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CHAPTER 3

ASBESTOS CARCINOGENESIS*

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I. INTRODUCTION

A. Cancer in Western Man

In countries where vital statistics concerning causes of death have been kept and mortality and morbidity data are validated by clinical and autopsy information, cancer deaths have increased dramatically (e.g., the United States, Silverberg and Holleb, 1972.(*1*) Some forms of malignant tumors have increased at alarming rates; such is the case for lung cancer, colon cancer, and bladder cancer. As an example, lung cancer now accounts for one of every six male deaths in Glasgow, Scotland (Haddow, 1970).(*2*). Some investigators have attributed the increasing cancer incidence to the inadvertent introduction of carcinogenic agents into the environment as pollutants (Boyland, 1969).(*3*) These agents, which represent low-level, long-term insult to the host, may represent one of the most important factors in the etiology of malignant neoplasms in the general population (Saffioti, 1970).(*4*)

The suggested correlation between exposure to an agent in the environment and occurrence of excess neoplasms in the general population has come about from the study of occupational cancers. The first such cancer was described over 200 years ago, and focused on the occurrence of scrotal cancers among chimney sweeps in London

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(Pott, 1976).(5) Since this time, many occupations have been scrutinized and studied, and agents found that have been correlated with the etiology of specific malignant neoplasms (Hunter, 1969).(6) Some of these tumors also have been observed in populations which are only indirectly exposed to the agent, or which simply lived in the locality where the agent was used. This has been defined in some detail for one such substance; asbestos (Selikoff and Hammond, 1968),(7) which is shown to occur as an air pollutant (Selikoff et al., 1972)(8) and which commonly occurs as fibers in the lung tissues of individuals from the general population (e.g., Langer, et al., 1971;(9) Pooley et al., 1970, 1976).(10,11)

B. Inorganic Particles and Disease

Exposure to inorganic dusts in the workplace is capable of producing disease that may cause disability or premature death. These facts have been known since antiquity, and have been the subject of early extensive treatises (e.g., Agricola, 1556).(12) Pneumoconiosis, a term that is recent in origin, was recognized first as a dust disease of the lungs (Zenker, 1867).(13) Indeed, it was not until the early 20th century that the most common of the dust diseases (silicosis) began to be understood on a sound basis (Collis, 1915).(14) Since this time, a number of other dust diseases have been recognized as related to exposure to inorganic agents. These have been reviewed extensively [Rosen, 1943;(15) Holt, 1957;(16) Hunter, 1969;(6) Aponte, 1970;(17) Langer and Mackler, 1972(18)]. Interestingly, not only have these diseases been reported in the areas where the materials are mined, but also in workplaces where milling, processing, and even use of the materials occur.

Not all inorganic dusts produce only lung scarring; some disseminate throughout the host, producing reactions in extra-pulmonary organs, as is the case with silica (Holt, 1957).(16). In addition to scar-tissue development, a number of inorganic dusts have been implicated in the etiology of malignant neoplasms. During the last 25 to 30 years, a number of inorganic particles have been implicated as the agent (or agents) responsible for malignant neoplasms in the workplace: chromium ores (Mackle and Gregorius, 1948);(19) heavy metals, especially lead, zinc, and copper (Breslow, 1954);(20) hematite ore, in the presence of free silica and other silicates (Lamy, et al., 1959;(21) talc (Kleinfeld et al., 1967);(22) uranium ore (Holaday, 1969).(23) Perhaps the best studied inorganic agent is asbestos. It is of particular importance because it is now used throughout the world, in thousands of products, so that it pervades all society (IARC, 1977).(24) To appreciate this great preoccupation with asbestos one must understand its disease potential.

C. Asbestos Exposure and Human Disease

Asbestos has been identified as a biologically active agent in relatively recent times. Asbestos fiber was first recognized as a potent fibrogenic agent capable of producing a fatal lung scarring with intensity and severity, as observed in cases of silicosis, when Cooke, in 1927 (25) noted several cases of fibrosis among asbestos workers. Several years later, there were two separate reports of primary bronchogenic carcinoma in individuals with asbestosis (Lynch and Smith, 1935, U. S. A.; (26) Gloyne, 1935, U. K.). (27) These observations were regarded as "curiosities" in that they may have been merely coincidental occurrences; i.e., asbestos and lung cancer. However, Wedler, in 1943 (28,29) later reported several autopsy series in which the occurrence of lung cancer among asbestos workers was far in excess of a similar matched autopsy series among individuals from the general population presumed to have not been exposed to asbestos. With the work of Doll in 1955 (30), a firm epidemiological basis was established for the occurrence of lung cancer in workmen exposed to asbestos fiber.

In 1960, Wagner et al. (31) reported that a tumor, heretofore considered rare, frequently occurred among asbestos-exposed workers in the Northwest Cape Province of South Africa. They reported the occurrence of pleural mesothelioma, which they suggested was a risk not only among occupationally exposed asbestos workers, but to individuals who merely lived in the area where asbestos was being mined and milled. Similar tumors were reported to have occurred intra-abdominally in factory workmen in the United Kingdom (Keal, 1960). (32) In addition to mesotheliomas, Selikoff et al. in 1964 (33) reported the occurrence of excess gastrointestinal tumors among asbestos-exposed workmen as well. Less than 15 years ago, data accumulated suggesting that occupational exposure to asbestos fiber resulted in excess risk to asbestosis, pleural and peritoneal mesothelioma, lung cancer, and gastrointestinal cancer.

