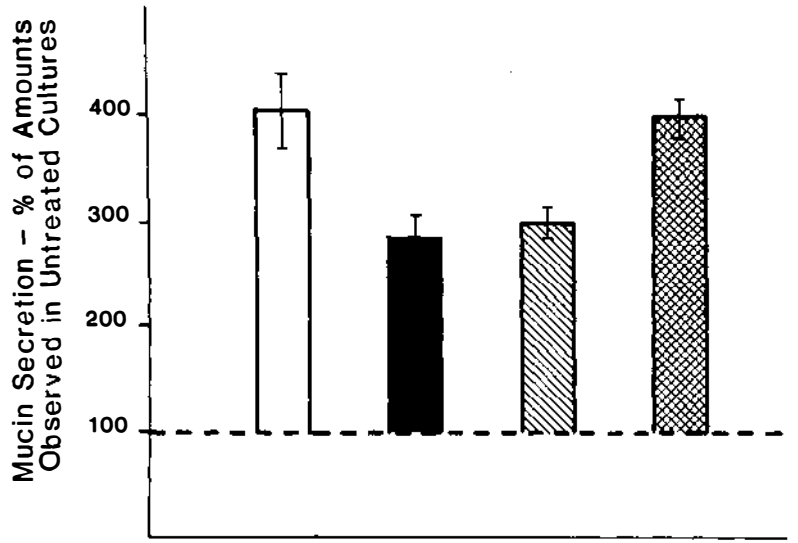

b

and carboxypeptidase A, but not neuraminidase, inhibited chrysotile-induced hypersecretion in a dose-dependent manner, although the normal secretion rate of mucin was not altered (Figure 4*b*). Interpreted in context, these results indicate that chrysotile interacts with glycoproteins and glycolipids containing terminal residues of α-D-mannose and α-D-glucose to induce hypersecretion.

Our negative results with neuraminidase, an enzyme that inhibits chrysotile-induced hemolysis, suggest that receptor-like mechanisms triggering cellular responses to asbestos can differ for various cell types. However, surface charge still appears to be an important determinant of both hemolytic and hypersecretory activity. For example, crocidolite and leached chrysotile do not cause hypersecretion, but montmorillonite, a nonfibrous mineral with a positively charged surface edge, and various polycations do (43).

Cytotoxic Alterations in Mammalian Cells In Vitro

Although the association of asbestos with the plasma membrane causes lysis of RBC, mammalian cells can adjust to fiber-induced damage by various mechanisms. Most investigators have explored the interaction of asbestos with isolated alveolar macrophages, a cell type that phagocytizes asbestos after inhalation (44). When added to cultures of macrophages, chrysotile initially causes a depolarization of cells, indicating an increase in membrane permeability (45). Repolarization occurs with time, suggesting repair and recovery processes. Selective release of lysosomal and cytoplasmic enzymes also occurs after addition of asbestos to cell cultures (46, 47). In macrophage-like cells, the degree of cell contact and phagocytosis, documented by time-lapse microcinematography, correlates directly with cytotoxicity, as measured by a decrease in the number of viable cells. In contrast, cultures of fibroblasts, a typically nonphagocytic cell type, are relatively resistant to asbestos (48).

Chrysotile and montmorillonite are more lethal to cells than a variety of uncharged minerals (49), but toxicity is reduced substantially when larger fibers and particles are milled to a submicron size (50, 52). These results not only confirm again the importance of charge as a determinant of toxicity, but also implicate the importance of fiber dimension. The greater toxicity of longer fibers might be due to the inability of macrophages to engulf and inactivate charged sites on longer fibers (Figure 5a). Under these circumstances, alterations in membrane permeability and leakage of lysosomal and cytoplasmic enzymes appear to result in cell death (46, 47).

Prolonged exposure to asbestos results in accumulation of macrophages and inflammatory cells in the airspaces of the lung (44). One consequence of this inflammatory process is the release of oxygen free radicals, which

Figure 5 Long ($> 10\mu m$) fibers of asbestos are phagocytized incompletely by macrophages. A scanning electron micrograph of crocidolite fibers (arrows) in macrophages on the surface of a tracheal explant.

causes peroxidation of membranes and damage to macromolecules such as DNA (53). To determine if oxygen free radicals are important in asbestos-induced cytotoxicity, superoxide dismutase (SOD), an enzyme that converts the superoxide radical (O_2^-) to H_2O_2 and oxygen, and catalase, which converts H_2O_2 to O_2 and H_2O, were added alone and in combination with sized preparations of long ($> 10\mu m$) and short ($< 2\mu m$) chrysotile to cultures of tracheal epithelial cells. Control cultures received chrysotile alone. Cytotoxicity then was measured at 24 h intervals by determining the release of ^{75}Selenium, an indicator of membrane damage, from prelabelled cells. In addition, we measured the amount of protein in cultures as an index of cell growth.

