Tim D. Oury · Thomas A. Sporn
Victor L. Roggli *Editors*

# Pathology of Asbestos-Associated Diseases

Third Edition

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The original version of the book backmatter was revised. An erratum to it can be found at DOI 10.1007/978-3-642-41193-9_14

ISBN 978-3-642-41192-2 ISBN 978-3-642-41193-9 (eBook)
DOI 10.1007/978-3-642-41193-9
Springer Heidelberg New York Dordrecht London

Library of Congress Control Number: 2014931788



Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

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# The Mineralogy of Asbestos

1

Thomas A. Sporn

## 1.1 Introduction and Historical Background

Minerals are naturally occurring inorganic compounds of specific chemical composition and crystal structure. Their nomenclature typically stems as an honorific, to indicate a pertinent geographic area or to highlight a distinctive characteristic of the compound. The term asbestos collectively references a group of naturally occurring fibrous minerals which have been exploited in numerous commercial and industrial settings and applications dating to antiquity. Its myriad uses as a "miracle mineral" owe to its remarkable properties of extreme resistance to thermal and chemical breakdown, tensile strength, and fibrous habit which allows it to be spun and woven into textiles. Abundant in nature, it has been mined considerably, and in all continents save Antarctica. The nomenclature concerning asbestos and its related species is complex, owing to the interest held therein by scientific disciplines such as geology, mineralogy and medicine, as well as legal and regulatory authorities. The silicate minerals may have fibrous and nonfibrous habits. The group of asbestos and "asbestiform" minerals shares the common features of occurrence as flexible polyfilamentous bundles, long fiber length, and small fiber diameter. As fibrous silicates, asbestos minerals are broadly classified into the serpentine (chrysotile) and amphibole (crocidolite, amosite, tremolite, anthophyllite, actinolite) series, both of which may also contain allied but nonfibrous forms of similar or even identical chemical composition, nonpathogenic to humans. As such, amphibole minerals in the non-polyfilamentous habit are not classified as asbestos, nor are some other asbestiform amphiboles which are not commercially exploitable. Although generally grouped, classified, and regulated generically as asbestos, the serpentine and amphibole groups have different geologic occurrences and, more importantly, significant differences in crystalline structures and chemical compositions. These in turn impart differences in fiber structure and dimension, as well as biopersistence, leading to marked differences in relative potency for causing disease in humans for the group of minerals known as asbestos. Derived from the Greek term for "unquenchable" or "indestructible," asbestos is the collective term for a family of naturally occurring fibrous silicates that exist in metamorphic, altered basic, or ultra basic igneous rock. Asbestos and asbestiform minerals are narrowly defined and classified, as will be discussed below. The asbestos minerals have found much utility owing to their common properties of thermochemical and electrical resistance, high tensile strength, and flexibility. Insoluble in water and organic solvents, its fine fibers may be spun and woven into textiles and incorporated into many

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T.D. Oury et al. (eds.), *Pathology of Asbestos-Associated Diseases*,
DOI 10.1007/978-3-642-41193-9_1, 

other types of materials; asbestos has seen literally thousands of industrial applications. The usage of asbestos dates through fact and fable to thousands of years ago. Once believed to have almost magical capabilities, first descriptions document its usage in the manufacture of pottery in Finland ca. 2500 B.C. Additional historical attributions for early asbestos usage include cremation garments for royalty and for embalming the pharaohs of ancient Egypt. Emperor Charlemagne reportedly astonished his guests at a feast by throwing table cloths made from asbestos into a fire from which the garments would be removed clean and unharmed. Medieval alchemists termed the mineral "salamander stone" referring to a mythical fireproof animal, and during these times asbestos was used in suits of armor [1]. Deposits of asbestos in the Ural Mountains led to the development of factories producing asbestos textiles in 1720. In the seventeenth century, fibrous minerals discovered in Germany termed Bergflachs or Bergleder likely contained amphibole asbestos, and by the mid-nineteenth century, some 20 asbestos mines were operating in Europe [2]. In colonial America, asbestos deposits were discovered in Pennsylvania and New England, where it was woven into textiles, and chrysotile was discovered in Quebec, Canada, in 1860 [2]. Significant commercial usage of asbestos did not occur until the latter part of the nineteenth century, with the development of demand for insulation for the burgeoning steam technology. At the turn of the twentieth century, additional applications for the useful minerals had been developed, deposits of amphibole asbestos species had been discovered in South Africa, and asbestos was once more being mined in the Urals, this time in large quantities. Commercial exploitation of asbestos was now global and full blown, and by 1980 over 100 million tons of asbestos had been mined worldwide [2], accompanied by the development of serious health concerns related to its usage. The purpose of this chapter is to describe what the groups of minerals classified as asbestos are from a mineralogic perspective, where they occur, and what are the important distinctions that allow relative differences within members of the asbestos group to have differing potencies on the basis of such differences in terms of inducing injury and producing disease following inhalation. It is well known from animal models that the oncogenic potential of fibrous dust increases following reductions in fiber diameter and decreases with reduction in fiber length, and these considerations are generally more important than the chemical composition of the fibers themselves [3–6]. The longer fibers have more potency to induce cell injury, proliferation, oxidant release, and inflammation. It is also the durability of the fibrous dust that confers biopersistence and the potential to induce malignant disease following deposition of fibers in the peripheral airways and migration of fibers to the serosal membrane. Contemporary usage of asbestos has been curtailed following its wide recognition as a most dangerous substance; it is noteworthy that the health hazards of asbestos date to antiquity as well. Pliny the Elder cautioned against the purchase of quarry slaves from asbestos mines, noting that they tended to die young [1]. Contemporary usage of asbestos is highly variable, although global demand still measures in the millions of metric tons. The European Union, Australia, and Japan are examples of states which enforce national bans on asbestos products; other countries allow its usage and enforce variably stringent regulations on fiber type and permitted levels of exposure. In 2006, six countries (the Russian Federation, the People's Republic of China, Kazakhstan, Brazil, Canada, and Zimbabwe) contributed to 96 % of the world's production of asbestos [7, 8] In the USA, asbestos consumption fell to 1,730 metric tons in 2007, chiefly in the form of chrysotile-containing roofing products [8].

## 1.2 Geologic and Mineralogic Features

Asbestos is properly considered a commercial and legal rather than a mineralogic term for a group of fibrous silicate minerals with crystalline structure and by definition have lengths >5 μm and aspect (length/diameter) ratios of three or greater. In the USA, the asbestos nomenclature as defined by the Environmental Protection Agency

encompasses six unique mineral species, conventionally divided into two distinct groups: the amphiboles and the serpentines [9]. Chrysotile is the sole member of the latter group and, as of the year 2000, accounted for virtually 100 % of the asbestos used commercially. Historically, at least 90 % of commercially used asbestos has been chrysotile. The amphibole group contains grunerite-cummingtonite (amosite, vide infra), crocidolite (a fibrous variant of riebeckite), tremolite, actinolite, and anthophyllite. The name amosite is derived from the acronym AMOSA—Asbestos Mines of South Africa—giving reference to the company in the Transvaal Province of South Africa, the sole mine producing the mineral. As such, amosite, too, is a commercial, rather than a true mineralogic term, but by convention, amosite is used synonymously for the fibrous forms of grunerite-cummingtonite, just as crocidolite is for the fibrous form of riebeckite. Among the amphiboles, only crocidolite and amosite have undergone significant commercial exploitation in industrialized countries and collectively account for less than 10 % of asbestos utilized in the last century. Fiber characteristics influence commercial exploitation. Long fibers are useful as insulation materials and textiles, medium-length fibers have been used in asbestos cement and friction products, and short fibers are used as reinforcing agents in floor tiles, joint compounds, and roofing material. Highly resistant to acid and salt water, large amounts of amosite were imported into the USA during World War II for usage in warship and merchant vessel insulation. The high tensile strength and extreme thermal stability of crocidolite allowed its usage as insulation material at very high temperatures, as well as packings and gaskets. The so-called noncommercial amphiboles, actinolite, tremolite, and anthophyllite, are common mineral species with wide distribution. They are relevant insofar as they are contaminants of other commercially useful mineral species such as talc and vermiculite, as well as chrysotile, and have been implicated in the induction of disease in humans. The asbestos minerals have nonpathogenic, non-asbestiform mineral counterparts of identical chemical composition. The noncommercial species of amphiboles all require the word "asbestos" after their mineral name for the purpose of distinguishing them from the non-asbestos forms. This is not necessary for crocidolite, amosite, and chrysotile as the non-asbestos forms have different names as discussed above (see Fig 1.1).

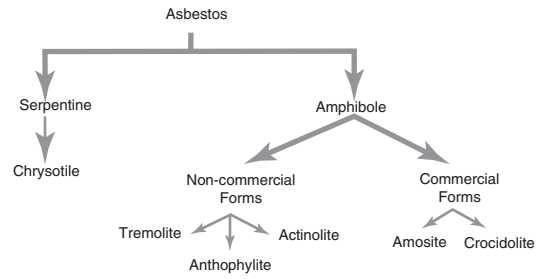


Fig. 1.1 Classification of asbestos and asbestiform silicates

Asbestos minerals owe their fibrous habit to the parallel growth of very fine and elongate crystals, producing bundles. The amphiboles may also occur as nonfibrous, chunky, acicular, and shard-like forms. Nonfibrous serpentine minerals include antigorite and lizardite. The nonfibrous forms of both serpentine and amphibole minerals are more common and widespread than the asbestiform species.

Deposits of commercial asbestos are to be found in four types of rocks: the banded ironstones, containing amosite and crocidolite; the alpine-type ultramafic rocks, containing chrysotile, anthophyllite, and tremolite; the stratiform ultramafic inclusions, containing chrysotile and tremolite; and the serpentized limestone (chrysotile) [2]. Recently in the USA, fibrous amphiboles not historically classified or regulated as asbestos (winchite, richterite) have been implicated in the causation of serious disease due to their profusion as natural contaminants (along with tremolite) of vermiculite, a commercially useful and nonfibrous silicate mineral [10, 11], vide infra. Other "asbestiform" minerals include the fibrous zeolites such as erionite. Erionite, found naturally in volcanic tuff in some areas of Turkey where it has been used as a construction material, has physicochemical characteristics resembling those of the amphiboles such as high aspect ratio and fiber diameters less than 0.25 um [12]. Fibrous erionite induces mesothelioma in

animal models and has been implicated in both benign and malignant pleural disease in humans [13–15].

## 1.3 Distribution and Physicochemical Properties of Chrysotile

Chrysotile is a common serpentine mineral with worldwide distribution and the only one of this series mined as asbestos. The type 1 (alpine-type ultramafic rock) deposits are the most important sources of chrysotile asbestos, with principal localities occurring in the Ural Mountains of Russia and the Appalachian Mountains of the Canadian province of Quebec and the state of Vermont in the USA, as well as the state of California. Chrysotile has also been mined in the Italian Alps, Cypress, Zimbabwe, and the People's Republic of China [2] (Table 1.1). Commercially useful chrysotile is prepared from chrysotile ore in the milling process, with extracted long fiber chrysotile finding usage in textiles and shorter fibers used in construction materials such as joint compound. Among the commercially exploited seams of the mineral, geographic variations are to be expected both in terms of physical characteristics of the fibers, type, as well as proximity to fibrous species of noncommercial amphiboles. For example, the rich chrysotile ores quarried at the Coalinga, California, mines yield fibers almost exclusively less than 5 μm [16]. There is also variance in the presence of other potentially dangerous minerals even within neighboring seams. McDonald et al. attributed the difference in reported deaths due to mesothelioma among workers in several different mines within the province of Quebec to be attributable to local variances in the amount of tremolite contamination known to exist within the various mines [17]. The topic of chrysotile purity following milling and the potential contamination by noncommercial species is frequently argued in the ongoing asbestos litigation in the USA.

The basic chemical unit of all silicate materials is the silicate tetrahedron, Si04. The actual number and configuration of tetrahedral within the crystal structure provides the basis for the classification of silicate minerals. Silicates may be classified on the basis of the polymerization type of the silicate ions and the variance in crystalline structure that occurs through association of various cations. Chrysotile is a hydrated (approximately 13 % water as a crystal) phyllosilicate (sheet silicate) with chemical composition $Mg_3Si_2O_5(OH)4$, containing the typical $(Si_2O_5)n^{-2}$ building block typical of the serpentine group of minerals [18] (Fig 1.2). Whereas other serpentines and other layered silicates (clays, mica) form flat sheets, spatial imbalances between magnesium and silica ions within the tetrahedral and octahedral sheets of chrysotile cause the layers to roll to form concentric hollow cylinders. Chrysotile fibers will thus appear scroll like when viewed end on (Fig. 1.3), containing a central capillary with 2–4.5 nm in diameter. The milling of chrysotile ore yields bundles of fibers of variable length, and some fibers may exceed 100 μm. The fibers may be curvilinear ("serpentine"), often with splayed ends due to the separation of fibers into individual and smaller fibrillar units (Fig. 1.4). Some very long chrysotile fibers may be quite thin, but the diameter of chrysotile fibers tends to increase with increasing fiber length. Magnesium is an important constituent of both chrysotile and the amphiboles; the presence of soluble magnesium molecules on the outside of the curled chrysotile structure permits its leaching at the surface, facilitating the breakdown of fibers within lung tissue into successively smaller, fragile fibrils, which are then readily cleared from the body. Loss of magnesium changes the surface charge from positive to

**Table 1.1** Geographic distribution of asbestos species

| Asbestos mineral | Geographic distribution |
|---|---|
| Chrysotile | Canada (QC), USA (Vermont, CA), Russia, China |
| Crocidolite | South Africa (NW Cape Province, Transvaal), Western Australia |
| Amosite | South Africa |
| Tremolite | Turkey, Cyprus, Greece |
| Anthophyllite | Finland, USA |
| Actinolite | South Africa (Cape Province) |
| Winchite/Richterite[a] | USA (MT) |

[a]Asbestiform amphibole species, not classified as asbestos

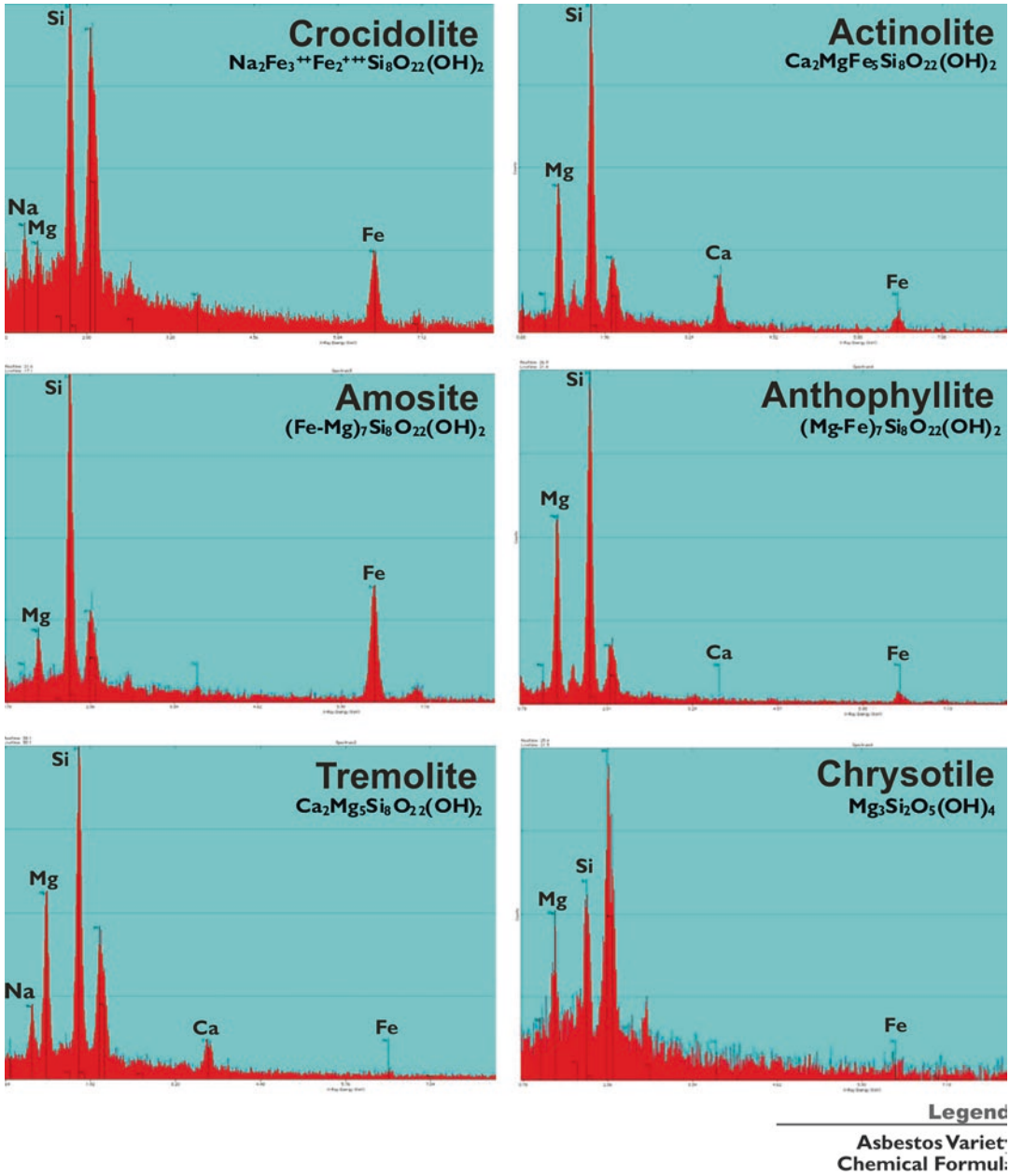


**Fig. 1.2** Chemical composition and elemental spectra of asbestos

negative, which diminishes the oncogenic potential [4]. The clearance half-time of inhaled chrysotile within the lower respiratory tract is measured in only weeks and may be much less. For example, with a clearance half-time measured in hours, the chrysotile from the Coalinga mine in California is among the mineral fibers with the most rapid clearance from the lung. Other chrysotile may have biopersistence similar to the range reported for glass and stone wools [19]. Thermoresistant to a degree, 70 % of the chrysotile structure is lost at 575 °C, with complete loss of the structure occurring at 650 °C [20]. Such high temperatures may be observed in the automotive braking process, causing pyrolysis and conversion to the nonfibrous, nonpathogenic

silicate mineral forsterite [20]. Due to its physicochemical characteristics, chrysotile has a greatly reduced biopersistence in contrast to the amphibole species, and those features as described above provide a likely explanation for the reported reductions in oncogenicity for this species in humans in contrast to the amphiboles [21, 22] and for the epidemiologic studies that conclude that motor vehicle mechanics performing brake repair are not at increased risk for developing mesothelioma [23].

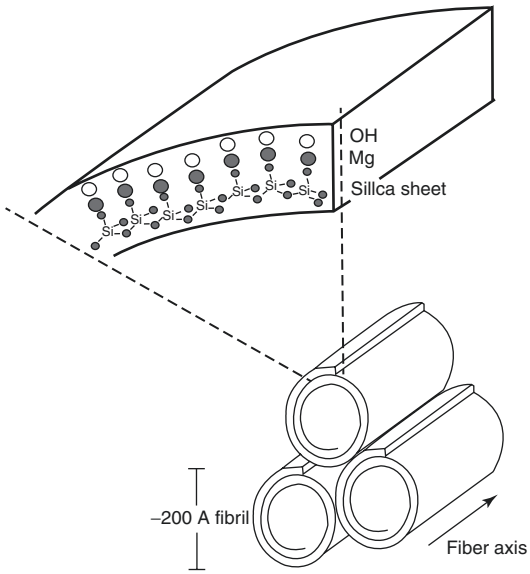


**Fig. 1.3** Crystalline structure of chrysotile (Schematic diagram modified)

## 1.4 Distribution and Physicochemical Properties of the Amphibole Species

The amphibole asbestos minerals crocidolite, amosite, anthophyllite, tremolite, and actinolite are inosilicates, or chain silicates, indicating the arrangement and alignment of the silicate tetrahedra. Tremolite, actinolite, and anthophyllite are grouped together with chrysotile as "white asbestos" and classified under the United Nations chemical identification schema as UN2590. Amosite "brown asbestos" and crocidolite "blue asbestos" are classified as UN2212. Amphiboles typically occur when veins of the mineral are created when cracks form in rocks during movement of the earth. These conditions help provide the environment necessary for massive amphibole crystallization and transformation to the fibrous form. The amphibole minerals are common, but their occurrence as exploitable forms is limited to certain locations where they obtain the proper physicochemical characteristics and abundance to be used as commercial asbestos. The major deposits of commercial amphiboles have generally been limited to the banded ironstones of

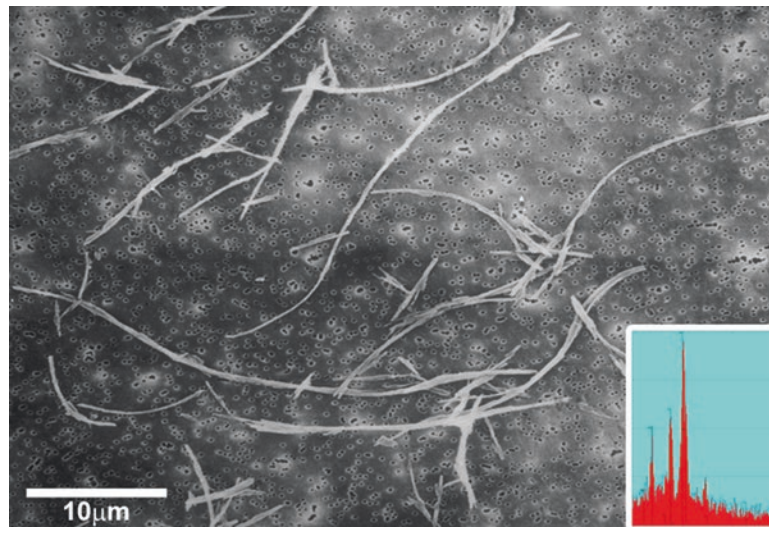


**Fig. 1.4** Chrysotile asbestos fibers, scanning electron photomicrograph. Note long fibers of variable thickness and curvilinear "serpentine" morphology

Western Australia and the Transvaal and Cape Provinces of South Africa. Alpine-type and stratiform ultramafic rocks are sources of chrysotile, as well as the noncommercial amphiboles tremolite, actinolite, and anthophyllite, the major source for the latter occurring in Finland with smaller deposits in rocky outcrops of the USA [2]. Some minerals aside from the commercial amphiboles may form polyfilamentous, asbestiform crystals. An example of this type of asbestiform amphiboles is to be found in the area around Libby, Montana, USA. Libby is the site of the largest mined deposit of vermiculite in the world, and the alkaline-ultramafic rock is rich in amphiboles, chiefly richterite and winchite (sodic-calcic tremolite), all of which can exist in asbestiform or fibrous habit [24, 25]. The latter species are not listed in the US federal regulations governing asbestos, but their recognition is important in view of the abnormally high number of asbestos-related diseases and deaths in former vermiculite miners and millers and residents of this area, and the potency of the Libby amphibole in terms of inducing mesothelioma is reported to be similar to crocidolite [26, 27]. Anthophyllite, tremolite, and actinolite are common constituents of the earth's crust, but have not been exploited commercially in industrialized countries, and are frequently associated with serpentine minerals, vermiculite, and talc. The noncommercial amphiboles may assume a variety of forms, including nonfibrous forms.