In addition to workmen incurring increased neoplastic risk, it appeared that individuals indirectly exposed to asbestos were also among the "at risk" population. Several studies of shipyard workers on the European continent demonstrated that asbestos disease carried over to all trades, rather than being confined to the insulation categories (or "laggers") (Harries, 1968; (34) Bohlig et al., 1970; (35) Stumphius, 1971; (36) Edge, 1976). (37) To add to these worrisome observations, a number of papers also reported the occurrence of asbestos-related tumors among individuals who merely lived in the environment of an asbestos plant (Wagner, et al., 1960; (31) Newhouse and Thompson, 1965; (38) Lieben and Pistawka, 1967). (39) Also, individuals who merely lived within a short distance of the workplace where asbestos was being processed or used, and members of the households of asbestos workers also incurred increased neoplastic risk (Anderson et al., 1976). (40)

One can readily understand why asbestos has been investigated so intensively. The literature concerning this material has so increased in the last decade that extensive review papers and bibliographies have been written (Harington, 1967; (41) IARC Monographs, 1972, 1973, (24) Harington et al., 1975). (43) Indeed, asbestos has become one of the important 20th century carcinogens.

D. Asbestos Studies in Animals

Utilizing a variety of animal species including substrains and colonies: rat, mouse, rabbit, guinea pig, gerbel, hamster; and different routes of administration, such as oral, inhalation, intrapleural, intratracheal, intraperitoneal, subcutaneous, all asbestos fiber varieties have produced malignant tumors in animals (Tables 14-21, reference 24). All asbestos varieties have induced mesotheliomas, and most have produced other carcinomas. (43) Doses, exposure intensities, fiber types, and species of animals have been varied to establish carcinogenicity and dose-response curves for each fiber type. (24)

E. *In Vitro* Studies of Asbestos

As the number of animal studies has grown, so have *in vitro* test systems. Most of these systems have dealt with relative toxicity of the fiber types and their ability to stimulate fibroblast growth and form scar tissue. Membrane systems (hemolysis) have provided a rough index of cytotoxicity, which appears to correlate well with relative fibrogenicity of the fibers. (43) However, little relevance has been gained concerning carcinogenicity. Cell systems have concentrated on hemolysis of mature erythrocytes, alveolar macrophage systems (viability, lysosomal enzyme changes, phagocytotic properties, etc.), other cell lines, especially dividing cells; organ culture involving specific tissues, e.g., tracheal transplant; and others; e.g., Davis, 1967; (44) Koshi et al., 1968; (45) Bey and Harington, 1971; (46) Allison, 1971, 1972; (47,48) Miller and Harington, 1972). (49) Whereas the animal studies determined the fibrogenicity and carcinogenicity of the fiber types, so the *in vitro* test systems were oriented toward determination of mechanisms. An extensive review of these papers may be found in Harington et al. (43)

F. Status Concerning Asbestos Activity

There continue to be many clinical and epidemiological studies concerning asbestos disease in workmen, an extensive literature concerning experimental carcinogenesis in animal models, and an ever growing literature in the area of mechanisms of interaction of asbestos fiber in cellular systems, all reflecting the importance of asbestos as a diverse and complex agent in human disease, and a widespread environmental contaminant. Significant quantitative and qualitative questions remain including the relative hazards associated with each of the asbestos fiber types, and those mechanisms leading to disease.

II. ASBESTOS AS A MATERIAL

A. The N[illegible] of Asbestos

Asbes[illegible] the name given to a group of naturally occuring silicate m[illegible] fibers in the serpentine and amphibole series. Normally, [illegible] six of these fibrous silicates are recognized as asbestos, although some rare forms of other mineral fibers may be used as well, e.g., fibrous clays (Whittaker, 1960).(*50*) The most commonly used fiber in the United States is the serpentine mineral chrysotile, sometimes referred to as white asbestos. Five amphibole silicate fibers make up the other common varieties: actinolite, amosite (brown asbestos), anthophyllite, crocidolite (blue asbestos), and tremolite. The mineral nature of asbestos is extensively reviewed in Speil and Leineweber, 1969;(*51*) and in the IARC Monograph, 1977.(*24*)

1. Atomic Structure - Chrysotile has been shown to be a curled sheet silicate that spirals around a central capillary. This has been determined on the basis of X-ray diffraction, single-crystal X-ray crystallographic, transmission electron microscopic, and selected area electron diffraction studies (Bates et al., 1950; (*52*) Jagodzinski and Kunze, 1954;(*53*) Whittaker, 1956;(*54*) Zussman et al., 1957;(*55*) Kalousec and Muttart, 1957;(*56*) Bates, 1959;(*57*) Maser, et al., 1960;(*58*) Whittaker, 1960, 1963;(*50,59,60*) Huggins and Shell, 1965;(*61*) Clifton et al., 1966; (*62*) Yada, 1967, 1971; (*63,64*) Langer et al., 1974).(*65*) These investigators determined the structure of chrysotile to be the magnesium analog of kaolin in which planar-linked silica tetrahedra face an adjoining brucite sheet composed of magnesium ions coordinated octahedrally with oxygens and hydroxyl groups. Two of the three apical oxygens in the silica sheet replace the hydroxyl groups in the brucite sheet to complete the chrysotile structure. The distance between these adjacent sheets are on the order of 7.3 angstroms, with symmetry repetition every 14.6 angstroms. The curvature of chrysotile is a function of the structural mismatch of the larger brucite sheet "stretched over" the smaller silica sheet to structurally satisfy the cation accommodations. There are several structural types that characterize the crystallography of chrysotile, the most important of which occurs in the monoclinic system and is called clinochrysotile (Zussman et al., 1957).(*55*)