Exogenous SOD prevented both the release of ^{75}Selenium and decreased amounts of protein observed with use of long, but not short chrysotile (Figures 6*a*, *b*). In contrast, catalase was not protective. These results suggest that longer chrysotile fibers, which are more cytotoxic than shorter fibers at equal concentrations, facilitate the generation of superoxide. Alternatively, levels of endogenous SOD could be altered after exposure to asbestos. For example, SOD is decreased initially after addition of chryso-

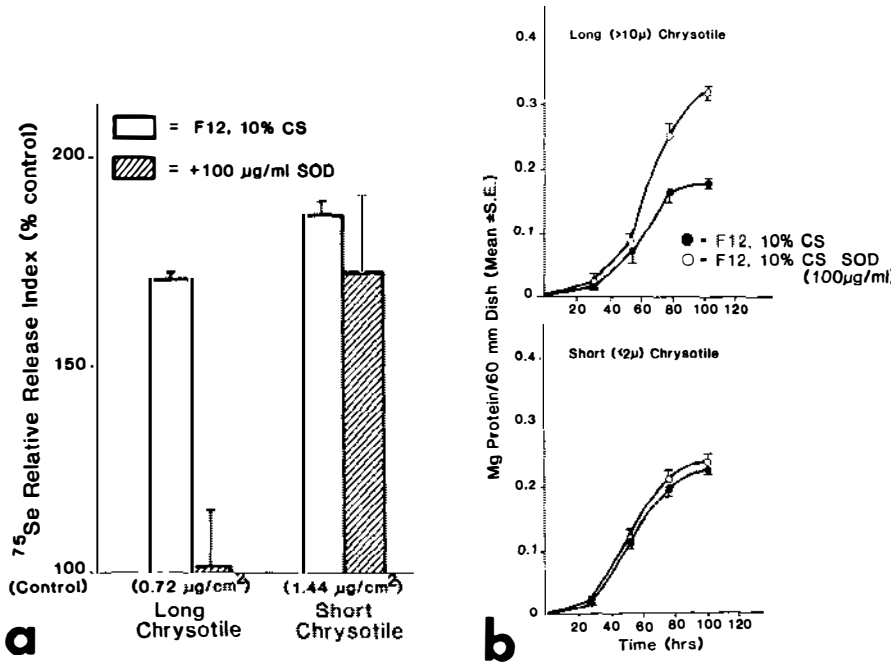

Figure 6 After addition of superoxide dismutase (SOD) to cultures of tracheal epithelial cells, the cytotoxicity of long (>10 μm), but not short (< 2 μm), fibers of chrysotile is inhibited as determined by a decrease in asbestos-induced release of ^{75}Selenium, an indication of membrane damage (*a*). The growth inhibitory effects of longer fibers also are prevented (*b*).

tile and crocidolite to cultures of tracheal epithelial cells, but activity increases above normal levels at 96 h after exposure (54).

TUMOR-PROMOTING EFFECTS OF ASBESTOS IN THE RESPIRATORY TRACT

A consequence of injury to epithelial cells of the airways is their replacement by basal cells, the presumed progenitors of bronchogenic carcinoma. In the next section, we discuss the properties of asbestos that suggest its classification as a tumor promoter in the respiratory tract.

Carcinogenesis is thought to be a multifaceted process consisting of sequential stages of initiation and promotion. These processes were first shown in the mouse skin, but are believed to occur in a variety of organs including liver and respiratory tract (55). An initiator is defined as an agent capable of interacting with DNA, an event resulting in a cell that is prepared for progression to malignancy. In contrast, promoters are substances

that lack significant carcinogenic activity but result in the enhancement of tumors when applied after a subcarcinogenic dose of an initiator.

Topping & Nettesheim (31) have shown the promoting effect of chrysotile on tumor development in rodent tracheal grafts exposed to the PAH, dimethylbenz(o)anthracene (DMBA). In these studies, asbestos enhances the tumor incidence of subcarcinogenic amounts of DMBA, although it is not carcinogenic alone at these doses. However, when asbestos is applied to tracheal grafts at much higher concentrations, a low incidence (5%) of squamous cell carcinoma is observed (56).