The chemical and crystalline structures of the amphiboles are highly similar and generally may be distinguished only on the basis of chemical composition and in specific the cation constituents (Fig. 1.2). Crystalline amphibole minerals demonstrate perfect prismatic cleavage, with direction of the cleavage parallel to the length of the silicate chains [28]. The silicate chains are formed by linear arrays of $SiO_4$ tetrahedra linked by octagonal groups of cations and may be of significant length (Fig. 1.5). The crystalline amphibole fibers are substantially more brittle than chrysotile, limiting their potential for fabrication. These mineralogic attributes confer the potential for great fiber length and, accordingly, significant pathogenicity following deposition in the lung

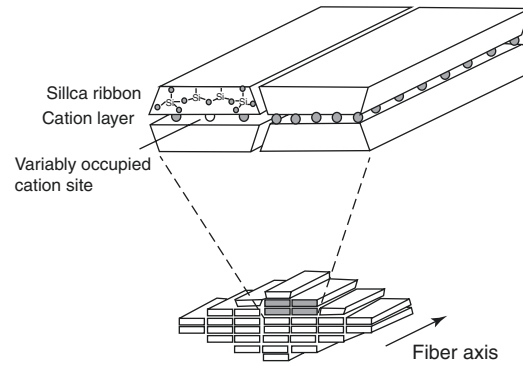


**Fig. 1.5** CrystallChemical composition and elemental spectra of asbestoscture of amphibole asbestos (Schematic diagram modified from Roggli and Coin (2004))

(Figs. 1.6, 1.7, 1.8, and 1.9). As their straight, broad fibers are resistant to fiber fragmentation and chemical degradation in the body, the biopersistence of the amphiboles is much greater than chrysotile, and their clearance half-time is generally measured in decades. The crystalline structure of the amphiboles also contains less water than chrysotile, and there is greater resistance to pyrolysis. Amphibole fibers are less flexible than chrysotile, permitting greater friability with potential to release respirable particles.

## 1.5 Identification and Characterization of Asbestos

Several techniques are available for the identification of asbestos fibers, making use of the morphologic, chemical composition and crystallogic features of the mineral [29]. The techniques include phase-contrast microscopy, polarizing microscopy with dispersion staining, infrared spectroscopy, x-ray and electron diffraction, and analytical electron microscopy. Each technique has its own advantages and disadvantages; Chap. 11 offers additional description of these. In brief, phase-contrast microscopy is a relatively inexpensive means to permit basic quantitative analysis of tissue fiber burden and is used to demonstrate the morphologic features of fibers such as size, shape, and aspect ratio. It is seldom used owing to the limits of the resolution of light microscopy, its

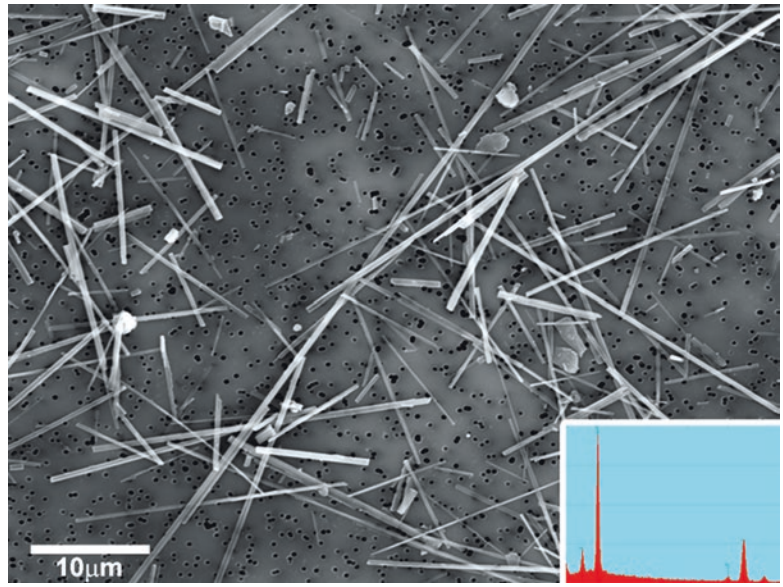


**Fig. 1.6** Amphibole asbestos fibers, scanning electron photomicrograph. Note long, straight, and slender fiber morphology

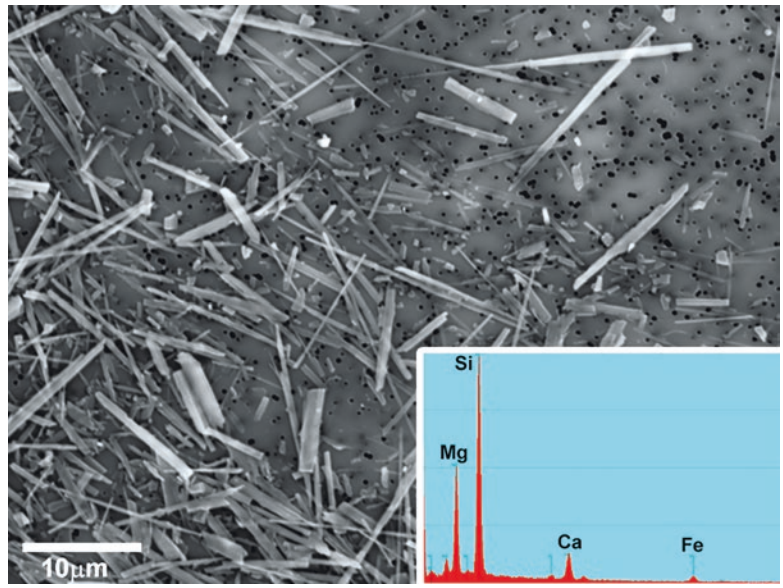


**Fig. 1.7** Libby asbestiform amphibole asbestos fibers, scanning electron photomicrograph. Note varying fiber morphologies, with thick, thin, short, and long fibers all represented

inability to distinguish asbestos fibers from non-asbestos mineral fibers or provide information regarding the chemical composition of fibers. Polarizing microscopy provides information pertaining to the basic crystalline structure of fibers and may be used to distinguish among the various asbestos fiber species and to make the distinction between asbestos and non-asbestos fibers. This technique is also limited by the resolution of light microscopy. Infrared spectrophotometry is a bulk analytical technique unable to examine individual fibers and is used to identify the characteristic spectra of the asbestos minerals. It is not generally used to identify asbestos in tissue or environmental samples. X-ray diffraction is also a bulk analytical technique which identifies diffraction patterns produced as x-rays pass through various crystalline materials [30]. It is generally considered a qualitative technique to measure the quantity of asbestos within a sample.

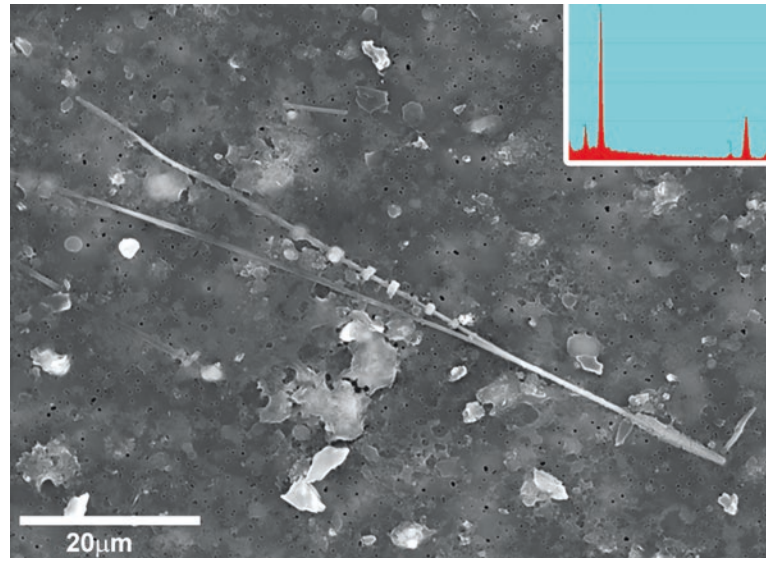


**Fig. 1.8** Amosite asbestos body. Note longitudinal cleavage of long, slender fiber

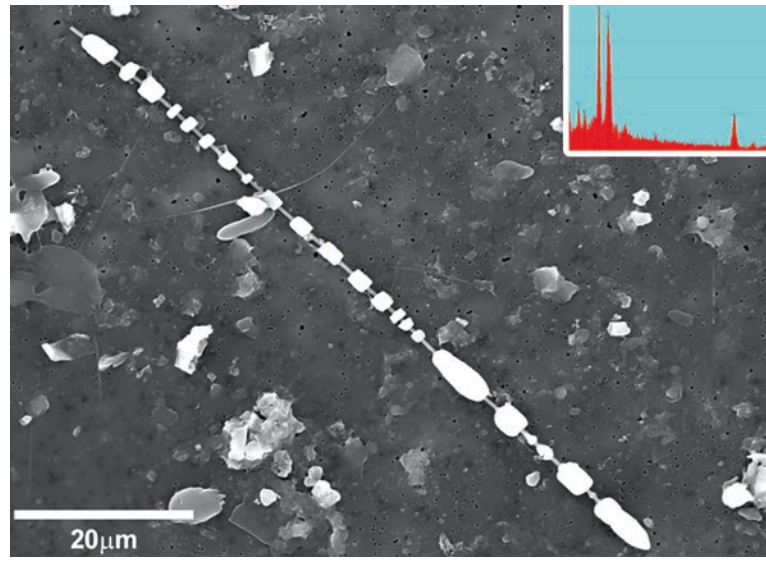


**Fig. 1.9** Crocidolite asbestos body. Note characteristic long slender fiber undergoing ferruginization

Most investigators prefer some form of analytical electron microscopy for the identification of asbestos. AEM has the ability to provide high-resolution images of the details of the smallest of fibers and to provide crystallographic compositional data for individual fibers through selected area electron diffraction and elemental composition information through energy dispersive spectrometry (EDS). EDS focuses an electron beam on individual particles and observes the x-ray spectra produced by the beam and the atoms within the particle. The spectra so produced consist of peaks distributed according to the energies of the x-rays, which are in turn related to the elemental composition of the fiber or particle being studied. Such spectra may be then compared to standards for confirmation of identification (Fig. 1.2). Analytical scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are both useful, albeit expensive and time-consuming. Our lab uses SEM to measure the number and dimensions of both fibrous and nonfibrous

crystalline material and provide both qualitative and quantitative analysis of fiber types and their proportionality. TEM generally offers superior resolution as well as the identification of very fine fibers and small fibrils, but the preparatory techniques are more complicated.

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# Occupational and Environmental Exposure to Asbestos

2

Dennis J. Darcey and Cynthia Feltner

## 2.1 Introduction

The usefulness of asbestos as an industrial material must be considered to understand the breadth of its public health impact. Since its discovery as an indestructible material centuries ago, it has found countless applications. Few substances rival its engineering and commercial performance.

Asbestos applications result from its many unique physical attributes. Its high tensile strength stabilizes mixtures with concrete, asphalt, and plastic. Asbestos also offers a stable material for frictional use, such as brake surfaces. Because of the length and pliability of its fibers, it has been incorporated into specially manufactured products, including gaskets, pads, fabric sheets, and asbestos paper with intrinsic properties of resistance and strength. Because it blocks heat transfer and is itself fireproof, it represents an ideal insulation material. Mixed into a slurry, it has been applied in economical fashion to building surfaces for fire protection and heat retention. In both its fabric and compacted-brick forms, it has been used to encase furnaces and kilns.

The economic advantages of asbestos explain its widespread application. As a natural (mined) rather than a manufactured substance, it was more available and its use not as closely evaluated by producers or consumers. Present in natural deposits on several continents, it has remained easily available for construction and industrial use by many nations, both industrialized and developing. Its production cost, as a truly raw material, has always been far less than substitute agents, which require manufacture and even technology licensing.

## 2.2 Historical Origin and Applications

### 2.2.1 Preindustrial Applications

The first recorded use of asbestos is as a wick material for oil lamps in ancient times. The material's name originates from the Greek for "inextinguishable" or "indestructible" [1]. Woven into cloth, asbestos provided nearly miraculous resistance to fire, especially impressive for shrouds of deceased whose cremation was open to public display.

Combining asbestos with clay and other malleable materials is also cited as one of the earliest applications of the material. In Finland in 2500 B.C., asbestos was added to clay pots for greater strength. Asbestos as a fortifying additive

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T.D. Oury et al. (eds.), *Pathology of Asbestos-Associated Diseases*,
DOI 10.1007/978-3-642-41193-9_2, © Springer-Verlag Berlin Heidelberg 2014

remains its major present-day use as a component of cement, concrete, paint, vinyl, and tar mixtures, accounting for 70 % of current applications worldwide.

### 2.2.2 The Modern Period

The past decades have witnessed a drastic change in America's patterns of asbestos use. Regulatory and health issues, rather than direct economic and engineering factors, now dominate its production and consumption. In developed nations, regulatory concern regarding asbestos' use and continued presence continues to grow. A ban on the use of asbestos was proposed by the US Environmental Protection Agency (EPA) in 1989 to prohibit the manufacture, importation, processing and distribution, and commerce of certain asbestos-containing products [2]. This provision also called for labeling requirements. However, in October 1991, the US Court of Appeals for the 5th Circuit vacated and remanded most of the EPA Asbestos Rule. The legal implications of the court's decision forced the EPA to revise its rule under the Clean Air Act (CAA) and Toxic Substances Control Act (TSCA). The products currently banned under TSCA include (1) corrugated paper, (2) roll board, (3) commercial paper, (4) specialty paper, (5) flooring, and (6) new uses of asbestos. Products not currently banned include asbestos-cement products, roofing felt and coatings, asbestos-cement shingle, millboard, asbestos automatic transmission components, clutch facings, friction materials, disc brake pads, and brake linings. Under the Clean Air Act, most spray-applied surfacing asbestos-containing materials containing more than 1 % asbestos are banned as well as wet-applied and preformed asbestos pipe insulation [3].

The Collegium Ramazzini, an international, nongovernmental organization that promotes public policy on occupational and environmental issues, first proposed an international ban on asbestos in the 1990s [4]. Some have criticized this proposal, arguing that the risks of continued asbestos use have been exaggerated and that health studies have not established the risk of substitute materials [5]. A renewed focus on an international ban continues and, to date, 52 countries have banned all forms of asbestos [6]. In 2006, the WHO called for the elimination of diseases associated with asbestos and vowed to support individual countries in developing national plans to ban asbestos. The International Labour Organization passed a resolution in 2006 to promote a worldwide asbestos ban. These movements to ban the use of asbestos maintain that morbidity and mortality related to asbestos exposure are preventable and that safer substitutes exist and have been successfully implemented in many cases [6].

The late Dr. Irving J. Selikoff, whose scientific, clinical, and public affairs careers are synonymous with asbestos and its health effects, categorized the societal impact of asbestos disease into three population "waves" of asbestos exposure and consequent clinical disease. Because of the well-documented latent interval for asbestos-related disease, the public health impact from each period of asbestos disease trails the period of exposure by 30–50 years.

The first wave of asbestos exposure comprises the workers whose activities actually generate asbestos for use, including the miners and packagers who transformed an ore into an industrial material. This exposure period, involving relatively few workers, extends from the initial use of the mineral into the early twentieth century. These workers, in countries where asbestos was first processed, such as Canada and South Africa, prompted the initial recognition of the diseases that required a latent period of decades to manifest themselves [7].

The second wave of asbestos-induced disease represents the impact of the manufacturing and construction use of the material. The most important peak in Western society's exposure to asbestos occurred during the period of rapid economic expansion surrounding World War II. Intense and high-volume ship construction, structural insulation, and the industrial fabrication of asbestos-containing products created a huge cohort of exposed workers during the mid-twentieth century. The ensuing period of public health impact manifested itself in the 1970s through the 1990s.

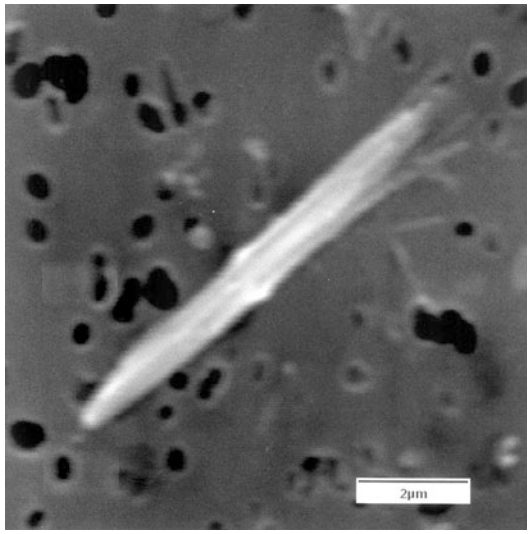


**Fig. 2.1** Scanning electron micrograph of chrysotile bundle isolated from bronchoalveolar lavage fluid from a New York City firefighter working on site for 2 weeks after the World Trade Center towers collapse on September 11, 2001. Nuclepore filter preparation, magnified ×14,000

The third wave of asbestos exposure and disease generates the most controversy and conjecture regarding both its size and the intensity of its public health impact. This comprises the cohort of citizens exposed to asbestos already in place. This population is likely to be exposed during the disruption of pre-applied asbestos insulation in homes and commercial buildings. Specific groups exposed to the highest dose of the mineral during this phase include building maintenance workers, construction workers, electricians, custodians, and the workforce employed specifically for asbestos abatement. The public health impact is seen at the present time. Disasters such as the collapse of the World Trade Center on September 11, 2001 raise concerns regarding the release of in-place asbestos into the ambient air and possible health effects of such exposures (Figs. 2.1 and 2.2).

Worldwide production of asbestos declined between 1980 and 2010, but worldwide use of asbestos remains sizable despite the increased recognition of its health consequences. Asbestos has not been mined in the USA since 2002, and imports, mostly from Canada, satisfied the needs of domestic consumers. US apparent consumption declined to 1,040 metric tons (t) in 2010. World production was 2.01 million metric tons in 2010 (Fig. 2.3), a decrease from 2.30 Mt in 2007 [8].

## 2.3 Occupational Exposure to Asbestos

### 2.3.1 Asbestos Processing

In the United States, for geological reasons, asbestos production has never been an important commercial enterprise. Even before restrictions for asbestos' use, the combined workforce involved in mining and milling of asbestos was known to be less than 600 [9]. Mining of asbestos creates exposure levels that are surprisingly low when compared to those of materials manufactured, averaging 0.9 fibers/cm$^3$. Because of the way the ore is handled, the fibers remain consolidated and have not yet become individualized. In contrast, the subsequent operation of mineral refining and milling (usually designed to "open" the bundles into individual fibers) generates worker exposure levels of 6.0–12.1 fibers/cm$^3$ [1].

Asbestos is shipped in bags, historically made of porous cloth, but recently of paper and plastic. The handling of this material in secondary industries routinely began with cutting open these bags and manually emptying them into hoppers, e.g., for mixture with concrete. Since this material is both dry and non-aggregated, the likelihood of dispersal is then at its maximum. The waste packaging material constitutes a source of exposure separate from the intended construction or industrial application.

### 2.3.2 Manufacture of Asbestos-Containing Products

The exposures that occur during the manufacture of asbestos products are extremely variable. Production of asbestos textiles involved higher exposure than other products. Carding and conventional spinning produced extreme air concentrations, resistant to environmental controls. Methods of manufacture utilizing liquid dispersion rather than dry asbestos are more successful at controlling potential exposure.

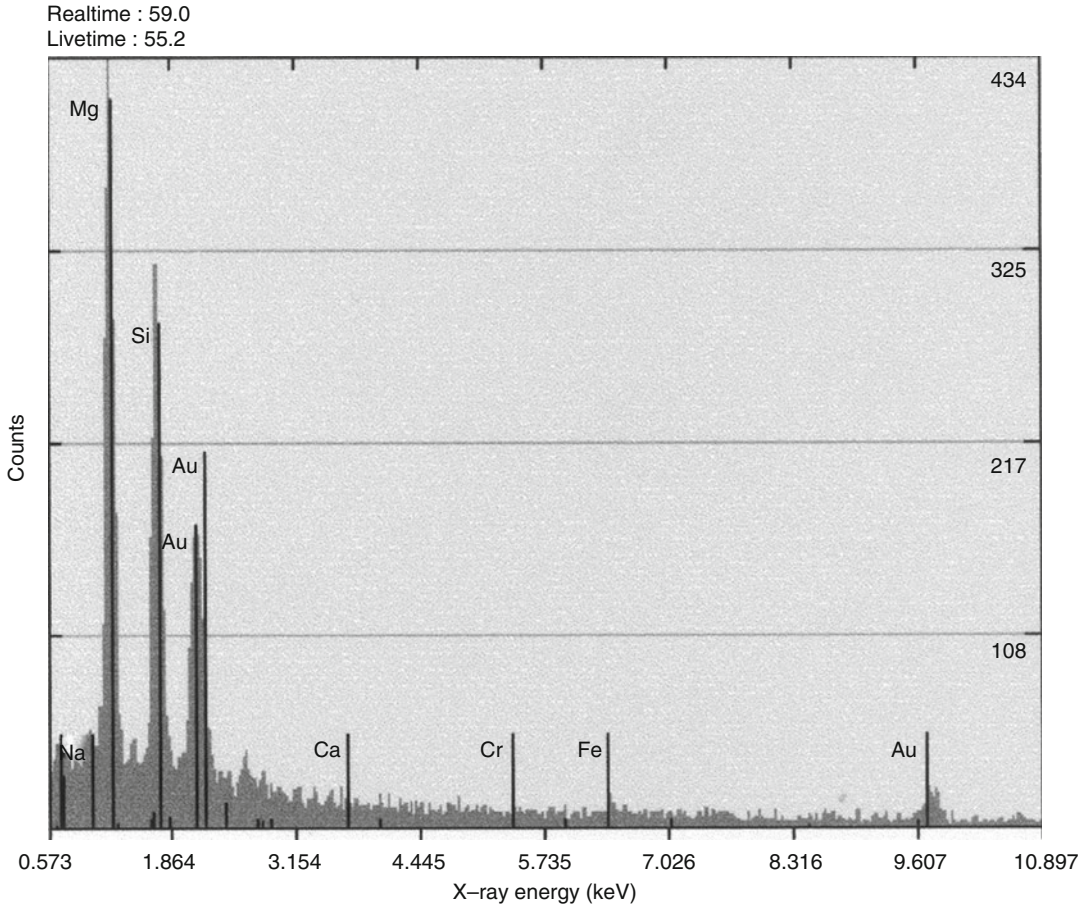


**Fig. 2.2** Energy dispersive spectrum from fiber shown in Fig. 2.1. Note the large peaks for magnesium and silicon, characteristic for chrysotile asbestos

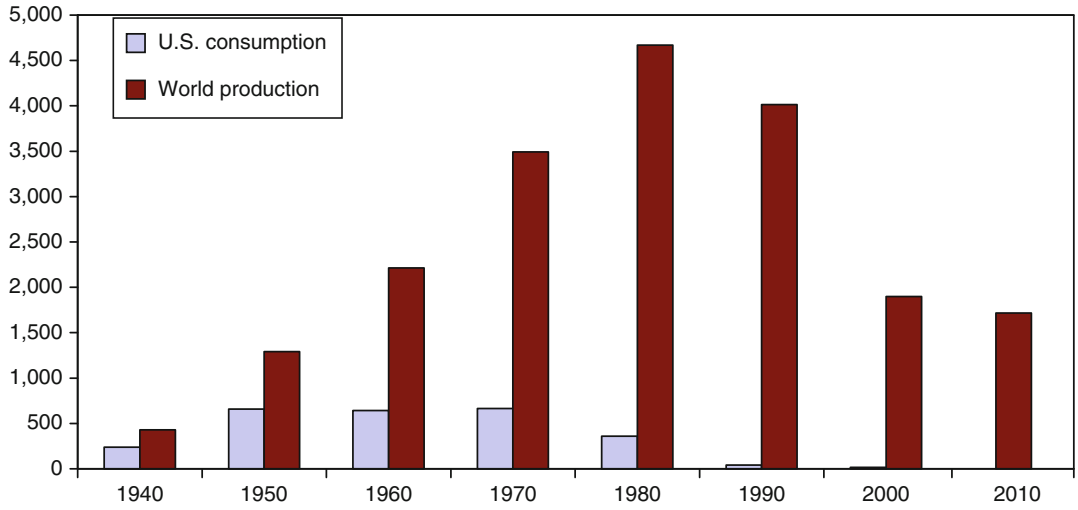


**Fig. 2.3** Asbestos consumption in the United States and world production of asbestos, which is used as a guide to world consumption. Peak US consumption of asbestos was 801,000 metric tons in 1973. Peak world production was 5.09 million metric tons in 1975 (Data from *Minerals yearbook*, v. 1 (published by the US Bureau of Mines until 1995 and by the US Geological Survey after 1995, with permission))

Work with material where the asbestos fibers were already entrapped (e.g., in roofing materials, floor tile, or cement pipe) presented considerable exposure opportunities, but only when such products are broken, thereby releasing respirable fibers. Information on job title provides some basis to assess actual exposure, but is often incomplete or misleading in estimating the degree of exposure. Certain jobs are more variable than others; for example, exposures for "inspectors" in manufacturing depend on the amount of loose asbestos dust remaining on the finished product.