The structure of the amphibole minerals is more complex. These minerals are termed inosilicates, occurring as double chains of linked silica tetrahedra that are cross-linked with bridging cations. The amphib[illegible] chain is formed by coplanar sharing of two of the three oxygens at[illegible] base of the silica tetrahedra to form an infinite chain axis. The double chain is completed by the third basal oxygen that is sh[illegible] between two opposite-facing, single-chain structures. These basic units make up the asbestos fiber axis (Deer et al.,

1967; (66) Whittaker; (50) Ernst, 1968; (67) Speil and Leineweber. (51) The different amphibole asbestos fibers possess the same basic structure with small modifications brought about by chemical variation. Substitutions within this structure alter interplanar spacings and the angle at which the adjacent units are stacked within the structure (referred to as the beta angle, forming the inclined plane of the monoclinic structure).

2. Fiber Unit - Electron micrographs demonstrate that single chrysotile fibers (called fibrils) are formed as hollow tubes (the internal capillaries surrounded by the spiraling sheet). These structures have been demonstrated many times. Studies of their dimensional characteristics indicate that the single fibril may range considerably with diameters ranging from 100-600 Angstroms (e.g., Langer and Pooley, 1973; (63) Langer et al., 1974). (65) Bundles of individual fibrils form the chrysotile fiber. These may range in diameter and in length, from millimeters to centimeters.

Amphibole asbestos fibers appear to consist of single crystals, twinned crystals, and stack-faulted fibers, in which the width distribution tends to be different. It has been observed that the different amphibole varieties behave differently when comminuted to form width distributions that are unique and characteristic for each mineral species (Timbrell et al., 1970, 1971; (69,70) Timbrell, 1972). (71) These studies demonstrated that crocidolite forms the shortest and thinnest fibers, followed by amosite, followed by anthophyllite. The log normal distribution of such width distribution appears to center at about 0.12 μ and smaller for crocidolite, 0.16 μ for amosite, and a polymodal distribution, greater than 0.20 μ for anthophyllite. The importance of such characteristics will be discussed in the section on inhalation potential. However, all of the amphibole fibers appear generally as straight rods when examined by electron microscopical techniques, with diffraction contrast figures resulting from flexing of the structure under the electron beam; curved fibers are noted as well. (65) It has been suggested that some of the internal structures of amphiboles, a fine, 20-50 Angstrom internal lamellar structure parallel to the fiber axis, may reflect twinning which imparts the unusual optical and tensile strength characteristic of the fiber (Seshan and Zoltai, 1976). (72)

3. Chemistry - The empirical chemical composition of chrysotile is $Mg_3Si_2O_5(OH)_4$. Although magnesium predominantly occupies the octahedral site within the structure, it is also common to have iron, nickel, and manganese substituting within this site up to several tenths of a percent. Occasionally, aluminum may substitute for silicon in structure, and fluorine may occupy several of the hydroxyl positions. In the Canadian chrysotile samples, originating from ultramafic rock types, the high iron content may be attributable to intergrowths of the mineral magnetite within the chrysotile

fiber bundle. Serpentine deposits that originate from silicified dolomites tend to have high calcium oxide content, reflecting intergrowth of carbonate minerals. Other trace metals that may have biological significance are nickel, chromium, and cobalt, which have been identified in concentrations up to several thousand parts per million in chrysotile originating from ultramafic rocks. The chemistry of chrysotile has been discussed at length in several papers: Pundsack, 1956;(73) Pundsack and Reimschussel, 1956;(74) Nagy and Faust, 1956;(75) Brindley and Zussman, 1957;(76) Gaze, 1965;(77) Harington, 1965;(78) Speil and Leineweber;(51) and Table 1.(24)

The chemistry of the amphibole asbestos minerals is far more complex than the chrysotile mineral. Generally, it may be described by the structural chemical formula:(67)

$$W_{0-1}\ X_2\ Y_5\ (Si_4O_{11})_2\ (O,OH,F)_2$$

For anthophyllite, no cations occupy the W structural site, and the X and Y cation sites are theoretically filled with magnesium. There is limited substitution of iron for magnesium in the structure, and some limited substitution of aluminum for silicon. Amosite has no cations in the W site also, and contains almost total iron in the X and Y sites. Some magnesium does substitute for iron, as well as manganese. Actinolite and tremolite, both forming the end members of a solid solution series, may contain some calcium, sodium, or potassium in the W site, in limited amounts. The X site is filled with calcium and the Y site with magnesium and iron (for actinolite the iron is greater than the magnesium, for tremolite the magnesium is greater than iron). Manganese may occur in these sites as well. Crocidolite may have traces of calcium and potassium in the W site, contain sodium in the X cation site, and iron in the Y site. Anthophyllite may contain traces of aluminum which substitutes for silicon in the basic chain structure. Crocidolite may contain substantial amounts of magnesium and occasionally traces of aluminum as well. Originating from different rock sources, there are a number of different trace metals that may be associated with the amphibole asbestos varieties, again a function of provenance and geological origin. This is reviewed in Whittaker;(50) Ernst;(67) Speil and Leineweber;(51) and in Tables 5, 6, 7.(24)