To study the tumor-promoting properties of asbestos in greater detail, we have used tracheal organ cultures and cloned lines of tracheal epithelial cells (57) to document morphologic and biochemical interactions between asbestos and respiratory epithelium. After precipitation of nontoxic amounts of either chrysotile or crocidolite on tracheal explants, larger fibers are removed by mucociliary action, whereas smaller fibers occasionally lodge on the surfaces of nonciliated cells (58). These fibers are either phagocytized by epithelial cells or transported between superficial cells to basal cells and ultimately the submucosa where fiber-laden macrophages often are observed (Figure 7). Thus, both types of asbestos penetrate the intact respiratory epithelium and reach basal cells from which neoplasms can originate. Phagocytosis of asbestos by alveolar epithelial cells in vivo has, in fact, been shown by Suzuki (59, 60).

Tracheal epithelia in organ cultures exposed chronically to asbestos exhibit basal cell hyperplasia. With chrysotile, proliferative changes are transient, and sloughing of superficial cells is followed by replacement with a normal mucociliary epithelium. Crocidolite, however, encourages the progression of basal cell hyperplasia to squamous metaplasia, i.e. the conversion of a differentiated epithelium to a squamous, cornified layer (61). Metaplasia is observed also with other rodlike fibers, including borosilicate glass (62) and amosite asbestos (61).

The appearance of squamous metaplasia has ominous implications for the development of bronchogenic carcinoma. Interruption of the mucociliary escalator might increase retention of asbestos and carcinogens, in general, in the respiratory tract. Longer fibers normally cleared by ciliary action then are more apt to penetrate the squamous epithelium and come in contact with basal cells. Should one of these cells be initiated (i.e. premalignant), a stimulus to proliferate might give it a selective advantage over normal cells.

Morphologic evidence of tumor promotion by asbestos is accompanied by biochemical changes in epithelial cells. After exposure to asbestos, increased incorporation of ^{3}H-thymidine by tracheal epithelium occurs, indicating increased DNA synthesis (61). Chrysotile and crocidolite also

Figure 7 Asbestos (arrows) is phagocytized by airway epithelial cells in vitro (*a*). Thereafter, asbestos-containing phagosomes fuse with lysosomes (L), which stain positively for acid phosphatase (*b*). Fibers are transported both intracellularly and through widened gaps between cells to the basal layer (*c*). At this juncture, fibers enter the submucosa (S) and are phagocytized by macrophages (M) (*d*).

produce dose-dependent increases in ornithine decarboxylase (ODC) activity in tracheal epithelial cells (63). ODC, the first and rate-limiting enzyme in the synthesis of polyamines, has been associated with other tumor promoters (64) and cell proliferation in general. An increase in ODC activity occurs concomitantly with a mitogenic response in tracheal epithelial cells exposed to asbestos and is potentiated when fibers are added in fresh serum-containing medium. Thus, some nutritional components appear to have a synergistic role in asbestos-induced proliferation.

Although squamous metaplasia often is considered a premalignant alteration, this lesion can be reversible. It has been reported that analogs of vitamin A (i.e. retinoids) are necessary for the maintenance of a differentiated respiratory epithelium (65). Moreover, retinoids can prevent the development of various types of chemically induced tumors in rodents (66). We have added a vitamin analog, retinyl methyl ether (RME), to tracheal organ cultures over a 2 to 4 week period, the time when asbestos-induced metaplasia is most apparent (61). RME produced a dose-dependent inhibition of both the squamous metaplasia and the increased incorporation of ^{3}H-thymidine induced by asbestos (Figure 8). These results are encouraging in light of future clinical studies using retinoids for prophylactic or therapeutic treatment of workers exposed to asbestos and/or cigarette smoke.

INTERACTIONS BETWEEN POLYCYCLIC AROMATIC HYDROCARBONS (PAH) AND ASBESTOS

Although asbestos exhibits many of the characteristics of a tumor promoter in the respiratory tract, additional mechanisms of carcinogenesis must be considered. Because PAH are products of combustion, they are ubiquitous in the urban environment and are found in association with airborne particulate matter (67). When adsorbed to the surface of particles such as fly ash, the normal photooxidative degradation of PAH is prevented (68). Thus, their biologic activity is prolonged.

In experimental models of pulmonary carcinogenesis, a number of investigators have used particles such as hematite, aluminum oxide, and carbon as vehicles for the delivery of adsorbed PAH to the respiratory tract (reviewed in 69). After intratracheal instillation of PAH on various particles, the chemical carcinogen appears to be eluted and enters the tracheobronchial epithelium (70). Under these circumstances, numbers of tumors appearing are increased above numbers observed with use of PAH alone.