### 2.3.3 Asbestos Insulation Materials

During the 1940s, 1950s, and 1960s, covering boilers and furnaces with asbestos was universal. Before the health effects of asbestos exposure were well recognized, the use of asbestos insulation material was considered an effective safety practice, preventing burns, heat release, and fire. Boiler makers and pipe coverers constitute the most important and widely evaluated cohort of exposed workers. Selikoff's 1964 study of New York insulation workers was one of the earliest US reports of the health consequences of this work. Among the 255 deaths evaluated in this mortality study, 18 % were due to lung cancer, 11 % to direct pulmonary damage from the dust, and 1.2 % to mesothelioma. This staggering impact was an early demonstration of asbestos exposure risk [10].

Construction industry application of asbestos coating to structural steel beams increased the societal scope of this exposure. The spraying of asbestos-cement mixtures was initiated in 1935 and, from 1958 to 1978, was widely employed for railway carriages, naval ships, and newly constructed buildings. By one estimate, 1.2 billion square feet of asbestos-containing insulation (averaging 14 % in concentration) is present in 190,000 American buildings [11]. The process was actually employed more rather than less frequently in the final years of this period, until the practice was halted when health issues became widely known.

### 2.3.4 Friction Materials

The use of asbestos for vehicular brakes takes advantage of its heat resistance and material strength. Asbestos concentrations in these materials are sizable, ranging from 30 to 80 %. Because manufacture and repair of automotive wheels is geographically widely distributed, this application exposes individuals in a wide variety of trades and geography. The practices of "blowing out" brake surfaces and beveling or grinding brake shoes produce modest airborne fiber concentrations, for considerable periods of time and at distances extending many feet from the actual operation. Another potential source of asbestos release to air is from clutches and brakes on cars and trucks; a wide range of air concentrations of asbestos fibers (0.004–16.0 f/cm$^3$) has been reported in numerous air sampling studies of workplaces during maintenance and replacement of vehicle brakes [12].

### 2.3.5 Construction Materials

In floor tile and in roof shingles and coatings, asbestos mixtures utilize the flexibility and strength of the mineral additive as an important stabilizing feature. Since these materials are popular for home improvement activities, this application provides additional opportunity for exposure to nonprofessional workers, who lack specific occupational monitoring or training. Ordinarily, exposures are quite low and require considerable disruption of the product's integrity to release respirable particles with asbestos content.

### 2.3.6 Shipyards

Shipbuilding makes unusually intense use of insulation materials because of the nature of the construction. Noise and heat from the immediate proximity of a shipboard power plant create an important need for effective thermal and acoustic insulation. Since ships have greater vulnerability to fire because of their isolation and confined

spaces, this insulation must be fireproof. Shipbuilding also brings workers not necessarily directly involved with asbestos work (e.g., electricians, metalworkers) into an asbestos-containing closed environment for the entire duration of the project. This closed-space exposure, by its nature, is difficult to control with usual industrial measures, such as ventilation, wetting of the fiber sources, and containment.

Because workplace safety efforts were relaxed during the establishment of the wartime economy of the 1940s, the massive shipbuilding effort of that period put the largest segment of workers at risk for subsequent asbestos-related disease. The conditions of enclosed, poorly ventilated, and unmonitored assignments produced prolonged and heavy exposure to all interior ship workers.

### 2.3.7 Asbestos Removal

As a result of the regulatory recommendation that asbestos must be removed from schools, industrial work sites, and residences, the most significant and identifiable current exposure to asbestos occurs during asbestos abatement [13]. In the removal of pure asbestos lagging, for example, potential exposures of 62–159 fibers/cm$^3$ have been reported [14]. This process often takes place in considerable disorder, because the surfaces are no longer easily accessible, and the work site is either in demand or in current use. Geographic isolation, soaking of the asbestos source, and personal containment represent the most important strategies for reduction of exposure.

The safety advantage in this process is that workers are required to be trained and become aware of the nature of the task and its hazards. Current regulations pertaining to asbestos removal provide clear guidelines for the handling of asbestos materials, contrasting with the historically careless handling of the same material.

The administrative demands of asbestos worker protection are extensive. Currently, workers involved in asbestos abatement are required to undergo a preemployment evaluation of their ability to work wearing a HEPA (high-efficiency particulate air) filter respirator and impermeable (thus hot and humid) disposable clothing [15]. Baseline and periodic chest radiographs are taken and measurement of pulmonary function. Before initiation of asbestos work, these individuals receive mandatory instruction regarding the health effects of asbestos-related disease and the means of dust and exposure control. Educational opportunities regarding the multiplicative effect of tobacco smoking on the risks from asbestos exposure are now a required component of asbestos worker training.

The area for asbestos removal is enclosed with a plastic barrier of specified 6-mil-thick polyethylene sheeting and by toxic-hazard warning signs. The site is kept at negative barometric pressure (relative to the surrounding area) by having fans blow air outward through HEPA filters. If possible, asbestos-containing material is covered in plastic bags to encase escaping fragments. Additionally, workers wear intensive personal protective gear (mask, gown, and gloves, as in Fig. 2.4). Throughout removal, every effort is made to keep the material soaked so that respirable dust is minimized. Waste products are labeled and are handled with special care. Monitoring for airborne asbestos concentration is performed outside the confined asbestos-abatement area. Following each work period, workers are required to discard all outer clothing and shower, to prevent secondary contamination from work clothes. Periodic medical monitoring is also required, although the decades-long latency of asbestos-related disease makes these sessions more appropriately an opportunity for discussions of health risk and for counseling on smoking cessation.

### 2.3.8 Current Occupational Exposure in the USA

Very limited information is available on the number of workers still exposed to asbestos in the USA. Overall, exposure patterns have changed from historically chronic exposures (manufacturing processes, cement pipe fabrication) to short-term, intermittent exposure occurring through maintenance and remediation work. Although asbestos is no longer mined in the USA, NIOSH

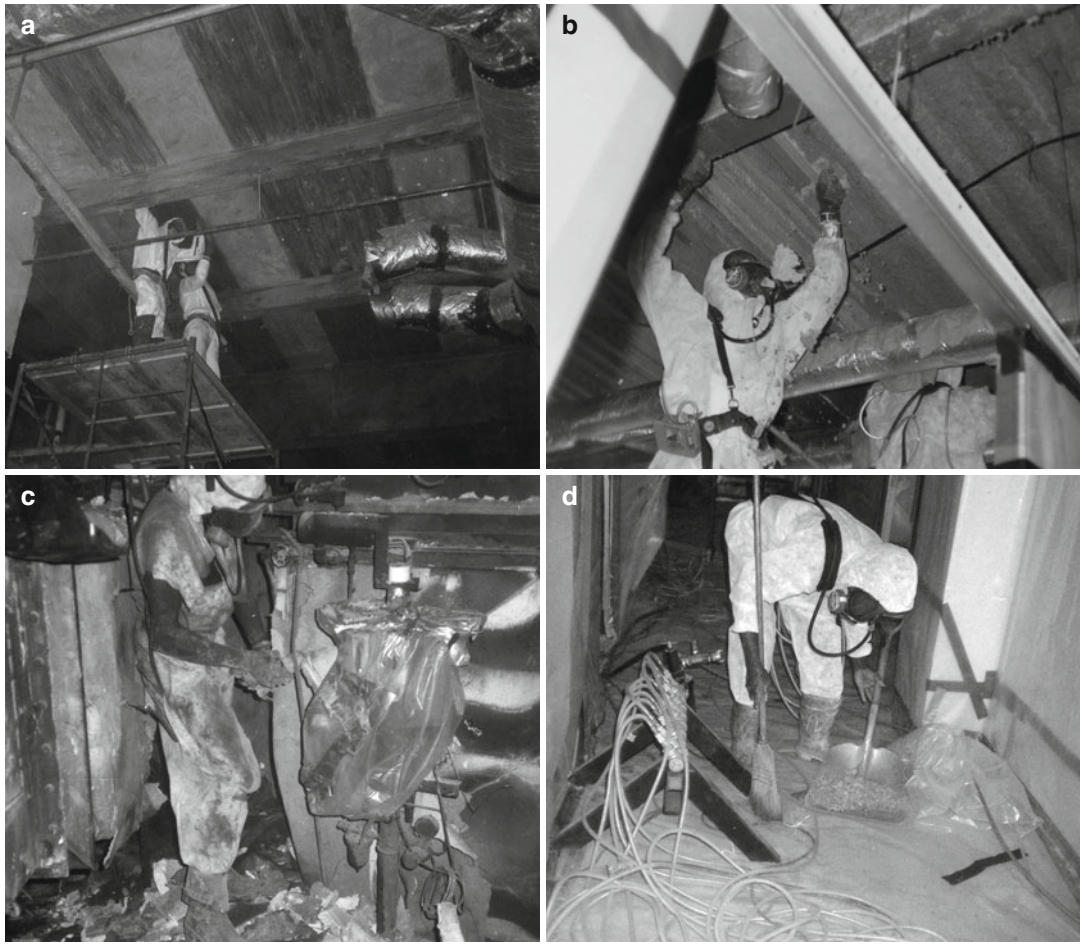


**Fig. 2.4** Workers in the North Carolina asbestos-abatement program. Asbestos removal occurs within confined spaces. Note the respiratory equipment and special protective clothing

estimates that 44,000 miners and other mine workers may be exposed to asbestos or amphibole cleavage fragments during the mining of some mineral commodities [16]. Recently, OSHA has estimated that 1.3 million employees in construction and general industry face significant asbestos exposure on the job [16].

### 2.3.9 Occupational Exposure in Developing Countries

The asbestos industry has reorganized at a global level; some have characterized this process as a "risk transfer" of hazardous exposure from industrialized to less-developed countries [17]. While more developed countries are limiting the production and use of asbestos, marketing of asbestos and asbestos-based products within less-developed countries continues.

Russia is now the leading producer of asbestos worldwide, followed by China, Kazakhstan, Brazil, Canada, Zimbabwe, and Colombia. These six countries accounted for 96 % of the world production of asbestos in 2007 [6]. More than 85 % of the world production of asbestos is used today to manufacture products in Asia and Eastern Europe [6]. Often these countries have limited healthcare for laborers. In addition, regulatory mechanisms for use, handling, and disposal of asbestos and associated waste are often lacking [18]. This problem is confounded by the

lack of epidemiological data on asbestos health effects and extent of local exposure. Without data on local situations, diseases such as asbestosis and mesothelioma may remain obscure and not attract attention from regulatory agencies [18].

## 2.4 Nonoccupational Exposure to Asbestos

Exposure to asbestos in the ambient indoor and outdoor environments results from many sources, both natural and man-made. Chrysotile asbestos, which accounts for over 90–95 % of the asbestos used in the United States, has become a ubiquitous contaminant of ambient air. It has been noted that asbestos fiber can be found in the lungs of almost everyone in the American population [19]. Natural sources of asbestos fiber release include weathering and erosion of asbestos-containing rock and of road surfaces composed of asbestos ores. If the primary areas of source rock are compared with high population density, the most critical areas for emissions from natural sources appear to be eastern Pennsylvania, southeastern New York, southwestern Connecticut, and greater Los Angeles and San Francisco.

Manufactured sources of exposure in the past have included off-site releases from mining, milling, and manufacture of asbestos products, exposing residents in nearby communities. In addition to workers' exposures, their families also have the potential for secondhand exposure to asbestos. Families have been exposed to asbestos when workers were engaged in mining, shipbuilding, insulating, maintenance and repair of boilers and vehicles, and asbestos-removal operations. Most documented cases of asbestos-related disease among workers' family members have occurred in households where women were exposed during home laundering of contaminated work clothing or in cases where children have been exposed by playing in areas where asbestos-contaminated shoes and work clothes were located or where asbestos-containing materials were stored [16]. Family members have been found to be at increased risk of malignant mesothelioma, lung cancer, cancer of the gastrointestinal tract, asbestosis, and nonmalignant pleural abnormalities [16].

Weathering of asbestos-cement wall and roofing materials is a relatively minor environmental source of exposure from man-made construction materials. However, off-site release from construction sites (primarily from sprayed-on asbestos fireproofing) has resulted in ambient asbestos levels 100 times background levels [20].Asbestos brake and clutch pads in automobiles contribute to the environmental load of asbestos. However, it is uncertain how much respirable fiber is released, because thermal degradation occurs at the high temperatures generated during braking.

Waste disposal has become a growing source of potential exposure to asbestos fibers, and promises to continue as removal, abatement, and renovation occur in the existing building stock. Consumer products, water supplies, and food sources have been contaminated with asbestos-containing materials in the past. These man-made sources of exposure have been significantly reduced by regulatory activity over the past 40 years and will continue to decline.

Currently, the most important source of nonoccupational exposure is the release of fibers from existing asbestos-containing surface materials in schools, residences, and public buildings or from sprayed asbestos-containing fireproofing in high-rise office buildings. The greatest potential for future exposure will be determined by the asbestos released during the maintenance, repair, and removal of these structures. The implementation of the Asbestos Hazard Emergency Response Act (AHERA), requiring inspection of the nation's public and private schools for asbestos, has resulted in an explosive commercial growth of the industry involved in asbestos identification and removal. Some have argued that removal itself presents more of an exposure hazard than leaving the materials undisturbed or encapsulated [21].

## 2.5 Measuring Exposure

Different techniques have been developed for measuring the concentration of asbestos in ambient air and in the workplace. The phase-contrast

**Table 2.1** Summary of asbestos exposure samples in different environments

| Sample set | Sample no. | Measured concentration (ng/m$^3$) | | Equivalent concentration (fibers/cm$^3$)[a] | |
|---|---|---|---|---|---|
| | | Median | 90th %ile | Median | 90th %ile |
| Air of 48 US cities | 187 | 1.6 | 6.8 | 0.00005 | 0.00023 |
| Air in US school rooms (asbestos) | 31 | 16.3 | 72.7 | 0.00054 | 0.00242 |
| Air in Paris bldgs (asbestos surfaces) | 135 | 1.8 | 32.2 | 0.00006 | 0.00107 |
| Air in US bldgs (cementitious asbestos) | 28 | 7.9 | 19.1 | 0.00026 | 0.00064 |
| Air in US bldgs (friable asbestos) | 54 | 19.2 | 96.2 | 0.00064 | 0.00321 |

Source: Modified from Ref. [22]
[a]Based on conversion factor of 30 μg/m$^3$ = 1 fiber/cm$^3$

light microscope for counting fibers in the workplace has been less useful in the ambient environment, where fiber identity and character are usually unknown, fibers are too small to be seen by light microscopy, and concentrations expressed as mass are usually hundreds or thousands of times lower than those in the workplace.

Fiber concentrations in the workplace have generally been measured as the number of fibers longer than 5 μm and an aspect ratio of 3:1 or greater. Ambient concentrations are now determined by transmission electron microscopy and usually are expressed as mass per unit volume (nanogram per cubic meter). Because of intrinsic variability in the unit weight of individual fibers, the conversion factors relating mass concentration to optical fiber concentration range widely from 5 to 150 μg/m$^3$/f/cm$^3$ [20].

Measurements via transmission electron microscopy have established background concentrations of asbestos in urban ambient air at generally less than 1 ng/m$^3$ (0.00003 fibers/cm$^3$) and rarely more than 10 ng/m$^3$ (0.00033 fibers/cm$^3$) [22]. Table 2.1 summarizes fiber concentration data from a variety of studies in both urban and rural areas.

Asbestos concentrations in buildings, on the other hand, are more variable, revealing a threefold variability among arithmetic mean concentrations [22]. Earlier studies often focused on buildings in which asbestos surface materials were visibly damaged and friable, which were not representative. In buildings with evidence of severe damage or deterioration, the probability of detecting excessive asbestos levels over background was high. If the asbestos-containing surface materials or thermal insulation was undamaged or encapsulated, lower air concentrations were observed.

Table 2.2 shows summary statistics for average airborne fiber concentrations near schools and buildings. Levels are comparable to outdoor air and are several orders of magnitude lower than current workplace standards (OSHA permissible exposure level (PEL) of 0.1 fibers/cm$^3$).

Asbestos-abatement work is a significant potential source of asbestos exposure, particularly in schools and public buildings. Although current procedures specified by the EPA should minimize building contamination following renovation, removal, enclosure, or encapsulation of asbestos materials, these procedures may be violated and lead to high-risk exposures.

The EPA has monitored the efficacy of the specified controls and cleanup procedures. Table 2.3 presents the results of one study of five schools where removal and encapsulation of asbestos-containing surfaces followed EPA procedures [22]. Although escape of asbestos fibers did occur during encapsulation and removal, there appeared to be a net reduction in fiber levels after encapsulation. Little improvement occurred in asbestos fiber levels following physical removal, with pre-and post-abatement fiber levels being virtually the same. These results have brought into question both the health risk-benefit and the cost-benefit considerations of removal versus encapsulation. Currently, widespread removal of asbestos is not frequently recommended, and encapsulation is preferred in many situations.

**Table 2.2** Summary statistics for average airborne fiber concentrations in US schools and buildings

| | | | Public buildings | | |
|---|---|---|---|---|---|
| | Schools | Outdoor air | Category 1 | Category 2 | Category 3 |
| Statistic | (71) | (48) | (6) | (6) | (37) |
| Median | | 0.00000 | 0.00010 | 0.00040 | 0.00058 |
| Mean | 0.00024[a] | 0.00039 | 0.00099 | 0.00059 | 0.00073 |
| Standard deviation | 0.00053 | 0.00096 | 0.00198 | 0.00052 | 0.00072 |

Source: From Ref. [23], with permission
The data used in the calculation of each statistic are the average concentrations (expressed as number of fibers greater than 5 μm in length per cubic centimeter of air) in a building (for indoor samples) or the concentration outside each building (for outdoor samples). By visual inspection, category 1 buildings contained no asbestos-containing material (ACM), category 2 buildings contained ACM in primarily good condition, and buildings in category 3 showed at least one area of significantly damaged ACM. In the study on public buildings, 387 indoor and 48 outdoor air samples were evaluated. No asbestos fibers were detected in 83 % of the 387 samples. The sample size is given in parentheses below each heading
[a]80th percentile = 0.00045; 90th percentile = 0.00083

**Table 2.3** Geometric mean of chrysotile fiber and mass concentrations before, during, and after asbestos abatement

| | Concentration | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Before abatement | | During abatement[a] | | Immediately after abatement | | Afterschool resumed | |
| Sampling location | (f/l)[b] | ($ng/m^3$) | (f/l) | ($ng/m^3$) | (f/l) | ($ng/m^3$) | (f/l) | ($ng/m^3$) |
| *Encapsulation* | | | | | | | | |
| Rooms with unpainted asbestos | 1,423.6 | 6.7 | 117.2 | 0.6 | 13.7 | 0.1 | 248.1 | 1.2 |
| Rooms with painted asbestos | 622.9 | 2.7 | – | – | 0.8 | 0.0 | 187.2 | 0.8 |
| Asbestos-free rooms | 250.6 | 1.2 | 0.5 | 0.0 | 9.3 | 0.0 | 30.7 | 0.2 |
| Outdoors | 3.5 | 0.0 | 0.0 | 0.0 | 6.5 | 0.0 | 2.8 | 0.0 |
| *Removal* | | | | | | | | |
| Rooms with asbestos | 31.2 | 0.2 | 1736.0 | 14.4 | 5.6 | 0.1 | 23.9 | 0.2 |
| Asbestos-free rooms | 6.1 | 0.1 | 12.0 | 0.1 | 1.6 | 0.0 | 18.1 | 0.1 |
| Outdoors | 12.6 | 0.1 | 1.3 | 0.0 | 20.0 | 0.1 | 7.9 | 0.0 |

Source: Reprinted from Ref. [22], with permission
[a]Measured outside work containment areas
[b]Fibers of all lengths

## 2.6 Regulatory Activity

Public health concern over the occupational and nonoccupational sources of asbestos exposure has created a vast array of governmental regulatory activity and the phasing out of asbestos production and its use in consumer products. This marked reduction in use is the result of regulatory activities in the 1970s and 1980s, during which time five government agencies invoked statutory authority to regulate asbestos.

The Occupational Safety and Health Administration (OSHA) regulates workplace exposure to asbestos and has set a permissible exposure level (PEL) (an 8-h time-weighted average for a 40-h-per-week work shift) for occupational exposures. The PEL has been steadily lowered, as concern over health hazards and better monitoring methods have become established (Table 2.4). The first permanent standard, set in 1972, was 5 fibers/$cm^3$. This was lowered in 1976 to 2 fibers/$cm^3$ and in 1986 to the lowest agreed to be technologically feasible at that time, 0.2 fibers/$cm^3$. The National Institute for Occupational Safety and Health (NIOSH) recommended a recommended exposure level (REL) of 0.1 fibers/$cm^3$. This level was also proposed as a regulatory standard by OSHA in 1990 and adopted in 1993.

**Table 2.4** US asbestos regulatory activity

| Year | Agency | Regulation |
|---|---|---|
| 1971 | EPA | Asbestos listed as hazardous air pollutant |
| 1972 | OSHA | 5 fibers/cm$^3$ STEL |
| 1973 | EPA | No visible asbestos emissions, milling, and manufacturing and ban on spray application of friable materials containing more than 1 % asbestos |
| 1976 | OSHA | 2 fibers/cm$^3$ TWA, 10 fibers/cm$^3$ STEL |
| 1976 | NIOSH | 0.1 fibers/cm$^3$ TWA |
| 1986 | OSHA | 0.2 fibers/cm$^3$ TWA |
| 1988 | OSHA | 1.0 fibers/cm$^3$ STEL |
| 1988 | EPA | Ban on asbestos cloth, felt, tile, gaskets, brakes, after market brakes, air conditioning pipe, shingles, roofing materials to be phased out over several years[a] |
| 1993 | OSHA | 0.1 fibers/cm$^3$ TWA |
| 1993 | EPA | Revision on ban on asbestos, vacating and remanding most of 1989 rule |
| 2000 | EPA | Asbestos worker protection rule, cross-reference to OSHA standards to protect state and local government employees[b] |

[a]Federal Register [2]
[b]Federal Register [31]
*STEL* short term exposure limit

NIOSH defined "airborne asbestos fibers" to encompass not only fibers from the six previously listed asbestos minerals (chrysotile, crocidolite, amosite, anthophyllite asbestos, tremolite asbestos, and actinolite asbestos) but also elongate mineral particles (EMPs) from their non-asbestiform analogs. This definition has led to controversy and confusion, and NIOSH published a clarification of the REL for airborne asbestos fibers and related EMPs in 2011 [16]. The REL and method for counting fibers was not changed; only the definition was clarified to make clear that EMPs included in the count are not necessarily asbestos fibers [16]. NIOSH has also called for additional research on the health effects of non-asbestiform EMPs and better sampling techniques to help guide future policies.