It is of interest to note that both chrysotile and the amphibole asbestos varieties may be contaminated with hydrocarbon traces, originating either from the geologic environment at the time of mineral formation (or migration of hydrocarbons after rock formation) or hydrocarbon contamination from processing and storage (Gibbs, 1969, 1970;(79,80) Commins and Gibbs, 1969;(81) Gibbs and Hui, 1971).(82) In addition to these sorbed hydrocarbons, occasionally impurities from the metals encountered during processing might contaminate the asbestos samples as well (Ayer and Lynch, 1967;(83) Baddolet, 1952;(84) Baddolet and Edgerton, 1961(85)).

4. Surface Charge - Chrysotile asbestos has a surface layer of magnesium hydroxide groups bonded to the silica layer through the sharing [redacted]xygens. The surface hydroxyl layer, having protons outermost, [redacted]s a net positive charge to the fibril surface. Zeta potent[redacted]easurements show a surface charge of +40 to +100 mv from pH 4 t[redacted]h 8.(73) The amphibole asbestos fibers tend to have surfac[redacted]ers composed predominantly of silica, with oxygens on the surface rendering a negative charge. Surface charge measurements of these fibers have been studied as a function of pH, ionic strengths, and differing cation concentrations of the media (Prasad and Pooley, 1973).(86) The negative range of zeta potential for the amphiboles is similar to that of quartz, but smaller in magnitude.

5. Mineral Stability - It is known that chrysotile asbestos possesses a chemical stability only in liquid media with pH centered at 10.8. Contact with solutions below this pH value results in magnesium loss from the fiber (Hargreaves and Taylor, 1946).(87) This instability has been noted in the mineralogical literature and was demonstrated to take place when the fiber was retained in residence in living tissues (Morgan and Holmes, 1970;(88) Langer et al., 1970, 1972;(89,90) Morgan et al., 1975).(91) Chemical degradation of the amphibole minerals *in vivo* has also been observed, mostly by electron microprobe characterization of fibers in tissues.

III. FACTORS TO BE CONSIDERED IN ASBESTOS CARCINOGENESIS

A. Fiber Types and Malignant Disease in Humans

Currently, all asbestos fiber types have been associated with malignant diseases: chrysotile exposure; e.g., McDonald and McDonald, 1976,(92) amosite exposure;(8,9) and anthophyllite exposure (Meurman et al., 1974).(93) A detailed summary, reflecting the world literature concerning human populations studied, is provided in Table 22.(24)

Exposure to all asbestos fiber types, with the exception of anthophyllite, has been observed to be associated with mesothelioma (both pleural and peritoneal); all asbestos fiber types have been implicated as an agent with bronchogenic carcinoma; chrysotile, crocidolite, and amosite have been associated with excess gastrointestinal cancers. In addition to these tumors, chrysotile and/or the amphibole fibers are implicated in excess malignancies of the trachea, kidney, and central nervous system among insulation workers who use all fiber types. In addition to implication of all fibers with many tumor types, multiple primary tumors also have been found in single in[redacted]iduals exposed to asbestos.(94)

Multipl[redacted]mors in the same individual and in different individuals point to the fact that asbestos fibers disseminate once in-

haled and/or ingested. (95,96,97,98,99,100,101,102). Such information and data point to the fact that a number of different organs exist as targets for asbestos, as well as at least two major cell types (epithelial and mesenchymal cells).

B. Fibers in Tissues

A number of studies have been made demonstrating the presence of asbestos fibers in the lung and extra-pulmonary tissues, of workemn exposed to asbestos fiber. (11,89,90,102,103,104,105,106) Most of these studies demonstrated the prevalence of submicroscopic fibers in relation to those that may be observed by light microscopy. More importantly, asbestos fibers were found in greater abundance in individuals exposed to the material in the work environment, as compared with those in the general population (which tended to have fewer and smaller fibers).

C. Retention of Fibers

The presence of fibers in tissues, many years after cessation of exposure, points to retention for long periods of time. Retention rates experimentally determined appear to be related to the animal model, route of administration, and fiber type involved in exposure. For example, rats exposed to milligram amounts of crocidolite by inhalation were observed to retain about 35%, with about 9% left 30 days later. (107) In inhalation studies in rats by Wagner et al., 1974(108) amphibole minerals (crocidolite and amosite) were retained, as much as 26% of the original amount being present 18 months after cessation of exposure; however, up to 59% of the original dosage of the larger anthophyllite fiber was retained. Elimination rates for chrysotile were not calculated because extremely low concentrations were found as compared to the amphibole fibers. Since it is known that the chrysotile fiber degrades *in vivo*, it tends to become lost in this manner, thus complicating retention estimation.