In comparative studies, we adsorbed identical amounts of the radioactively tagged PAH, 3-methylcholanthrene (3MC), to the surfaces of various particulates including crocidolite, hematite, kaolin, and carbon, before their

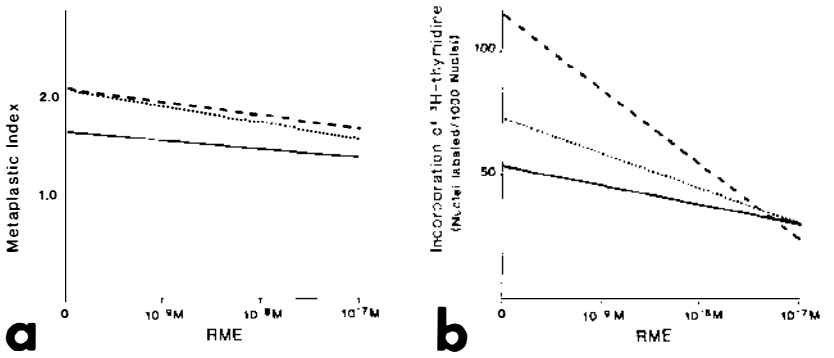

Figure 8 Asbestos-induced metaplastic changes (*a*) and incorporation of ^{3}H-thymidine by tracheal epithelial cells (*b*) can be inhibited ($p < .01$) with addition of the vitamin A analogue, retinyl methyl ether (RME) to hamster tracheal organ cultures (N=608). The extent of squamous metaplasia in the epithelium was measured in histological sections using a 4-point ordinal scale (1=differentiated normal epithelium; 2=focal metaplasia affecting < 15% of the epithelium; 3=15–50% affected epithelium; 4=> 50% affected epithelium).

precipitation on tracheal organ cultures (29). Particulates without 3MC were added to other tissues. After maintenance in vitro for 4 weeks, explants (N= 15 to 30 per group) were examined morphologically or grafted subcutaneously on syngeneic recipient animals. Results show the development of tumors in a fashion related directly to the amount of 3MC on tissues. All minerals with adsorbed 3 MC were carcinogenic, whereas minerals without hydrocarbon did not induce tumors. At highest amounts of dusts, more malignancies were observed using crocidolite than other materials.

To determine whether crocidolite adsorbed and/or released more hydrocarbon than other dusts, we evaluated the binding to and release of ^{14}C-3MC from minerals comparatively. The enhanced carcinogenicity of crocidolite could not be related to either increased adsorbance of 3MC or elution of the hydrocarbon into culture medium. For example, on an equal weight basis, carbon adsorbed more PAH than other dusts, but release into medium was minimal. In contrast, hematite adsorbed more 3MC than other minerals and eluted appreciable quantities of the hydrocarbon. We were perplexed by these observations and initiated experiments to determine the influence of asbestos on uptake and metabolism of PAH by tracheal epithelial cells.

PAH are incomplete chemical carcinogens in their natural state and must be activated by the microsomal mixed-function oxidases of mammalian cells to forms that can interact with DNA. Lakowicz and colleagues document by fluorimetry the increased transfer of PAH to artificial membranes and microsomes when hydrocarbons are adsorbed to a variety of particulates

(71–73). In contrast, dispersions and particles of PAH alone do not transfer readily to membranes. In comparison to nonfibrous materials such as hematite, silica, and carbon, various types of asbestos are most effective in facilitating a rapid uptake by membranes.

We used a different approach to demonstrate cellular incorporation and retention of the PAH, benzo(a)pyrene (BaP) after it is coated on asbestos fibers (74, 75). When ^{3}H-BaP is adsorbed to either crocidolite or chrysotile before their addition to cultures of tracheal epithelial cells, a rapid and efficient transfer of the chemical can be documented by autoradiography and scintillation spectrometry (Figure 9). Approximately 70% of the total BaP introduced enters the cell within 1 h, and 50% remains intracellular although unmetabolized at 8 h. In contrast, if identical amounts of BaP are added directly to medium, an initial influx of 20% is observed and cells retain only 5% of the initial amount at 8 h. Increased uptake and retention are not observed when BaP is added 1 h after the addition of asbestos.

A small but reproducible decrease in water-soluble metabolites of BaP occurs when fibers are coated with BaP. These results are consistent with those of Kandaswami (76), which show inhibition of metabolism of BaP when it is added with asbestos to rat liver microsomes. In contrast, increased metabolism of BaP is observed in cultures of human skin fibroblasts when chrysotile is added 24 h prior to the hydrocarbon (77).