The Mine Safety and Health Administration regulates the mining and milling of asbestos ore. The Food and Drug Administration (FDA) is responsible for regulating asbestos in food, drugs, and cosmetics. Consumer product bans on the use of asbestos in garments, dry-wall patching compounds, and fireplace emberizing materials have been implemented by the Consumer Product Safety Commission. Despite these selected events, most of the regulatory activity has emanated from the Environmental Protection Agency.

Through the National Emissions Standards for Hazardous Air Pollutants (NESHAP) program, the EPA regulates external emissions from asbestos mills and from manufacturing and fabrication operations. The EPA also regulates the use of asbestos in roadway surfacing and in insulation materials and has banned most uses of sprayed-on asbestos materials and pipe wrapping. These standards also require specific work practices during demolition and renovation involving asbestos materials and regulate the removal, transport, and disposal of asbestos-containing materials. The EPA has also established programs to evaluate and certify asbestos-removal contractors and established work rules to protect workers during asbestos-abatement activities.

Since 1982, when the EPA issued the Asbestos and Schools Identification and Notification Rule, the agency has required all local education agencies to inspect for friable asbestos materials, to notify parents and teachers if such materials are found, to place warning signs in schools where asbestos is found, and to keep accurate records of their actions eliminating this problem. With Congressional approval of the Asbestos School Hazards Abatement Act of 1984, the EPA was given responsibility for providing both financial and technical assistance to local education agencies.

## 2.7 Assessing Nonoccupational Risk

Asbestos-related disease resulting from nonoccupational exposure to asbestos has been recognized in published reports of mesothelioma among household contacts of asbestos workers and in residents living near asbestos mines and factories. An increase in the prevalence of malignant mesothelioma and asbestos-related disease has been reported in nonoccupationally exposed populations in Turkey, Cyprus, the Metsovo region of Greece, and Northeast Corsica. The causal factor for at least some of the excess mesothelioma in Turkey may be due to the geologic presence of a non-asbestos mineral fiber, erionite (see Chap. 5).

A meta-analysis of eight published studies conducted in populations with relatively high household and neighborhood exposure to asbestos showed significantly elevated relative risks for developing pleural mesothelioma. In the neighborhood exposure groups, the risk ranged between 5.1 and 9.3. In the household exposure groups, the risk ranged between 4.0 and 23.7 [24].

However, these study populations were exposed to ambient concentrations much higher than those observed in US homes and public buildings, and these data are insufficient to estimate the magnitude of the excess risk for pleural mesothelioma at levels of environmental exposure commonly encountered by the general population in industrialized countries.

In an effort to assess the health risk of nonoccupational exposure to asbestos in buildings and schools, numerous international panels have been convened. In the absence of undisputed evidence, several mathematical models have been proposed to assess the lifetime risk of lung cancer and mesothelioma. Underlying these varying risk assessment models are assumptions and uncertainties making the interpretation of these risk estimates inherently difficult.

The estimation of risk is based upon extrapolation from high-dose workplace exposures in the past to low doses found in buildings and the ambient environment. Modern ambient exposures are orders of magnitude less than even today's OSHA permissible exposure level of 0.1 fibers/cm$^3$. Estimates of exposure assigned to these retrospective worker cohorts cannot be fully characterized, due in part to poor sampling and analytical methodology and the use of surrogate exposure categories based on job title. Mass-to-fiber conversions utilized in these models add substantial uncertainty. Models that include an assumption of a linear dose response assume that exposure to one fiber of asbestos carries an inherent and finite risk for lung cancer and mesothelioma and that the risk is cumulative for each fiber to which an individual is exposed. There appears to be no evidence of a threshold level below which there is no risk of mesothelioma [25]. This hypothesis is still debated.

In a review of potential health risk associated with working in buildings constructed of asbestos-containing materials, the lifetime risk for premature cancer death was estimated to be four per million for those exposed for 20 years working in office buildings (estimated exposure levels ranging from 0.0002 to 0.002 fibers/cm$^3$). For those exposed for 15 years in schools, the risk was estimated to be one per million (estimated exposure levels ranging from 0.0005 to 0.005 fibers/cm$^3$) [26]. In comparison the risk associated with the OSHA permissible exposure level of 0.1 fibers/cm$^3$ for 20 years was estimated at 2,000 per million exposed. The risk estimates associated with building exposure to asbestos are orders of magnitude lower than some commonplace risks from drowning, motor vehicle accidents, and household accidents. They are also far less than the background estimate of mesothelioma of 1–2 cases per million population per year.

The EPA uses mathematical models, based on human and animal studies, to estimate the probability of a person developing cancer from breathing air containing a specified concentration of a chemical [27]. The EPA calculated an inhalation unit risk estimate of $2.3 \times 10^{-1}$ (fibers/cm$^3$)$^{-1}$. The EPA estimates that if an individual were to continuously breathe air containing asbestos at an average of 0.000004 fibers/cm$^3$ over his or her entire lifetime, that person would theoretically have no more than a one-in-a-million increased

chance of developing cancer as a direct result of breathing air containing this chemical. Similarly, the EPA estimates that breathing air containing 0.00004 fibers/cm$^3$ would result in not greater than a one-in-a-hundred thousand increased chance of developing cancer, and air containing 0.0004 fibers/cm$^3$ would result in not greater than a one-in-ten-thousand increased chance of developing cancer. The risk assessment model used by the EPA was called into question in a study by Camus et al. of nonoccupational exposure to chrysotile asbestos [28]. Attempts to stratify risk of disease by potency of fiber type have been proposed but recently abandoned by the EPA [29]. The uncertainties center around limitations of the exposure data—primarily the difficulty in trying to classify risk by exposure subgroups when these groups cannot be well defined and when there are multiple exposures present [29].

Some studies of asbestos workers have observed an increased risk of cancer at other sites, including the gastrointestinal tract, larynx, esophagus, and kidney (see Chap. 8). There has been controversy over these findings given the limited nature of this body of evidence. However, more recently the evidence for the carcinogenicity of asbestos has been evaluated by several bodies including the Institute of Medicine (IOM) and National Academy of Sciences (NAS) in 2006 and by the International Agency for Research on Cancer (IARC) in 2009. The IARC concluded that there was sufficient evidence from epidemiological studies that asbestos caused cancer of the larynx and ovary as well as limited evidence that it caused cancer of the colorectum, pharynx, and stomach [30]. These conclusions were consistent with the IOM evaluation.

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# Asbestos Bodies and Non-asbestos Ferruginous Bodies

3

Victor L. Roggli

## 3.1 Introduction

Asbestos bodies are the histologic hallmark of exposure to asbestos [1–4]. These structures are golden brown, beaded or segmented, dumbbell-shaped objects that have a characteristic microscopic appearance that is readily recognized by the pathologist. Their identification in histologic sections is an important component of the pathologic diagnosis of asbestosis (see Chap. 4), and their presence serves to alert the pathologist that the patient has been exposed to airborne asbestos fibers. It is the purpose of this chapter to discuss the structure and development of asbestos bodies as well as their occurrence and distribution within human tissues. In addition, techniques for the quantification of asbestos bodies are reviewed, along with the relationship of asbestos body formation to the various types of asbestos fibers. Finally, the distinction of asbestos bodies from other ferruginous bodies based on light microscopic and analytical electron microscopic observations is emphasized. The identification and significance of asbestos bodies in cytologic specimens is discussed in Chap. 9, and the relationship between asbestos body concentrations in pulmonary tissues and the various asbestos-associated diseases is reviewed in Chap. 11.

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## 3.2 Historical Background

Asbestos bodies were first described in the lung by Marchand in 1906 [5]. He called them peculiar "pigmented crystals" and did not recognize their relationship to asbestos fibers. Eight years later, the German pathologist T. Fahr also took note of peculiar crystals in the lungs of an asbestos worker with pulmonary interstitial fibrosis [6]. W. E. Cooke described these structures as "curious bodies" [7], and by 1929 Stewart and Haddow had coined the term "asbestosis bodies" [8]. By this time Cooke [9] and Gloyne [10] recognized that these curious bodies had asbestos fibers at their core, although as late as 1930 in this country, they were confused with fungal hyphae [11]. The term asbestosis body was later changed to asbestos body when it was discovered that they also occurred in the lungs of workers who did not have asbestosis [12, 13]. Experimental animal studies in the 1960s showed that structures resembling asbestos bodies were formed when a number of different types of fibrous dusts (fibrous aluminum silicate, silicon carbide whiskers, cosmetic talc, and fibrous glass) were instilled intratracheally into the lungs of hamsters [14]. As a result, it was suggested that the noncommittal term "ferruginous body" be used when the precise nature of the fibrous core was not known [14, 15]. It then remained for Churg and Warnock [16, 17] to show by means of energy dispersive spectrometry and electron diffraction that ferruginous bodies isolated from human lungs and having a thin, translucent fibrous core were virtually always true asbestos bodies.

T.D. Oury et al. (eds.), *Pathology of Asbestos-Associated Diseases*,
DOI 10.1007/978-3-642-41193-9_3, 

## 3.3 Structure and Development of Asbestos Bodies

Asbestos bodies form when an asbestos fiber is inhaled and deposited in the distal regions of the lung parenchyma [13]. Here the free alveolar macrophages phagocytose the fiber (Fig. 3.1). Subsequently, through a process which is poorly understood, the fiber becomes covered with a layer of iron-protein-mucopolysaccharide material [19–21]. It has been proposed that this process is a means of host defense, since in vivo [22] as well as in vitro [23] studies have shown that asbestos bodies are nonfibrogenic and noncytotoxic in comparison to uncoated asbestos fibers. Furthermore, the iron coating is bound in such a way that it does not efficiently participate in the generation of reactive oxygen species [24, 25]. Indeed, Ghio et al. have proposed that coating process is a marker for particle-induced oxidative stress [26].

The coated asbestos fiber, or asbestos body, has a characteristic golden brown appearance, which is due to the iron component of the coat-

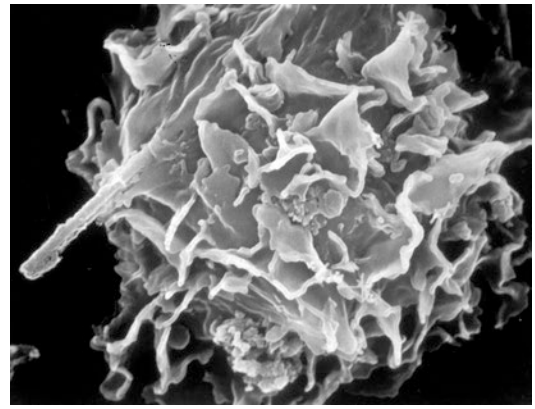

**Fig. 3.1** Scanning electron micrograph of a human free alveolar macrophage phagocytizing an amosite asbestos fiber. Magnified ×2,000 (Reprinted from Greenberg [18] with permission)

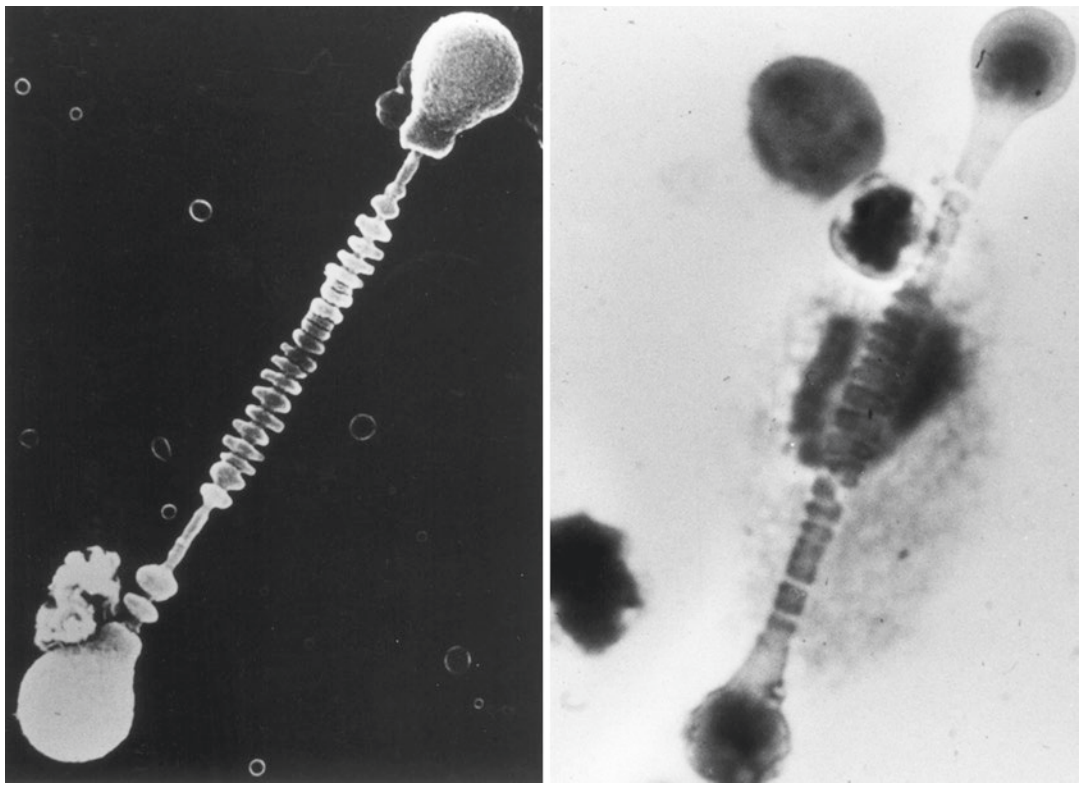

**Fig. 3.2** Side-by-side scanning electron micrograph of an asbestos body to the left with bronchoalveolar lavage-recovered asbestos body and free alveolar macrophage to the right (SEM, magnified ×2,000; Papanicolaou, magnified ×600) (Reprinted from Ref. [2], with permission)

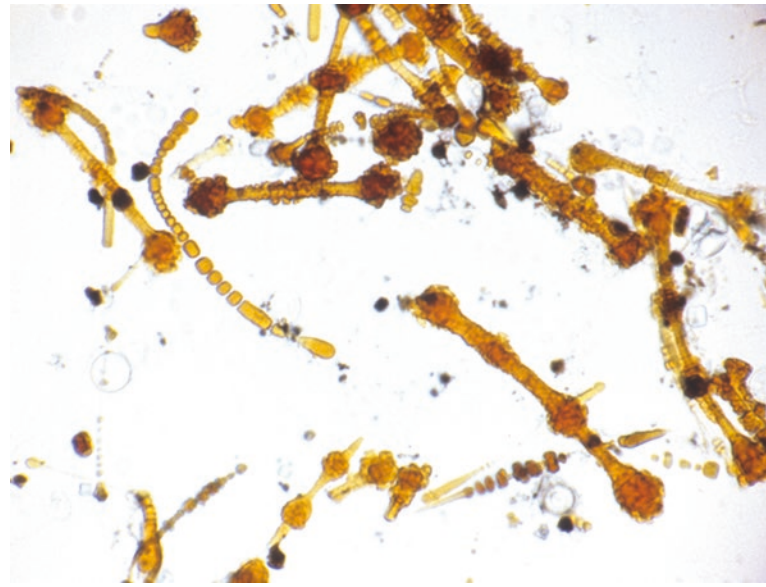

**Fig. 3.3** Asbestos bodies on a Nuclepore filter show the range of morphologic appearances, including dumbbell shapes, beaded structures, and lancet forms. Note the variable quantity of iron coating and the visible asbestos core fibers

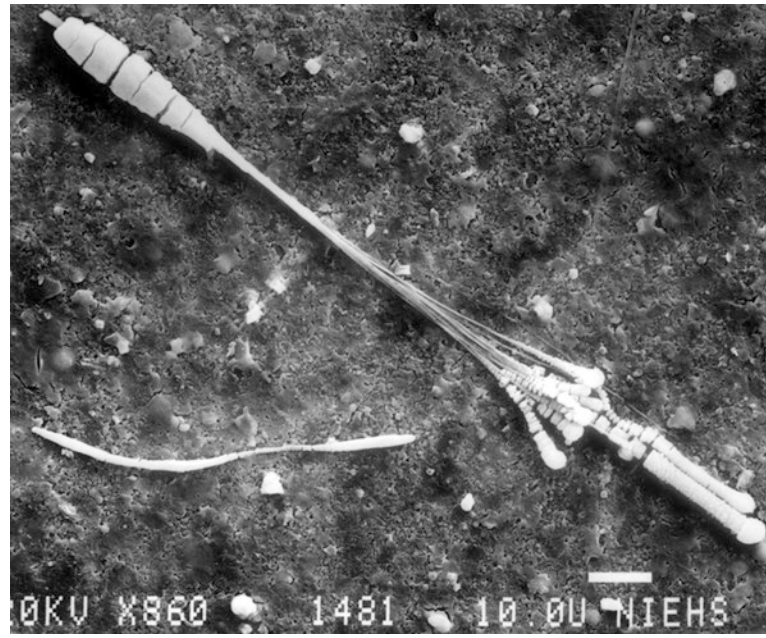


**Fig. 3.4** Scanning electron micrograph of an asbestos body with splaying of one end of the core fiber. Each splayed fiber has its own ferroprotein coating. Magnified ×860

ing. These structures thus give a strong positive reaction with the Prussian blue stain. In histologic sections, asbestos bodies have a beaded, segmented, dumbbell, or lancet shape, which is especially well appreciated in cytologic preparations (Fig. 3.2) and in Nuclepore filter preparations of lung tissue digests (Fig. 3.3). Branched forms, which result from the deposition of coating material on a splayed fiber, may also occur (Fig. 3.4). Curved or circular asbestos bodies may also be observed (Fig. 3.5), and these are usually found to have very thin core fibers (average core diameter of 0.2 μ) [17]. Asbestos bodies are generally 20–50 μ in length [21], with an average length of about 35 μ [27]. However, they may exceed 200 μ in length and some examples

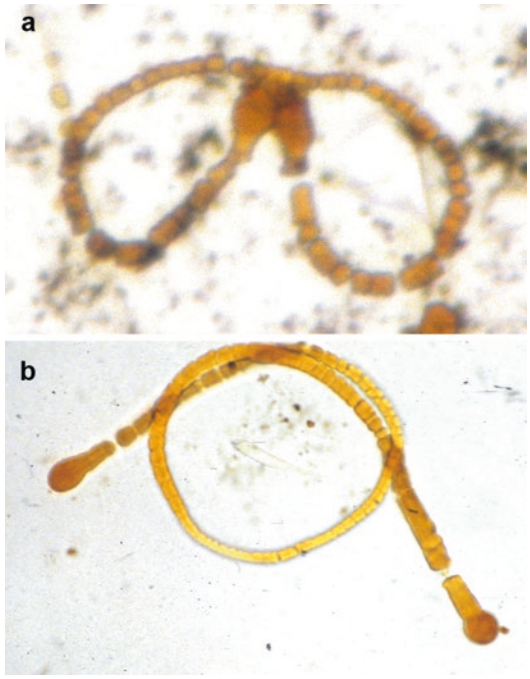


**Fig. 3.5** Examples of curved asbestos bodies with thin amphibole cores. (**a**) Pair of asbestos "spectacles." (**b**) This asbestos body isolated from bronchoalveolar lavage fluid appears to be tied in a knot

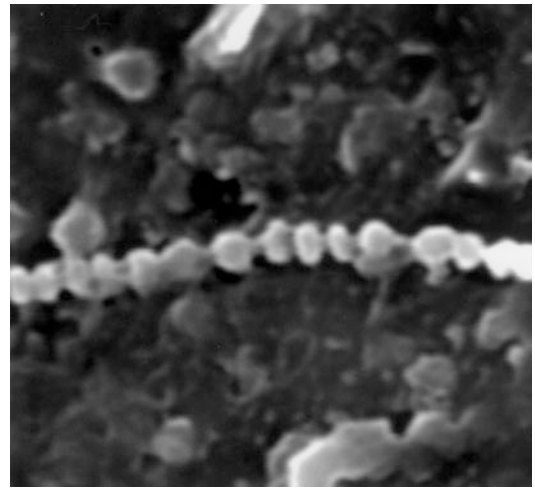

**Fig. 3.6** Scanning electron micrograph of an asbestos body with very thin coating, measuring approximately 0.5 **μ** in diameter. The core fiber is less than 0.2 **μ** in diameter. Such a body would be difficult to visualize with light microscopy. Magnified ×14,000

approaching 0.5 mm (500 μ) have been reported [28]. Asbestos bodies are usually 2–5 μ in diameter [21], although by scanning electron microscopy, the author has observed rare bodies which were only 0.5 μ in diameter (Fig. 3.6) [13].

Only a small percentage of asbestos fibers found within the lung at any single point in time are coated, and there are a number of factors which determine whether an individual fiber will become coated to form an asbestos body. These factors include both characteristics of the inhaled dust and characteristics pertaining to the host. Regarding the former, fiber dimensions are important factors in asbestos body formation. Morgan and Holmes [29] found that in humans, fibers less than 20 μ in length rarely become coated, while virtually all fibers 80 μ or greater in length are coated. Fiber diameter is also an important factor, with thicker fibers being more likely to become coated than thinner fibers [30]. Dodson et al. [31] suggested that fiber surface irregularities, such as etching, fracture, fraying, and multifibrillar composition, may also influence the coating process, with uncoated fibers having much smoother surface features. The type of fiber is also important (vide infra), with the vast majority of asbestos bodies isolated from human lungs possessing an amphibole asbestos core [13, 16, 17, 21, 30]. The proportion of fibers 5 μ or more in length that are coated appears to increase as the tissue fiber burden increases (Fig. 3.7). The presence of other dusts in the lung may also influence the coating process. For example, the author has observed that welders, who have heavy burdens of iron-oxide particles in their lungs, tend to have a high percentage of coated fibers (median value of 26 % for 15 welders [range, 7.1–45 %], as compared to 15.2 % for 757 other asbestos-exposed individuals [range, 0.22–80.3 %]).

With regard to host factors, coating efficiency depends upon the animal species exposed to the asbestos fibers. Humans, hamsters, and guinea pigs form asbestos bodies efficiently, whereas cats, rabbits, and mice do so much less readily, and rats and dogs are poor asbestos body formers [22]. There is also individual variability in coating efficiency, with some individuals appearing to be poor asbestos body formers [32, 33].

