THE ROLE OF ANALYTICAL TECHNIQUES IN THE DIAGNOSIS OF ASBESTOS-ASSOCIATED DISEASE

Authors: Val Vallyathan
Francis H. Y. Green
Pathology Section
National Institute for Occupational Safety and Health
Morgantown, West Virginia

Referee: Jerrold L. Abraham
Department of Pathology
State University of New York
Upstate Medical Center
Syracuse, New York

I. INTRODUCTION

Asbestos is a general term used to describe a variety of commercially mined fibrous silicates known as serpentines and amphiboles. Serpentine asbestos is a sheet silicate and the amphiboles are composed of double chains of tetrahedra.[1] These fibrous silicate minerals have been called "wooly rock" because of their appearance and have been used in various products for nearly 4500 years. The fire-resistant and apparent indestructible properties of asbestos were familiar to ancient civilizations. Clay pottery discovered in Finland, approximately 4800 years old, has been found to contain anthophyllite fibers, presumably added to increase strength.[2] Its use in cremation cloth (445 to 25 B.C.) and wicks of lamps of the Vestal Virgins have been cited.[2] It was used as an acoustic insulator by a Greek doctor.[2] Although uses of asbestos in various products can be traced back many years, large-scale commercial production and utilization are relatively recent phenomena following its discovery in rocky outcrops after the Canadian forest fires in 1877. During the industrial revolution in the 19th century, multiple uses for asbestos were found and minerals were exploited on a commercial basis. An 80,000-fold increase in production and utilization was reported from 1877 to 1967.[3] World production of asbestos has risen from less than 100 thousand tons in 1900 to 5317 thousand tons in 1978.[4] Although its use has dramatically increased during the last 100 years, the recognition of various forms of disease associated with asbestos exposure is relatively recent.

Asbestos has been aptly called "the lethal dust" because of its potential to cause disabling respiratory disease and malignant tumors.[5] Although Pliny (c. 75 A.D.) mentioned the use of masks in the protection of weavers producing wicks, the first record of a case of asbestos-associated lung disease was reported by Murray at Charing Cross Hospital, London, in 1906.[6] Occupational lung disease was recognized before this time and the term "pneumonokoniosis" was introduced by Zenker in 1867.[7] The recognition of the various forms of asbestos-associated diseases has been relatively slow, and reflects the long latency period between exposure and occurrence of disease. Although in 1918 a roentgenographic study of 15 individuals exposed to asbestos reported X-ray changes consistent with pneumoconiosis,[8] a positive association of exposure to asbestos and pulmonary fibrosis was not confirmed until 1924 by Cooke,[9] and later by Merewether and Price in 1930[10] and Cooke in 1927.[11] Mills reported the first case of asbestosis in the U.S. in 1930.[12]

An association of asbestos exposure with bronchogenic carcinoma was first reported in a case by Lynch and Smith in 1935[13] and Gloyne.[14] During the next 20 years, substantial evidence conclusively established a cause and effect relationship between asbestos exposure and lung cancer.[15] Mesothelioma was first described by Klemperer and Rabin in 1931,[16] however, an etiologic relationship with asbestos exposure was only established in 1960.[17] Asbestos exposure is now known to be associated with increased morbidity and mortality from asbestosis, and cancers of the lung, pleura (mesothelioma), larynx, esophagus, stomach, kidney, colon, and ovary.[3,18-24]

The exposed population is large. It has been estimated that from 1940 to 1979 close to 27.5 million people in the U.S. had potential asbestos exposure at work[25] and close to 1.56 million people are actively employed in asbestos-related works.[26] In addition to this occupationally exposed group, asbestos fibers are present in urban air, homes, schools, water supplies, beverages, and drugs, making exposure to some degree unavoidable. The true incidence of asbestos-induced disease is therefore difficult to assess due to the widespread contamination of the environment.

Asbestos-associated diseases are characterized by long latency periods, usually in excess of 20 years. One of the common and earliest disease manifestations of asbestos exposure is pleural effusion followed by thickening of the parietal pleura. Both these entities are benign and do not contribute significantly to morbidity and mortality; however, they are useful markers of exposure and therefore important from the epidemiologic point of view. The majority of mortality and morbidity from asbestos exposure is derived from asbestosis, lung cancer, and mesothelioma. It is estimated that from the years 1980 to 2009, approximately 12,000 cases of mesothelioma and 55,000 cases of lung cancer will arise in U.S. men with nontrivial occupational exposures to asbestos.[27] Other estimates have projected 8200 asbestos-related cancer deaths annually rising to 9700 by the year 2000.[25] From an occupational point of view, exposures to high doses of asbestos are controllable and new cases are preventable. From a public health point of view, low dose disease may be with us for many years to come.

Because many of the clinical and pathologic features of asbestos-associated diseases resemble diseases due to other causes, it is often necessary to document exposure by demonstrating asbestos fibers in tissue samples. There is growing interest in the use of electron microscopic and analytic techniques for documenting asbestos exposure. In this article, the roles of light microscopy (LM), scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray energy spectrometry (XES), selected area electron diffraction (SAED), and X-ray diffractometry (XRD) — as adjunct techniques in the identification of asbestos bodies and fibers in tissues — are reviewed. This is preceded by a brief discussion of mineralogy, sources of possible exposure, and diseases associated with asbestos exposure.

II. ASBESTOS MINERALS

Before describing the biological effects of asbestos, it is important to know the different types of asbestos minerals and their physical and chemical characteristics. From the biological point of view, knowledge of the type of asbestos to which a person has been exposed is important, since the risk of mesothelioma and other tumors may be different for the different asbestos types.

Asbestos is the general term given to a group of naturally occurring fibrous minerals which are relatively indestructible and resistant to fire. When these fibrous hydrated silicate minerals are mined or reduced to very minute particles, they maintain their fibrous nature, with an aspect ratio of 3 or more. Asbestiform minerals fall into two major commercial subdivisions — the serpentines and amphiboles. Chrysotile is the

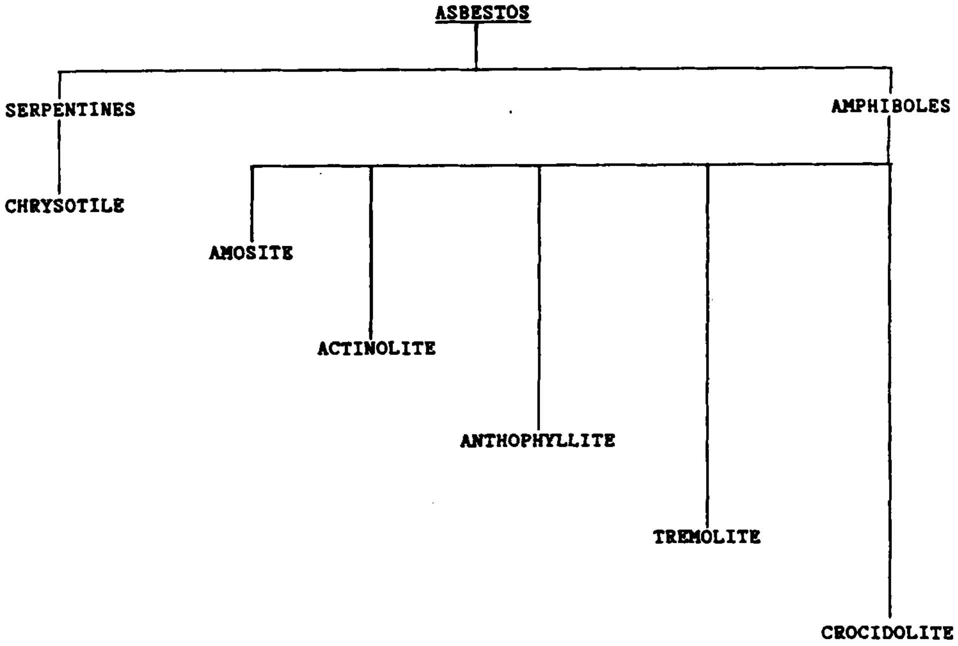

FIGURE 1. Classification of the major commercial types of asbestos.

Table 1
ASBESTOS IMPORTS INTO THE U.S. (JANUARY TO OCTOBER 1982)

Type and country	Short tons
Amosite (South Africa)	410
Crocidolite (South Africa)	5987
Chrysotile (Canada)	200718
Chrysotile (Mexico, South Africa, Zimbabwe, Italy, Japan, Norway)	2800
Asbestos, other types (Canada, Germany, India, Mexico, South Africa, U.K., Zimbabwe)	21511
Total	231426

Data taken from *Asbestos*, February, 1983.

major serpentine asbestos and amphiboles include amosite, actinolite, tremolite, anthophyllite, and crocidolite (Figure 1). Table 1 shows the relative proportions of the different commercial forms of asbestos imported into the U.S.

Chrysotile consists of fibrils with silicate layers fomed into scrolls or spirals with a tubular or cylindric structure.[1] Individual chrysotile fibrils have magnesium hydroxide on the outer surface of the silicate layers. Many such fibrils bonded together constitute fibers. The high concentrations of magnesium hydroxide on the surface of chrysotile fibrils give them a strong positive surface charge.

Amphiboles consist of silicon dioxide tetrahedron layers arranged in parallel chains and linked laterally by various cations to form a three-dimensional crystal. The silicon-oxygen bonds along the chain are stronger than the cationic bonds and therefore the amphiboles break easily lengthwise.

A. Chrysotile

Chrysotile is a serpentine asbestos that accounts for more than 90% of all asbestos

FIGURE 2. **Scanning electron micrograph of commercial chrysotile asbestos. (Magnification × 3000.)**

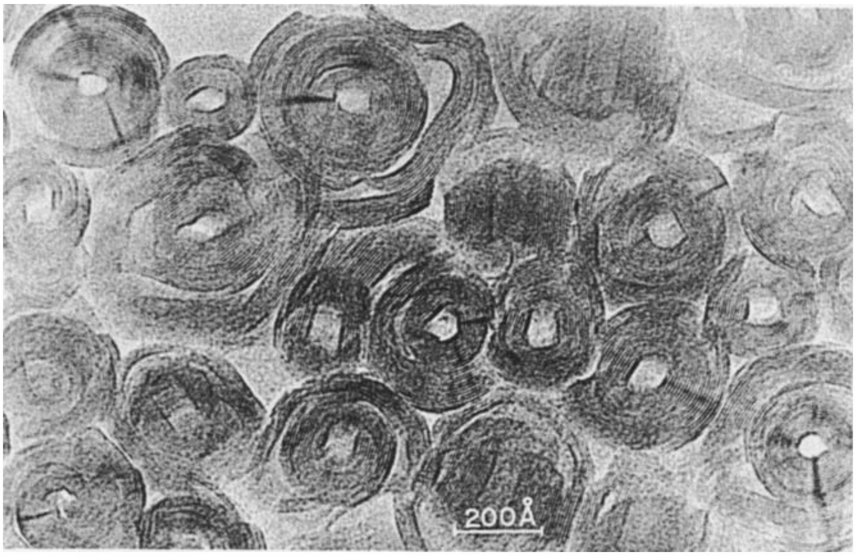

FIGURE 3. Transmission electron micrograph of a bundle of chrysotile fibrils. Note the hollow tubular structure. (From Yada, K., *Acat Crystallogr.*, 27, 659, 1971. With permission.)

used in this country. It is mined primarily in Canada and the Soviet Union and to a lesser extent in the U.S., South Africa, Australia, and elsewhere. Chrysotile fibrils and fibers are soft, flexible, curved, and white in color (Figure 2). The fibers have a very small diameter with a high tensile strength (28,500 kg/cm^2) and good spinnability. The fibers show a tubular hollow structure when examined under the transmission electron microscope (Figure 3), and this is considered a characteristic feature of chrysotile.[28] Chemically, it is a hydrated silicate with magnesium and iron in varying concentrations (Figure 7A). Chrysotile reacts weakly with water, salt water, body fluids, and acids. It tends to fragment with time and lose its magnesium content. It is highly resistant to heat and alkali.

B. Amosite

Amosite is an amphibole asbestos with a yellow to dark color, a pearly luster, and a coarse texture. It is mainly found in South Africa and India. Its tensile strength is lower and it is less pliable and spinnable than chrysotile (Figure 4). Iron is the dominant cation and it may contain magnesium and manganese depending on its source (Figure 7B). It is heat resistant and relatively unaffected by water, body fluids, seawater, and mild acids. It is, however, susceptible to strong alkali.

C. Actinolite

Actinolite is an amphibole asbestos found mainly in South Africa; it frequently contaminates amosite. It is greenish in color; the fibers have a silky luster and hard consistency. The tensile strength of actinolite is relatively low and it is not affected by water, seawater, body fluids, or mild acids. Actinolite contains calcium, magnesium, silicon, and iron. It is resistant to heat and susceptible to strong alkali.

D. Anthophyllite

Anthophyllite is an amphibole asbestos with a yellowish brown to pale gray color. It is found mainly in South Africa, Finland, Bulgaria, and the U.S. It has a low tensile

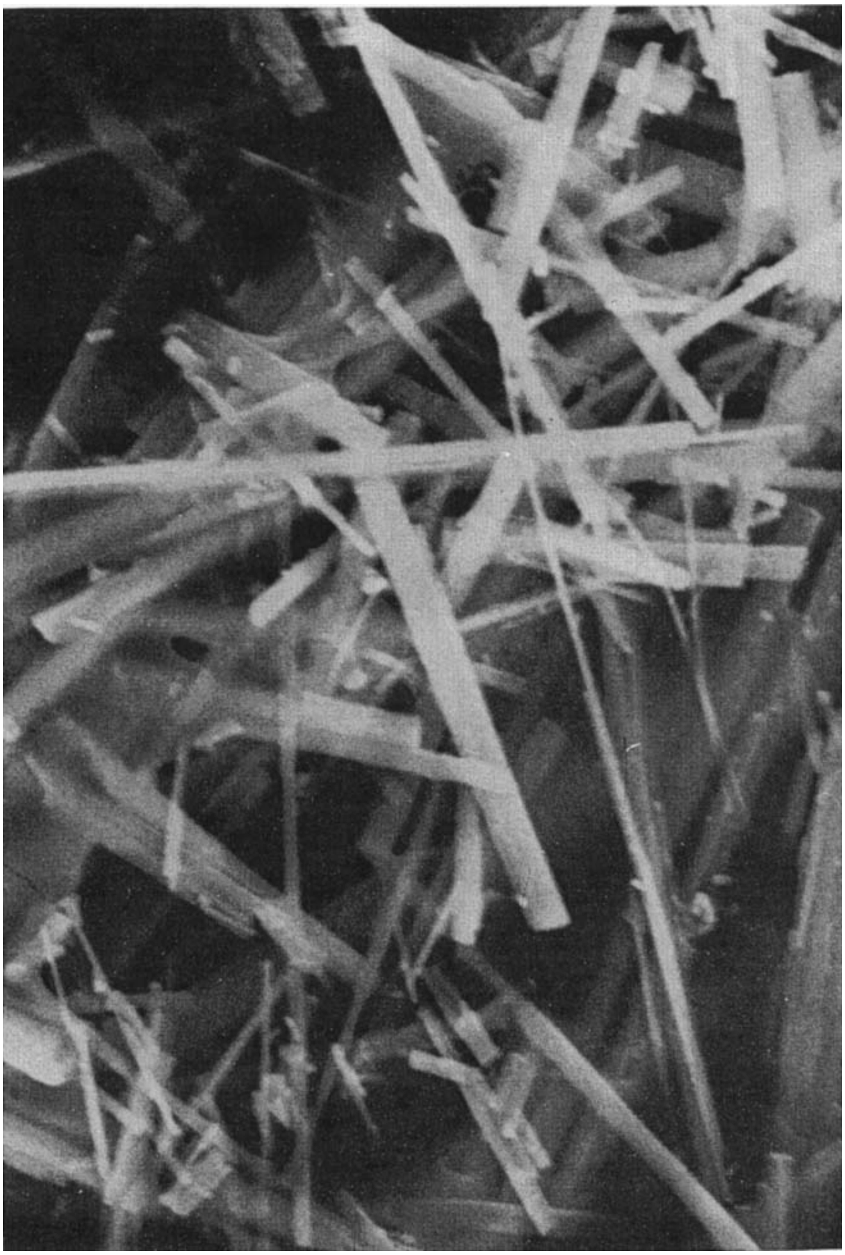

FIGURE 4. Scanning electron micrograph of commercial amosite. (Magnification × 3000.)

strength and poor flexibility and spinnability (Figure 5). Magnesium is the major cation found in anthophyllite, together with a small amount of iron (Figure 7C). It is highly resistant to heat, water, seawater, body fluids, and acids, but susceptible to alkali.

E. Tremolite

Tremolite has few commercial applications; its health significance is related to the fact that it frequently contaminates Canadian chrysotile and commercial talc mined in the northern U.S. It is found in western and northern U.S. and in Ontario, Quebec, Italy, Switzerland, and Austria. It is usually found in fibrous form as thin columnar aggregates. It is brittle in nature and contains magnesium, calcium, and silicon (Figure 7D). It is found in white, gray, brown, or colorless forms.