Because interaction of BaP with DNA is thought to be a critical event in BaP-induced transformation, we measured alkylation of DNA by BaP under various circumstances. For as long as 5 days after introduction of BaP adsorbed to asbestos, alkylation is increased, which suggests impaired

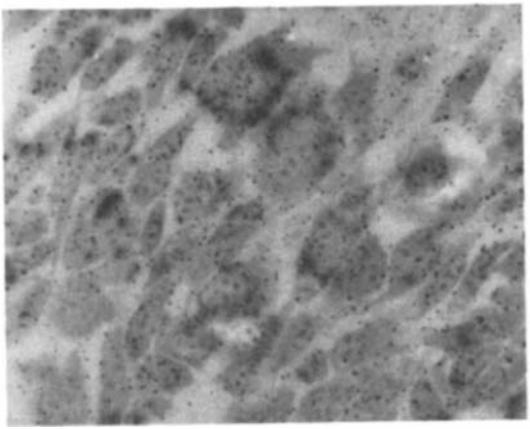

Figure 9 An autoradiograph of tracheal epithelial cells 1 h after exposure to ^{3}H-benzo(a)pyrene (BaP)-coated crocidolite. Note the concentration of ^{3}H-BaP (dark grains) in the cells. In contrast, ^{3}H-BaP has dissociated from the fibers.

removal of BaP-DNA bound adducts. In contrast, enhanced alkylation of BaP is not observed when it is added to medium alone or after exposure of cells to asbestos. Although further confirmatory experiments are required, the concept of altered DNA repair in asbestos-exposed cells is attractive in distinguishing the effects of this mineral from a variety of particulates that can act as carriers of chemicals but are not implicated as carcinogens in man.

Trace metals associated with asbestos fibers also have been implicated in the metabolism of PAH (78, 79). For example, certain metals such as copper and nickel increase the activation of mixed function oxidases and others (e.g. chromium, nickel, cadmium) are carcinogenic either in the simple ionic state or when complexed with macromolecules (i.e. iron-dextran, ferrous glutamate) (80).

CONCLUSIONS

Asbestos-induced cell damage is initiated by a reaction at the plasma membrane. For certain interactions, the degree of reactivity is dependent primarily on the charge of the fiber, a property that can be altered after periods of time in the respiratory tract. However, dimensional characteristics become important in determining cellular responses such as phagocytosis.

Although longer, thinner fibers are more carcinogenic in the induction of mesothelioma and more cytotoxic in general to a variety of cells, there is no clear relationship between fiber size, cytotoxicity, and carcinogenicity in the development of bronchogenic carcinoma. Clearly, various cell types respond differently to asbestos and this might be linked to their respective predilection towards transformation. In this regard, asbestos causes chromosomal breakage and abnormalities in some cells (81–83) but not in others (75, 84, 85). In mesothelial cells and fibroblasts, the progenitors of mesotheliomas and pleural sarcomas, asbestos is a complete carcinogen. Under these circumstances, the carcinogenic process resembles tumor induction by a variety of plastics and other chemically inert materials [i.e. an "Oppenheimer effect" (86)] and (co)carcinogenic influences (i.e. PAH, trace metals), are not necessary. For example, the carcinogenic potential of asbestos in mesothelium is not altered after extraction of naturally occurring organic contaminants and trace metals (12). In contrast, the role of asbestos in bronchogenic carcinoma appears epigenetic and can be compared to that of a classical tumor promoter after initiation of cells by PAH or other chemical carcinogens.

Despite its hazardous nature, the production of asbestos has increased exponentially in the 20th century. It is irreplaceable in friction materials and has over 1000 commercial uses (87). Moreover, millions of tons of

asbestos now are incorporated into the insulation of buildings and water systems. Because the biological and carcinogenic effects of asbestos are attributed, in general, to its fibrous nature, concern arises with the increasing development and use by industry of man-made mineral fibers and a variety of naturally occurring fibers such as attapulgite and sepiolite (88). The recent outbreak of mesothelioma in the Anatolian region of Turkey (89), an area in which fibrous zeolites are used in construction, suggests the possibility that other fibers of fine dimension are carcinogenic in man. In confirmatory experiments using rodents, intraperitoneal injection of the fibrous zeolite, erionite, produces mesothelioma whereas its nonfibrous analog, mordenite, is without effect (90).

Since the latency of mineral-induced lung disease is protracted, the accumulation of definitive epidemiologic data on man-made mineral fibers is unlikely before the year 2000. Therefore, we must rely on experimental models to test this hypothesis. Clearly, because exposure to naturally occurring asbestiform fibers is unavoidable, more emphasis should be placed not only on mechanistic studies, but also on possible chemopreventive and therapeutic approaches to fiber-induced disease.

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