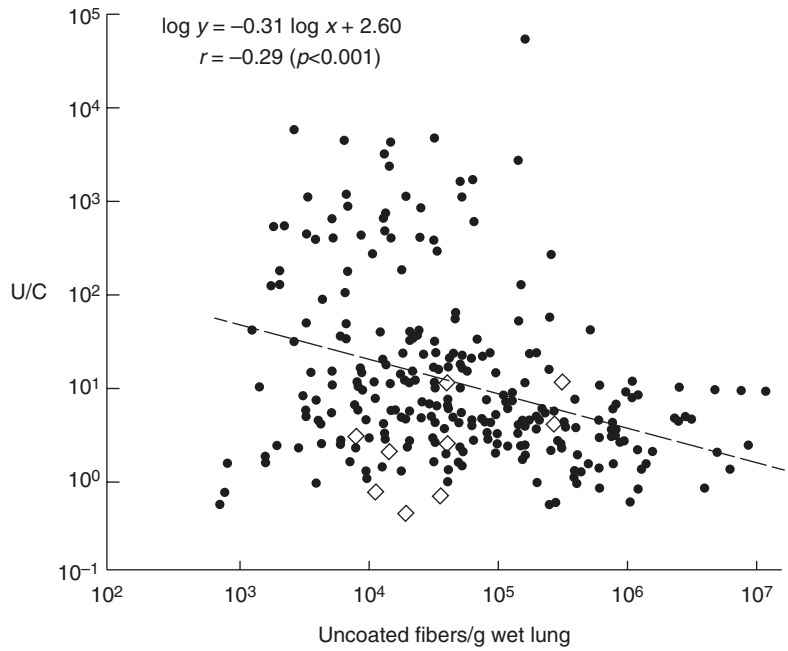


**Fig. 3.7** Graph showing the relationship between the pulmonary burden of uncoated fibers 5 μ or greater in length and the ratio of uncoated to coated fibers (*U*/*C*) isolated from the lung, as determined by scanning electron microscopy of 263 cases. The percentage of fibers which are coated increases significantly as the pulmonary asbestos burden increases. Welders (◇) tend to have especially low U/C ratios (i.e., high percentage of coated fibers)

Considerable variation in coating efficiency has even been observed in different areas of the lung from a single individual [30]. In the author's laboratory, the percentage of fibers 5 μ or greater in length which are coated (as determined by scanning electron microscopy) has ranged from 0.002 to 72 %, with a median value of 11.8 %. This latter value is very similar to the 11 % coated fibers reported by Morgan and Holmes [30] using phase-contrast light microscopy. Finally, fiber clearance may be reduced in individuals with asbestosis, so that increased numbers of short fibers are retained and the proportion of fibers which become coated is greatly reduced [30].

The mechanism of formation of asbestos bodies was studied in detail by Suzuki and Churg [34]. Asbestos fibers deposited in the distal regions of the lung parenchyma are phagocytosed by free alveolar macrophages. Those fibers that are approximately 20 μ or greater in length cannot be completely ingested by a single cell, and by poorly understood mechanisms, this "frustrated phagocytosis" then triggers the coating process. Within 16 days of initial exposure, the iron micelles appear in the cytoplasm of the macrophages in close proximity to the ingested fibers, and by continuous accretion of these micelles embedded in a homogeneous matrix material, the typical asbestos bodies recognizable by light microscopy are eventually formed [34]. The asbestos fiber is separated from the cytoplasm of the macrophage by a lysosomal limiting membrane. Koerten et al. demonstrated that the process of asbestos body formation may occur extracellularly and is analogous to the process of bone resorption by osteoclasts [35]. The source of the iron which coats the fiber is unknown, but is probably derived from either hemoglobin or plasma transferrin. More recent studies using synchrotron soft x-ray imaging have demonstrated that magnesium also participates along with iron in the coating process [36]. Studies in which asbestos bodies were recovered by exploiting their magnetic properties (as opposed to harsh chemical techniques that destroy the proteinaceous component of the coating) have shown that albumin and ferritin are the major proteins associated with asbestos bodies [37].

In experimental animals, asbestos bodies can be recognized by light microscopy within 2 or 3 months of exposure [30]. The finding of asbestos bodies in lung tissue digests of infants from 3 to 12 months of age [38] suggests that the time course for the formation of asbestos bodies is

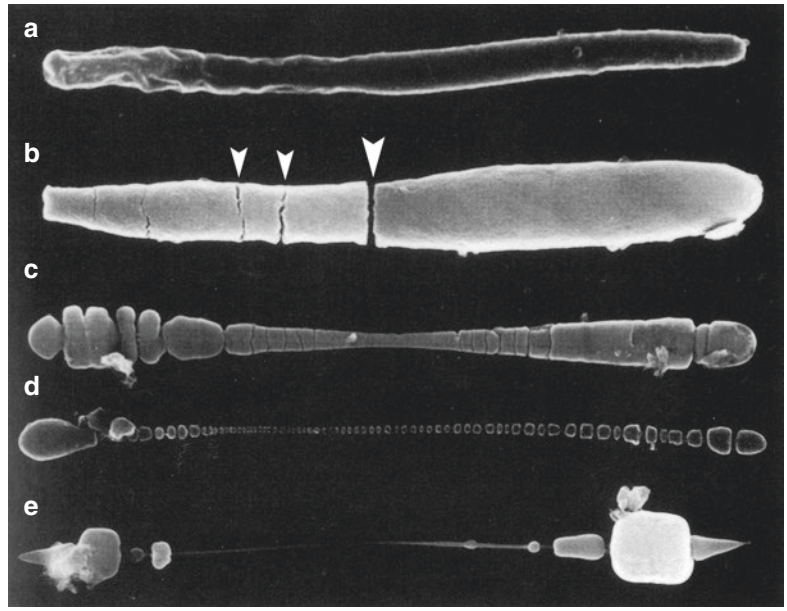


**Fig. 3.8** A composite SEM demonstrating the proposed sequence of events in asbestos body segmentation. (**a**) Membrane limited smooth coating. (**b**) Partial (*small arrowheads*) and complete (*large arrowheads*) cracks in a coated fiber. (**c**) Erosion of the sharp edges of cracked regions to form a smooth contour along an asbestos body. (**d**) Extensive beading along the axis of an asbestos body. (**e**) A bizarre form, with an extensive central uncoated fiber region capped by heavily eroded ends (Reprinted from Ref. [41], with permission)

similar in humans. It has been suggested that the peculiar segmentation of asbestos bodies is due to the fragmentation of the rigid, sheathlike coating and that further "weathering" and dissolution of the coating eventually occur [39, 40]. This sequence of events has been supported by scanning electron microscopic observations of asbestos bodies isolated from human tissues [41] (Fig. 3.8). However, Koerten et al. have shown that typical, segmented asbestos bodies can be formed in vitro in a mouse peritoneal macrophage culture system [42], casting doubt on the "weathering" mechanism of asbestos body segmentation.

It should be noted that not all asbestos bodies have a ferruginous coating. De Vuyst et al. [43] described a case in which amosite asbestos fibers coated with calcium oxalate crystals were recovered by bronchoalveolar lavage. Similar observations were reported by Le Bouffant et al. [44], who described "enrobant" forms in which entire asbestos bodies are encased within an oxalate crystal. Ghio et al. reported three additional cases (Fig. 3.9), including one with longstanding renal failure [45]. However, systemic disturbance in oxalate metabolism cannot be identified in some cases [43–45]. Ghio et al. reported in vitro studies which indicated that iron-catalyzed production of oxalate from ascorbate can account for deposition of this crystal on ferruginous bodies [45]. Coating of asbestos fibers with spherules of calcium phosphate has also been observed in humans and experimental animals [35, 46] (Fig. 3.10). The formation of calcium phosphate salts in association with interstitial asbestos fibers appears to be a common reaction to injury in the white rat [47]. The calcium phosphate coatings are distinctive by virtue of their large size, spherical shape, and wide separation between deposits on an individual fiber. Intra-alveolar calcium carbonate concretions (pulmonary "blue bodies") have also been reported in association with asbestos exposure [48], but have not been described as a coating material on asbestos fibers. It must be emphasized that calcium phosphate and calcium oxalate bodies are rare occurrences and that ferruginized asbestos fibers are by far the most common form of asbestos body.

## 3.4 Occurrence and Distribution of Asbestos Bodies

In 1963, Thomson et al. [49] reported that asbestos bodies could be found in scrapings of autopsy lungs in 24 % of urban residents in

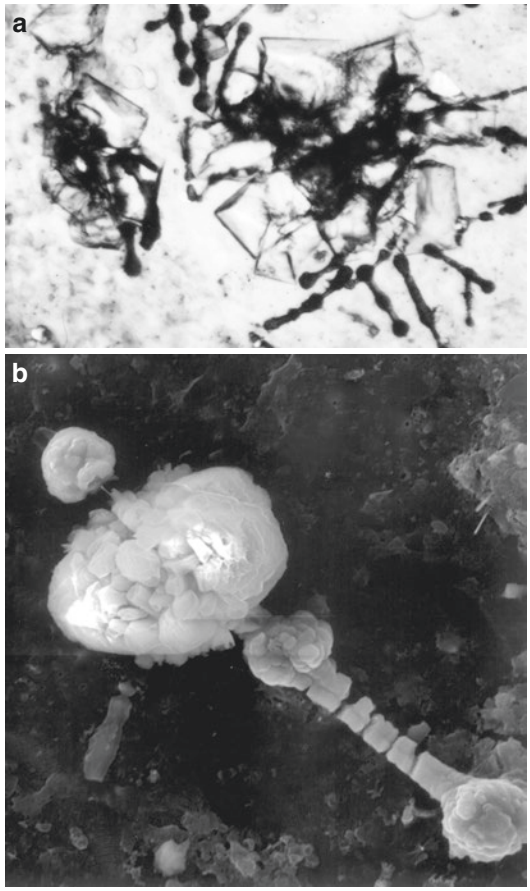


**Fig. 3.9** Calcium oxalate bodies. (**a**) Light micrograph of cluster of asbestos bodies in sputum associated with numerous crystals of calcium oxalate dihydrate. (**b**) Scanning electron micrograph of asbestos body with a bulbous deposit of calcium oxalate crystals near one end. The crystals are platy and yielded peaks for calcium only by EDXA ((**a**) Courtesy of Dr. Robert Moore of the Richmond VA Medical Center. (**b**) Magnified ×1,900)

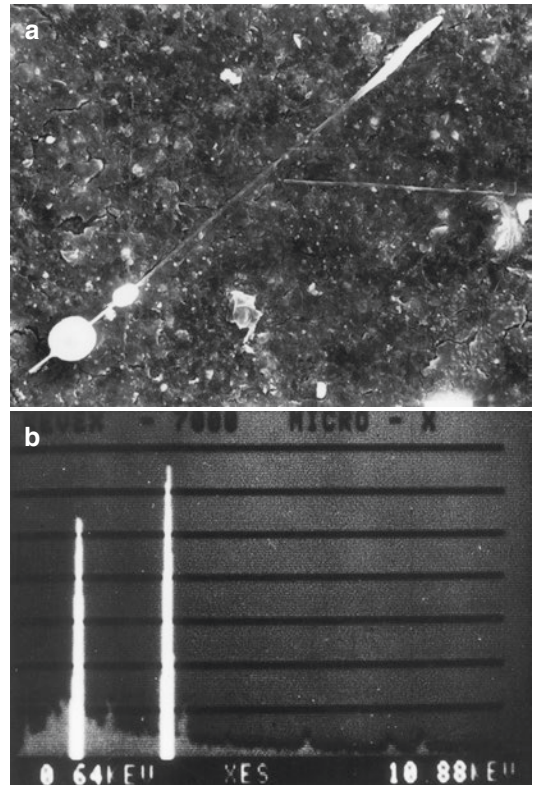


**Fig. 3.10** (**a**) Calcium phosphate-coated asbestos fiber isolated from the lungs of a construction worker with asbestosis and squamous carcinoma of the lung. Note the uncoated fibers. Magnified ×1,100. (**b**) Energy dispersive x-ray spectrum of the large spherical globule in A shows peaks for calcium and phosphorus but not for iron (Reprinted from Ref. [46], with permission)

South Africa. Since that time, a number of studies have demonstrated that when digestion-concentration techniques are employed to analyze sufficient quantities of lung tissue, some asbestos bodies can be recovered from the lungs of virtually all adults in industrialized nations [1, 50–60]. The percentage of patients from a general autopsy adult population with asbestos bodies in their lungs has ranged from as low as 21 % in East Texas in a study using 0.3 g samples of lung tissue [59] to as high as 100 % in a study from the USA employing 5 g samples [51]. The median value for the 12 cited studies is 90 % (Table 3.1). Correlations with occupational data indicate that "blue-collar" men tend to have the highest counts [55], reflecting some occupational exposure to asbestos for many of these individuals. Lower counts are often found in women as compared to men [52, 55, 56, 61, 62], indicating that men are more likely to have jobs with some asbestos exposure. In addition, smokers appear to have higher lung asbestos body counts than nonsmokers [55, 62]. Environmental asbestos contamination is not confined to urban areas, since rural dwellers are found to have asbestos bodies in their lungs just as often as urban dwellers (95 % versus 91 %), although the levels tend to be higher in urban areas [53, 62, 63]. An increasing prevalence and

**Table 3.1** Occurrence of asbestos bodies in the general population as determined by tissue digestion

| Authors | Year | Country | No. of cases | Percent[a] |
|---|---|---|---|---|
| Bignon et al. [50] | 1970 | France | 100 | 100 % |
| Smith and Naylor [51] | 1972 | United States | 100 | 100 % |
| Rosen et al. [52] | 1972 | United States | 86 | 90 % |
| Breedin and Buss [53] | 1976 | United States | 124 | 93 % |
| Bhagavan and Koss [54] | 1976 | United States | 145 | 91 % |
| Churg and Warnock [55] | 1977 | United States | 252 | 96 % |
| Roggli et al. [1] | 1980 | United States | 52 | 92 % |
| King and Wong [60] | 1996 | United States | 135 | 80 % |
| Steele and Thomson [56] | 1982 | United Kingdom | 106 | 80 % |
| | | New Zealand | 248 | 75 % |
| Rogers [57] | 1984 | Australia | 128 | 37 % |
| Kobayashi et al. [58] | 1986 | Japan | 656 | 33 % |
| Dodson et al. [59] | 1999 | United States | 33 | 21 % |

[a]Percent indicates the percentage of cases in which asbestos bodies were recovered from autopsy lung tissue by digestion

concentration of asbestos bodies in autopsy lungs during the past several decades has also been reported. Bhagavan and Koss reported an increase in asbestos body prevalence in the USA from 41 % in the 1940s to 91 % of cases in 1970–1972 [54]. Arenas-Huertero et al. reported an increase in average asbestos body concentration in lung samples from Mexico from 4.2/g in 1975 to 42.5/g in 1988 [62]. Bhagavan and Koss also found a significant increase in the proportion of lungs containing asbestos bodies with increasing age [54], although others have found no increase in asbestos body content with age [52, 55, 56]. Indeed, studies by Haque et al. [38] who reported the isolation of asbestos bodies from the lungs of infants indicate that exposure to asbestos in our industrialized society begins within the first year of life.

A few studies have examined the topographic distribution of asbestos bodies within the lung. Sebastien et al. [64] examined autopsy lung tissue from six patients with no known asbestos exposure and found no consistent relationship between the concentration of asbestos bodies in the upper versus lower lobes or central versus peripheral lung parenchyma. Rosen et al. [52] reported on results from 14 cases in which lung tissue was analyzed for asbestos body content from more than one site and again found no consistent relationship between asbestos body content in the upper versus lower lobes or right versus left lung. Gylseth and Baunan [65] described the asbestos body content in two asbestos workers and found considerable variation from site to site within the lungs. These observations are consistent with the data from the author's laboratory involving 41 cases for which tissue was available for digestion from two or more sites. The asbestos body concentration in the upper lobe exceeded that in the lower lobe in 17 instances, whereas the reverse was true in 15 instances. Similarly, the asbestos body concentration in the right lung exceeded that in the left lung in 24 cases, whereas the opposite was found in 17 cases. This variability in asbestos body concentration from one site to another within the lung was dramatically demonstrated in the studies of Morgan and Holmes [66, 67], who extensively sampled lung tissue from one insulator and two Finnish anthophyllite mine workers. Their data show a five- to tenfold variation in asbestos body concentration in adjacent blocks of tissue. Experimental animal studies suggest that this site-to-site variability in asbestos content may be related to airway path lengths and branching patterns [68].

## 3.5 Quantification of Asbestos Bodies

Since a few asbestos bodies can be found in the lungs of virtually everyone in industrialized nations, quantitative studies are required in order to draw inferences relative to exposure and various disease processes. A number of techniques have been devised for quantification of asbestos bodies in tissues, and these are

reviewed in the following sections. They include quantification in histologic sections, lung tissue digests, lymph nodes, and extrapulmonary tissues.

### 3.5.1 Histologic Sections

Paraffin sections are routinely used by pathologists for diagnostic purposes, so it is only natural that histologic sections have played an important role with respect to identification and quantification of asbestos bodies in tissues. In early studies investigating the prevalence of asbestos bodies in the general population, 30 μ thick paraffin sections were employed [69]. Selikoff and Hammond used basal smears and ashed tissue sections to study the prevalence of asbestos bodies in the lungs of New York City residents [70]. However, there was little attempt to actually quantitate the numbers of asbestos bodies in histologic sections. A semiquantitative study was reported in 1980 by Roggli et al. [1] who concluded that 5,000 or more asbestos bodies per gram of wet lung tissue were required before bodies were likely to be encountered in ten random high-power fields of iron-stained sections. Churg observed that roughly 500 asbestos bodies per gram of wet lung needed to be present before any bodies could be found in tissue sections [71]. Subsequently, Roggli and Pratt [27] reported a quantitative study relating the numbers of asbestos bodies observed in iron-stained tissue sections to asbestos body counts in lung tissue digests. The observations in this study were validated using a more rigorous mathematical mode1 [72], and similar results have subsequently been reported by others [33, 58].

A key factor in the calculation of the numbers of asbestos bodies per gram of wet lung tissue from the numbers observed in histologic sections is the recognition that the same asbestos body may be observed in several serial sections [27]. This is due to the fact that the average asbestos body is considerably longer than the average section is thick. Thus there is a finite probability that an asbestos body will be oriented in the block in such a way that it will appear on two or more adjacent sections. This concept is depicted schematically in Fig. 3.11. Once the orientation of the asbestos body in the paraffin block has been accounted for, it is a simple matter to calculate asbestos bodies per gram, using a conversion factor from volume of paraffin-embedded tissue to wet weight of lung. The relevant formulas are as follows [27]:

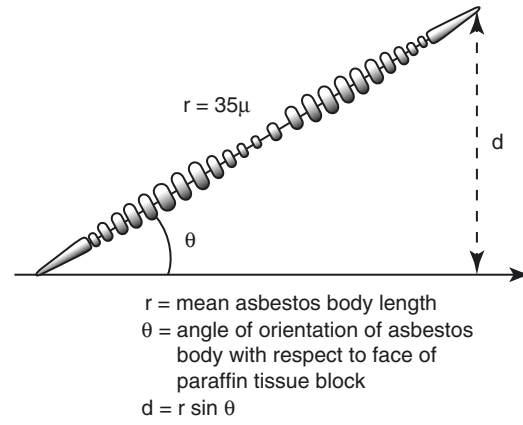


**Fig. 3.11** Model for determining the orientation correction factor for counting asbestos bodies in tissue sections. Bodies are assumed to be rigid, straight structures with a mean length of 35 μ. The abscissa is parallel to the paraffin block face; $\theta$ is the angle between the asbestos body and the plane of the block face, which can range from 0° to 90°; and d is the projection of the asbestos body (in μ) in a direction perpendicular to the plane of the tissue section. As d increases, so does the probability that the asbestos body will be observed in two or more serial sections (Reprinted from Ref. [27], with permission)

$$N_g = \frac{N_c}{A_s t \cdot O_c \cdot R} \tag{3.1}$$

where:

$N_g$ = number of asbestos bodies per gram of wet lung tissue

$N_c$ = number of asbestos bodies counted on iron-stained tissue section

$A_s$ = area of tissue section in $mm^2$

$t$ = thickness of tissue section in mm

$O_c$ = orientation correction factor (see Ref. [27] for details)

$R$ = ratio of wet weight of fixed lung tissue to volume of paraffin-embedded lung tissue

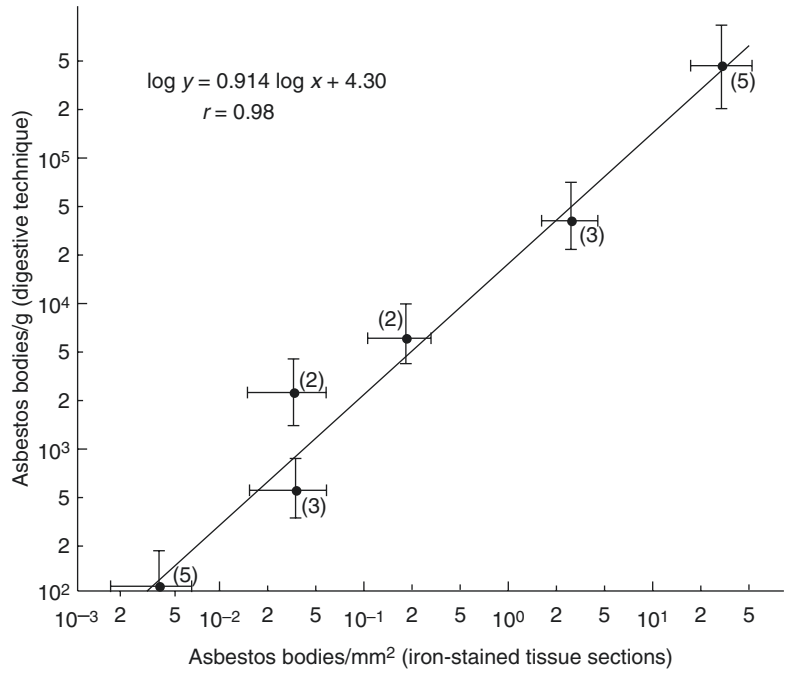


**Fig. 3.12** Relation (log-log scale) of the number of asbestos bodies seen in iron-stained sections (in units of bodies per mm$^2$) to the number measured by a tissue digestion technique (in units of asbestos bodies per gram of wet, formalin-fixed lung tissue). The number of sections evaluated per case is shown in parentheses, and each data point represents the mean result for one case, with error bars indicating one standard deviation. The least squares fitted regression equation is shown at upper left, and the correlation coefficient is significant at the $p<0.001$ level (Reprinted from Ref. [27], with permission)

Typical values for these variables in our laboratory are as follows:

$t = 5\ \mu m = 0.005$ mm

$O_c = 2.56$ for an average asbestos body length of 35 μm

$R = 2.1$ g/cm$^3$ (includes a factor for shrinkage of lung during paraffin embedding [27])

Therefore,

$$N_g = \frac{N_c}{A_s} \times 37{,}200\,\text{mm}^2 / g \qquad (3.2)$$

It should be noted that Eq. (3.2) is only applicable to sections cut at 5 μm thickness and an average asbestos body length of 35 μm. Also, these formulas were derived using iron-stained sections examined at 200× magnification using a mechanical stage [27]. Since asbestos bodies are not necessarily distributed uniformly through tissue sections, the more sections and the more total area examined, the greater the accuracy of the estimated asbestos body concentration. Similar results can be obtained by using the regression line in Fig. 3.12 (in lieu of Eq. 3.2) to estimate the asbestos body concentration per gram of wet lung from the numbers of asbestos bodies per mm$^2$ of tissue section [27]. Also, Table 3.2 shows the number of 400× microscopic fields that have to be examined on the average to find the first asbestos body for a given tissue asbestos body concentration [72]. These calculations indicate that asbestos body detection in tissue sections (i.e., one asbestos body per 4 cm$^2$ section area) requires 100 or more asbestos bodies per gram of wet lung tissue [27, 72].