F. Crocidolite

Crocidolite is an important commercial amphibole found mainly in South Africa, Australia, China, and Bolivia. It has a blue color with a silky dull luster and is both flexible and spinnable (Figure 6). Crocidolite contains sodium, magnesium, silicon and iron (Figure 7E). It is relatively resistant to acids and heat and has a very high tensile *strength of 35,000 kg/cm^2. Crocidolite contains sodium, magnesium, and iron.*

III. USES OF ASBESTOS

Approximately 700,000 tons of asbestos is used annually in the U.S. It is estimated that more than 3000 commercial products contain asbestos. The large number of uses of asbestos results from its desirable physical properties, low cost, and ready availability. Although all asbestos types are resistant to thermal degradation, the specific commercial uses are determined by their other properties, such as acid or alkali resistance, spinnability, or extreme heat resistance. For example, the highly flexible chrysotile is useful in the manufacture of woven textile products. The acid-resistant properties and high tensile strength of the amphiboles are useful in the ship building industry and in the manufacture of battery casings.

The following gives a partial listing of the major uses of asbestos in free form and asbestos products used in commercial applications. Raw asbestos is used in yarn, textiles, felt, rope, high temperature insulation sheets, filters, wicks, rugs, gaskets, tape, masks, and insulation. Major asbestos-containing products are cement, clutch facings, gaskets, brake lining, asphalt, paints, moldings, caulking, wallboards, roofing materials, paper, electrical molds, flooring materials, pipe, boiler insulation, fine shields, pottery, etc. Among the various commercial uses, manufacture of asbestos cement products constitutes the single largest use of asbestos in the U.S.

IV. SOURCES OF EXPOSURE

Since asbestos-related diseases tend to have long latency intervals, obtaining detailed occupational and environmental histories is essential. A history should document each and every job and place of residence where the patient has worked and lived, however short. Materials used or handled in the workplace and home, and the occupation of the spouse and/or other people sharing the household, should be elicited. Since intermittent and apparently trivial exposures to asbestos can occur to bystanders in the workplace, detailed descriptions of materials handled by other workers in common work areas are also important. If an accurate occupational and environmental history is available, it may be possible to determine the type of asbestos exposure and correlate it with the relative pathogenecity of the various asbestos types. A detailed smoking

FIGURE 5. Scanning electron micrograph of anthophyllite asbestos. (Magnification × 3000.)

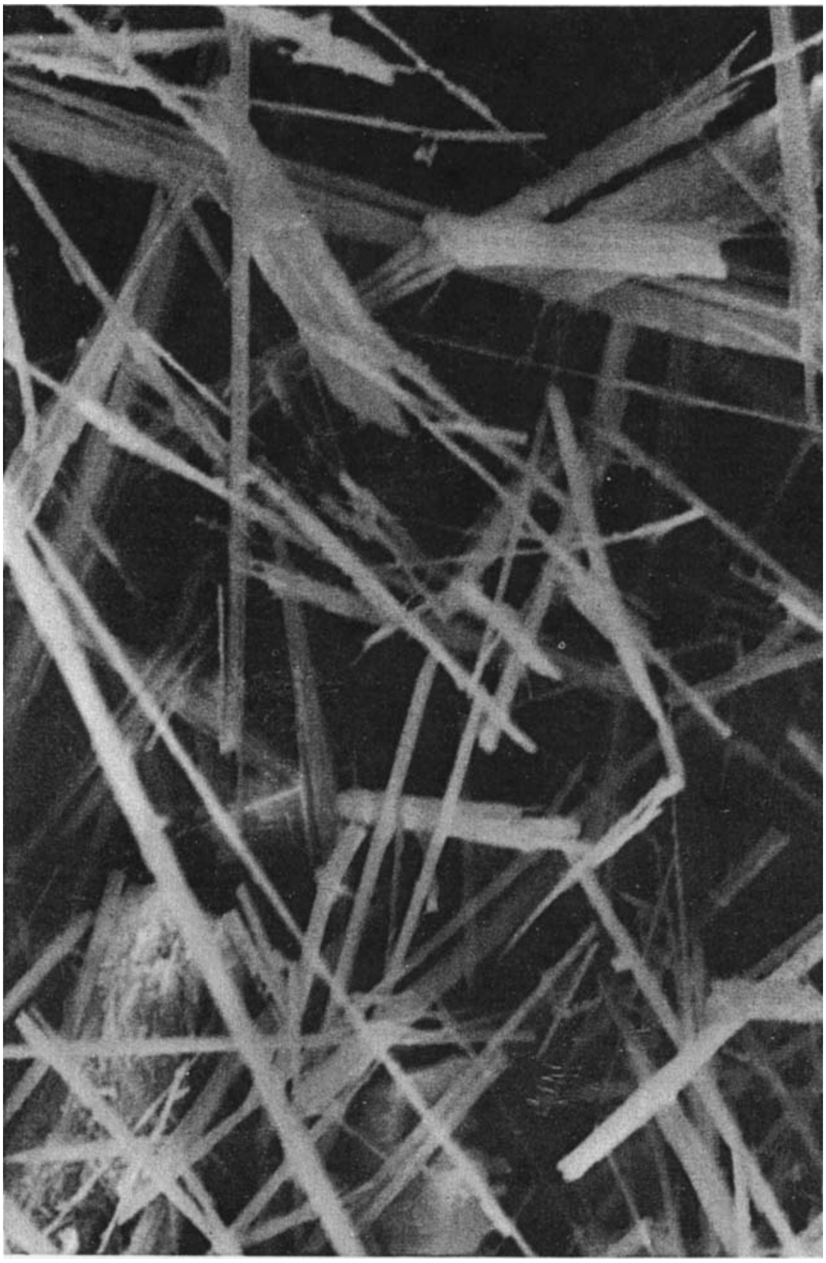

FIGURE 6. Scanning electron micrograph of crocidolite asbestos. (Magnification × 3000.)

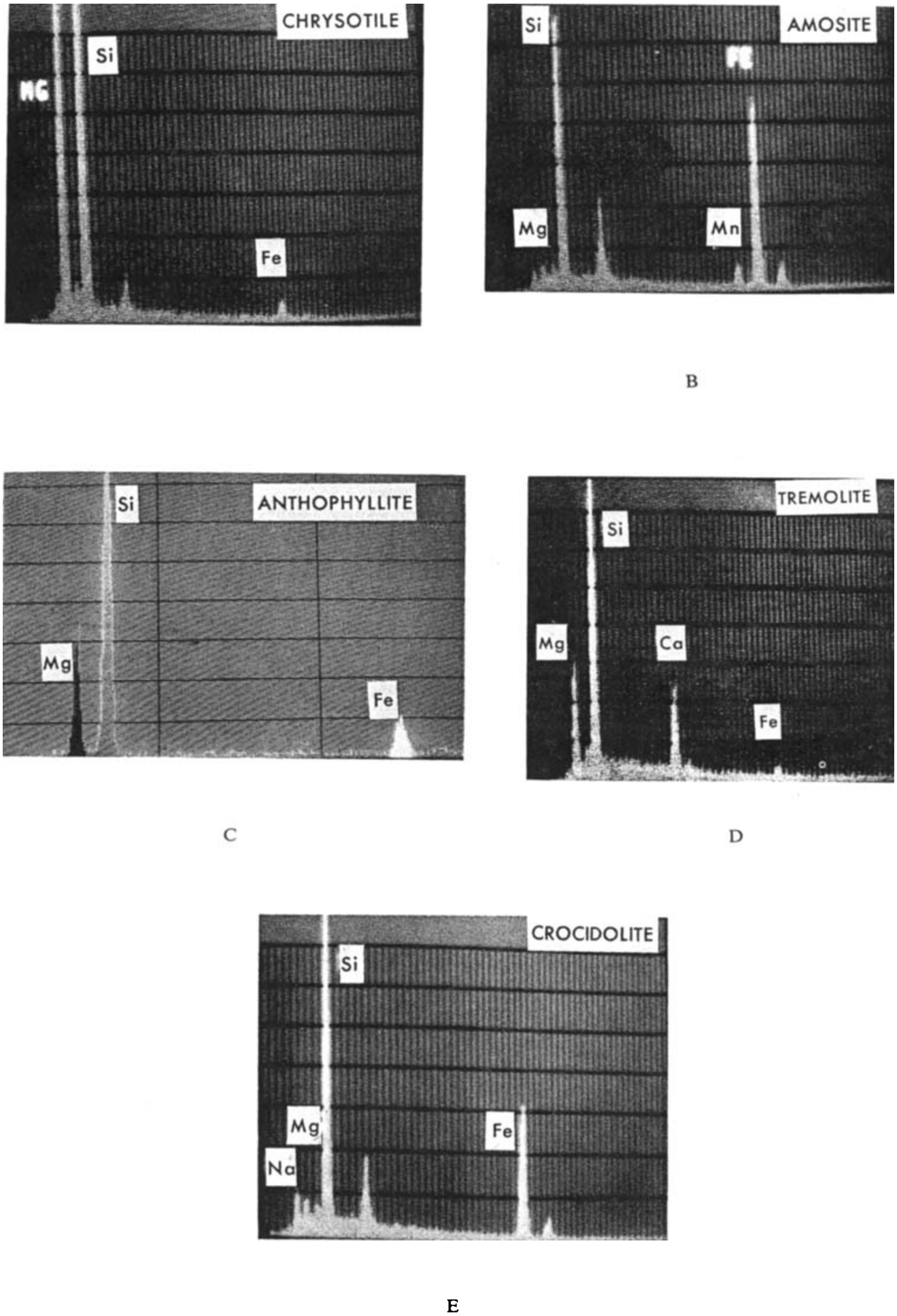

FIGURE 7. X-ray energy spectrometric analysis spectra of major commercially important types of asbestos. Height of peaks indicate the relative proportions of elements present. Unlabeled peaks are either from instrument or stub. (A) Chrysotile (Mg, Si, Fe), (B) Amosite (Mg, Si, Mn, Fe), (C) Anthophyllite (Mg, Si, Fe). (D) Tremolite (Mg, Si, Ca, Fe). (E) Crocidolite (Na, Mg, Si, Fe).A

history should also be obtained in view of the synergism between cigarette smoke and asbestos in the pathogenesis of lung cancer.

Exposure to asbestos occurs in three main settings: (1) occupational, (2) avocational, and (3) environmental.

A. Occupational Exposure

Exposure to asbestos may occur in a large number of occupations associated with the mining, milling, and manufacturing of asbestos and its products. The major occupations at risk in primary production are miners, millers, crushers, loaders, lumpers, transport workers, and processors. In the secondary and manufacturing industries, occupations such as insulation workers, construction workers, spray insulators, textile workers, loggers, painters, welders, shipyard workers, auto mechanics, electricians, plumbers, carpenters, masons, heating equipment workers, railroad engine workers, steam fitters, auto body repairmen, caulkers, and cement pipe manufacturers are particularly at risk of exposure to high levels of asbestos.

B. Avocational Exposures

Overt diseases of an occupational nature found in individuals without occupational histories are often considered medical curiosities. Avocational exposure to asbestos may occur in individuals undertaking do-it-yourself repairs including home construction, plumbing, insulation, painting, and auto body repairs. Therefore, when investigating a suspected case of asbestos-associated disease, these activities should be specifically investigated.

C. Environmental Exposures

Several studies have led to the recognition of indirect domestic exposure as a cause of mesothelioma in persons without occupational exposure.[29] Residents in urban areas, those living near asbestos mines, processing factories, demolition and construction sites, and dumping sites are the most likely candidates to have environmental exposures of an intermittent nature. Additionally, family members and launderers are likely to have exposures from handling contaminated clothes worn by asbestos workers. In former years, asbestos was used indiscriminantly as a spray fire retardant on ceilings, walls, and metal girders in public buildings, schools, and hospitals. It is estimated that over 1 million tons of asbestos has been incorporated into buildings, ships, power plants, refineries, and other facilities. It has become a major source of concern as building deterioration releases large amounts of respirable asbestos into the ambient air. In one investigation of the Yale University library, levels of asbestos greater than those currently allowed for workers in the asbestos industry were recorded.[30] Environmental exposure to asbestos is also possible for the residents of large cities from the demolition of buildings and the release of fibers from auto brake and clutch linings. Recently, asbestos-insulated hair dryers have been incriminated as a possible source of exposure. Asbestos fibers are also present in drinking water due to use of asbestos cement pipes and the dumping of asbestos tailings. Beverages, drugs, and medicines may also be contaminated by asbestos.[31] Although autopsy studies have shown a higher concentration of asbestos bodies and fibers in the lungs of urban as compared to rural dwellers, there is no definite evidence of harmful effects of these ambient exposures.

V. CLINICAL AND RADIOLOGICAL FEATURES OF ASBESTOS-ASSOCIATED DISEASES

Clinical features of asbestosis are nonspecific and indistinguishable from other forms of interstitial pulmonary fibrosis. Pulmonary functional changes that occur in asbestosis include diminished lung volume, diminished forced vital capacity, diminished lung compliance, diffusion defects, and changes characteristic of small airway obstruction.[3] In early asbestosis end inspiratory rales at the lung bases may be present. Exertional dyspnea, finger clubbing, and cyanosis are often seen in the late stages of the disease. Cough, either dry or with sputum, may occur in asbestos-exposed workers.

The earliest radiological abnormalities described in association with asbestos exposure are bilateral pleural effusions.[32,33] They are usually small and asymptomatic. When exudative effusions are present in the absence of other predisposing conditions, they are of diagnostic value. The fluid exudate is generally hemorrhagic and contains, in addition, proteins, eosinophils, and lymphocytes. Systematic pathologic studies of the exudate have not been undertaken in exposed populations. Parietal and diaphragmatic pleural plaques are important radiologic markers of asbestos exposure. They frequently undergo calcification and this feature may aid in their differentiation from other conditions. Radiologic features of asbestosis are not different from those seen in other forms of interstitial lung diseases. Early radiographic features associated with asbestosis are often found in the lower lung zones. Small fine irregular or linear opacities are found bilaterally with or without pleural shadows. Short horizontal septal linear shadows (Kerley B-lines) are common in asbestosis. With the progression of disease, the middle and upper lung zones become involved and the opacities become more profuse. Honeycomb lung and generalized visceral pleural thickening are also common with advanced disease.

VI. PATHOLOGY OF ASBESTOS-ASSOCIATED PULMONARY DISEASES

A. Pleural Plaques

Pleural plaques are generally considered by radiologists to be indicative of asbestos exposure. The prevalence of roentgenographic pleural abnormalities in the general population is less than 1%.[35] In asbestos-exposed populations, the prevalence ranges from 1 to 40%.[35,36] All commercial types of asbestos are associated with plaque formation, but the highest recorded prevalence was in Finnish anthophyllite workers.[34] Factors that may be responsible for the large range in prevalence rates in exposed individuals include type of asbestos, cumulative dose, length of exposure, and variations in individual responsiveness.

Hyaline pleural plaques occur exclusively on the parietal pleura and need to be distinguished from thickening of the parietal and visceral pleura caused by inflammation and exposure to other dusts. The plaques appear as well-defined, ivory-colored elevations of the pleural surface (Figure 8). They may be smooth or nodular and range in size from a few millimeters to several centimeters in diameter. They are often bilaterally symmetrical and tend to follow the lines of the lower ribs. Diaphragmatic involvement is common. Histologically, the plaques are composed of laminated bundles of collagen fibrils. The fibrils lie parallel to the surface and are characteristically arranged in an open "basket weave" pattern (Figure 9). Calcification is common. Asbestos bodies or coated fibers are rarely found in plaques, however, uncoated fibers are reported present in the plaques following ashing and electron microscopy.

B. Asbestosis

Asbestosis is defined as pulmonary fibrosis caused by the inhalation of asbestos. It is a progressive disease which may develop within a few years of first exposure and progress after cessation of exposure. An association between asbestos exposure and pulmonary fibrosis was noted as early as 1906.[6] Since then, numerous epidemiologic

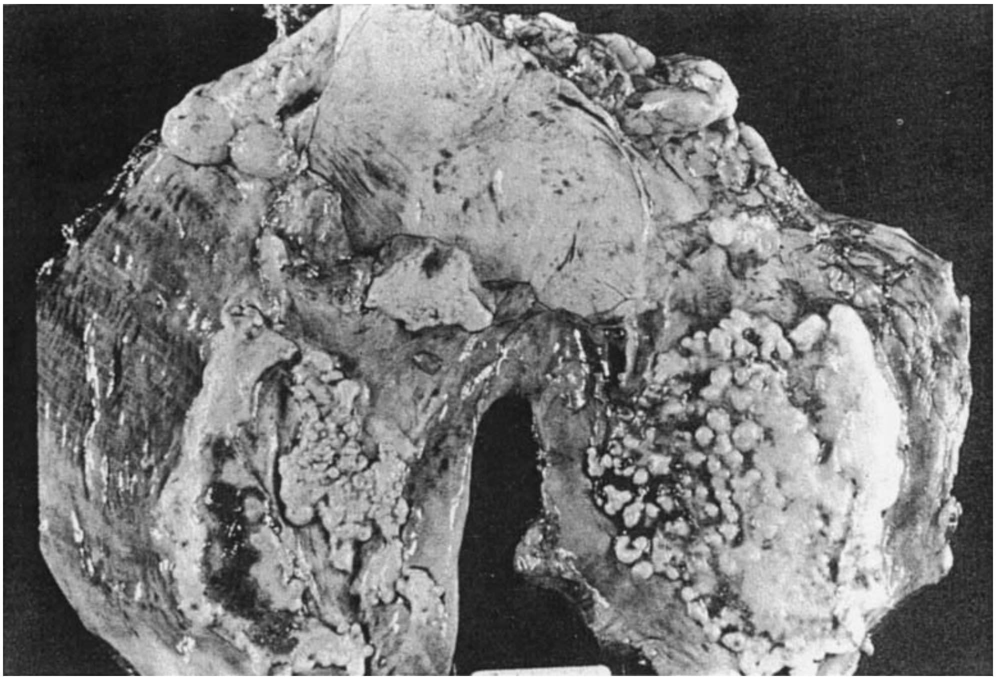

FIGURE 8. Micrograph illustrates the gross features of a pleural plaque on the diaphragmatic pleura of an asbestos worker. Note the nodularity of the plaque. (Courtesy of Milton Hales, M.D.)

and pathologic studies have confirmed this finding. All commercial types of asbestos are associated with asbestosis and a dose-response relationship has been well documented. Moderate or severe asbestosis is associated with a marked reduction in lung volume. The pleural surface is usually thickened and shows loss of its normal translucency. In mild or early forms of the disease, the cut surface of the lungs shows a fine pale gray, reticular fibrosis which is most evident in the lower parts of the lungs. As the disease progresses, the fibrosis becomes more coarse with the formation of enlarged air spaces (honeycombing) ranging in size from 3 to 15 mm in diameter (Figure 10). Honeycombing represents the most severe form of the disease and may involve zones of parenchyma ranging from a few square millimeters to an entire lobe.