D. Dose Response and Latent Period

Utilizing human studies in retrospective-prospective epidemiological studies, it has become apparent that the different malignant tumors possess different latent periods between first onset of exposure to dust and clinical appearance of disease. Lung cancer appears to peak after some 25-30 years from onset of exposure to the mineral dust; pleural mesothelioma, 25-30 years; peritoneal mesothelioma, 35-40 years. (109,110,111) Latent period is also influenced by dose, in that the latent period for the above disease increases as the intensity of exposure decreases. This was observed for factory workers exposed to amosite. (8,9) In this latter study, holding all other factors equal, demonstrable neoplastic risk was also found to decrease as the exposure decreased and the latency period increased; that is, those individuals exposed for short time

periods had a significantly decreased attributable risk to neoplastic disease which, when it occurred, appeared only after a greatly extended time period.

It is of interest to note that several investigators, in experimental animal systems, have produced data suggesting that a dose-response exists for lung scarring, but not for tumor production.(*112*) This was also suggested by Gold in 1970(*113*) who correlated the number of asbestos bodies in lung tissue with severity and extent of fibrosis, but no such correlation existed in individuals with tumors. Importantly, an "excess" dose which may produce asbestosis in animal or man, may produce death in the organism before neoplastic change can occur. Thus, competitive risk between asbestosis and cancer suggests that high exposures may result in asbestosis, whereas lower exposures would produce an increased life span, and a long enough latent period for the tumors to become manifest.

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On the basis of examination of the best cohort studies available, coupled with exposure data, Schneiderman, in 1974(*114*) concluded that a zero cancer response may exist only at a zero exposure level.

E. Cofactors in Disease

On the basis of a number of epidemiological studies of asbestos workers, it has been demonstrated that those individuals who smoke cigarettes and are exposed to asbestos incur a significantly elevated risk of developing lung cancer.(*7,115,116,117*) Indeed, the risk of bronchogenic carcinoma has been demonstrated to be greater than 90 times more for the cigarette-smoking asbestos worker than for a noncigarette-smoking individual from the general population who does not work with asbestos. Cofactors in the induction of tumors in animals have been investigated in a number of laboratory studies as well.(*118,119,120*) As in the human studies, it has been demonstrated that polycyclic aromatic hydrocarbons, in addition to asbestos exposure, greatly enhances tumor risk in living organisms.

The bronchogenic carcinomas in cigarette-smoking asbestos workmen are positioned unlike the bronchogenic carcinomas found in cigarette smokers; first, they tend to occur more peripherally and not in the main bronchus or bronchi of the individual; the tumor cell type tends to be more undifferentiated and of the more virulent "oat cell" variety.

OK, maybe

F. Size, Shape Characteristics

Fiber size, including both diameter and length, influences the relative "toxicity" of the dust indirectly and directly. Particle

size affects many important properties to be considered in the biological activity of dusts: stability of the aerosol; inhalation potential; site of reaction; migration potential; type of cellular response; and surface area.

1. Aerosols - Small asbestos fibers form stable aerosols in the work environment. Asbestos fibers are aerodynamically stable because their falling speed is primarily determined by fiber diameter.(*121*) Fiber diameters for the asbestos minerals tend to be in the tenth micron range, which compares with falling speeds of spherical particles in the submicron size range; therefore, asbestos fibers tend to form stable aerosols, thereby increasing inhalation potential and dosage.

2. Inhalation Potential - As asbestos dust persists in the work environment, the inhalation potential is increased. Depending upon the concentration of the dust, the duration of exposure, and the individuality factors involved; e.g., physiology of the individual, etc.; the risk associated with each dust appears to be primarily related to particle size.(*122*)

3. Site of Reaction - With smaller particle size (primarily length) there is deeper penetration to the alveolar spaces and greater retention by means of both diffusion and inertial impaction mechanisms.(*71*) This latter study demonstrated that long fibers (>8μm) that come to rest in the trachea and tracheal bronchial tree are by and large eliminated, or cause fibrosis, whereas the smaller fibers (<8μm) penetrate to the alveolar spaces and are retained. It is of interest to note that the smallest fibers are those that reach the mesothelial lining of both the visceral and parietal pleural surfaces.

4. Migration Potential - Small fibers tend to be those that migrate and that are found in extra-pulmonary organs.(*102*) These observations have been made by other investigators as well, suggesting that small fibers migrate more efficiently than large ones.

5. Type of Cellular Response - A number of investigations has demonstrated that the longer asbestos fibers tend to come to rest in the upper and middle bronchial tree, whereas the majority of shorter fibers lodge in the alveolar spaces. Long and short fibers in the tracheal bronchial tree tend to be eliminated in greater amounts than those that come to rest in the more peripheral areas. Complete phagocytosis of short fibers and incomplete phagocytosis of long fibers, which Kuschner and Wright(*123*) have termed "frustrated phagocytosis," occurs. This incomplete phagocytosis may lead to "leakage" of lysosomal enzymes and cell death, followed by collagen development. Observations, on both an experimental and a human basis, have demonstrated that asbestosis tends to occur with

long fibers.(*124*) Although peribronchiolar fibrosis is observed in humans (long fibers only?) the characteristic scar pattern developed involves sept[illegible]ells as well. Long and short fibers are implicated, althou[illegible]ome investigators consider long fibers more potent. Long fiber a[illegible]ity has carried over into the carcinogenesis area in that some [illegible]stigators consider long fibers responsible for carcinogenes[illegible]s well.(*125,126*) Indeed, a number of experimental pathology studies utilizing short fiber have reported no responses in laboratory animals, thereby dismissing short fiber (< 5 μ in length) as biologically innocuous.(*124,127,128,129,130,131*) It is of interest to note that each of these investigators produced short asbestos fiber by vigorous mechanical methods (e.g., ball-milling) which may have significantly altered the fiber surface and biological properties.(*132*)