**Table 3.2** Average number ($N$) of 400× microscopic fields examined to find first asbestos body for a given asbestos body concentration (AB/g)

| $N$ | AB/g |
|---|---|
| 1 | 181,000 |
| 5 | 36,200 |
| 10 | 18,100 |
| 18 | 10,000 |
| 25 | 7,240 |
| 36 | 5,000 |
| 50 | 3,620 |
| 100 | 1,810 |
| 181 | 1,000 |
| 362 | 500 |
| 1,810 | 100 |

Modified after Ref. [72], with permission

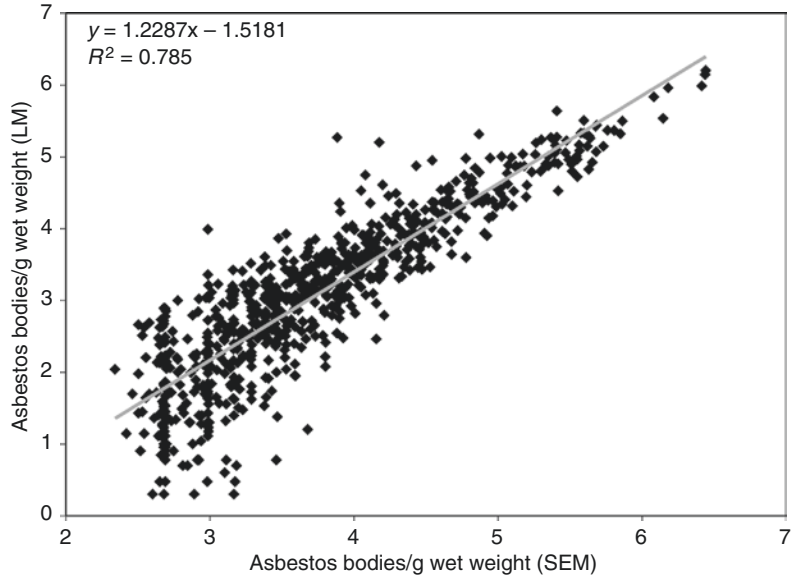


**Fig. 3.13** Correlation between asbestos body counts by light and scanning electron microscopy in 767 cases. Each diamond represents one case. The linear regression line has a correlation coefficient of 0.89 ($p < 0.0001$). Note log-log scale

### 3.5.2 Lung Tissue Digests

A variety of techniques have been described for the extraction of asbestos bodies from lung tissue for subsequent quantification or identification [14, 21, 30, 50–58, 73–80]. Most of these techniques employ wet chemical digestion, although low-temperature plasma ashing techniques have been used as wel1 [60, 78, 79]. The inorganic residue remaining after digestion is then suspended in ethanol and collected on an acetate or polycarbonate filter with an appropriate pore size (0.45 µm or less). If the intent of the study is to quantify asbestos bodies alone, then the filter can be examined by light microscopy at a magnification of 200–400×. However, scanning electron microscopy (SEM) can be used to count asbestos bodies just as well [46, 60], and there is an excellent correlation between asbestos body concentrations determined by light microscopy and those determined by SEM (Fig. 3.13). Once the number of asbestos bodies on the filter has been determined, the asbestos body concentration per gram of wet lung [1, 46, 76], gram of dry lung [21, 33, 74], or $cm^3$ of lung tissue [50, 59] can be calculated. The relationship between these three ways of reporting results varies somewhat from case to case, but a useful rule of thumb for comparative purposes is

$$1\,\text{AB / g wet wt.} \sim 1\,\text{AB / cm}^3 \sim 10\,\text{AB / g dry wt.} \tag{3.3}$$

Digestion studies must be carefully performed, as there are a number of potential sources of error. Asbestos bodies (and fibers) may be lost during the extraction process through adhesion to glass surfaces, and this can result in substantial underestimation of the actual tissue concentration [79]. However, Corn et al. [81] have shown that with their bleach digestion technique, the percentage error due to adherence of fibers or bodies to glass surfaces in cases with a heavy tissue asbestos burden is negligible. Whether this is true for low or moderate tissue asbestos burdens is unknown. Ashing of the specimen (especially in a muffle furnace at 400–500 °C) causes tissue shrinkage, resulting in fracture of long fibers, which increases the asbestos body count [60, 79]. Morgan and Holmes have also reported that dicing of the tissue sample prior to bleach digestion results in a decrease in median asbestos body length and thus an apparent increase in asbestos body concentration [67]. However, this effect on asbestos body counts appears to be of the same order of magnitude as the coefficient of variation for counting different aliquots of the same sample (i.e., about 10 %) and is substantially less

than the five- to tenfold variation which can occur from sampling different sites in the same lung [66, 67]. This serves to emphasize the importance of sampling multiple sites for digestion whenever this is feasible.

The asbestos body concentrations which have been reported on lung samples from the general population as well as from individuals with various asbestos-related diseases are discussed in Chap. 11. The range of values observed spans at least nine orders of magnitude (from 0.1 to $10^7$ AB/g wet lung tissue). It should be noted that there is fairly good agreement in the determination of tissue asbestos body concentrations among different laboratories employing different analytical techniques [82], with a reported interobserver correlation coefficient of 0.8975 [83]. The agreement is considerably worse for the determination of uncoated asbestos fiber concentrations [82]. However, there may be significant variation in tissue asbestos body content from one region of the country to another [1, 21, 46, 55, 76]. Therefore, it is preferable for laboratories engaged in such determinations to calculate their own normal range of asbestos body concentrations.

It is important to recognize that asbestos body counts in decomposed human lungs decrease over time. This was shown in a study by Mollo et al. [84] in eight cases where asbestos bodies were measured shortly after death and again after 1–18 months of decomposition. This is probably due to loss of proteins in the matrix of the ferruginous material, so that the coating becomes brittle and shatters off during the recovery process.

### 3.5.3 Lymph Nodes

Gloyne in 1933 described asbestos bodies in histologic sections of lymph nodes and noted that when present, they are usually found in areas of the node containing pigment [85]. Godwin and Jagatic reported asbestos bodies in the regional lymph nodes of 6 of 7 patients with malignant mesothelioma [86]. Others have also mentioned the presence of asbestos bodies in histologic sections of lymph nodes [21]. Roggli and Benning reported asbestos bodies in histologic sections in 20 cases [87] (Fig. 3.14). Seventeen of these patients had histologically confirmed asbestosis, and all were heavily exposed to asbestos for durations ranging from 4 to 40 years. The median asbestos body concentration in the lung parenchyma as determined by light microscopy of lung tissue digests was more than 1,000 times our upper limit of normal [87]. In four cases, lymph node tissue was also available for digestion, and

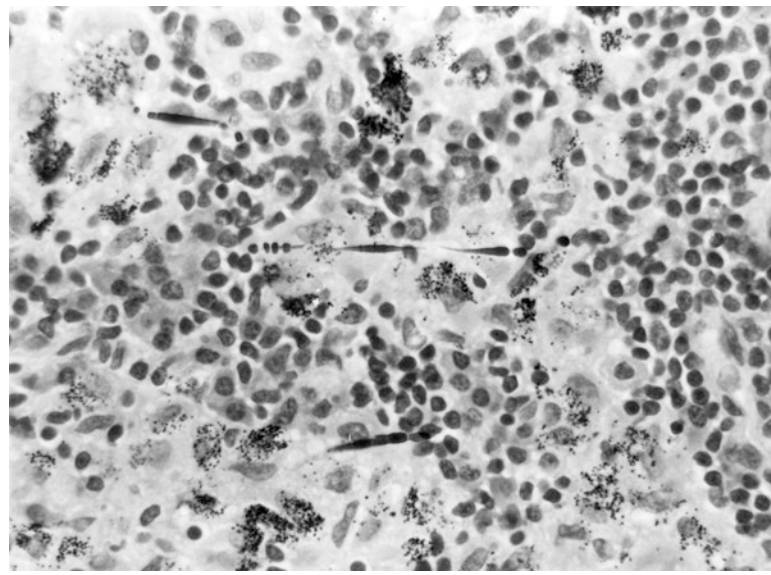

**Fig. 3.14** Asbestos bodies within a histologic section of a hilar lymph node from an insulator with asbestosis and squamous cell carcinoma of the right upper lobe. Hematoxylin and eosin, magnified ×520 (Reprinted from Ref. [87], with permission)

the asbestos body concentration in the lymph nodes ranged from 3,000 to more than 300,000 asbestos bodies per g wet weight of lymph node. Asbestos bodies were not observed in iron-stained sections of lymph nodes in 14 autopsied controls, all of which had lung asbestos body counts within our normal range of 0–20 AB/g. However, a few asbestos bodies were found in lymph node digests in 6 of 14 controls. The range of values for the lymph nodes was roughly the same as for normal lung parenchyma (0–17 AB/g of wet lymph node tissue) [87]. Dodson and Huang found asbestos (ferruginous) bodies in lymph node digests from 2 of 21 nonoccupationally exposed individuals [88]. Dodson et al. reported asbestos body counts in thoracic lymph nodes obtained from various stations in 11 individuals. The authors concluded that there were reproducible patterns of asbestos in various lymph nodes but variations in the amounts of asbestos found in the sites sampled [89].

Considerations similar to those used for the determination of asbestos body concentrations from asbestos body counts in tissue sections of lung (see above) can be used to estimate the minimum asbestos body concentration necessary in lymph node tissue for asbestos bodies to be observed in lymph node histologic sections. For an average lymph node measuring 1.0 by 0.5 cm, average asbestos body length of 35 μ, tissue section thickness of 6 μ, and lymph node density of 1 g/cm$^3$, the finding of one asbestos body in an iron-stained section of lymph node is equivalent to approximately 1,600 asbestos bodies per gram wet weight of lymph node [87] (Fig. 3.15). Several conclusions can be drawn from these observations. First, the finding of asbestos bodies in histologic sections of lymph nodes is indicative of a heavy asbestos body burden within the node and is associated with considerably elevated lung asbestos body burdens. Second, a few asbestos bodies can be found in digests of lymph nodes in many individuals with no known exposure to asbestos, indicating transport of some long fibers to the lymph nodes even at low tissue asbestos burdens. Finally, in some cases, the asbestos body content of the hilar nodes exceeds that of the lung parenchyma at both low and high tissue asbestos burdens.

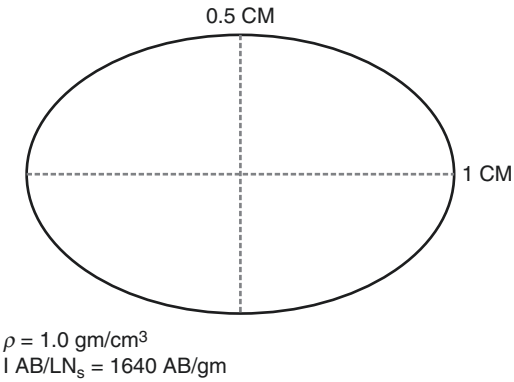


**Fig. 3.15** Schematic diagram of a typical hilar lymph node section, measuring 1 × 0.5 cm and with approximate density ($\rho$) of 1.0 g/cm$^3$. The finding of one asbestos body in such a paraffin section is equivalent to roughly 1,600 asbestos bodies per gram of wet fixed nodal tissue (Reprinted from Ref. [87], with permission)

### 3.5.4 Extrapulmonary Tissues

As early as 1933, Gloyne had observed that asbestos bodies are readily transported from place to place on a scalpel or microtome blade and can easily be carried over from one specimen jar to another [85]. It is common practice for pathologists to place portions of multiple organs in a single container of formalin. The author has recovered scores of asbestos bodies from one cc of formalin within a container in which lungs (and other organs) from an individual with a heavy pulmonary asbestos burden had been placed. In addition, asbestos bodies may adhere to glassware used in the digestion procedure and thus potentially be carried over from one case to the next [79, 90]. All of these sources of contamination would have to be considered in studies of asbestos bodies in extrapulmonary tissues, especially since the tissue concentrations would be so low that confirmation by means of histologic sections would be lacking [27]. Nevertheless, most investigators reporting asbestos bodies in extrapulmonary sites have failed to take these considerations into account. Therefore, the reader should keep these confounding factors in mind when considering the literature on this subject.

Extrapulmonary organs from which asbestos bodies have been recovered are listed in Table 3.3.

**Table 3.3** Extrapulmonary tissues from which asbestos bodies have been recovered

| | |
|---|---|
| Adrenal gland | Mesentery |
| Bone marrow | Omentum |
| Brain | Pancreas |
| Esophagus | Prostate |
| Heart | Small intestine |
| Kidney | Stomach |
| Larynx | Spleen |
| Liver | Thyroid |
| Large intestine | Urinary bladder |

From Refs. [91–94]

Auerbach et al. [91] reported the occurrence of asbestos bodies in extrapulmonary sites in 37 cases, including 19 with asbestosis and 18 with parietal pleural plaques. These investigators recovered 20 $mm^3$ of tissue from paraffin blocks deparaffinized in xylene and digested in potassium hydroxide, with the residue collected on an ashless paper filter and ashed on a glass slide within a low-temperature plasma asher. Asbestos bodies were recovered from the kidney, heart, liver, spleen, adrenals, pancreas, brain, prostate, and thyroid. The authors concluded that in individuals with heavy pulmonary asbestos body burdens, asbestos bodies are likely to be present in other organs as well [86]. Kobayashi et al. [92] reported a similar study of 26 cases with varying levels of pulmonary asbestos body burden. They used up to 5 g of formalin-fixed tissue which was digested with potassium hydroxide and the residue collected on a membrane filter. Asbestos bodies were found in the esophagus, stomach, small and large intestine, spleen, pancreas, liver, heart, kidney, urinary bladder, bone marrow, thyroid, and adrenals. These investigators also noted that the incidence and the number of asbestos bodies in extrapulmonary organs tend to increase as the pulmonary asbestos burden increases [92]. However, this observation is also consistent with contamination of formalin by pulmonary asbestos bodies.

Ehrlich et al. [95] reported a case of an asbestos insulator with asbestosis who underwent a resection for carcinoma of the colon. Asbestos bodies were recovered from digests of 3–5 g of tumor, adjacent normal bowel, mesentery, and serosal fat. In contrast, Rosen et al. [96] found no asbestos bodies in digests of colonic tissue from 21 cases of colon cancer from the general population. Dodson et al. [93] studied 20 cases with mesothelioma and found asbestos bodies in mesentery samples from five and in the omentum from two. Roggli et al. [94] recovered asbestos bodies from digests of laryngeal mucosa in two of five asbestos workers, but in none of ten autopsy controls. The occurrence of asbestos bodies in the upper airway and the gastrointestinal tract is not unexpected, since asbestos bodies may be found in mucus from the lower respiratory tract, which is then coughed up and swallowed (see Chap. 9). Although asbestos bodies in other extrapulmonary sites may be artifactual (see above), there is some data to indicate that vascular transport of dust from the lungs can occur [97, 98]. Once an asbestos fiber gains access to the intravascular compartment, hematogenous transport to any of the organs listed in Table 3.3 could theoretically occur. However, one would then expect to find the largest numbers of asbestos bodies in the organs which receive the greatest percentage of systemic blood flow, i.e., the brain, the heart, and the kidneys. This has not been the case in the reported studies [91, 92].

## 3.6 Asbestos Bodies and Fiber Type

The vast majority of asbestos bodies isolated from human lungs have been found to have an amphibole asbestos core [13, 16, 17, 21, 32, 76, 99–102]. Asbestos workers [46, 76] and men from the general population [103] generally have the commercial amphiboles, amosite or crocidolite, forming the cores of asbestos bodies within their lungs. On the other hand, women from the general population are more likely to have one of the noncommercial amphiboles, tremolite or anthophyllite, as the core to asbestos bodies found in their lungs [103]. This latter finding may be related to contamination of commercial talcum powder with tremolite and anthophyllite [104]. The predominance of amphibole asbestos body cores is somewhat curious, considering that

the bulk of asbestos used commercially is chrysotile [105] (Fig. 3.16). Chrysotile asbestos bodies do occur, however (Fig. 3.17), and account for about 0.5 % of all asbestos bodies that have been analyzed by our laboratory [46, 106] and about 2.0 % by others [21, 107]. Moulin et al. [108] reported that chrysotile asbestos bodies accounted for 10 % of bodies analyzed from asbestos-exposed workers but only 3 % of bodies from members of the Belgian urban population. They are especially likely to occur in individuals exposed to long fibers of chrysotile, such as asbestos textile workers or chrysotile miners or millers. In the latter group of workers, most asbestos bodies isolated from lung tissue have chrysotile asbestos cores [109]. Thicker chrysotile bundles are more likely to become coated than thin chrysotile fibrils [30]. The rarity of chrysotile asbestos bodies apparently results from the ready fragmentation of chrysotile into shorter fibrils and the fact that asbestos bodies tend to form only on fibers which are 20 μm or

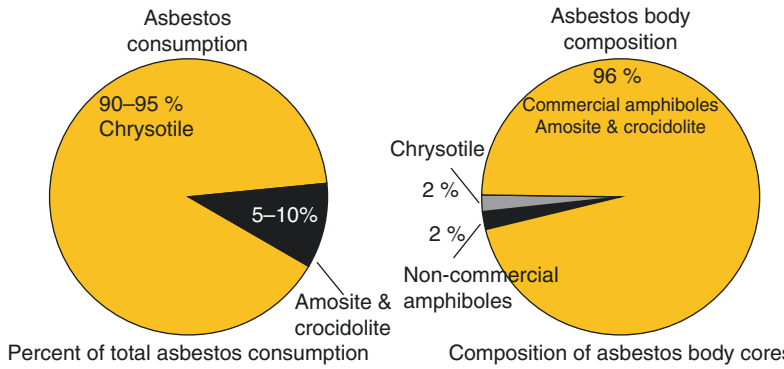


**Fig. 3.16** Diagram showing the proportion of the various types of asbestos fibers that are consumed commercially (*left*) versus the composition of asbestos body cores (*right*). Although chrysotile accounts for the great bulk (90–95 %) of asbestos consumed commercially, asbestos bodies infrequently (approximately 2 %) have a chrysotile core (Reprinted from Ref. [106], with permission)

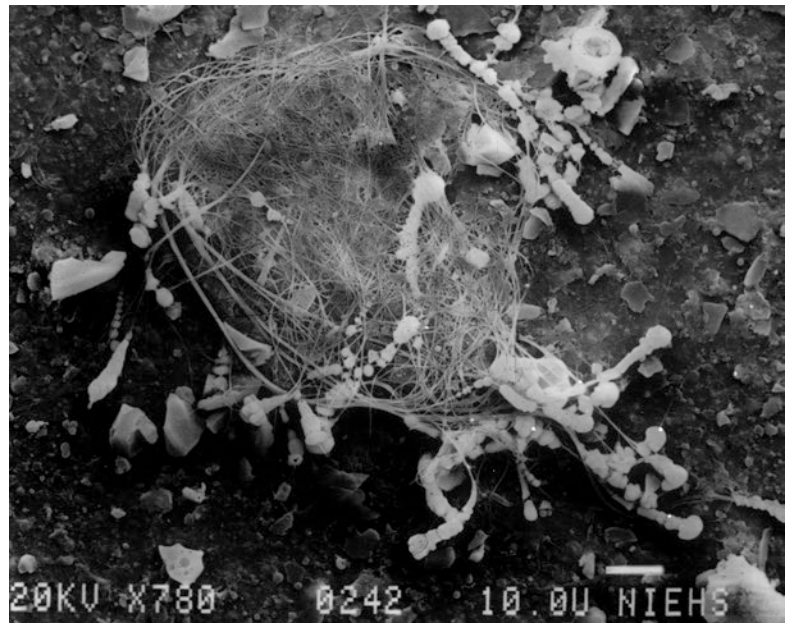


**Fig. 3.17** Cluster of chrysotile asbestos fibers on a Nuclepore filter isolated from the lungs of an asbestos textile worker with a pleural mesothelioma. The fiber ends appear to spin off the central mass like the arms of a spiral galaxy and have become coated to form numerous chrysotile asbestos bodies. Scanning electron microscopy, magnified ×850 (Reprinted from Ref. [46], with permission)

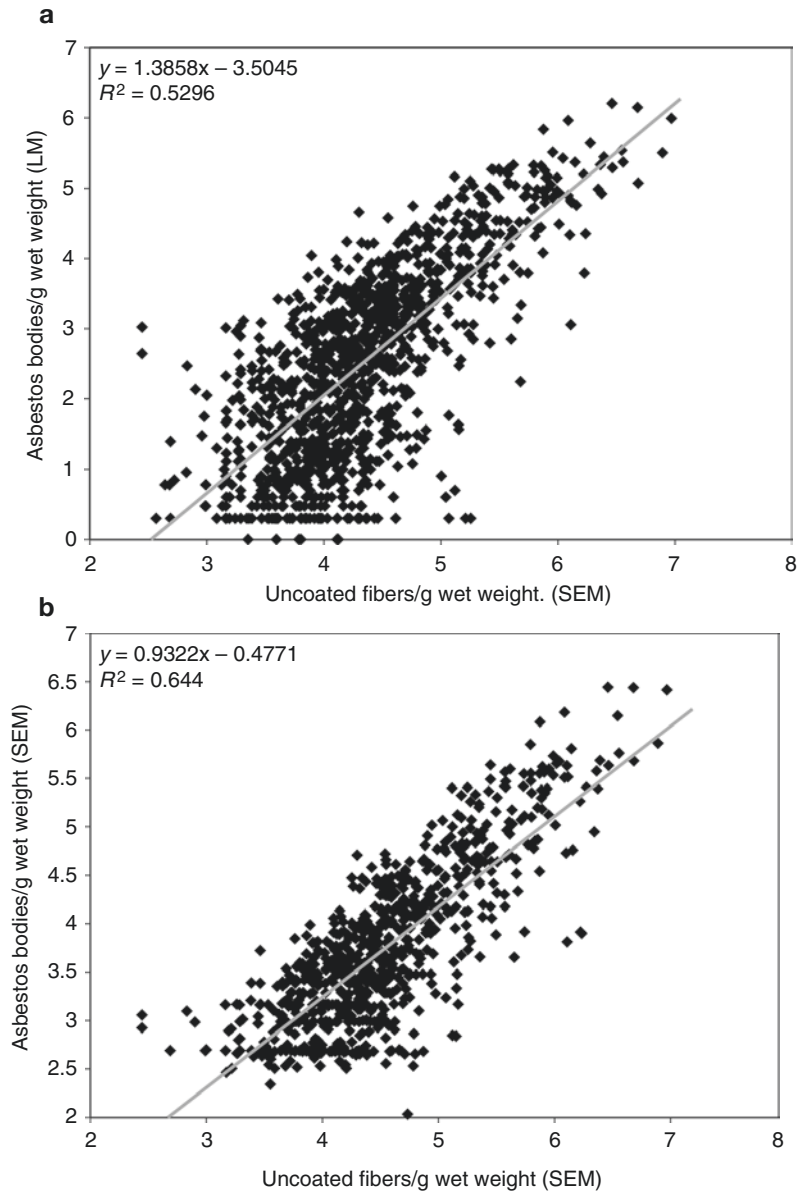


**Fig. 3.18** (**a**) Scattergram showing the relation between asbestos body counts by light microscopy and uncoated fiber counts by scanning electron microscopy, for 1,135 cases. The correlation coefficient for the least squares fitted linear regression line is 0.73 ($p<0.0001$). (**b**) Correlation between asbestos body and uncoated fiber counts by scanning electron microscopy in 780 cases ($r=0.80$, $p<0.0001$). Note log-log scale

more in length [29, 30]. As a result, asbestos bodies are generally a poor indicator of the pulmonary chrysotile asbestos burden [21, 33, 74, 75, 110]. On the other hand, the pulmonary asbestos body content correlates very well with the burden of uncoated fibers 5 μm or greater in length (Fig. 3.18) [46, 66, 67, 76, 111, 112]. Among individuals exposed occupationally to asbestos, most fibers in this size range are commercial amphiboles [46, 76]. An obvious exception to this is the relatively small percentage of asbestos workers exposed exclusively to chrysotile.