Histologically, the lung parenchyma shows varying degrees of fibrosis. In mild cases, the fibrosis is predominantly seen around the bronchioles (Figure 11). More severe cases show a diffuse interstitial fibrosis (Figure 12) and in advanced cases, the pulmonary architecture has been revised to form fibrous-walled cysts lined by flattened or cuboidal epithelium (honeycombing) (Figure 13). The lung may show a chronic inflammatory cell infiltrate; however, this is a nonspecific feature. There are many causes of pulmonary fibrosis, some of which resemble that produced by asbestos dusts. The diagnosis of asbestosis, therefore, depends upon the recognition of asbestos bodies in tissue sections in conjunction with these fibrotic lesions.[37] It should be stressed that the pathological diagnosis of asbestosis can only be made by light microscopy. The use of the advanced techniques described later provides supportive evidence concerning the type and degree of exposure, but cannot be used currently to make the diagnosis.

C. Asbestos Bodies and Fibers

Asbestos bodies were recognized by light microscopy in the lungs of individuals exposed to asbestos as early as 1900.[10] Morphologically, typical asbestos bodies are club-

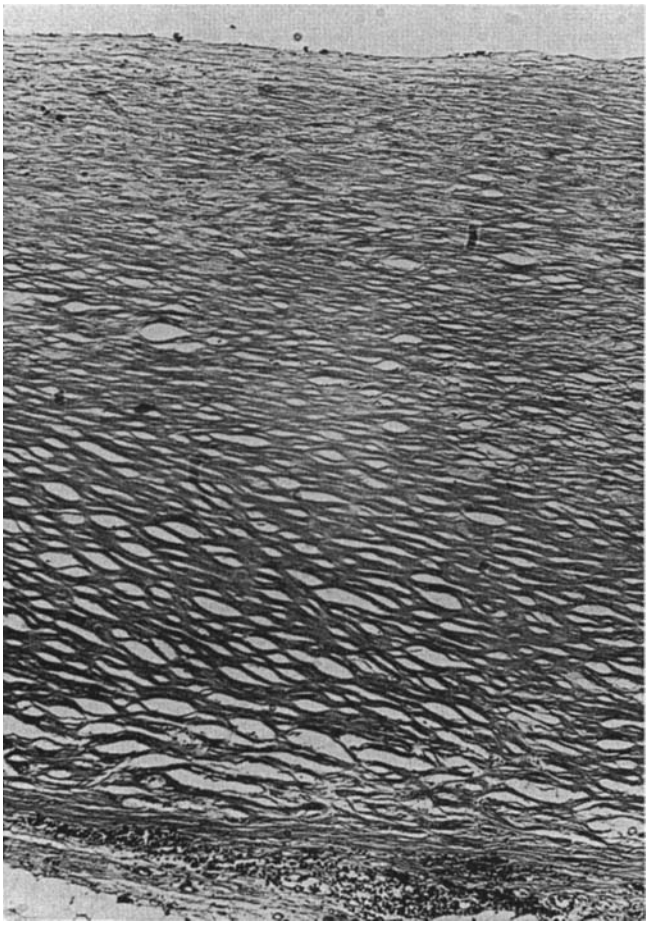

FIGURE 9. Photomicrograph of a pleural plaque showing the "basket weave" pattern. Note the acellular, avascular network of collagen fibrils.

shaped or beaded structures with a clear central core fiber (Figure 14). They may be associated with phagocytic giant cells (Figure 15). Occasional coated asbestos fibers are seen which do not have the beaded or club-shaped configuration. In hematoxylin-eosin stained sections they appear golden-brown in color and with Perl's iron stain they appear blue. Asbestos bodies are usually formed on fibers longer than 10 μm and range up to several hundred microns in length. Asbestos bodies are usually less than 3.0 μm in overall width. Core fibers vary from 0.1 to 1 or more microns in diameter. Asbestos bodies are formed by the depositon of endogenous organic iron compounds and mucopolysaccharides on the core fiber. Alveolar macrophages are thought to be instrumental in this process. Structures similar to asbestos bodies are formed by a variety of fibrous minerals other than asbestos such as talc, aluminum silicates, carbon whiskers, fibrous glass, and zeolite.[38,39] These are called "ferruginous bodies." However, the core fibers are usually irregular in shape, frequently opaque, and do not show the clear central core of a typical asbestos body. Analytical studies indicate that more than 95% of bodies showing the typical morphological features of asbestos bodies contains an asbestos fiber.[40,41] Thus, continued use of the term asbestos body is justified. Selected

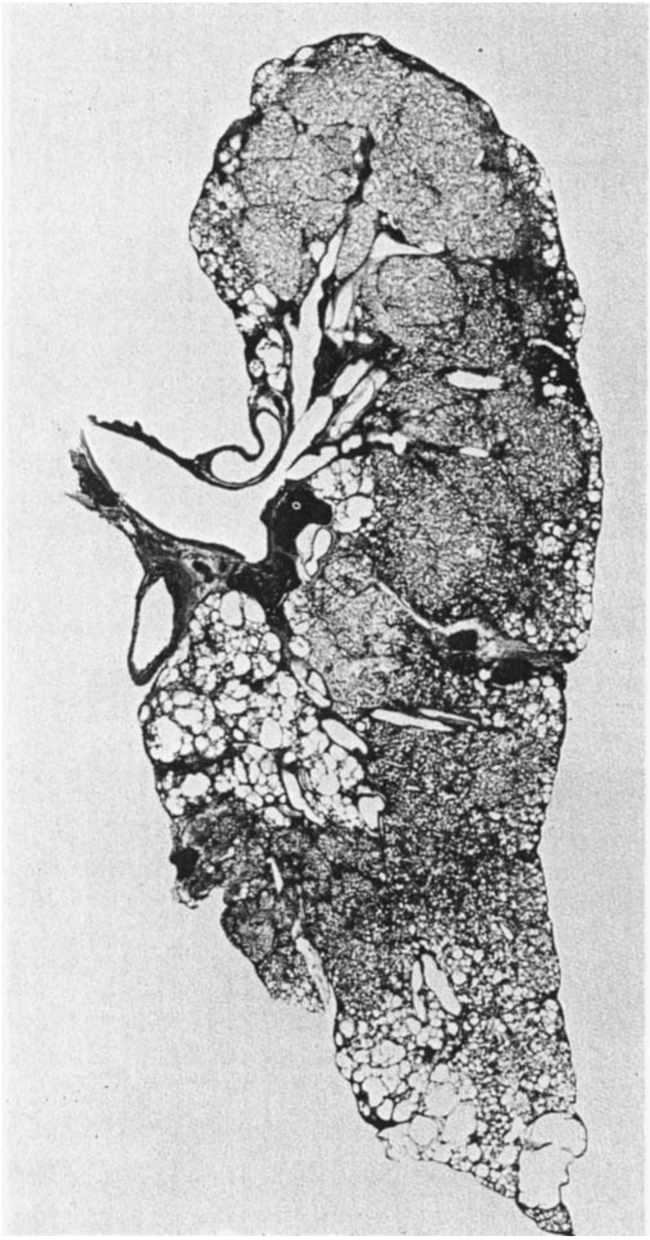

FIGURE 10. Freeze-dried lung section from an asbestos worker showing the gross features of advanced asbestosis. The disease is most prominent in the lower zones and subpleural areas of the lung.

area electron diffraction and elemental analysis of core fibers are necessary for positive identification of the fiber.

All major types of asbestos are known to produce asbestos bodies. However, electron microscopical studies on autopsy lung tissue of Canadian chrysotile asbestos workers and nonoccupationally exposed U.S. urban dwellers have shown that the majority of asbestos bodies have amphibole cores.[42] These findings suggest that asbestos bodies are formed on amphibole fibers more readily than on serpentine fibers. The

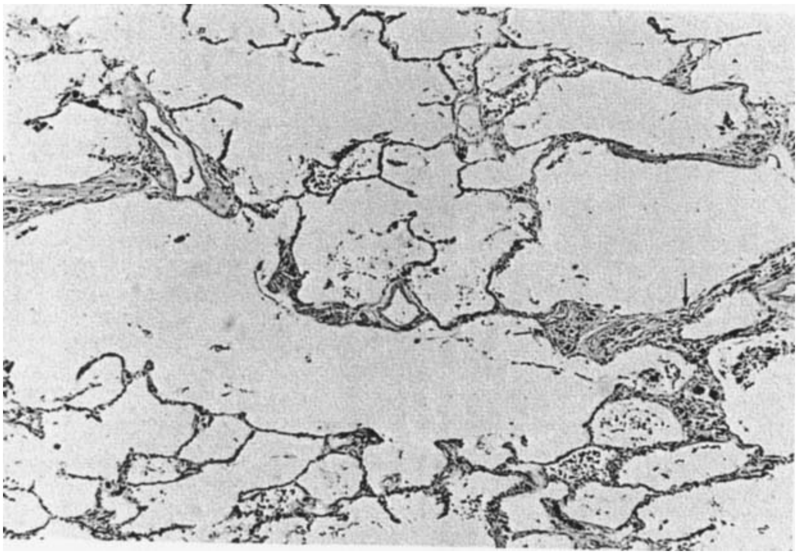

FIGURE 11. Photomicrograph of a lung section showing the early stage of asbestosis. The lesion is characterized by peribronchiolar fibrosis with mild thickening of the adjacent alveolar walls and asbestos bodies (arrows).

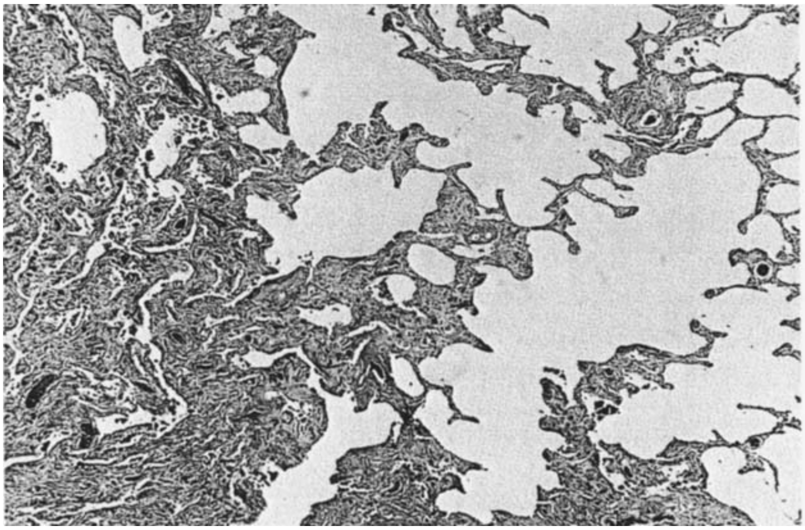

FIGURE 12. Asbestosis of severe degree showing widespread interstitial fibrosis, and asbestos bodies.

previously mentioned tendency of chrysotile asbestos to fragment into submicroscopic fibrils and to leach magnesium may account for its poor ability to form asbestos bodies.

Asbestos bodies represent only a small fraction (less than 1%) of the fibers in the lung.[42,43] Uncoated fibers similar to the inhaled particle are often present in large numbers. The majority of these fibers are too small to be resolved by the light microscope. Electron microscopic studies indicate that in asbestosis, several million fibers may be

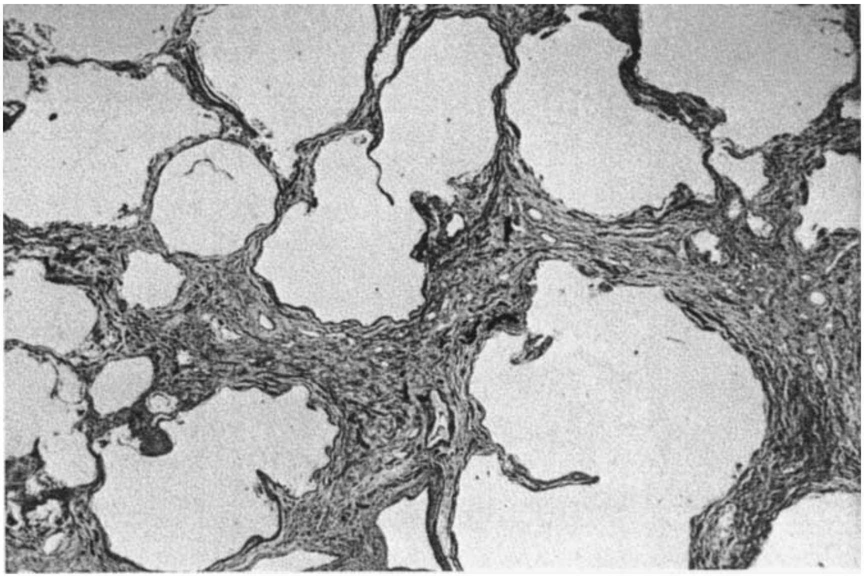

FIGURE 13. Asbestosis with honeycombing and diffuse fibrosis.

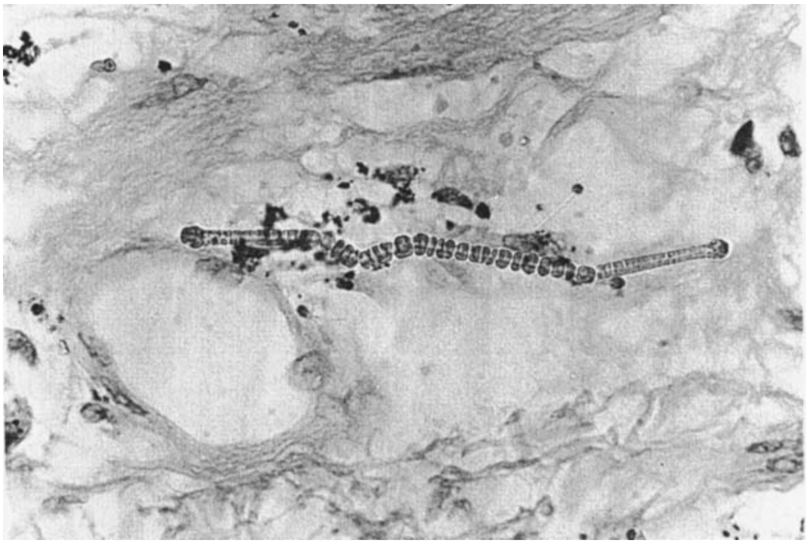

FIGURE 14. Typical asbestos body. Note the characteristic hollow core fiber with the beaded iron protein coat.

retrieved from a gram of lung tissue. The role of these small fibers in the pathogenesis of asbestosis and lung cancer has not been established.

D. Bronchogenic Carcinoma

There are inherent difficulties in establishing an association between exposure to asbestos and lung cancer. The multitude of other carcinogens present in the environment (including cigarette smoke) together with their possible synergistic effect with

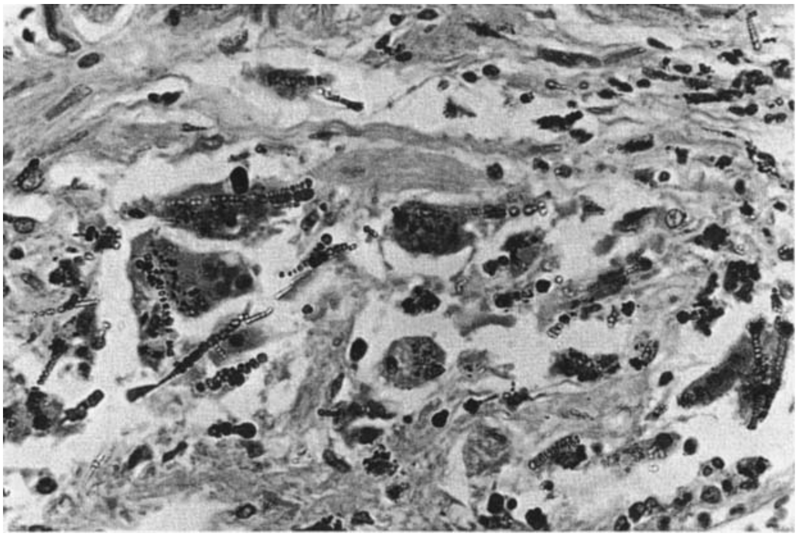

FIGURE 15. Typical asbestos bodies within phagocytic multinucleate giant cells.

asbestos complicate the picture. Furthermore, exposure effect and dose are difficult to assess in view of the long latency or induction period between asbestos exposure and the development of lung cancer.