Indeed, a number of workers has demonstrated short fibers to be extremely active in a number of animal models.(*133,134,135*) These investigators produced short fibers using special techniques that prevented surface destruction. They remarked that most of the disease processes were intracellular in nature and required small fibers at the onset. Pott et al., 1972(*136*) and Pott and Friedrichs, 1972(*112*) noted the importance of short fibers in terms of human carcinogenesis. Suzuki and Churg, in 1969(*137*) noted cellular response with chrysotile fibers less than one micron in length. These were also phagocytized by attached epithelial cells and were observed to be present in alveolar septa where fibrosis and thickened membranes indicated marked activity. Wagner, in 1968(*138*), and Wagner and Berry in 1969(*139*) produced more mesotheliomas with a superfine chrysotile fiber (92% < 6μ in length) than with any other fiber used experimentally. Indeed, this group reports that the smaller the fiber, the greater its carcinogenic potential.(*135*)

These investigations are supported in part by human data that suggests that the finer crocidolite fiber encountered in the Northwest Cape Province of South Africa produces more mesotheliomas as compared with similar fibers in the Transvaal of South Africa.(*71*) This feature seems to support the concept that small fibers are active in terms of carcinogenic potential.

G. Structure of the Fiber Bundle

It has been proposed that the physical character of asbestos fiber is responsible for its biological acitvity; i.e., its polyfilamentous character.(*140*) Chrysotile asbestos was examined in various states and it was concluded that only when chrysotile was used in fiber bundle form did it possess biological activity. However, monofil[illegible]entous fibrous glass has been demonstrated to produce tumors i[illegible]nimals;(*125*) chrysotile reduced to single fibrils by vigorous m[illegible]anical methods produced no response in animals;(*127, 124*) which may be explained by alteration of its surface activity;

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(*132*) "monofilamentous" chrysotile asbestos fibers used by Gross and Har[redacted] in 1973(*140*) to demonstrate lack of activity was produced [redacted] extreme heat, and thus dehydroxylated and recrystallized into a[redacted]irely different mineral phase.(*132,51*)

1. Surface Area - It has been estimated that several of the asbestos types, especially chrysotile, may be comminuted until surface areas reach 90-100 square meters per gram of material. Since the surface of the alveolar spaces is about 100 square meters, vast interaction phenomenon can be possible. Large surface areas with small particle sizes provide great potential for cellular interaction.

2. Fiber Chemistry - It has been demonstrated that chrysotile asbestos is markedly unstable in an acid environment and readily releases magnesium in aqueous media with pH less than 10.8.(*141, 142,143,144*) The *in vivo* degradation of asbestos has been documented as well, with magnesium loss from both chrysotile and amphibole fibers observed.(*90*) Thus, fiber chemistry may play a significant role in carcinogenesis.

3. Magnesium - Magnesium may be an important ion in controlling such reactions as cell membrane lysis (especially the red cell). For example, sialic acid groups protrude on the surface of the cell, with carboxyl units outermost. These units are hydrophilic, with a high net negative charge. It was postulated that magnesium ion, lost from asbestos fibers, interacts with these carboxyl groups and by pulling on the oppositely charged glycoproteins, cross-links several of these groups at the surface, forming channels that tend to increase the passive permeability of both potassium and sodium ions far in excess of the normal transport system.(*145*) This induces an osmotic force within the cell, causing sodium and water accumulation, cellular swelling and bursting. Such an effect, it seems to us, might not necessitate removal of magnesium from the fiber. In fact, magnesium structurally confined by the fiber crystalline matrix would have the ability to stereochemically interact at membrane surfaces at a higher effective concentration. Experimentally, the membrane system, hemolysis, is used to relate to cytotoxicity.(*45,146*) Fiber type and hemolytic activity are apparently related to the mesothelioma yield as observed in chrysotile-exposed rats by Wagner, in 1973.(*135*) The direct relationship between these membrane studies and carcinogenesis is presently unknown.

Macnab and Harington, in 1967(*148*) suggested that the relative activ[redacted]ies observed for the asbestos fiber types may be related to their [redacted]gnesium concentrations. Using chelating agents specifically for m[redacted]esium, treated fibers were observed to be far less hemolytic than [redacted]reated fibers. The leaching of magnesium from the fiber also greatly decreased the hemolytic potency. The use of several

varieties of chelating agent, calcium specific versus magnesium specific, demonstrated again the importance of magnesium in membrane interaction.(48) A number of adsorbed chemical agents have been used to antagonize the hemolytic response.(146,148,149,150) Again, these investigations suggested the importance of magnesium. However, the chelation and adsorption studies do not preclude more complex surface mechanisms such as those involving silicate or hydroxyl groups.