## 3.7 Non-asbestos Ferruginous Bodies

As noted in the section on "Historical Background," fibrous dusts other than asbestos can become coated with iron, or ferruginized, so

**Table 3.4** Composition of 89 non-asbestos ferruginous bodies examined by scanning electron microscopy/energy dispersive spectrometry

| Core composition | Total (%) |
|---|---|
| Talc | 29 (33) |
| Iron | 16 (18) |
| Silica | 10 (11) |
| Fe K Al Si | 8 (9) |
| Aluminum silicate | 5 (6) |
| Iron chromium (stainless steel) | 4 (4) |
| Potassium aluminum silicate | 4 (4) |
| Rutile | 3 (3) |
| Fibrous glass | 3 (3) |
| Magnesium aluminum silicate | 3 (3) |
| Aluminum | 2 (2) |
| Fe K Ca Mg Al Si | 1 (1) |
| Unknown[a] | 1 (1) |

[a]This body was too heavily coated to identify the nature of the core fiber

that one must be cautious in identifying asbestos bodies by light microscopy [14, 15]. Fortunately, most of the non-asbestos ferruginous bodies, or pseudoasbestos bodies, can be distinguished from true asbestos bodies at the light microscopic level [13, 17, 21, 113]. The author has observed non-asbestos ferruginous bodies in tissue digests from 311 out of 1,357 cases (23 %) in which ferruginous bodies were identified by light microscopy and in 55 of 752 cases (7.3 %) with lung tissue digests in which ferruginous bodies were observed by SEM. Only rarely are they found in numbers approaching those of true asbestos bodies [21]. The results of the analysis of 89 non-asbestos ferruginous bodies by SEM in the author's laboratory are shown in Table 3.4. The morphologic features of the various types of non-asbestos ferruginous bodies are reviewed below.

### 3.7.1 Sheet Silicates

These structures may form ferruginous bodies with a distinctive broad, yellow core. Churg et al. [17] described two patterns of ferruginous body formation with sheet silicate cores: bodies with a highly irregular shape and platy structure with irregular, often sparse, coating (Fig. 3.19) and bodies with a rectangular shape, more uniform coating, and diameter only slightly greater than that of true asbestos bodies. The second pattern may be confused with true asbestos bodies, and when the core is particularly thin, this distinction may not be possible at the light microscopic level. Electron diffraction shows a pseudohexagonal pattern, and energy dispersive spectrometry shows that most of these bodies have cores of talc, mica, or kaolinite [21]. They are commonly found in the lungs of roofers and rubber factory workers, who are exposed to substantial amounts of talc [21], and the author has observed them commonly in the lungs of shipyard welders. In the general population, they may contribute up to 20 % of the total ferruginous body burden [17].

### 3.7.2 Carbon Fibers

Some ferruginous bodies have black cores, ranging from uniform, very thin black filaments to broader, more irregular platy forms. Gross described ferruginous bodies of this type from human lungs and suggested that they had carbon cores [115]. The coating on these bodies is also variable and may be segmented and sheathlike or form right-angle branches [17]. Electron diffraction shows that these core fibers are amorphous, and energy dispersive spectrometry indicates that there are no elements with atomic number greater than or equal to that of sodium ($Z=11$) [21]. These observations are consistent with the carbonaceous nature of the cores. We have observed bodies of this type in the lungs of coal miners [116] and also in the lungs of a woman with an unusual exposure to woodstove dust [117] (Fig. 3.20). In the general population, they may contribute to as much as 90 % of the total ferruginous body burden in some cases [21], and in our case with the unusual woodstove dust exposure, they accounted for 100 % of the ferruginous bodies that were analyzed [117]. Although not yet described as the cores of ferruginous bodies, the dimensions of carbon nanotubes, especially the multiwalled type, are such that they could possibly form the cores of ferruginous bodies [119].

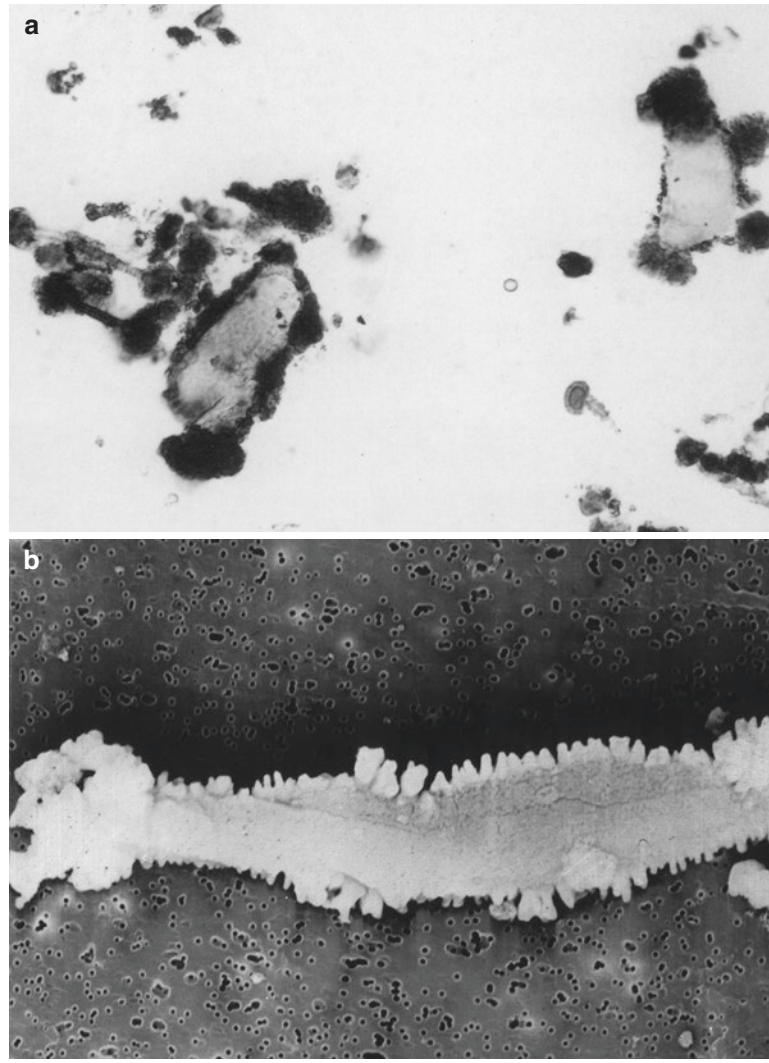


**Fig. 3.19** (**a**) Pseudoasbestos body of the sheet silicate type has a broad yellow core. True asbestos body is also present (*lower right*). Nuclepore filter preparation, ×520. (**b**) Scanning electron micrograph of a sheet silicate pseudoasbestos body. Note the heavy coating on the ends and the serrated edges. Magnified ×1,500 ((**a**) Reprinted from Butnor and Roggli [114])

### 3.7.3 Metal Oxides

Fibrous forms of a variety of metal oxides can form ferruginous bodies with dark brown to black cores [13]. These structures usually have a core with a uniform diameter and segmented coating which, except for the color of the core, otherwise resemble typical asbestos bodies (Fig. 3.21). We have identified such ferruginous bodies with cores of titanium, iron, chromium, and aluminum (Table 3.4). These are presumably in the form of the metal oxide. Titanium particles and fibers are commonly found in lung specimens [46, 116], and when they reach a certain critical length [29], they may then become coated to form a ferruginous body. Dodson et al. [120] described ferruginous bodies with iron-rich core fibers isolated from the lungs of a worker at an iron reclamation and manufacturing facility. We have observed ferruginous bodies with iron-rich cores and rarely with aluminum-rich cores from the lungs of shipyard welders. These individuals have large numbers of nonfibrous iron and aluminum oxide particles in their lungs. Finally, a unique case has been reported in which chromium-rich cores were identified in ferruginous bodies isolated from the lungs of a metal polisher [118].

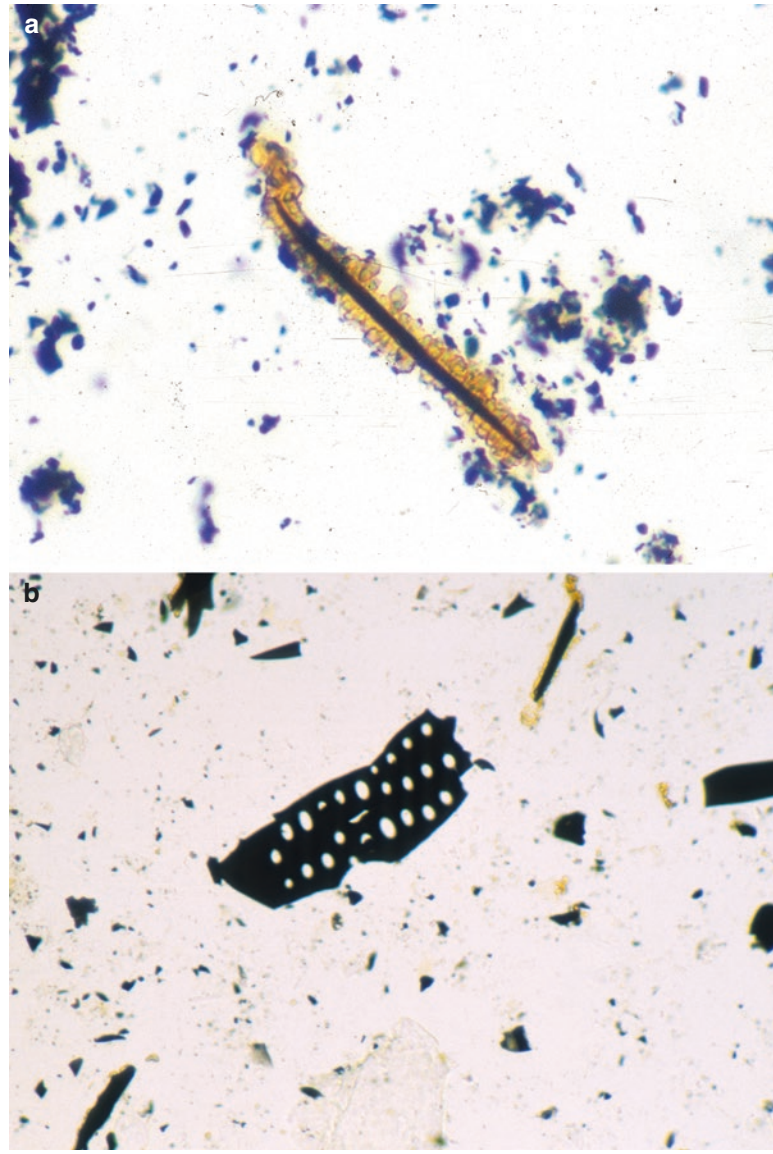


**Fig. 3.20** (**a**) Pseudoasbestos body isolated from the lungs of a coal worker has a black carbon core which is coated with segmented ferroprotein material. Magnified ×800. (**b**) Dust recovered from bronchoalveolar lavage fluid from a woman exposed to woodstove dust. Fiber in upper middle portion of field is iron coated. Note grid-like structure left of center. Nuclepore filter preparation, magnified ×330 ((**a**) Reprinted from Ref. [116], (**b**) reprinted from Ref. [118], with permission)

### 3.7.4 Man-Made Mineral Fibers

Man-made mineral fibers are commonly used in insulation materials and can form ferruginous bodies in experimental animals [14]. Therefore, it would not be unexpected to find such fibers at the cores of ferruginous bodies isolated from human lungs. Langer et al. [100] studied 50 ferruginous bodies isolated from the lungs of members of the general population of New York City and concluded that the cores in most were either degraded chrysotile or fibrous glass. Roggli et al. [121] examined 90 ferruginous bodies isolated from the lungs of six individuals with malignant pleural mesothelioma and three with asbestosis and found two with cores that had a chemical composition consistent with fibrous glass. Although fibrous glass may occasionally be found in samples of human lung tissue [116] (Fig. 3.22), it is uncommonly

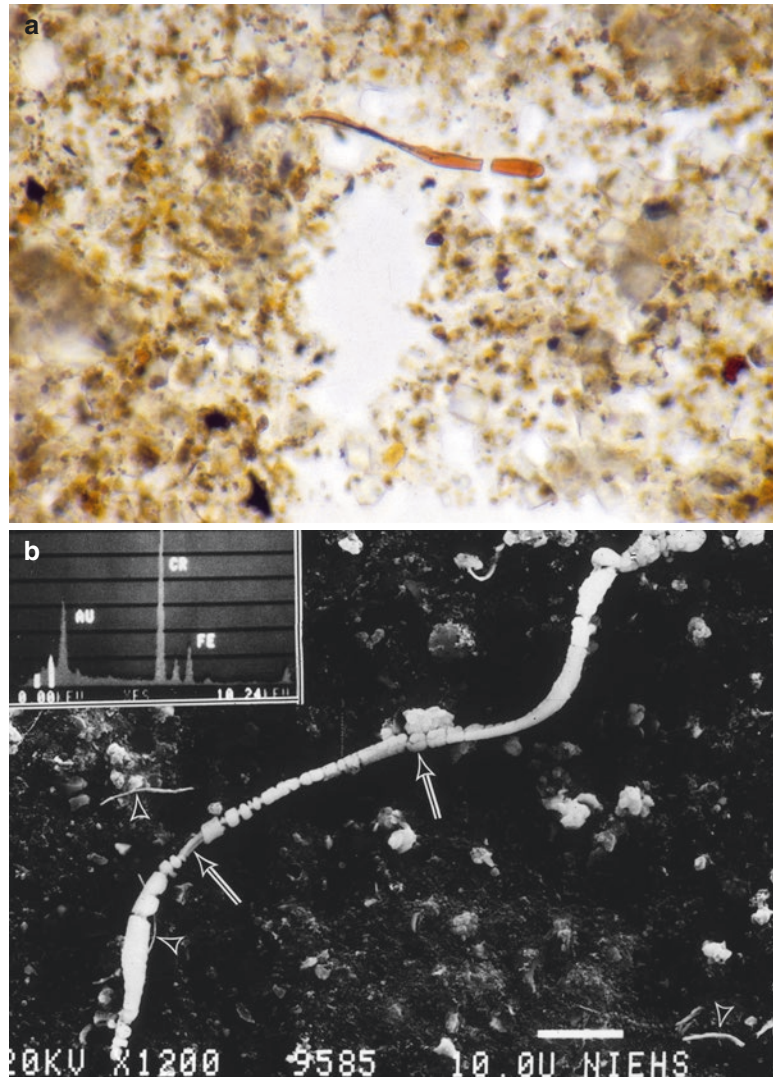


**Fig. 3.21** (**a**) Pseudoasbestos body with black core fiber recovered from lungs of a 76-year-old metal polisher. Magnified ×500. (**b**) Scanning electron micrograph of pseudoasbestos body, showing beaded iron coating as well as bare area revealing the core fiber (*arrows*). Additional uncoated fibers are present (*arrowheads*). *Inset*: EDXA spectrum from bare area of coated fiber, showing prominent peaks for chromium and a smaller peak for iron. Au peak is due to sputter coating. Magnified ×1,200 (Reprinted from Ref. [118], with permission)

identified as the core of ferruginous bodies. This observation is most likely due to the brittleness of these fibers so that they tend to break transversely producing shorter fibers [122] and to the tendency for fibrous glass to dissolve in vivo [123]. On the other hand, refractory ceramic fibers tend to be more biopersistent [124] and thus are more likely to form ferruginous bodies. Such bodies are indistinguishable from true asbestos bodies at the light microscopic level, but have aluminum silicate cores when examined by energy dispersive spectrometry [125] (Fig. 3.23).

### 3.7.5 Diatomaceous Earth

Ferruginous bodies with cores of diatomaceous earth are infrequently encountered. By light microscopy, they are large, broad, segmented, and frequently serpiginous. They do not have the clubbed ends so often observed in true asbestos

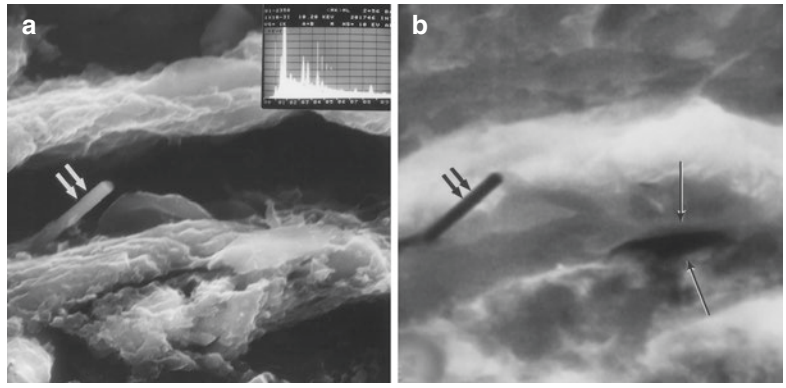


**Fig. 3.22** Lung tissue from a young woman with interstitial fibrosis. (**a**) Secondary electron image, showing a fiber protruding from an alveolar septum (*double arrow*). (**b**) Backscattered electron image of the same field shown in (a), demonstrating the fiber (*double arrow*) and an additional particle of talc (magnesium silicate) embedded in the tissue (*single arrows*). *Inset*: EDXA spectrum obtained from the fiber shown in (a, b), indicating a chemical composition of Na-Al-Si-K-Ca-Ba. This composition is consistent with fibrous glass. (**a**, **b**) magnified ×3,300 (Reprinted from Ref. [116], with permission)

bodies, and their color varies from golden yellow to deep orange brown [17]. In some examples, the sieve-like skeletal pattern of the diatom can be observed by electron microscopy [17, 116] (Fig. 3.24). Since the diatom skeleton is composed of amorphous silica, the cores would be silicon-rich with energy dispersive spectrometry and show no pattern with electron diffraction. Some silica "fibers" encountered in the lung may be acicular cleavage fragments of quartz, and such fragments, if of the proper dimension, can form the cores of ferruginous bodies and give a peak for silicon only with energy dispersive spectrometry (Table 3.4).

### 3.7.6 Zeolite Bodies

Zeolites are hydrated aluminum silicates which are naturally occurring, and some forms of zeolite, such as erionite, are fibrous. Erionite is found in volcanic tuff in Turkey and has physical characteristics which closely resemble those of amphibole asbestos. Sebastien et al. [126] have isolated ferruginous bodies with erionite cores from the lungs of individuals from Turkish villages situated on volcanic tuff rich in erionite. By light microscopy, they are indistinguishable from typical asbestos bodies. The villagers in this region of Turkey have a high incidence of pleural mesothelioma (see Chap. 5) and pleural fibrosis and calcification (see Chap. 6). Zeolite bodies have also been reported in lung tissue from a mesothelioma case in North America [127]. This individual had lived for 20–25 years in Mexico but had no history of travel to Turkey.

### 3.7.7 Others

Silicon carbide ceramic fibers can form ferruginous bodies in experimental animals [14], and ferruginous bodies with black cores have been observed in the lung tissues of silicon carbide workers [128–130]. In addition, elastic fibers under certain conditions can undergo fragmentation and ferruginization, and hence form the cores of ferruginous bodies [21]. Ghio et al. described ferruginous bodies associated with synthetic fibers in two patients with interstitial lung disease who were employed in textile mills [131]. In consideration of the wide variety of non-asbestos mineral fibers which can be recovered from human lungs (see Chap. 11), it is likely that non-asbestos ferruginous bodies of types other than those described above will be reported in the future.

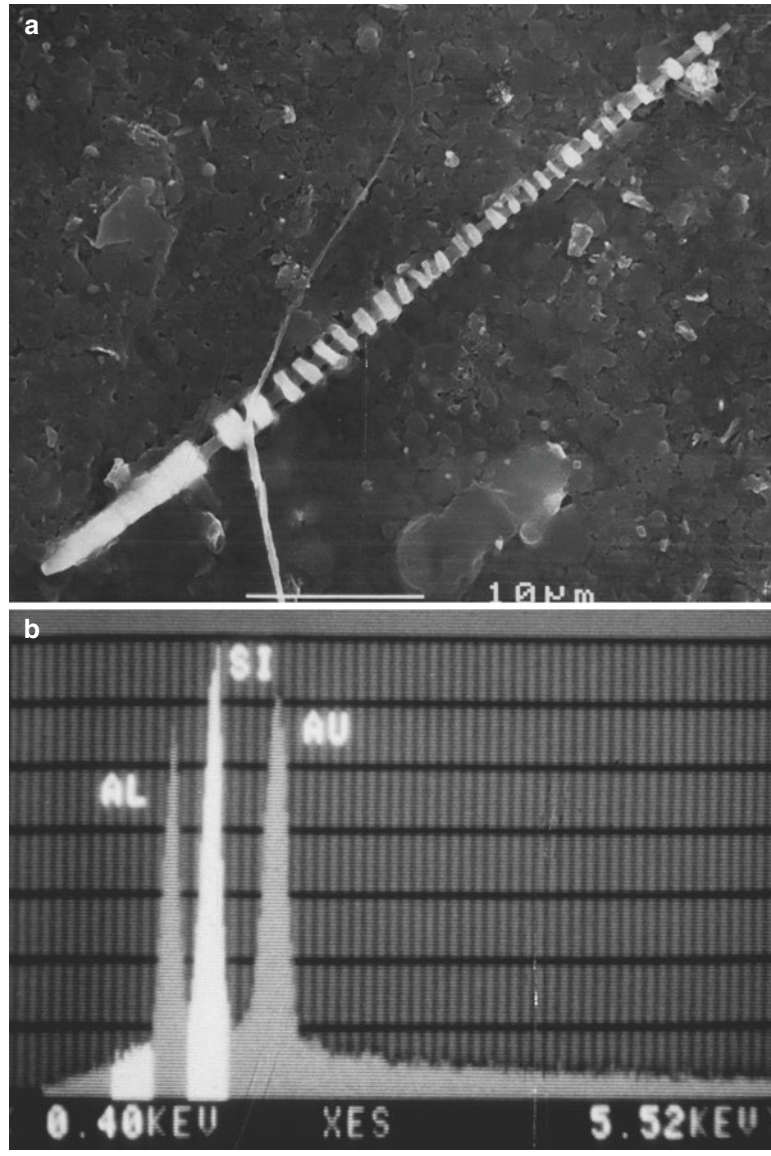


**Fig. 3.23** (**a**) Ferruginous body isolated from the lungs of a refractory ceramic fiber worker. Magnified ×2,300. (**b**) EDXA spectrum from bare area of coated fiber, showing peaks for aluminum and silicon. Au peak is due to sputter coating

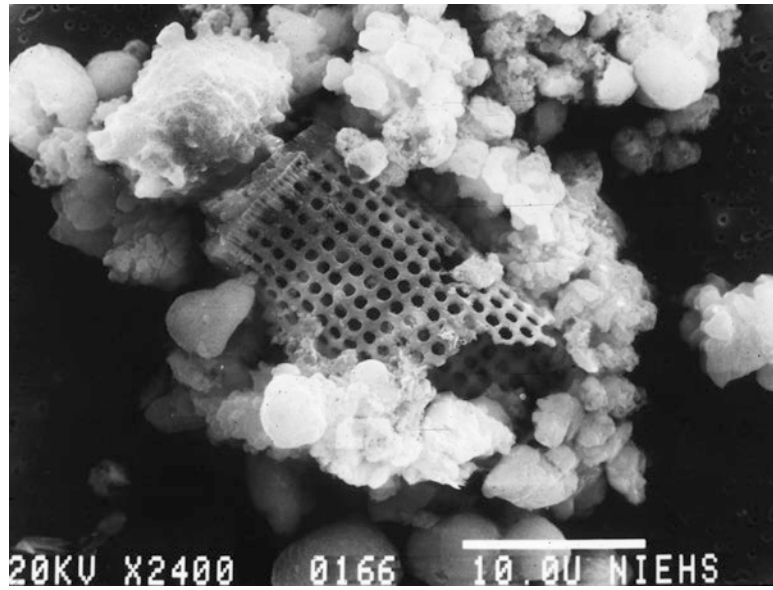


**Fig. 3.24** Diatomaceous earth pseudoasbestos body isolated from the lungs of an asbestos insulator in a shipyard. The diatom fragment with symmetrically aligned holes represents the silicon-containing diatom skeleton, which is surrounded by irregular granules of hemosiderin. Magnified ×2,400 (Reprinted from Ref. [116], with permission)

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# Asbestosis

# 4

Thomas A. Sporn and Victor L. Roggli

## 4.1 Introduction

The term pneumoconiosis dates to Zenker's 1866 description of pulmonary disease processes related to the inhalation of dusts [1]. As some dust, including asbestos fibers, may be found in the lungs of virtually all adults from the general population, pneumoconiosis now refers to the accumulation of excessive amounts of dust in the parenchyma of the lung and the pathologic response to its presence [2]. Asbestosis, the form of pneumoconiosis related to excessive amounts of asbestos fibers in the substance of the lung, is the prototype of diseases caused by inhalation of mineral fibers. *Asbestos* is a commercial, legal, and regulatory term, rather than a strictly mineralogical one, that encompasses a group of naturally occurring fibrous silicates whose differing physicochemical attributes confer a spectrum of pathologic properties upon their inhalation and deposition into the lung. Much has been learned from experimental models about the pathogenesis of asbestos-induced lung injury, which is reviewed in detail in Chap. 10. The reader is directed to Chap. 3 for a discussion of asbestos bodies, the histologic emblem of asbestos exposure, and a requisite component of the pathologic diagnosis of asbestosis. Chapter 11 discusses the methodology and results of quantitative tissue analysis for asbestosis, other asbestos-related diseases, as well as normal and disease control populations. The present chapter describes the morphologic features of asbestosis and relates them to the clinical and radiographic features of the disease.