Lung cancer is the most common asbestos-associated neoplasm. It has been reported that one in five asbestos insulation workers die from cancer of the lung.[44] An association between asbestos exposure and bronchogenic carcinoma was first suspected in the 1930s from case reports.[13,45] Subsequent autopsy studies conducted independently in the U.S. and U.K. reported an unusual number of cases of lung cancer in a series of patients dying of asbestosis.[46,47] Epidemiological studies confirmed this carcinogenic effect of asbestos.[15,48] Lung cancers associated with asbestos exposure have a long latency period (in excess of 20 years) and occur, on the average, at an earlier age than lung cancer in nonasbestos-exposed individuals.[44] The greater the cumulative exposure to asbestos, the higher the risk of dying of lung cancer.[49-51] All types of asbestos appear to be carcinogenic. Chrysotile, amosite, and anthophyllite are known to be associated with increased risk of lung cancer.[44] Mortality data on workers in the asbestos cement manufacturing industry suggest that crocidolite exposure also carries a risk for lung cancer.[50] Animal experimental studies support the epidemiologic findings.[52]

Several epidemiological studies have strongly implicated that asbestos workers who smoke have a very high incidence of lung cancer.[48,53,54] For example, it has been reported that smoking asbestos insulation workers have an approximately 50-fold risk of lung cancer compared to nonsmoking nonasbestos workers.[53,55] The high incidence of lung cancer in smoking asbestos workers cannot be explained as a result of an additive effect of two independent carcinogens. Asbestos and cigarette smoke are therefore considered as independent carcinogens acting synergistically.[56]

Autopsy studies have shown that the occurrence of bronchial carcinoma in patients with asbestosis is high. Bronchial carcinomas associated with asbestosis have no unique features and thus cannot be separately identified. The majority of these tumors, however, arise in areas of fibrosis and thus tend to be located in the lower lobes[57,58] (Figure 16). In contrast, lung tumors in the general population are more common in the upper lung fields. Asbestos-related tumors are frequently peripheral and while all histological types are represented, a predominance of adenocarcinomas has been recorded.[59] Early

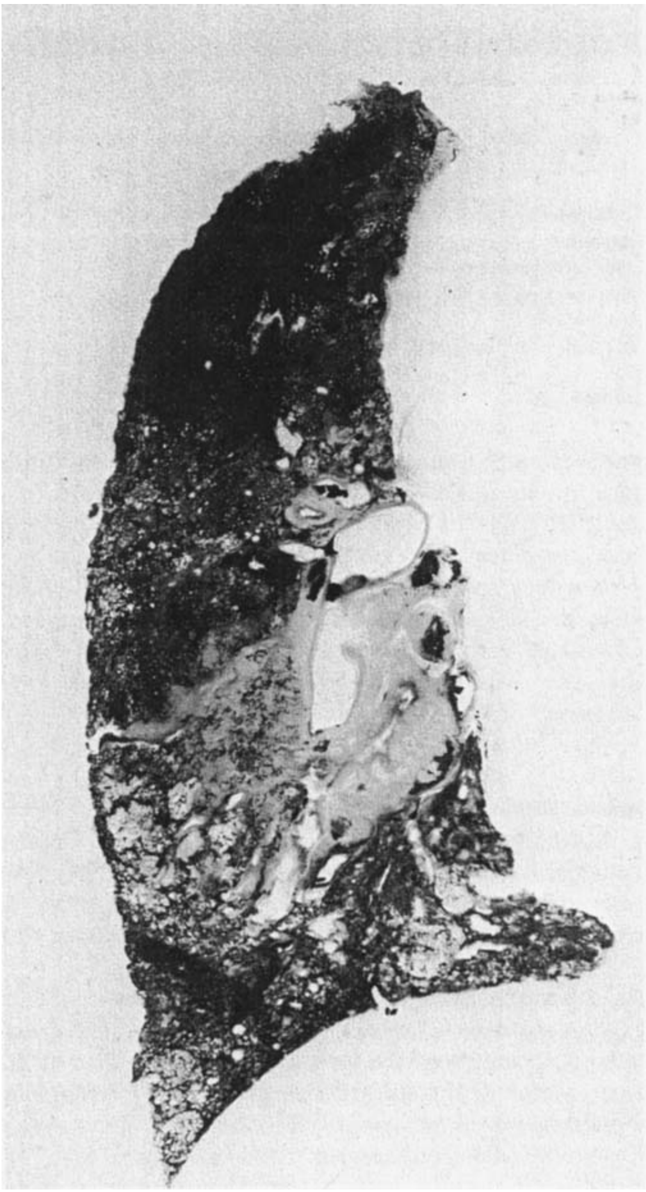

FIGURE 16. Peripheral, lower lobe adenocarcinoma in patient exposed to asbestos. (Courtesy of Russell Harley, M.D.)

pleural involvement is characteristic and this may pose some difficulty in making a distinction from pleural mesothelioma.

E. Malignant Mesothelioma

Mesothelioma is a malignant tumor that arises from the coelomic mesothelial cells that line the pleural, pericardial, and peritoneal cavities. Although exceedingly rare in the general population, mesotheliomas of the pleura and peritoneum have been shown to account for as much as 8 to 10% of asbestos workers' deaths.[20,44,60] Both peritoneal and pleural mesotheliomas are common following occupational exposure, whereas

Table 2
DIFFERENTIAL DIAGNOSIS OF MESOTHELIOMA AND ADENOCARCINOMA

Stain	Adenocarcinoma	Mesothelioma
PAS	+	±
PAS/diastase	+	−
Mucicarmine	+	−
Colloidal iron/alcian blue	+	+
Colloidal iron/alcian blue with hyaluronidase	+	−
Carcinoembryonic antigen (CEA)	+	−
Keratin	−	−
Hyaluronic acid	−	+

pleural mesothelioma is more frequently observed following nonoccupational or environmental exposure to asbestos.[44,61] It has been estimated that 85 to 90% of all mesotheliomas is related to asbestos exposure and for this reason mesothelioma is often considered a "signal neoplasm."[44] Very short and even low levels of exposure to asbestos may lead to the development of mesothelioma.[17,62,63] In occasional cases of mesothelioma, a history of exposure to asbestos cannot be demonstrated. While all types of asbestos are associated with mesothelioma, the association is stronger with crocidolite and amosite than with chrysotile asbestos.[17,64] The tumor has a long latency period, typically in excess of 20 years. There is no relationship with cigarette smoking. Presently there is only limited evidence to suggest a dose-response relationship for mesothelioma.

The tumor appears pale gray to yellow-gray in color and forms a rubbery mass encasing the lung. It usually extends into the interlobar fissures. Direct invasion of the lungs or thoracic cage occurs late in the disease and death is usually related to the local effects of the tumor rather than to metastases which occur in approximately 50% of cases. Mesotheliomas of the peritoneum have a similar appearance to those arising in the pleura.[65]

Microscopically, the tumor can be classified into three broad histological types: (1) tubopapillary, (2) sarcomatous, (3) mixed.[65] Differentiation of the tubopapillary type from peripheral adenocarcinoma of the lung or metastic tumor may be difficult. Special stains are often useful in the differential diagnosis of mesothelioma. Mesotheliomas usually contain intracellular hyaluronic acid which stains with alcian blue and Hale's colloidal iron. The hyaluronic acid can be removed by pretreatment with hyaluronidase. Mesotheliomas usually do not stain with periodic acid Schiff when pretreated with diastase. Adenocarcinomas, on the other hand, usually contain intracellular diastase resistant PAS positive, mucicarmine positive droplets (Table 2).

F. Cancer of Other Organs

Several mortality studies of asbestos insulation workers, mining and milling workers, and secondary users have shown a greater than expected risk for all types of cancers. Gastrointestinal cancer is increased two- or threefold compared to the expected rate in the general population. Asbestos exposure is also associated with increased occurrence of cancer of the larynx and esophagus in smokers.[55] In addition to this, there is some evidence that asbestos exposure is associated with increased frequency of cancers of the kidney, ovary, pancreas, and other organs.[3,18-24,66] Nonoccupational (environmental) exposure to asbestos has also been etiologically related to cancers of the gastrointestinal organs.[67]

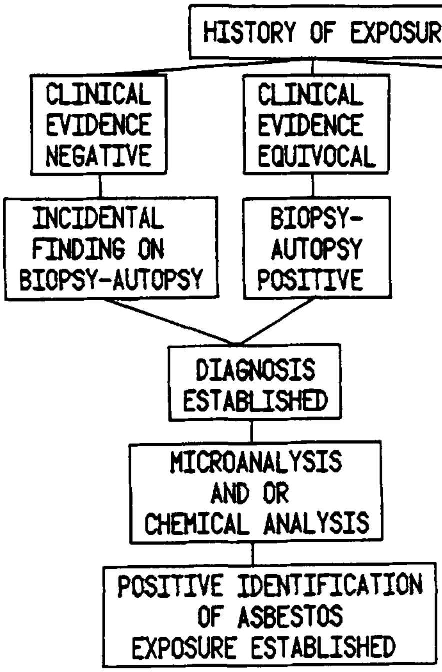

FIGURE 17. Algorithm for the diagnosis of asbestosis and establishment of exposure to asbestos.

The increased risk for cancers of the larynx, esophagus, and other sites in the gastrointestinal tract may be related to direct exposure from ingested or expectorated fibers. It is not readily apparent how asbestos exposure increases the risk of cancer of internal organs, though several studies have shown that asbestos bodies and fibers are widely distributed throughout the body tissues.[68]

VII. ROLE OF ADJUNCT TECHNIQUES IN DIAGNOSIS OF ASBESTOS-ASSOCIATED DISEASE

The relationship of the adjunct techniques to the diagnostic process is shown in Figures 17 and 18. In practice, a history of exposure together with clinical and/or pathological evidence of asbestosis are sufficient evidence to make the diagnosis of asbestosis. In situations where the diagnosis is suspected but a positive exposure history cannot be obtained, the adjunct techniques play an essential role in characterizing exposure. This is particularly relevant to the rare case where the patient has a history of exposure to asbestos, shows typical pulmonary fibrosis on lung biopsy, but does not have asbestos bodies in tissue sections. Microanalytical studies may be indicated in patients with pleuropulmonary malignant disease, especially if there is a history of exposure to asbestos. The adjunct techniques are also valuable in epidemiological studies to (1) provide information on the asbestos burden in relation to suspected or known health effects; (2) provide information on dose response and threshold levels; (3) monitor changes in environmental pollution with time; (4) monitor the effectiveness of regulatory controls; and (5) enable the recognition of new or unsuspected diseases associated with asbestos exposure.

Microanalytical techniques alone cannot be used to make a diagnosis of asbestos-associated disease. This is due to a number of reasons. First, the relationship between

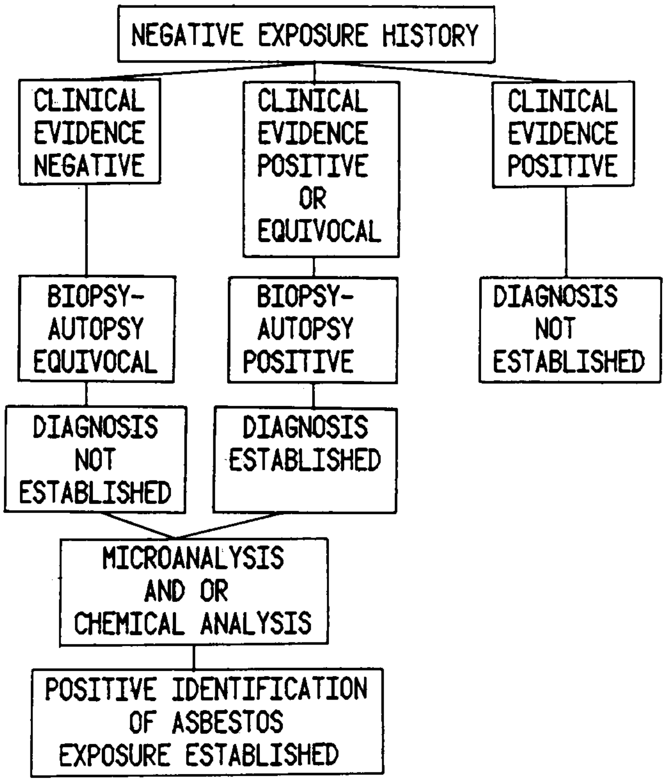

FIGURE 18. Algorithm for the diagnosis of asbestosis and establishment of exposure to asbestos.

asbestos exposure and disease is highly complex and is influenced by factors such as asbestos type, cumulative dose, disease latency, variabilities in individual susceptibility, and synergisms with extraneous factors, e.g., cigarette smoking. Second, because fibers are slowly cleared from the lung, microanalytical studies will tend to reflect more recent exposures. Third, different types of asbestos are cleared at different rates. Thus the asbestos recovered from the lung may not necessarily be the most important in the pathogenesis of the observed disease process. Fourth, almost all individuals, irrespective of their occupation, have asbestos bodies and fibers in their lungs. It is therefore important to compare the test results with those from controls matched for age, sex, and geographic region. Finally, the analytic techniques have not been adequately standardized between laboratories; therefore, comparison of values obtained in different laboratories may be misleading. Despite these limitations, it is generally recognized that the asbestos fiber burdens in asbestos workers and in patients with asbestos-related pleuropulmonary disease (with the possible exception of some cases with mesothelioma) are usually orders of magnitude higher than those observed in the general population.

Although there are many techniques available for the identification of asbestos minerals, only a few of them are of practical value in clinical practice. Choice of technique will depend upon a number of factors: (1) the concentration and distribution of asbestos in the tissues; (2) the amount of tissue available; (3) the type of information required — qualitative or quantitative; and (4) the availability of equipment and expertise.

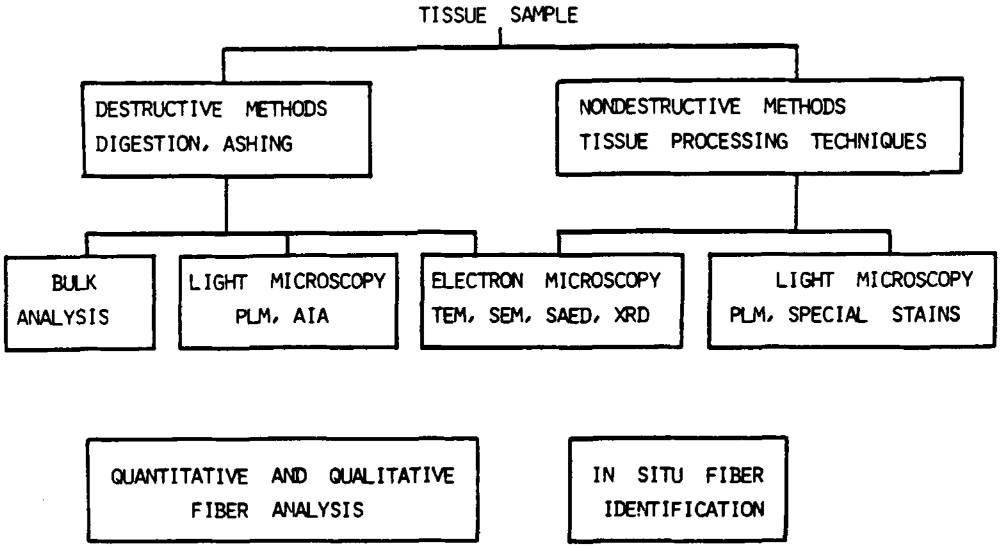

FIGURE 19. Identification of asbestos fibers and bodies in tissue samples.

There are two basic approaches to microanalysis: destructive and nondestructive.[69] The destructive approach using digestion or ashing is preferable for quantitative studies, however, larger quantities of tissue are required for analysis. The nondestructive approach is of value when the amount of tissue available is small (e.g., a single tissue section) and for correlating fiber burden with anatomical features of disease. The two approaches provide complementary information. A schematic approach to microanalysis showing the relationships between the various techniques is shown in Figure 19.

Light microscopy is of value in the identification of asbestos fibers in environmental samples and for recognizing asbestos bodies in tissue sections and digestates.[43] It is of virtually no use for the recognition of bare fibers in tissue sections as they tend to be obscured by tissue components and are usually smaller than the limits of detectability by the light microscope.

Definitive identification of asbestos fibers and bodies requires information on particle morphology, crystal structure, and chemical composition. This capability exists in transmission electron microscopes equipped with X-ray energy spectrometry (XES) and selected area electron diffraction (SAED). Scanning electron microscopy (SEM) is of lesser value in view of the fact that it is not equipped with electron diffraction capability. Similarly, bulk chemical analysis using X-ray diffraction is of limited value due to overlapping spectral patterns produced by nonasbestos silicates commonly found in the lungs.