4. Iron - A number of investigators have considered iron and several of its compounds as possibly being involved in carcinogenesis. Some have suggested that iron plays a role in enzyme blockage, in interference with the immune system, and in upsetting iron-sensitive cellular energy dynamics and enzymatic transformations through interference with $iron^{+2}$: $iron^{+3}$ equilibrium.(151,152,153) As is the case with magnesium, the iron contents of the various asbestos fibers range greatly, and those iron-rich fibers tend to keep this cation structurally bound when in biological residence. The low iron content of certain biologically quite active fibers speaks against its being the only factor in carcinogenesis.

5. Silicon - The question may be posed whether silicon as a silicate entity is a chemical carcinogen. Several investigators consider this unlikely in that silicon is normally present in man at concentrations that are detectable.(154) However, exposed silica surfaces (whic can exist in amphibole asbestos minerals, and in chrysotile that has been degraded by organic acids *in vivo*) may act as powerful hydrogen-bonding agents.(155) Nash et al. suggested that hydrogen donors, especially those of the phenol configuration, can be extremely damaging to living cells. Silicic acid, as well as other weak acids, may act as hydrogen donors.

6. Hydroxyl Groups - The concept of hydrogen donors in the form of "phenolic-like" groups as damaging to living cells as discussed by Nash et al. may be the fundamental, or at least initial approach to understanding biological interaction of asbestos, which inevitably contains active surface or interlayer hydroxyl groups in the crystalline structure. Pundsack(73) estimated that 7% of the total hydroxyl groups of chrysotile were on the surface of the fiber. Allison(145) described such hydroxyls as phenolic-like in activity, and we have found that these surface hydroxyls interact with a number of organic compounds to reduce or oxidize them.(132) We have observed chemisorption, free radical and acid-base interactions at the surface of the fiber which suggest that the surface of chrysotile is amphoteric (as befits magnesium hydroxide). Such reactions are attributed to surface activity related to the phenolic groups. Both phenomena vary greatly as a function of the pretreatment of the fiber (especially heating and other manipulations which alters the surface configuration of the phenolic groups). These

reactions occur with other magnesium silicates and amphiboles, usually to a [illegible]h smaller degree, which can be accounted for at least qualit[illegible]vely by the stereochemical and electronic presentation of th[illegible]-OH groups. Such reactions have been seen at highly reactive [illegible]lytic surfaces, e.g., for zeolites.

7. Trace Metals - As indicated previously, most of the asbestos fibers contain significant quantities of trace metals (ranging up to several thousand ppm). It is of interest to note that the concept of nickel, chromium, and cobalt activity, implicated early in asbestos carcinogenesis research as one of the mechanisms of action, is now less important. The idea lost favor mainly because of experiments wherein the same number of tumors were produced in animals utilizing fibers with and without detectable amounts of trace metals. (*139,135*) However, the role of trace metals may be important, and can be considered in the category of iron.

8. Adsorbed Hydrocarbons - A number of studies has suggested that the adsorbed hydrocarbons on the surfaces of the asbestos fibers are responsible for their carcinogenic properties. Again, this theory cannot account entirely for the general phenomenon of asbestos carcinogenesis, since tumors were produced in animals utilizing fiber with and without adsorbed hydrocarbons. (*139,147,156*) However, this concept should not be discarded since it represents many experimentally substantiated synergistic carcinogenesis studies, which parallels strikingly the asbestos-cigarette smoking lung cancer incidence in man. Furthermore, in the light of the very active chemical redox reactions observed by us and others, it is clear that the benzopyrene-asbestos synergism may be less important than interactions with more potent electron donors and acceptors, exogenous and endogenous.

It is also of interest to consider the interference of asbestos with repair mechanisms in this regard, so that the presence of the active fiber would (a) prolong the insult of a proximate carcinogen; or (b) inhibit repair processes.

The potential carcinogenicity of the hydrocarbons adsorbed on asbestos has led many investigators to study these surface compounds. Polycyclic aromatic hydrocarbons such as phenanthrene, perylene, anthracene, and the carcinogen benzpyrene, adsorb on the surface of chrysotile. (*156*) Adsorption of various organic polymers has been studied extensively by Schnitzer et al. (*150*) Indeed, specific polymers adsorb more readily to the surface of chrysotile (through int[illegible]action with the "phenolic" surface groups) whereas, others prefe[illegible]he oxide surface of the amphibole asbestos fibers. Those polymer[illegible]that theoretically inactivate the surface hydroxyls or magnesium[illegible] their adsorption also reduce hemolytic potency.

9. Surface Sorption Properties - It has been noted that asbestos fibers; e.g., chrysotile, can adsorb five times more protein from human serum than many other mineral species; e.g., quartz.(147) The adsorption of specific substances at the surface of asbestos is well known. The physisorption of benzene, ethanol, hexane, and other organic liquids has been reported, with polar molecules possessing a great affinity for chrysotile as compared to the amphiboles. This subject has been investigated by Gorski and Stettler.(157) This aspect can be independent of those discussed here; for example, by causing an imbalance in cellular components.