The diagnosis of asbestos-related disease and the identification of asbestos in the tissues should be approached systematically. We have found the following tissue sampling procedure and microscopical methods useful in the diagnosis of asbestosis and the identification and quantitation of asbestos fibers and bodies in tissues.

A. Tissue Sampling

In order to make the diagnosis, grade the severity of the disease, and quantitate asbestos bodies and fibers in the lung, it is important to collect an appropriate number of samples from all major anatomical sites of the lung. Adequate tissue sampling for analysis is important, since in asbestosis the lesions are irregularly distributed and analysis of tissue samples from adjacent areas may give wide variability in results.

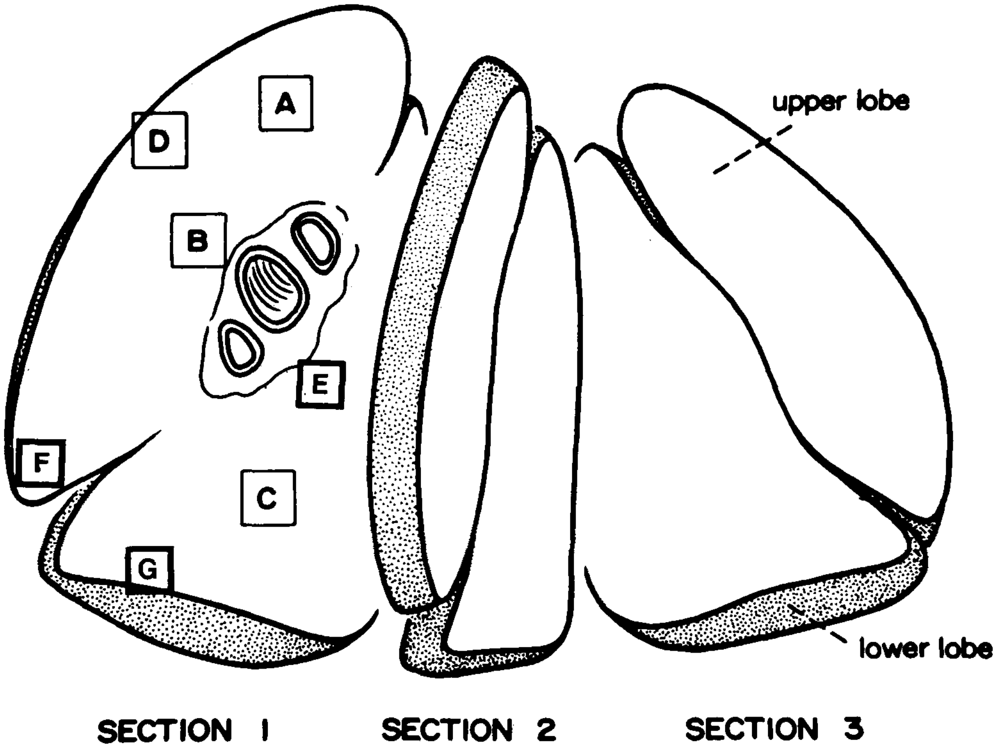

FIGURE 20. Left lung cut into three slices to show sites of tissue sampling (A to G).

B. Autopsy

The lungs at autopsy should be excised with the main stem bronchus and major blood vessels. The bronchus is cannulated and inflated with 10% filtered buffered formalin at a pressure of 25 cm of water for 1 hr. The bronchus is then tied and fixed in formalin for 3 days. The lung is sliced sagitally into three pieces making a center section 1-in. thick with two peripheral sections (Figure 20). Serial whole lung sections can be made from the center section according to the Whimster modification of the Gough and Wentworth technique.[70] Alternatively, whole lung, freeze-dried sections can be prepared. These latter procedures allow both gross evaluation of asbestosis and its radiological correlations.[71] Seven tissue samples (1 × 1.5 cm) are taken from predesignated areas (A to G) of the peripheral section (Figure 20). Ideally, tissue samples should be taken from all lobes of both lungs to include visceral pleura, subpleura, deep parenchyma, and portions of major bronchi.[37]

C. Lung Biopsy

Lung biopsy is not indicated in a typical case of asbestosis with a documented occupational exposure. When the clinical diagnosis is equivocal and/or exposure histories cannot be obtained, an open lung biopsy or thoracotomy may be appropriate.

Since lesions have an irregular distribution in the early stages of disease, adequate tissue sampling is important; needle biopsies are of little value due to the limited size of tissue sampled. An open lung biopsy technique which yields adequate portions of lung tissue has been described.[72] The lingular and angular parts of the lung should be avoided as these areas commonly show nonspecific fibrotic change. Tissues obtained at biopsy can be inflated to prevent collapse and maintain the parenchymal architecture.[73]

D. Light Microscopy (LM)

Light microscopic histopathologic evaluation is the most valuable technique available to the pathologist in the diagnosis of asbestosis and identification of asbestos bodies. In our laboratory, tissue samples obtained from the seven predesignated anatomical sites of the left lung are processed for light microscopy in the routine way. Serial 5-μm sections are obtained from each tissue block and labeled 1 to 10. Sections 1, 2, 5, 6, 9, and 10 are mounted on microscopic slides; sections 1 and 10 are stained with hematoxylin and eosin, sections 2 and 9 are stained with Perl's iron stain, and sections 5 and 6 are retained for special stains. Sections 3, 4, 7, and 8 are mounted on carbon planchets for scanning electron microscopic studies. In cases where asbestos bodies or coated fibers are not easily detected by routine histopathological evaluation of 5-μm sections, 30-μm-thick sections may be useful. In cases where the number of asbestos bodies is few, their location should be marked on the slide for future reference. Light microscopy is of value for counting asbestos bodies in both tissue sections and digestates. However, it has limited use in the identification of asbestos fibers in tissues due to their weak birefringence, small size, and poor contrast with tissue components. Phase contrast microscopy is also of little value in the assessment of tissue sections, however, it may be of value for studying tissue digestates or environmental samples. Because most of the fibers present in tissues are below the resolving capacity of the light microscope, quantitative information is limited to enumeration of fibers greater than 5 μm in length. Therefore, to obtain a better appreciation of the total lung burden, analytical electron microscopy with microchemical analysis and selected area electron diffraction studies are necessary.

E. Extraction of Asbestos Fibers and Bodies from Tissues

Sample preparation should be kept as simple as possible. Avoidance of specimen contamination must be given primary consideration when preparing samples for analysis. Prior to use, reagents and water should be filtered through a 0.2-μm membrane filter to remove any contaminating fibers or other particulate. Work on the sample and reagent preparation should be conducted in areas with high efficiency (HEPA) filtration or under a class IIb laminar flow biological safety cabinet. Ideally, several grams of wet tissue from different areas of the lung should be analyzed. Both fixed and unfixed tissues can be used. When fixed tissues are processed, a sample of the fixative should be analyzed. Embedded tissues can also be used following deparaffinization in xylene. The wet weight of the samples and of the whole lung should be recorded. The sample can then be dried and reweighed. Based on these data, estimates of the concentration of fibers in the lung and total fibers per gram of lung can be calculated. Because drying may result in fiber breakdown, it has been recommended that the dry weight of the tissue sample to be digested is estimated by drying and weighing an adjacent portion of tissue.

Wet chemical digestion techniques are usually preferred for the isolation of asbestos minerals as high temperature ashing procedures may alter their chemical composition. Although low temperature plasma ashing is satisfactory, expensive equipment and complex operational procedures limit the use of this technique in routine laboratory practice. Several types of acids, alkalies, and oxidizing agents (such as hydrochloric acid, nitric acid, sodium and potassium hydroxide, laundry bleach, hydrogen peroxide, etc.) are commonly used for the extraction of minerals from lung tissues. Some of these strong chemicals are known to react with asbestos and change their chemistry and crystalline properties; therefore milder extraction procedures are recommended. Laundry bleach and hydrogen peroxide are the most widely used reagents. From 1 to 2 g of tissue is sliced with a pair of scissors in a large plastic centrifuge tube and digested overnight with 20 to 30 m*ℓ* of filtered bleach (Clorox) at room temperature.[74] The digested material is sedimented at high speed 20,000 rpm for 30 min and the supernatant is removed with a Pasteur pipette. If digestion is incomplete, additional Clorox is added and the tube is heated to 60°C and left for a few hours. When digestion is complete, the sample is centrifuged and the sediment treated with a mixture of 1:1 chloroform and methanol to remove the lipids and carbonaceous material. If excess carbonaceous material is present, the sediment is treated with 30% hydrogen peroxide for a few hours at 60°C. The undigested residue is then diluted with distilled water and made up to a convenient volume depending on the anticipated burden of asbestos. A drop of "Tween 80" is added to prevent clumping of fibers and debris. Aliquots of 10 or more volumes are then filtered through a 0.2-μm 25-mm diameter Nuclepore filter. Uniform deposition of particles is obtained by prewetting the filter paper before filtration. The filters are then allowed to dry in Petri dishes and are screened by light microscopy to determine the asbestos load. If only a few fibers and bodies are detected, additional filters are prepared using larger volumes of the aliquot. Since the tissue digestion and all the preparative procedures are carried out in the same centrifuge tube, loss of fibers or bodies is minimal.

Asbestos bodies can be counted from the filter paper preparations by light microscopy. Also, an estimate of lung asbestos fibers by phase contrast microscopy can be made from these preparations. For the electron microscopic enumeration of asbestos fibers, pieces of filter are cut out and placed on formvar-carbon coated nickel grids. The Nuclepore filter is dissolved with acetone or chloroform leaving the dust particles on the EM grid. The grid is transferred to an electron microscope equipped with an energy dispersive X-ray analyzer and SAED. Alternatively, the filtered samples are dried and carbon coated. Portions of carbon-coated filter are transferred to Petri dishes containing chloroform or acetone to dissolve the filter. Particles, embedded in the carbon film, float on the surface of the solvent and can be transferred to EM grids.

F. Scanning Electron Microscopy (SEM) and X-Ray Energy Spectrometry (XES)

Serial histological sections (3, 4, 7, and 8) are mounted on 1-in. diameter carbon planchets. The sections are then deparaffinized in xylene, air dried in a filtered air chamber to avoid contamination, and coated with a 200-Å-thick layer of carbon using a vacuum evaporator. The sections are then studied in an SEM equipped with a backscattered electron detector and XES at magnifications of 500 to 5000×. Backscattered electron imaging is useful for identifying mineral inclusions in tissue sections as the signal generated (and hence contrast) is proportional to atomic number. Therefore, mineral inclusions, which usually have a higher average atomic number than the tissue background, are readily visualized.[69] Asbestos bodies and fibers identified by backscattered electron imaging are analyzed by X-ray energy spectrometry. The analysis is performed in the spot mode. Uncoated fibers and the core fibers of asbestos bodies can be analyzed by this technique. The morphology of the fiber together with its elemental profile can be used to make a reasonable estimate of identity. Estimates of fiber length are unreliable in tissue sections.

For the examination of samples prepared from tissue digestates, a known volume of digestate is dispersed on a carbon planchet; alternatively, a piece of Nuclepore filter may be mounted directly on a carbon planchet. The preparations are then coated with carbon and asbestos bodies and fibers are counted (Figure 21).

The major limitation to the use of the SEM is the resolution. Small asbestos fibers less than 0.05 μm in diameter are not resolved well in the SEM. In addition, because the SEM is not equipped with electron diffraction capabilities, information on crystal

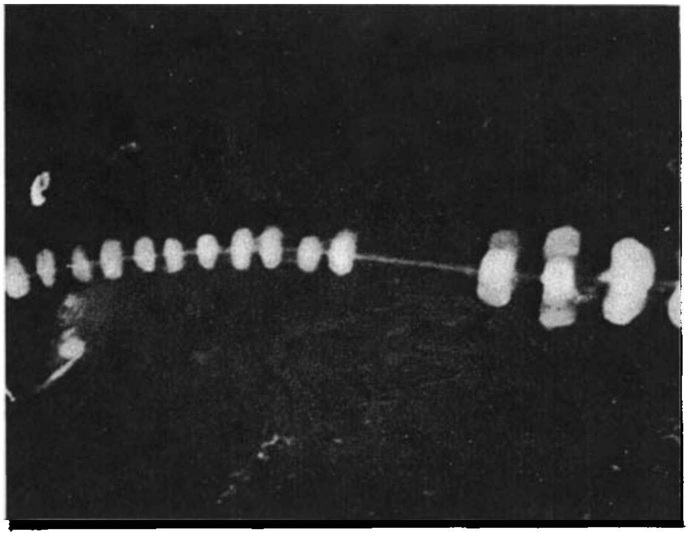

FIGURE 21. Scanning electron micrograph of lung tissue digestate on carbon planchet showing a beaded asbestos body with a central core fiber.

structure cannot be obtained. The major advantages are that large areas of tissue or digestate can be examined and backscattered imaging can be used to identify fibers and bodies in situ.

G. Transmission Electron Microscopy (TEM)

Transmission electron microscopes equipped with X-ray energy spectrometry and selected area electron diffraction enable a threefold examination of asbestos by morphology, chemistry, and crystalline structure. Such analytical instruments are essential for the accurate characterization and quantitation of asbestiform minerals. The major advantages of TEM are its high resolving power, enabling smaller asbestos fibers to be identified, and the ability to obtain information on crystalline structure. In addition, the morphological information obtainable through TEM may be very useful in the identification of certain types of asbestos such as chrysotile. Electron microscopic studies of tissue sections are not very useful in the study of asbestosis because of sampling limitations and loss of bodies and fibers in sectioning. Accordingly, for TEM studies, asbestos bodies and fibers are usually isolated from lung tissue by the Clorox digestion technique described earlier. Nuclepore membrane filter preparations are most suitable for TEM studies.

For elemental analysis it is necessary to use grids that do not interfere with the elements involved. For example, the identification of crocidolite by TEM requires the use of nickel, platinum, or nylon grids rather than copper grids so that the sodium energy peak ($K\alpha$) of crocidolite is not confused or overlapped by the $L\alpha$ copper peak.

Asbestos bodies and asbestos fibers are counted in a predetermined number of grid squares and multiplied by a correction factor to convert to the total filter area. The total number of asbestos bodies and fibers thus obtained for the preparation is then divided by the weight of the tissue used in the preparation of tissue digestate. The asbestos bodies and fibers are then expressed as number per gram of lung tissue.

H. Aspect Ratio Measurements (ARM)

Aspect ratio measurements (length/width) of asbestiform minerals are often used as

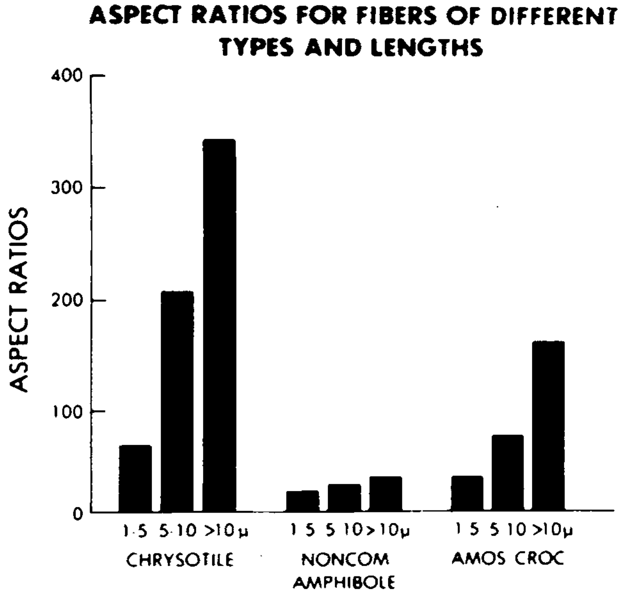

FIGURE 22. Aspect ratios for asbestos fibers found in the lungs of 21 patients from general population. (Reproduced with permission from Churg, A. and Warnock, M. L., *Am. Rev. Resp. Dis.*, 122, 669, 1980.)

a microscopic identification criterion.[75,76] For regulatory purposes, OSHA defines a fiber as having an aspect ratio of 3:1 or greater. This definition is relatively crude as it includes nonasbestos minerals and cleavage fragments that assume a fibrous habit; however, it is a simple and quick method for screening for asbestos. For these purposes light microscopy is used. However, for research studies the measurement is best performed in the TEM at magnifications of 20,000 ×. Length and width measurements are recorded for each fibrous particle. The majority of asbestos fibers have aspect ratios greatly in excess of 3:1, ranging up to 300:1 or more. In addition, aspect ratios vary with asbestos type (Figure 22).

I. Selected Area Electron Diffraction (SAED)

Interaction of the transmitted electron beam with a crystalline particle produces a diffraction pattern. As most minerals have distinct diffraction patterns, it is an important identification technique.[77-80] Diffraction patterns produced by fibrous minerals should be compared to UICC standard reference patterns. The technique can be made more accurate by recording two SAED patterns obtained by tilting into two distinct zone axis orientations.[78]

The technique has some limitations such as overlapping patterns produced by twinning or structural defects, instrument variabilities, and errors in interpretation.[81] Occasionally, fibers lose their crystallinity due to interactions with tissues or as a result of destructive analytic techniques. Because the fibers become contaminated by prolonged exposure to the electron beam, it is advisable to perform SAED before XES analysis. Despite these limitations, SAED is probably the most valuable technique available for the identification of asbestos fibers in tissue samples.[82-84] The diffraction pattern produced by chrysotile asbestos is specific[77,79,82] (Figure 23). This pattern taken in conjunction with a tubular morphology, an average fiber diameter of 250 to 500 Å units, and an elemental makeup of Mg, Si, Fe are characteristic of chrysotile asbestos. In

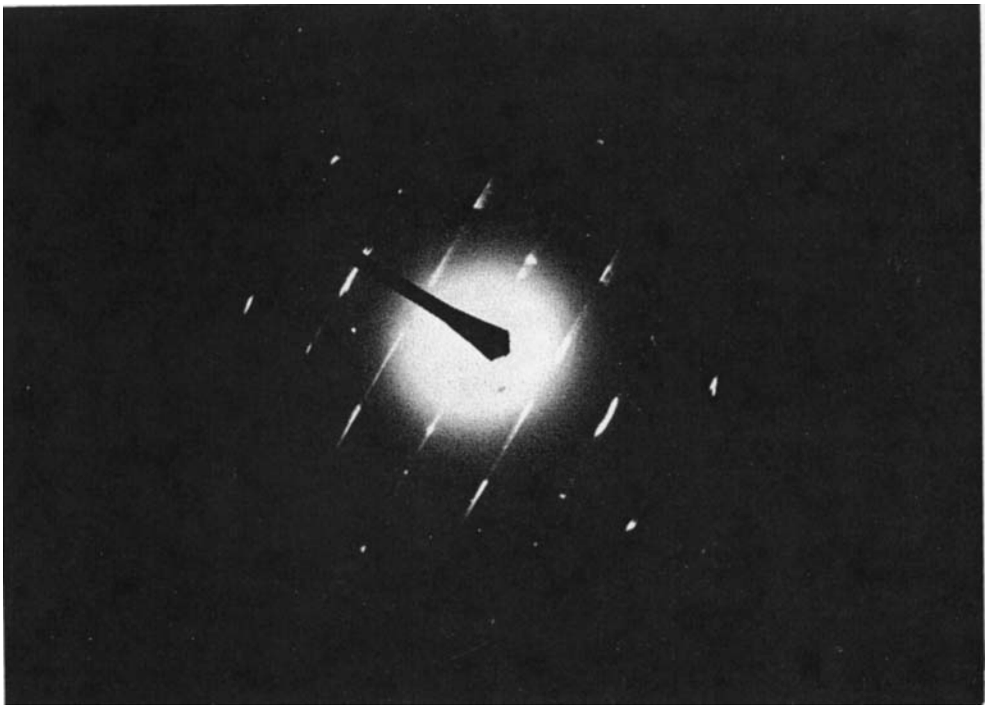

FIGURE 23. Selected area electron diffraction pattern for chrysotile asbestos. The diffraction pattern for chrysotile is specific.

contrast, the diffraction patterns produced by the amphiboles are only specific for the group as a whole and will not distinguish the individual types.[77,82] Thus, identification of amphibole minerals requires, in addition, chemical information derived from X-ray analysis.

J. Automated Image Analysis (AIA)

TEM and SEM interfaced with automated image and particle chemical analysis capabilities are now being increasingly used for particle characterization and identification.[85] Automated image analysis systems permit the rapid characterization of SEM and TEM images. Feature analysis may be programmed to determine size, number, perimeter, and chemical characterization by X-ray analysis. Low temperature ashed or chemically digested tissue aliquots are filtered through a 0.1-μm Nuclepore filter. For SEM interfaced systems, the filter is attached to a 25-mm carbon planchet with colloidal graphite and carbon coated. The plachet is then examined in an SEM equipped with computer-assisted image analysis and X-ray analysis systems. A suitable field of view with particles is selected and the image is converted to a binary image by the computer. The computer then scans the images and the particles detected are traced on a series of horizontal, vertical, and diagonal lines with the electron beam. Area, perimeter, width, length, and number are calculated from these line scans. X-ray spectrometric analysis of individual particles is then performed for a specified time. With a 5-sec analysis time, it is possible to characterize and analyze the chemistry of 600 to 650 particles per hour. In TEM systems, polycarbonate filters are used.

K. X-Ray Diffraction (XRD)

Quantitative X-ray powder diffraction is a very valuable method for the study of several minerals. It is of little use for quantitative estimation of tissue asbestos due to interfering spectral patterns produced by other minerals in the lung. However, XRD has been used sucessfully for the quantitative analysis of small environmental samples of chrysotile asbestos using computer-automated X-ray diffraction.[86] Briefly, dust samples collected on membrane filters are plasma ashed. The dust is suspended in alcohol and dispersed uniformly on silver filters. Measurements of integrated peak intensities and background intensities are made by a computer-automated X-ray diffractometer.

VIII. CLINICAL AND EPIDEMIOLOGICAL SIGNIFICANCE OF ASBESTOS BODIES AND FIBERS

The total numbers of fibers identified in the lung tissue samples will depend to some extent on the method employed for identification. Fiber counts based on light microscopic methods will greatly underestimate the total fiber burden compared with data based on electron microscopic studies, as the majority of fibers in tissues are less than 5 μm in length. Quantitative estimates of bodies and fibers in lung samples are usually expressed in terms of numbers per gram of wet or dry lung. Generally the weight ratio of wet lung to dry lung is approximately 10:1. Thus a conversion factor needs to be applied when comparing data presented in different units. The techniques and units are identified in the tables. A variety of epidemiologic data indicates that a positive linear dose-response relationship exists for asbestosis and asbestos-associated lung cancer.[49-51,87] A well-defined dose response for mesothelioma has not been demonstrated. Although the numbers of asbestos bodies and fibers in the lung reflect prior exposure, the exact relationship between exposure and lung fiber concentration cannot be calculated due to incomplete knowledge of the clearance and dissolution properties of asbestos fibers. Moreover, the distribution of asbestos bodies and fibers varies widely within the lung. Asbestos appears to be maximally concentrated in the immediate subpleural and lower lung zones[88-90] (Figure 24). For these reasons, quantitative and qualitative studies of pulmonary asbestos burden need to be interpreted with caution, especially where small biopsies are used.

There is a good correlation between the number of asbestos bodies observed in tissue sections compared to the number present in tissue digestates (Figure 25). It has been estimated that two asbestos bodies on a 2 × 2 cm, 5-μm-thick tissue section are equivalent to approximately 200 asbestos bodies per gram of wet fixed tissue.[91] A reasonably good correlation between numbers of asbestos bodies in sputum and numbers in lung tissue digestates has also been demonstrated.[92] Studies reported to date have focused on workers occupationally exposed to asbestos and groups of patients with disease associated with asbestos exposure. Comparison groups are usually derived from urban residents with no recorded occupational exposure to asbestos.

A. General Population and Asbestos Worker Population Studies

Asbestos bodies can be isolated from the lungs of approximately 90% of the general population. More bodies are found in urban dwellers[93,94] compared to rural dwellers and in blue collar workers compared to white collar workers.[95] The number of asbestos bodies obtained from autopsied lung in patients without known occupational exposure to asbestos varies, but usually lies within the range of 0 to 500 bodies per gram of lung (Table 3). A study of blue collar male workers, who presumably had low level exposure to asbestos but did not specifically work with asbestos, showed that 65% of the construction workers, 45% of steel workers, and 32% of other manual laborers had more than 100 asbestos bodies per gram of lung compared to a group of white collar workers

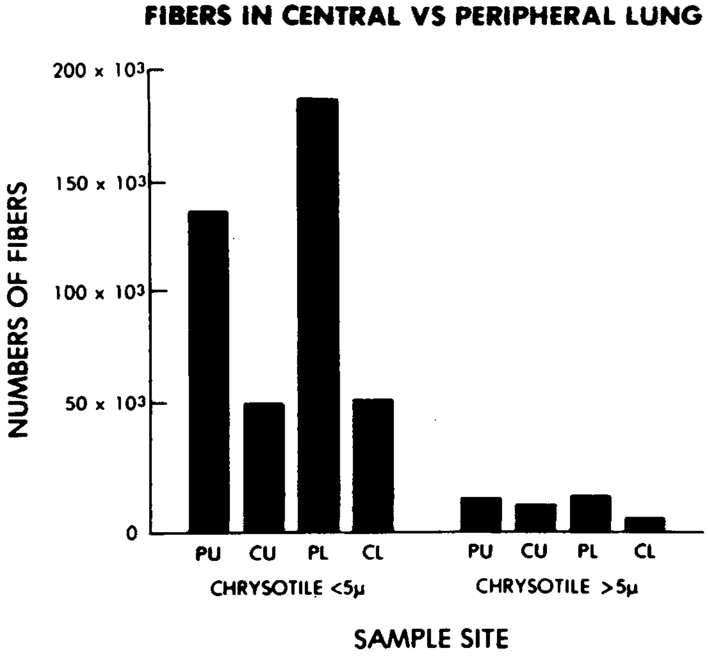

FIGURE 24. Differences in the distribution pattern of asbestos fibers in lungs of patients from general population. PU, peripheral lung; CU, central upper lung; PL, peripheral lower lobe; CL, central lower lobe. (Reproduced with permission from Churg, A. and Warnock, M. L., *Am. Rev. Resp. Dis.*, 122, 669, 1980.)

(men and women) in which 90% of the subjects had less than 100 asbestos bodies per gram of lung.[95] The number of bodies in the lungs of workers occupationally exposed to asbestos is usually in excess of 500 asbestos bodies per gram of lung (Table 3).

General population studies indicate that the number of uncoated fibers in the lung usually lies in the range of 0.01 to 1.3×10^6 per gram of lung (Table 3). The fibers are predominantly chrysotile, but substantial quantities of amphiboles are also present[89] (Figure 26). In occupationally exposed groups, the number of fibers may be as high as 1370×10^6 per gram of lung.[43] It appears that the commercial amphiboles — amosite and crocidolite — predominate in lung samples from occupationally exposed patients, whereas the noncommercial amphiboles — tremolite, actinolite, and anthophyllite — predominate in the general population.[41] The noncommercial amphiboles — anthophyllite and tremolite — are also more frequently observed in the cores of asbestos bodies from women. This may reflect amphibole contamination of cosmetic talc. Asbestos bodies and fibers have also been demonstrated in extrapulmonary sites including brain, spleen, kidney, heart, thyroid, adrenals, liver, prostate, and pancreas.[68] The significance of these bodies and fibers has not been determined.

In nonoccupationally exposed patients, approximately 50% of the fibers found in the lung are nonasbestos minerals. These are predominantly fibrous forms of apatite, talc, attapulgite, gypsum, silica, and other silicates.[96] Nonasbestos fibrous minerals have also been demonstrated in the lungs of patients formerly employed in the Quebec chrysotile mining industry.[97]

B. Pleural Plaques

Whitwell et al. demonstrated increased numbers of asbestos fibers in the lungs of 21

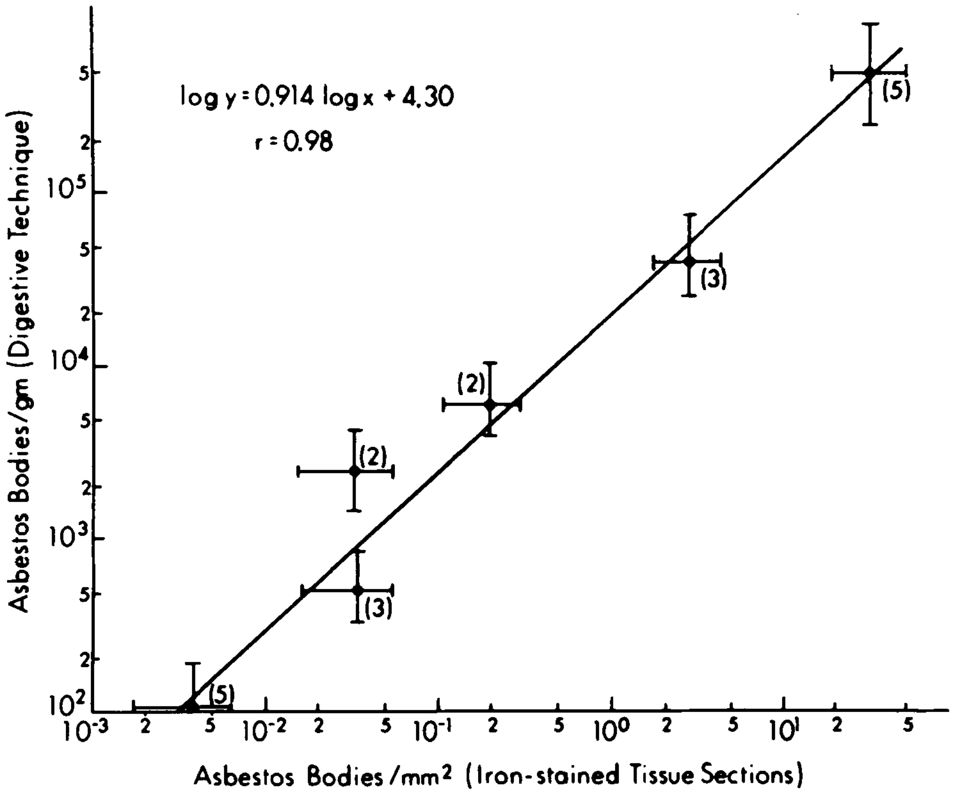

FIGURE 25. Relationship of the number of asbestos bodies in lung sections to the number counted by tissue digestion. Numbers in parentheses indicate the number of sections evaluated. (Reproduced with permission from Roggli, V. L. and Pratt, P. C., *Hum. Pathol.*, 14, 335, 1983.)

patients with bilateral pleural plaques as compared to 79 patients without plaques.[98] Gylseth et al. showed that the average concentration of asbestos fibers in the lungs of a group of 14 patients with pleural plaques was four times greater than in 12 control patients[99] (Table 4). Analyses of lung tissue in a group of 29 hospital autopsies selected on the basis of the presence of pleural plaques showed significantly increased numbers of asbestos bodies compared to a control population matched by age, sex, and smoking history.[100] In addition, the number of long commercial amphiboles, amosite and crocidolite, were also increased in the patients with pleural plaques. The number of short chrysotile fibers was similar in both groups implicating long amphibole fibers in the pathogenesis of these lesions.

C. Asbestosis

Patients with asbestosis usually have levels of asbestos bodies and fibers in their lungs similar or higher than those observed in patients with occupational exposure to asbestos.[98,101] In a general way, the number of bodies and fibers appears to correlate with the severity of asbestosis[43,98,102] (Figure 27; Table 5). These studies, taken together, support the epidemiologic evidence for a dose-response relationship with disease severity.

D. Mesothelioma

A majority of studies have shown increased numbers of asbestos bodies and fibers in the lungs of patients with mesothelioma[98,99,103-105] (Table 6). While it is true that

Table 3
ASBESTOS BODIES AND FIBERS IN POPULATIONS WITH AND WITHOUT OCCUPATIONAL EXPOSURE TO ASBESTOS

Patient population	Asbestos bodies (1 g lung)	Asbestos fibers (1 g lung)	Ref.
Patients without occupational exposure (50)	0.001×10^3 (wet — LM)	ND	104
Patients without occupational exposure (20)	0.3×10^3 (wet — TEM)	1.3×10^6 (wet — TEM)	89
Patients without occupational exposure (100)	ND	0.01×10^6 [a] (dry — LM)	98
Patients without occupational exposure (6)	0.4×10^3 (dry — LM)	0.2×10^6 (dry — LM)	103
Patients with no occupational history (12)	ND	0.7×10^6 (dry — SEM)	99
Patients with low level occupational exposure (21)	1.2×10^3 (wet — TEM)	ND	109
Workers in Canadian chrysotile industry (47)	ND	132×10^6 [a] (dry — TEM)	97
Patients with high level occupational exposure (6)	10×10^6 (dry — LM)	290×10^6 (dry — TEM)	88

[a] Data taken from Reference 42.