One cannot readily distinguish between true surface phenomenon, or physical-chemical interaction. It is known, for example, that proteins may be denatured at the surface of quartz particles by interacting with the surface oxide groups with the hydrogens on the amine groups of the proteins.(158) This mechanism of interaction may take place at the surface of the amphibole asbestos fibers, or at the surface of chemically degraded chrysotile. It has been reported that silicon may be chelated directly from the surface by serum proteins, causing possible changes in the organic materials.(159) Indeed, these investigators consider the surface of silica compounds as the key to biological interaction. In addition, such oxide surfaces provide secondary amide-hydrogen bonding to amino acids.(160) The extent of this interaction in terms of carcinogenesis is presently unknown. In addition, direct adsorption of substances including "tumor inhibitors" has been proposed by Oppenheimer et al.(161) and by Bischoff and Bryson.(154)

Asbestos fibers adsorb a number of organic compounds. These organic compounds are both endogenous to the organism and exogenous, derived from pollutants. These materials tend to be nonpolar for the amphibole asbestos types and polar for the serpentine asbestos variety. The mineral surfaces can act as solid-state catalysts and may reduce and/or oxidize these compounds, creating altered forms. It has been suggested that the denaturation of proteins may produce materials that are antigenic to the host, iniating an immune response. Protein or polysaccharide adsorption may simply cause an undesirable concentration gradient or stereochemical effect (e.g., membrane configuration). Adsorption of other biochemical substrates such as those leading to hemosiderin deposition may interfere with iron-dependent energetics (redox). It has been reported that silicotic nodules contain substances rich in gamma globulin.(162) As in the present instance, no specific antigen or immunological factor has been observed for the asbestos minerals.

In addition to the surface-controlled chemical phenomena, there appears to be chemisorption which may involve specific components of the organic compounds, producing alteration to specific sites of the organic molecule. Again, these reactions may involve

either oxidation or reduction reaction. Some surfaces may behave amphoterically and may change with time as chemical degradation of the surface proceeds, as is the case for chrysotile. (*132*)

IV. ASBESTOS ACTIVITY: CHRYSOTILE AS A MODEL

Chrysotile may comminute to particles that are only several hundred angstroms in diameter and a few thousand angstroms in length. The aerosol formed by such particles is extremely stable, durable in the environment, and has associated with it a high inhalation potential. The inhalation of such small fibers would produce deposition in the alveolar spaces and the peripheral areas of the respiratory tract. Such fibers may have only to migrate a short distance to reach the mesothelial surfaces of the lung and the parietal pleura. The fibers could be easily available to reaction to form both mesotheliomas and the peripheral lung carcinoma characteristic of asbestos exposure. In addition to its presence in the deep regions of the lung, chrysotile may migrate to other organs for interaction. We have observed fibers in liver, spleen, testicle, brain, and intestinal tissue (Langer et al., unpublished results).

Small particle sizes suggest that these fibers are easily phagocytized and removed from the lung tissue; hence, the levels of chrysotile observed in lung tissues (even in highly dosed experimental animals) are often very much less than observed in similar animals exposed to the amphibole fibers. Also, the chemical activity of chrysotile is such that one would suspect that both phagocytosis and chemical degradation would account for a significant loss of fiber from the lung.

This very process of chemical degradation of chrysotile, however, may provide several major mechanisms for cellular interaction. Once phagocytized by cells within the lung, the surface of chrysotile structure may readily oxidize and/or reduce organic compounds present within the cell. Release of magnesium may follow phenolic-like hydroxyl group interaction, causing alteration of membrane geometry, and cause lysis. Denaturing of proteins, alteration of macromolecules of various kinds, or adsorption of proteins involved in immunological processes also may take place on the surface of the mineral. Trace metals may be released, or trace iron within the structure be present, causing interference with enzyme systems (especially those that are governed by iron equilibrium).

Chrysotile asbestos offers continuous interaction throughout its chemical degradation ("phenolic" interaction at the surface, oxidation-reduction reaction, magnesium release, iron and trace metals release, silicic acid release) or even a reaction with macromolecules, thereby providing a "passive" mechanism for alteration.

Inde[redacted]all these processes may take part in its biological activity.

[redacted] greater membrane activity of chrysotile versus amphibole asb[redacted]os is, we hypothesize, a more complex event than any single potential interaction. With chrysotile, interaction may proceed through sequential layers: proton or hydrogen radical; hydroxyl ion or radical; oxide (Mg-O) ion or radical; magnesium ion; silicon-oxygen or hydroxyl group; silicon ion or radical; cation or anion substitutions (Fe, F^-). The various reactions through such layer stripping have extreme potential biological ramifications, especially when combined with the stereochemical control that can occur at its well-defined crystalline surfaces.

Amphibole fibers possess all of these molecular possibilities, in an entirely different sequence and chemical range.

These factors, coupled with factors that are related to the host conditions of work and the presence of other agents that might act synergistically with the fiber, underscore the complexity of the problem. Investigators in this field cannot help to amplify the observations of Bryson and Bischoff(*159*) who said:

> "Considering the extent to which silicates comprise man's environment on this planet, and bearing in mind the silicate-induced diseases (including cancer) had been recognized since antiquity, the sparsity of animal experimentation concerned with silicate carcinogenesis is striking and deplorable."

Asbestos research has gone beyond the "sparse" stage and has been increasingly detailed and focused. The problem is not so much the lack of experimentation as the complexity of mechanisms of carcinogenesis.

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