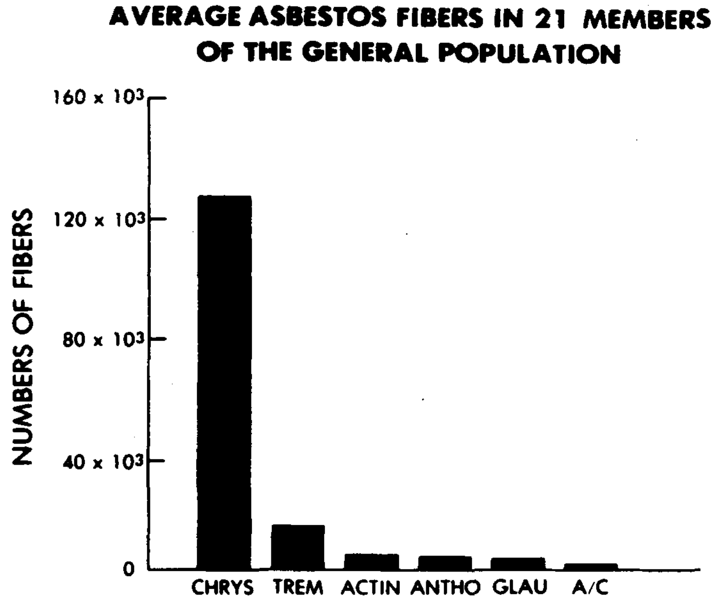

FIGURE 26. Average number of asbestos fibers found per gram of lung in patients from general population. CHRYS, chrysotile; TREM, tremolite; ACTIN, actinolite; ANTHO, anthophyllite; GLAUC, glaucophane; A/C, amosite/crocidolite. (Reproduced with permission from Churg, A. and Warnock, M. L., *Am. Rev. Resp. Dis.*, 122, 669, 1980.)

mesothelioma patients as a group have a greatly increased pulmonary asbestos burden, there is considerable variability from patient to patient. A sizeable number of patients has fiber counts within the range associated with the general population. It has also

Table 4
ASBESTOS BODIES AND FIBERS IN POPULATIONS WITH PLEURAL PLAQUES

Population	Asbestos bodies (1 g lung)	Asbestos fibers (1 g lung)	Ref.
Patients with pleural plaques (29)	1.7×10^3 (wet — TEM)	0.11×10^6 (wet — TEM)	100
Patients with pleural plaques (14)	ND	3.1×10^6 (dry — SEM)	99

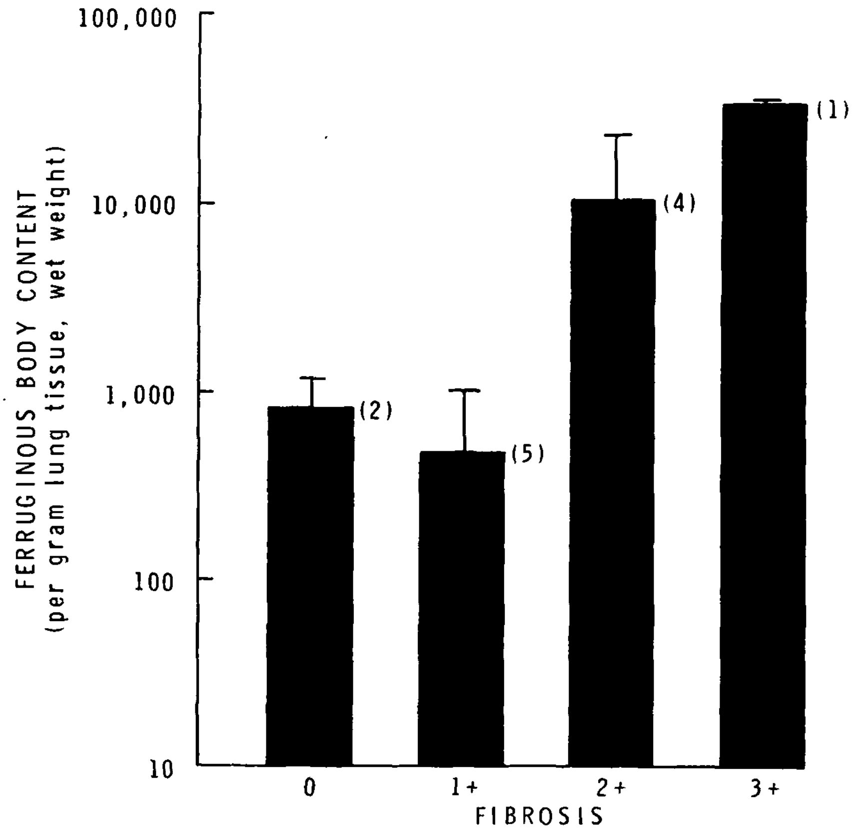

FIGURE 27. Ferruginous (asbestos) body count and severity of pulmonary fibrosis graded 0 to 3. Numbers in parentheses indicate the number of tissue sections with the severity of fibrosis. (Reproduced with permission from Roggli, V. L., Greenberg, S. D., Seitzman, L. H., McGavran, M. H., Hurst, G. A., Spivey, C. G., Nelson, K. G., and Hieger, L. R., *Am. J. Clin. Pathol.*, 73, 496, 1980.)

been shown that patients with peritoneal mesothelioma have fewer asbestos bodies in their lungs than patients with pleural mesothelioma[104] (Figure 28). Lung fiber analyses support the concept, derived from epidemiologic data, that the amphiboles are more potent inducers of mesothelioma, as McDonald et al. have shown, the lungs of 99 patients with mesothelioma contained more of the amphiboles amosite and crocidolite than did the lungs of 99 control patients, whereas the concentration of chrysotile fibers was similar in the two groups.[105]

Table 5
ASBESTOS BODIES AND FIBERS IN POPULATIONS WITH ASBESTOSIS

Population	Asbestos bodies (1 g lung)	Asbestos fibers (1 g lung)	Ref.
Asbestosis — grade 0 (13)	1.4×10^{6} (wet — LM)	2.4×10^{6} (wet — TEM)	43
Asbestosis — grade 1 (12)	7.7×10^{6} (wet — LM)	19.6×10^{6} (wet — TEM)	43
Asbestosis — grade 2 (5)	70.9×10^{6} (wet — LM)	203.4×10^{6} (wet — TEM)	43
Asbestosis — grade 1 and mesothelioma (9)	ND	8×10^{6} (dry — LM)	98
Asbestosis — grade 2 and mesothelioma (8)	ND	14×10^{6} (dry — LM)	98
Asbestosis — grade 3 and mesothelioma (6)	ND	37×10^{6} (dry — LM)	98
Asbestosis — grade 3	0.01×10^{6} (wet — LM)	ND	104

Table 6
ASBESTOS BODIES AND FIBERS IN MESOTHELIOMA POPULATIONS

Patient population	Asbestos bodies (1 g lung)	Asbestos fibers (1 g lung)	Ref.
Pleural mesothelioma patients with asbestos exposure (100)	ND	0.5×10^{6}* (dry — LM)	98
Male mesothelioma patients with asbestos exposure (76)	ND	193×10^{6} (dry — TEM)	105
Mesothelioma patients with asbestos exposure (10)	1783.7×10^{3} (dry — LM)	43.3×10^{6} (dry — LM)	103
Pleural mesothelioma patients (19)	3.0×10^{3} (wet — LM)	ND	104
Peritoneal mesothelioma patients (6)	0.1×10^{3} (wet — LM)	ND	104
Mesothelioma patients (15)	ND	87.1×10^{6}	99

* Data taken from Reference 42.

E. Lung Cancer

Several research groups have counted asbestos bodies and fibers in the lungs of lung cancer patients with and without occupational exposure to asbestos[93,103,106-110] (Table 7). The number of asbestos fibers in the lung cancer patients with occupational exposure asbestos was increased as expected.[103] However, no study to date has shown increased pulmonary asbestos fiber concentrations in lung cancer patients from the general population.

F. Other Cancers

Churg and Warnock counted asbestos bodies in the lungs of 50 patients with gastrointestinal neoplasms.[107] They found no increase in asbestos bodies compared to a matched control population.

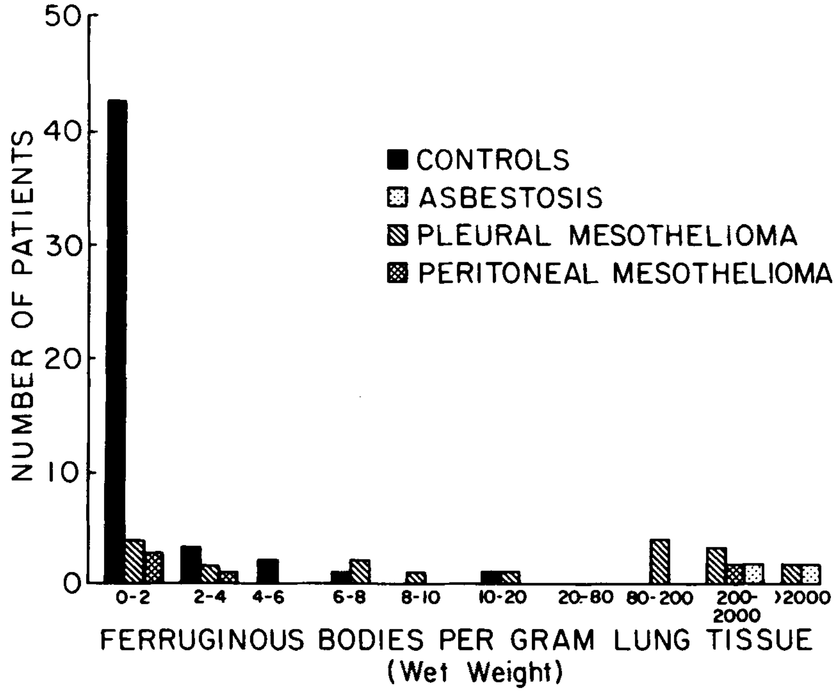

FIGURE 28. Ferruginous (asbestos) body counts for patients with pleural and peritoneal mesotheliomas, asbestosis, and controls. (Reproduced with permission from Roggli, V. L., McGavran, M. H., Subach, J., Sybers, H. D., and Grunberg, D., *Cancer*, 50, 2423, 1982.)

Table 7
ASBESTOS BODIES AND FIBERS IN POPULATIONS WITH LUNG CANCER

Patient population	Asbestos bodies (1 g lung)	Asbestos fibers (1 g lung)	Ref.
Lung cancer patients without occupational exposure (100)	ND	0.01×10^6 (dry — LM)	98
Lung cancer patients with low level asbestos exposure (11)	1.3×10^3 (wet — TEM)	ND	109
Lung cancer patients with asbestos exposure (3)	34.7×10^3 (dry — LM)	0.2×10^6 (dry — LM)	103

IX. MEDICOLEGAL CONSIDERATIONS

A. The Role of the Pathologist in Asbestos Litigation

In recent years, the medicolegal aspects of asbestos-related diseases have assumed increasing importance. For up to date discussions of historical background, public policy, and legal issues of asbestos litigation, the reader is referred to References 111 and 112. Most cases of asbestos-related disease are litigated.[112] It is important, therefore, that case material be adequately prepared by the pathologist. The pathologist's role as medicolegal consultant is threefold: (1) to describe and diagnose all important pathologic findings; (2) to prove or disprove a causal relationship between the important findings and impairment and death; and (3) to identify and explain the etiology of the pathologic findings.[112]

The pathologist should review the following materials when preparing a case for litigation*:

1. Detailed occupational history. This may be derived from the patient, next of kin, or the employer's records.
2. Environmental history. Urban or rural dweller, occupations of household dwellers, details of hobbies, home or auto repair work.
3. Smoking history with pack years, etc.
4. Medical records and results of clinical studies. These should be complete and photocopies should be readable. They may be available from hospital, industry, or union sources. Records should include a general medical evaluation together with details of specialized tests and procedures. Original data on pulmonary function and roentgenographs should be reviewed as well as their interpretation by both plaintiff and defense experts.
5. Pathological material. This may include cytology slides, surgical or autopsy slides and blocks, and wet tissue. All slides should be reviewed, including special stains, and each slide should be matched with the tissue blocks and the labels compared. If the material is inadequate for diagnosis, additional blocks and slides should be prepared. Results of all special evaluations, e.g., microanalytical studies and reports of referee groups (mesothelioma panels), should be reviewed. A record of which slides are originals and which are recuts should be kept as the latter will not necessarily show the same features.
6. Other materials. Before testimony it is important to be familiar with the natural history of asbestos-associated diseases, to review previous court records and depositions, and to obtain a copy of the death certificate (if relevant). Witness should also be familiar with legal definitions of disability, the estimated effects on life expectancy (from life tables), the problems associated with estimating exposure dose, and the confounding effects produced by smoking.

B. Precautions in Handling Materials

It is important to establish a chain of evidence for transmitted materials. Although use of certified mail with return receipt requested is currently adequate, hand-carried material with receipts is preferable. Records of all transmittals are essential. Additional precautions for handling pathological materials are needed. Fresh tissues should be sealed to avoid contamination and a sample of the fixative and tissue should be retained for possible future analysis. Blocks and slides can be photocopied so that comparison with the originals can be made at a later date.

X. CONCLUSIONS

Asbestos use and production have increased dramatically during the 20th century. Numerous studies have linked asbestos exposure with pulmonary fibrosis (asbestosis), cancers of the lung, larynx, gastrointestinal organs, and mesotheliomas of the pleura and peritoneum. Because most of these diseases have long latency intervals, the major impact of asbestos exposure on the U.S. population will be experienced in the closing decades of this century. In most patients, the diagnosis can be made by routine clinical, radiological, and pathological procedures. A relatively large residue of patients exists, however, in which the clinical or radiological features are nonspecific and/or a history

* Modified from personal communication with George E. Gantner, M.D., Division of Forensic and Environmental Pathology, University School of Medicine, St. Louis, Mo.

of asbestos exposure cannot be obtained. In these patients, microanalytical techniques will aid in diagnosis by providing information on exposure. Identification of specific types of asbestos in tissues can be used to provide information on the relative pathogenicity of the different types of asbestos. Quantitation of fiber burden can be used to elucidate the relationship between dose and severity of specific disease entities.

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REFERENCES

1. **Harington, M. S., Allison, A. C., and Badami, D. V.**, Mineral fibers: chemical, physiocochemical, and biological properties, *Adv. Pharmacol. Chemother.*, 12, 291, 1975.
2. **Rom, W. N. and Palmer, P. E. S.**, The spectrum of asbestos related diseases, *West. J. Med.*, 121, 10, 1974.
3. **Becklake, M. R.**, State of the art asbestos related diseases of the lung and other organs. Their epidemiology and implications for clinical practice, *Am. Rev. Resp. Dis.*, 114, 187, 1976.
4. **Clifton, R. A.**, Asbestos, Mineral Commodity Profiles, Bureau of Mines, U.S. Department of the Interior, Washington, D.C., 1979.
5. **Collins, T. F. B.**, Asbestos. The lethal duct, *S. Afr. Med. J.*, 41, 639, 1967.
6. **Murray, H. M.**, Report of the Departmental Committee on Compensation for Industrial Disease, Her Majesty's Stationery Office, London, 1907.
7. **Zenker, F. A.**, Iron lung: sclerosis pulmonum, *Dtsch. Arch. Klin. Med.*, 2, 116, 1867.
8. **Pancoast, H. K., Miller, T. G., and Landis, H. R. M.**, A roentgenologic study of the effects of dust inhalaion upon the lungs, *Am. J. Roentgenol. (N.S.)*, 5, 129, 1918.
9. **Cooke, W. E.**, Fibrosis of the lungs due to the inhalation of asbestos dust, *Br. Med. J.*, 2, 147, 1924.
10. **Merewether, E. R. A. and Price, C. V.**, Report on Effects of Asbestos Dust on the Lungs and Dust Suppression in the Asbestos Industry, Her Majesty's Stationery Office, London, 1930.
11. **Cooke, W. E.**, Pulmonary asbestosis, *Br. Med. J.*, 2, 1024, 1927.
12. **Mills, R. G.**, Pulmonary asbestosis; report of a case, *Minn. Med. J.*, 130, 495, 1930.
13. **Lynch, J. M. and Smith, W. A.**, Pulmonary asbestosis: carcinoma of the lung in asbesto-silicosis, *Am. J. Cancer*, 24, 56, 1935.
14. **Gloyne, S. R.**, Two cases of squamous carcinoma of the lung occurring in asbestosis, *Tubercle*, 17, 5, 1935.
15. **Doll, R.**, Mortality from lung cancer in asbestos workers, *Br. J. Ind. Med.*, 12, 81, 1955.
16. **Klemperer, P. and Rabin, C. B.**, Primary neoplasms of the pleura, *Arch. Pathol. Lab. Med.*, 11 385, 1931.
17. **Wagner, J. C., Sleggs, C. A., and Marchant, P.**, Diffuse pleural mesothelioma and asbestos exposure in North-Western Cape Province, *Br. J. Ind. Med.*, 17, 260, 1960.
18. **Selikoff, I. J. and Lee, D. H. K.**, Asbestos and Disease, New York, Academic Press, 1978, 101.
19. **Selikoff, I. J., Churg, J., and Hammond, E. C.**, Asbestos exposure and neoplasia, *JAMA*, 188, 22, 1964.
20. **Selikoff, I. J., Hammond, E. C., and Seidman, H.**, Mortality experience of insulation workers in the United States and Canada, 1943 to 1976, *Ann. N.Y. Acad. Sci.*, 330, 91, 1979.
21. **Selikoff, I. J. and Hammond, E. C.**, Asbestos-associated disease in United States shipyards, *Ca-A Cancer J. for Clinicians*, 28, 87, 1978.
22. **Guidotti, T. L., Abraham, J. L., and DeNee, P. B.**, Asbestos exposure and cancer of the larynx, *West. J. Med.*, 122, 75, 1975.
23. **Libshitz, H. I., Wershba, M. S., Atkinson, G. W., and Southard, M. E.**, Asbestos and carcinoma of larynx, *JAMA*, 228, 1571, 1976.
24. **Wagner, J. C., Gilson, J. C., and Berry, G.**, Epidemiology of asbestos cancers, *Br. Med. Bull.*, 27, 71, 1971.

25. Perkel, G., Nicholson, W. J., Selikoff, I. J., Anderson, H. A., Seidman, H., and Holstein, E. C., Population at risk, in *Disability Compensation for Asbestos-Associated Disease in the United States*, Selikoff, I. J., Ed., Mount Sinai School of Medicine, New York, 1982, 21.
26. U.S. Department of Health, Education and Welfare, National Occupational Hazard Survey, Vol. 3, DHEW (NIOSH) Publ. No. 78-114, Washington, D.C., 1978.
27. Walker, A. M., Loughlin, J. E., Friedlander, E. R., Rothman, K. J., and Dreyer, N. A., *J. Occup. Med.*, 25, 409, 1983.
28. Yada, K., A study of microstructures of chrysotile asbestos by high resolution microscopy, *Acta Crystallogr.*, 27, 659, 1971.
29. Newhouse, M. L. and Thompson, H., Mesothelioma of pleura and peritoneum following exposure in asbestos in the London area, *Br. J. Ind. Med.*, 22, 261, 1965.
30. Nicholson, W. J., Rohl, A. N., Weisman, I., and Selikoff, I. J., Environmental asbestos concentrations in the United States, in *Biological Effects of Mineral Fibers*, Wagner, J. C., Ed., IARC Scientific Publications, 1980, 823.
31. Cunningham, H. M. and Pontefract, R., Asbestos fibers in beverages and drinking water, *Science*, 232, 332, 1971.
32. Eisenstadt, H. B., Pleural asbestosis, *Am. Prac.*, 13, 573, 1962.
33. Eisenstadt, H. B., Asbestos pleurisy, *Dis. Chest*, 46, 78, 1964.
34. Meurman, L., Asbestos bodies and pleural plaques in a Finnish series of autopsy cases, *Acta Pathol. Microbiol. Scand. Suppl.*, 181, 1, 1966.
35. Kiviluoto, R., Pleural calcification as a roentgenologic sign of nonoccupational endemic anthophyllite asbestosis, *Acta Radiol. Suppl.*, 194, 1, 1960.
36. Harries, P. G., Mackenzie, F. A. F., Sheers, G. et al., Radiological survey of men exposed to asbestos in naval dockyards, *Br. J. Ind. Med.*, 29, 274, 1972.
37. Craighead, J. E., Abraham, J. L., Churg, A., Green, F. H. Y., Kleinerman, J., Pratt, P. C., Seemayer, T. A., Vallyathan, V., and Weill, H., Asbestos associated diseases, *Arch. Path. Lab. Med.*, 106, 543, 1982.
38. Gross, P., DeTreville, T. P., Cralley, L. J., and Davis, J. M. C., Pulmonary ferruginous bodies: development in response to filamentous dusts and a method of isolation and concentration, *Arch. Pathol.*, 85, 539, 1968.
39. Baris, I. Y. and Artvinli, M., Epidemiological, immunological and genetic aspects of asbestosis, *Arch. Immunol. Ther. Exp.*, 30, 3, 1982.
40. Gaensler, S. A. and Addington, W. W., Asbestos or ferruginous bodies, *N. Engl. J. Med.*, 280, 488, 1969.
41. Churg, A. M. and Warnock, M. L., Asbestos and other ferruginous bodies. Their formation and clinical significance, *Am. J. Pathol*, 102, 447, 1981.
42. Churg, A., Fiber counting and analysis in the diagnosis of asbestos-related diseases, *Hum. Pathol.*, 13, 381, 1982.
43. Ashcroft, T. G. and Heppleston, J., The optical and electron microscopic determination of pulmonary asbestos fibre concentration and its relation to the human pathologic reaction, *J. Clin. Pathol.*, 26, 224, 1973.
44. Selikoff, I. J., The asbestos health problem, in *Disability Compensation for Asbestos-Associated Disease in the United States*, Selikoff, J. J., Ed., Mount Sinai School of Medicine, New York, 1982, 1.
45. Egbert, D. S. and Geiger, A. J., Pulmonary asbestosis and carcinoma, *Am. Rev. Tuberc.*, 34, 143, 1936.
46. Gloyne, S. R., Pneumoconiosis: a histological survey of necropsy material in 1205 cases, *Lancet*, 1, 810, 1951.
47. Merewether, E. R. A., Asbestosis and carcinoma of the lung, Annual Report of the Chief Inspector of Factories for the year 1947, Her Majesty's Stationery Office, London, 1949.
48. Selikoff, I. J., Bader, R. A., Bader, M. E., Churg, J., and Hammond, E. C., Asbestosis and neoplasia, *Am. J. Med.*, 42, 487, 1967.
49. McDonald, J. D., Liddell, F. D. K., Gibbs, G. W., Eyssen, G. E., and McDonald, A. D., Dust exposure and mortality in chrysotile mining, 1910 to 1975, *Br. J. Ind. Med.*, 37, 11, 1980.
50. Weill, H., Hughes, J., and Waggenspack, C., Influence of dose and fiber type on respiratory malignancy risk in asbestos cement manufacturing, *Am. Rev. Resp. Dis.*, 120, 345, 1974.
51. Dement, J. M., Harris, R. L., Symons, M. J., and Shy, C., Estimates of dose response for respiratory cancer among chrysotile asbestos textile workers, *Ann. Occup. Hyg.*, 26, 869, 1982.
52. Wagner, J. C., Berry, G., Skidmore, J. W., and Timbrell, V., The effects of inhalation of asbestos in rats, *Br. J. Cancer*, 29, 252, 1974.
53. Selikoff, I. J., Hammond, E. C., and Churg, J., Asbestos exposure, smoking and neoplasia, *JAMA*, 204, 100, 1968.

54. Berry, G., Newhouse, M. L., and Turok, M., Combined effect of asbestos exposure and smoking on mortality from lung cancer in factory workers, *Lancet*, 2, 476, 1972.
55. Hammond, E. C., Selikoff, I. J., and Seidman, H., Asbestos exposure, cigarette smoking and death rates, *Ann. N.Y. Acad. Sci.*, 330, 473, 1979.
56. Saracci, R., Asbestos and lung cancer: an analysis of the epidemiological evidence on the asbestos smoking interaction, *Int. J. Cancer*, 20, 323, 1977.
57. Kannerstein, M. and Churg, J., Pathology of carcinoma of the lung associated with asbestos exposure, *Cancer*, 30, 14, 1972.
58. Sluiss-Cremer, G. R., The relationship between asbestosis and bronchial cancer, *Chest*, 78, 380, 1980.
59. Whitwell, F., Newhouse, M. L., and Bennett, D. R., A study of the histological cell types of lung cancer in workers suffering from asbestosis in the United Kingdom, *Br. J. Ind. Med.*, 31, 398, 1974.
60. Newhouse, M. L. and Berry, G., Predictions of mortality from mesothelial tumours in asbestos factory workers, *Br. J. Ind. Med.*, 33, 147, 1976.
61. Cochrane, J. C. and Webster, I., Mesothelioma in relation to asbestos fiber exposure, *S. Afr. Med. J.*, 54, 279, 1978.
62. Wagner, J. C., Gilson, J. C., Berry, G., and Timbrell, V., Epidemiology of asbestos cancers, *Br. Med. Bull.*, 27, 71, 1971.
63. Newhouse, N. L., Berry, G., Wagner, J. C., and Turok, M. E., A study of mortality of female asbestos workers, *Br. J. Ind. Med.*, 29, 134, 1972.
64. Selikoff, I. J., Hammond, E. C., and Churg, J., Carcinogenicity of amosite asbestos, *Arch. Environ. Health*, 25, 183, 1972.
65. Kannerstein, M., Churg, J., and McCaughey, W. T. E., Asbestos and mesothelioma: a review, *Pathol. Annu.*, 13, 81, 1978.
66. McDonald, J. C., McDonald, A. D., Gibbs, G. W., Siemiatycki, J., and Rossiter, C. E., Mortality in the chrysotile asbestos mines and mills of Quebec, *Arch. Environ. Health*, 22, 677, 1971.
67. Doniach, I., Swettenham, K. V., and Hathorn, M. S. K., Prevalence of asbestos bodies in a necropsy series in East London: association with disease, occupation, and domicilliary address, *Br. J. Ind. Med.*, 32, 16, 1975.
68. Auerbach, O., Conston, A. S., Garfinkel, L., Parks, V. R., Kaslow, H. D., and Hammond, E. C., Presence of asbestos bodies in organs other than the lung, *Chest*, 77, 133, 1980.
69. Green, F. H. Y., Tucker, J. H., and Vallyathan, V., Occupational diseases of the lung, *Lab. Med.*, 14, 103, 1983.
70. Whimster, W. F., Rapid giant paper sections of lungs, *Thorax*, 24, 737, 1969.
71. Lyophilized unfixed whole lungs for correlative roentgenologic pathologic microanalytic study of occupational respiratory disease, *Am. J. Forensic Med. Path.*, 1, 181, 1980.
72. Gaensler, E. A. and Carrington, C. B., Open biopsy for chronic diffuse infiltrative lung disease. Clinical, roentgenographic and physiological correlations in 502 patients, *Ann. Thorac. Surg.*, 30, 411, 1980.
73. Brody, A. R. and Craighead, J. E., Preparation of human lung biopsy specimens by perfusion fixation, *Am. Rev. Resp. Dis.*, 112, 645, 1975.
74. Smith, M. J. and Naylor, B., A method for extracting ferruginous bodies from sputum and pulmonary tissue, *Am. J. Clin. Pathol.*, 58, 250, 1972.
75. National Institute for Occupational Safety and Health, Revised Recommended Asbestos Standard, DHEW (NIOSH) Publ. No. 77-169, U.S. Department of Health, Education, and Welfare, Washington, D.C., 1976.
76. Wylie, A. G., Fiber length and aspect ratio of some selected asbestos samples, *Ann. N.Y. Acad. Sci.*, 330, 605, 1979.
77. Skikne, M. I., Talbot, J. H., and Rendall, R. E. G., Electron diffraction patterns of UICC asbestos samples, *Environ. Res.*, 4, 141, 1971.
78. Samundra, A. V., Optimum procedure for asbestos fibers identification from selected area electron diffraction patterns in a modern analytical electron microscope using tilted specimens, SEM. Vol. 1, IIT Research Institute, Chicago, 1977, 385.
79. Rudd, C. O., Barrett, C. S., Russel, P. A., and Clark, R. L., Selected area electron diffraction and energy dispersive x-ray analysis for identification of asbestos fibers. A comparison, *Micron*, 7, 115, 1976.
80. Smith, G. R., Stone, G. A., Stanley, R. L., Carpenter, L. M., Seshan, K., and Mueller, P. K., Identification of airborne asbestos by selected area electron diffraction, *in Proc. 31st Annu. Meet. Electron Microscopical Soc. Am.*, Acrenaux, C. J., Ed., Claitor's Publ. Div., Baton Rouge, La., 1973, 310.
81. Farrell, R. E., Paulson, C. G., and Walker, C. W., Pollutant identification by selected area electron diffraction. I. Method. II. The limitations, *in Proc. 32nd Annu. Meet. Electron Microscopy Soc. Am.*, Acrenaux, C. J., Ed., Claitor's Publ. Div., Baton Rouge, La.

82. Langer, A. M., Rohl, A. N., Wolf, M. S., Klimentidis, R., and Shirley, S. B., Review of current techniques for the analysis of fibers in talc, in *Proc. 1st FDA Office Sci.*, Pennsylvania State University, 1976, 28.
83. Agar, A. W., Accuracy of selected area micro-diffraction in electron microscope, *Br. J. Appl. Phys.*, 11, 185, 1960.
84. Lee, R. J. and Fisher, R. M., Identification of fibrous and non-fibrous amphiboles in the electron microscope, *Ann. N.Y. Acad. Sci.*, 330, 645, 1979.
85. Stettler, L. E., Groth, D. H., and Platek, S. F., The automated characterization of particles extracted from human lungs: three cases of tungsten carbide exposure, *SEM*, 1, 439, 1983.
86. Lange, B. A. and Haartz, J. C., Determination of microgram quantities of asbestos by x-ray diffraction. Chrysotile in the dust layers of matrix material, *Anal. Chem.*, 51, 5205, 1979.
87. Nicholson, W. J., Criteria in assessing the relation of asbestos-associated disease to work exposure, in *Disability Compensation for Asbestos Associated Disease in the United States*, Selikoff, I. J., Ed., Mount Sinai School of Medicine, New York, 198299.
88. Sebastien, P. A., Fondimare, J. Bignon, et al., Topographic distribution of asbestos fibres in human lung in relation to occupational and nonoccupational exposure, in *Inhaled Particles*, Vol. 4 (Part 2), Walton, W. H. and McGovern, B., Eds., Pergamon Press, New York, 1977, 435.
89. Churg, A. and Warnock, M. L., Asbestos fibers in the general population, *Am. Rev. Resp. Dis.*, 122, 669, 1980.
90. Churg, A., Asbestos fibers and pleural plagues in an autopsy population (abstract), *Am. Rev. Resp. Dis.*, 123, 135, 1981.
91. Roggli, V. L. and Pratt, P. C., Numbers of asbestos bodies on iron-stained tissue sections in relation to asbestos body counts in lung tissue digests, *Hum. Pathol.*, 14, 355, 1983.
92. Roggli, V., Greenburg, S. D., McLarty, J. W., Hurst, G. A., Hieger, L. R., Farley, M. L., and Mary, L. C., Comparison of sputum and lung asbestos body counts in former asbestos workers, *Am. Rev. Resp. Dis.*, 122, 941, 1980.
93. Breedin, P. H. and Buss, D. H., Ferruginous (asbestos) bodies in the lungs of rural dwellers, urban dwellers, and patients with pulmonary neoplasms, *South. Med. J.*, 69, 401, 1976.
94. Auerbach, O., Hammond, C., Selikoff, I., Parks, V. R., Kaslow, H. D., and Garfinkel, L., Asbestos bodies in lung parenchyma in relation to ingestion and inhalation of mineral fibers, *Environ. Res.*, 14, 286, 1977.
95. Churg, A. and Warnock, M. L., Analysis of the cores of ferruginous (asbestos) bodies from the general populations. III. Patients with environmental exposure, *Lab. Invest.*, 40, 622, 1979.
96. Churg, A., Nonasbestos pulmonary mineral fibers in the general population, *Environ. Res.*, 31, 189, 1983.
97. Rowlands, N., Gibbs, G. W., and McDonald, A. D., Asbestos fibers in the lungs of chrysotile miners and millers — a preliminary report, *Ann. Occup. Hyg.*, 26, 411, 1982.
98. Whitwell, F., Scott, J., and Grimshaw, M., Relationship between occupations and asbestos fiber content of the lungs in patients with pleural mesothelioma, lung cancer and other diseases, *Thorax*, 32, 377, 1977.
99. Gylseth, B., Mowe, G., Skaug, V., and Wannag, A., Inorganic fibers in lung tissue from patients with pleural plaques or malignant mesothelioma, *Scand. J. Work Environ. Health*, 7, 109, 1981.
100. Churg, A., Asbestos fibers and pleural plaques in a general autopsy population, *Am. J. Pathol.*, 109, 88, 1982.
101. Warnock, M. L., Prescott, B. T., and Kuwahara, T. J., Correlation of asbestos bodies and fibers in lungs of subjects with and without asbestosis, *SEM*, 845, 1982.
102. Roggli, V. L., Greenberg, S. D., Seitzman, L. H., McGavran, M. H., Hurst, G. A., Spivey, C. G., Nelson, K. G., and Hieger, L. R., Pulmonary fibrosis, carcinoma and ferruginous body counts in amosite asbestos workers, *Am. J. Clin. Pathol.*, 73, 496, 1980.
103. Morgan, A. and Holmes, A., Concentrations and dimensions of coated and uncoated asbestos fibers in the human lung, *Br. J. Ind. Med.*, 37, 95, 1982.
104. Roggli, V. L., McGavran, M. H., Subach, J., Sybers, H. D., and Grunberg, D., Pulmonary asbestos body counts and electron probe analysis of asbestos body cores in patients with mesothelioma. A study of 25 cases, *Cancer*, 50, 2423, 1982.
105. McDonald, A. D., McDonald, J. C., and Pooley, F. D., Mineral fiber content of lung in mesothelial tumors in North America, *Ann. Occup. Hyg.*, 26, 417, 1982.
106. Churg, A. and Warnock, M. L., Analysis of the cores of ferruginous (asbestos) bodies from the general population. I. Patients with and without lung cancer, *Lab. Invest.*, 37, 280, 1979.
107. Churg, A. and Warnock, M. L., Numbers of asbestos bodies in urban patients with lung cancer and gastrointestional cancer and matched controls, *Chest*, 76, 143, 1979.
108. Steele, R. H. and Thomson, K. J., Asbestos bodies in the lung: Southampton (U.K) and Wellington (New Zealand), *Br. J. Ind. Med.*, 39, 349, 1982.

109. Warnock, M. L., Kuwahara, T. J., and Wolery, G., The relation of asbestos burden to asbestosis and lung cancer, *Path. Annual,* Vol. 18, Sommers, S. C., Rosen, P. P., Eds., Appleton-Century-Crofts, New York, 1983, 109.
110. Churg, A. and Warnock, M. L., Analysis of the cores of asbestos bodies from members of the general population: patients with probable low-degree exposure to asbestos, *Am. Rev. Resp. Dis.,* 120, 781, 1979.
111. Weill, H., Asbestos-associated diseases. Science, public policy and litigation, *Chest,* 84, 601, 1983.
112. Richman, S. I., Medicolegal aspects of asbestos for the pathologist, *Arch. Pathol. Lab. Med.,* 107, 557, 1983.