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## 4.2 Historical Background

Asbestos usage dates to antiquity, as do observations regarding its attendant health hazards. With the widespread usage of asbestos after the Industrial Revolution, the number of individuals exposed to asbestos increased dramatically. Dr. H. Montague Murray is credited with the first description of asbestos-related pulmonary disease, which occurred in a 33-year-old man who had been working for 14 years in the carding section of an asbestos textile plant. This case was not published, but was reported to a British parliamentary committee in 1906 [3, 4]. Eight years later, the German pathologist Fahr described diffuse interstitial fibrosis in the lungs of a 35-year-old asbestos worker, which he attributed to the patient's exposure to asbestos. Fahr also called attention to crystals in the lung parenchyma [4, 5]. An additional report of pulmonary fibrosis

T.D. Oury et al. (eds.), *Pathology of Asbestos-Associated Diseases*,
DOI 10.1007/978-3-642-41193-9_4, © Springer-Verlag Berlin Heidelberg 2014

in an asbestos worker was recorded by Cooke in 1924, who subsequently coined the term asbestosis [6, 7]. Numerous cases of asbestosis have since been recorded, affecting workers exposed to asbestos through the processes of mining and milling asbestos-containing ores, the manufacture of asbestos-containing materials such as commercial insulation, or through the utilization of asbestos-containing products [4, 8].

The term *asbestosis* is properly defined as diffuse and bilateral pulmonary interstitial fibrosis caused by inhalation of asbestos. The incorporation of the asbestos-related pleural diseases under the heading of asbestosis is to be avoided, as this unnecessarily groups together diseases with different epidemiologic and pathophysiologic features, as well as different clinical features and outcomes [9, 10]. Historically, asbestosis has been defined in pathologic terms by a number of investigators, but pathologists have not applied these uniformly [11–13]. The most comprehensive descriptions of the pathologic features of human asbestosis are those reported by the Asbestosis Committee of the College of American Pathologists and the Pulmonary Pathology Society [14, 15]. These documents propose the minimal histologic criteria for the diagnosis of asbestosis. Histologic criteria are defined as those histopathologic observations that permit the definitive diagnosis without the requirement of clinical and radiographic data or exposure history. The identification of asbestos bodies within tissue sections remains the diagnostic sine qua non in view of the nonspecificity of interstitial fibrosis as a response to diffuse lung injury and the large number of occupational exposures and other disorders that may cause scarring in the lung [16–20].

## 4.3 Epidemiology

Asbestosis occurs in individuals exposed to large amounts of asbestos over long periods of time [21, 22]. Review of relevant epidemiologic studies indicates asbestosis development following such long-term and large-volume exposure, with threshold asbestos fiber dosage of between 25 and 100 fibers per cubic centimeter year [9]. Moreover, there is a direct relationship between intensity and duration of asbestos exposure and the prevalence of asbestosis [23]. Workers likely to sustain such high levels have historically included spray insulators and asbestos miners and millers. Asbestosis may result from exposure to any of the commercial forms of asbestos (chrysotile, amosite, and crocidolite) as well as the noncommercial amphibole anthophyllite [24]. The incidence is likely higher in cigarette smokers than nonsmokers with similar levels of asbestos exposure [25–29], may be due to the retention of larger amounts of asbestos in the lungs of smokers due to the inhibitory effect of cigarette smoking on pulmonary clearance mechanisms and direct enhancement of asbestos penetration into respiratory epithelium by cigarette smoking [30]. Experimental models have shown that cigarette smoke increases the degree of asbestos-induced pulmonary fibrosis, with increased asbestos fiber retention and rate of fiber penetration [31–33].

A case-control study of South African asbestos miners undergoing autopsy showed no positive association between smoking and histopathologic evidence of asbestosis, but reports using radiographic criteria have favored a role for cigarette smoking in the causation and progression of asbestosis [31–36]. Churg has shown that cigarette smoking causes increased retention of all types of fibers in respiratory epithelium [35]. Schwartz has posited a role for cigarette smoking in the causation of asbestosis by comparing the BAL cellular profile in smokers with asbestosis, attributing the increased inflammatory cell counts in lavage fluid to the effects of cigarette smoke [36]. Comparison of lavage cell counts in patients with asbestosis and asbestos-related pleural fibrosis showed that smoking strongly influenced lavage fluid cellularity, in particular the constituency of alveolar macrophages and eosinophils independent of the effects of asbestosis alone. Such changes are believed to play a role in the fibrogenesis seen in asbestosis [37].

Relatively brief, but intense, exposures to asbestos may be sufficient to attain the threshold tissue fiber burdens necessary to cause asbestosis. The development of asbestosis following a less than 3-year exposure to asbestos in the case of an

insulator has been reported, and asbestosis has been reported in a worker exposed to crocidolite for 9 months in the dusty process of manufacturing certain cigarette filters [21, 38]. The outcome of short-term exposure has also included the demonstration of 20 % prevalence of parenchymal opacities on radiographic studies in a cohort of amosite asbestos factory workers after as little as 1 month's employment [24]. While there is a direct relationship between intensity and duration of exposure to asbestos and the prevalence of asbestosis [23], it is interesting to note that not all those sustaining high-level exposure to asbestos will develop asbestosis. In addition to smoking habits, individual variance in asbestos fiber retention likely plays a role in this regard.

The interval of time between exposure to an injurious substance such as asbestos and the development of clinical disease is referred to as the latency period. In cases of asbestosis, latency periods are typically measured in decades and are generally inversely proportional to exposure intensity, with shorter latency periods following heavy exposure. Latency periods are rarely less than 15 years from the time of initial exposure [39]. Reasons for this include the fact that asbestos fibers, principally the biopersistent amphiboles, trapped within the pulmonary interstitium have a prolonged residence time in the lung, and asbestosis may develop and continue to progress many years after exposure has ceased [27].

Antao et al. describe the clear association between asbestos consumption and deaths from asbestosis. Per capita consumption of asbestosis in the USA peaked in 1951, but the persistence of the disease illustrates its latency period [40]. Between 1970 and 2004, Bang et al. described 25,413 deaths from asbestosis in the USA, with a maximum age-adjusted death rate of 6.9 per million population. The death rate for men was nearly 35-fold greater than for women [41]. The areas of the country with the highest death rates were the coastal regions, with the shipbuilding industry sustaining an expected proportionate mortality ratio. In this series, insulators and boilermakers had the highest asbestosis mortality rates. This is in keeping with the historical observation that occupations leading to heavy exposures caused asbestosis. It is believed that below the threshold "dose" of asbestos exposure, typically between 25 and 100 fibers per cc years, asbestosis is not observed. In this spectrum of exposures, commercial amphibole-induced asbestosis appears at lower dosages, whereas chrysotile-induced asbestosis seems to require the higher range of exposures [42]. This difference likely results from the lesser capacity of chrysotile to induce fibrogenesis in comparison to the amphiboles on a per-fiber basis, which in turn is most likely a function of the marked physicochemical differences in the fiber types, as discussed in Chap. 1.

## 4.4 Clinical Features

The clinical features of asbestosis are not unique to this entity and are those common to the entire spectrum of chronic pulmonary parenchymal fibrosing disorders. Dyspnea and dry cough are accompanied by basilar rales, and digital clubbing may be present in early as well as advanced disease. Late-stage disease is often indicated by the presence of constitutional symptoms and signs of cor pulmonale. In general, asbestosis differs from idiopathic pulmonary fibrosis (usual interstitial pneumonia, UIP) in that clinical manifestations are less severe and physiologic derangements milder in cases of the former. The rate of disease progression in asbestosis is generally slower than that seen with typical cases of UIP. Such physiologic derangements on pulmonary function studies are not specific to asbestosis and are those of restrictive ventilatory defects, with reduction in lung volumes, decreased diffusion capacity, and arterial hypoxemia. The reduction in forced vital capacity appears proportionate to the profusion of irregular opacities on chest radiographs, which may be further exaggerated by the presence of diffuse pleural thickening [43]. In cases of asbestosis, obstructive ventilatory defects, as characterized by reduction in the FEV1/FVC ratio, are not to be expected in the absence of emphysema resultant from smoking. There is some data suggesting that exposure to mineral dusts can contribute to the development of airflow obstruction, likely related to peribronchiolar fibrosis. A study of 17 nonsmoking asbestos workers demonstrated reduced airflow at low lung

volumes consistent with obstruction at the level of the small airways [44]. Another study contrasting the physiologic derangements in workers exposed to silica, asbestos, and coal dust found lower FEV1/FVC ratios in the cohort of coal workers, suggesting exposure to coal dust is responsible for the development of obstructive ventilatory defects, irrespective of the degree of any underlying coal workers pneumoconiosis. Restrictive ventilatory defects were observed in the cohort of asbestos workers, accompanied by increases in FEV1/FVC ratios as a function of progression of fibrosis and adjustment for smoking status, which the authors suggest is due to the salutary effects of fibrosis upon peripheral airflow conductance and elastic lung recoil [44]. It remains controversial whether peribronchiolar fibrosis related to asbestos causes clinically significant airflow obstruction in the absence of cigarette smoking. In the presence of smoking, the additive contribution of asbestos exposure to airflow obstruction is likely negligible [45–47]. There is no evidence that asbestos exposure alone contributes to clinically significant emphysema [8, 9].

Asbestosis is uncommonly associated with systemic manifestations. Kobayashi et al. described a case of asbestosis in which interstitial fibrosis was also observed in the liver, kidney, myocardium, and thyroid gland [48]. Asbestos fibers were also identified in each of these sites using electron microscopy. Such an occurrence is analogous to the phenomenon of extrapulmonary silicotic nodules, which may be found in the liver, spleen, bone marrow, and abdominal lymph nodes resulting from migration of dust-laden macrophages to those sites in patients with heavy exposure and advanced silicosis [49]. Experimental studies clearly showing the transport of asbestos fibers in sufficient quantity to cause fibrosis have not to our knowledge been reported. Kobayashi's case more likely represents coincident asbestosis and progressive systemic sclerosis (scleroderma). Scleroderma is an uncommon, poorly understood but well-recognized complication of exposure to crystalline silica, and it is possible that the development of systemic sclerosis in asbestosis shares a similar pathophysiology [50]. Asbestos exposure is known to produce immunologic alterations in the host that affect both humoral and cell-mediated immunity [51, 52], but it is unclear what clinical manifestations may result from these derangements. Immunoblastic lymphadenopathy and lymphoma have also been reported in association with asbestosis [53–55]. Cooke et al. described another patient with lymphomatoid granulomatosis and proliferative glomerulonephritis in which asbestos fibers were observed in the renal mesangial matrix [56].

Similar to other forms of diffuse pulmonary interstitial fibrosis, asbestosis results in significant morbidity and mortality. The immunosuppressive therapy which constitutes the mainstay of medical intervention for other forms of pulmonary interstitial fibrosis is ineffective. Historically, deaths in asbestosis have been due to the development of intractable respiratory failure. The prognosis associated with asbestosis is variable, but in general is associated with decreased life expectancy, in proportion to severity of disease [57–59]. In cases of heavy exposure, progression of disease generally occurs, which is also resistant to medical therapy. It is not clear whether such progression of fibrosis is inevitable in all cases. Studies from the UK and Finland document considerable and excessive mortality from carcinoma of the lung in workers with asbestosis [57, 60]. Other causes of death include cor pulmonale and mesothelioma. A review of 525 autopsy cases of asbestosis from Japan between the years 1958 and 1996 noted an incidence of malignancy in approximately 60 % of the entire autopsied series, represented chiefly by carcinomas of the lung and malignant mesothelioma. The highest rate of malignancy was observed in the cohort dying between 1990 and 1996, in which the rate of malignancy was 65 %, compared to a rate of 43 % observed in the cohort dying between 1958 and 1979 [61]. In a review of cohort studies, Weiss observed that asbestosis is a far better predictor of excessive risk of developing lung cancer than exposure to asbestos alone [62]. Selikoff et al. showed that the risk of lung cancer among insulators was increased fivefold relative to a population unexposed to asbestos with similar smoking histories [63]. Most of

these individuals had asbestosis radiographically or pathologically. McDonald and McDonald reported a relative risk for mesothelioma of 46 among insulators [64].

The continued recognition of the serious hazards posed by exposure to asbestos, in concert with the reduction in asbestos consumption, improvements in regulation, occupational and industrial hygiene, and the scientific understanding of asbestos-associated illness at the molecular level will hopefully result in a diminution of this scourge, just as deaths from other pneumoconioses are on the decline. But statistical modeling indicates that asbestosis deaths are not due to decrease sharply over the next 10–15 years. The disease is predicted to claim the lives in excess of 29,000 individuals, between the years 2005 and 2027 [40].

## 4.5 Mechanisms and Pathogenesis

The mechanisms and pathogenesis of asbestos-related fibrosis are much studied. Progression to pulmonary fibrosis, following asbestos fiber inhalation, entails the development of a protracted cycle of inflammatory cell recruitment, the generation of reactive oxygen species and release of proteases, epithelial injury, apoptosis, and fibroblast proliferation [65–68]. Upregulation of oncogenes and macrophage expression of cytokines and growth factors are also implicated. The suggested role for cigarette smoke in the causation and progression of asbestosis probably results from increased fiber retention. This follows enhanced fiber penetration in smokers, reduced pulmonary fiber clearance, and changes in alveolar inflammatory cell populations, resulting from smoking as reported in bronchoalveolar lavage fluid studies [66, 68].

Genetic factors are believed to play a significant role in the development of asbestosis [69–72]. Specifically, polymorphisms in the genes coding for glutathione-S-transferases (GST) have been implicated in the increased risk. GSTs are critical enzymes involved in the detoxification and inactivation of reactive oxygen and nitrogen species. These are involved in the inflammatory cascade following the phagocytosis of asbestos fibers by macrophages [73]. The GST family contains a number of cytosolic isoenzymes, and polymorphisms in GSTP1, M1, and T1 isoenzymes are associated with an increased risk for the development of asbestosis. Such polymorphisms are relatively common, with approximately 50 % of the Caucasian population affected with the null polymorphism of the GSTM1 and GSTT1 genes [74]. A detailed discussion of these pathways and the cellular and molecular basis for tissue injury related to inhalation of asbestos is to be found in Chap. 10.

## 4.6 Diagnosis

### 4.6.1 Radiographic Features

The radiologic findings in asbestosis are those of lower lung zone reticulonodular infiltrates and small irregular opacities, detectable on plain films (Fig. 4.1). The identification of bilateral pleural thickening and/or plaques heightens suspicion for asbestosis, as these findings are not typically observed in other forms of diffuse pulmonary interstitial fibrosis. In contrast, pleural changes may be observed in some 80 % of asbestosis cases on plain chest radiographs, which rises to 100 % using high-resolution CT imaging [75–77]. Predominantly mid- or upper lung zone distribution of infiltrates argues against the diagnosis of asbestosis. The principal radiographic differential diagnosis for asbestosis is usual interstitial pneumonia (idiopathic pulmonary fibrosis, UIP), as both entities feature predilection for the peripheral lower lung zones, with subpleural accentuation and progression to honeycomb changes at late stage. CT scanning is more sensitive at detecting the parenchymal changes of asbestosis at an early stage (Fig. 4.2). Such findings may be observed in the presence or absence of supportive clinical data and may not be evident on plain radiographs [75]. Other findings on the chest radiographs of patients with asbestosis include "shaggy" cardiac silhouettes and indistinct diaphragmatic contours [75]. The radiologic findings are crucial to the establishment of an acceptable clinical diagnosis of asbestosis. The International Labor Office (ILO) developed a

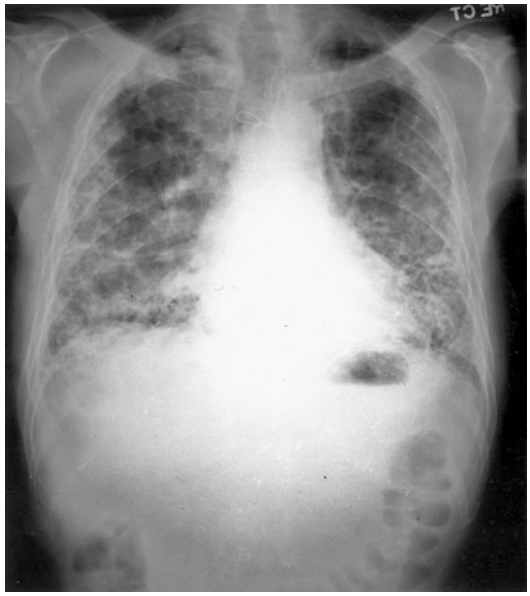

**Fig. 4.1** Posteroanterior chest radiograph from an insulator. Reduced lung volumes with peripheral bibasilar reticulonodular infiltrates typical of asbestosis are present (Reprinted from Ref. [8])

classification scheme for the radiographic assessment of pneumoconioses, based on the size and profusion of radiographically detectable opacities [78] (Table 4.1). The American Thoracic Society (ATS) has proposed that in the appropriate clinical setting, the ILO category of 1/1 reticulonodular opacities, accompanied by reductions in predicted FVC and diffusion capacity less than the lower limits of normal, was sufficient for the diagnosis of asbestosis [79]. Inexplicably, a more recent ATS document concerning the diagnosis of nonmalignant asbestos-related disease reduced the radiologic criteria for the diagnosis of asbestosis, thus decreasing specificity at a time when the disease is becoming more scarce [80]. While the advent of high-resolution computed tomography has certainly increased the sensitivity of detection of early asbestos-related parenchymal lesions, plain chest roentgenograms remain the radiographic gold standard. In view of the above, the clinical diagnosis of asbestosis in the setting of heavy asbestos

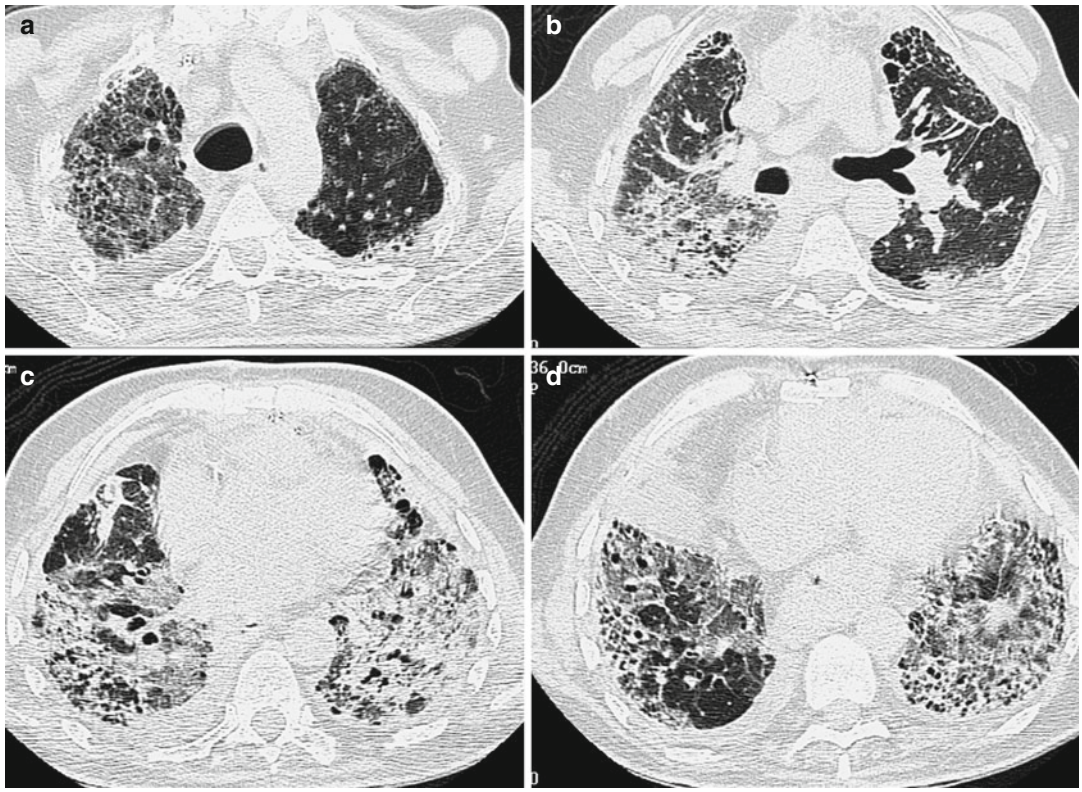


**Fig. 4.2** Computed axial tomography (CAT) of the thorax. Findings typical of and associated with asbestosis include visceral pleural fibrosis, prominent fibrotic interstitial markings with peripheral and bibasilar accentuation, traction bronchiectasis, and honeycombing

**Table 4.1** ILO international classification of radiographs of pneumoconioses

| **I. Parenchymal abnormalities** | | |
|---|---|---|
| Small and large opacities: descriptors include profusion, affected zones of lung, shape, and size | | |
| **Small opacities** | **Major** | **Minor/subcategory** |
| Profusion | Category 0: normal | 0/–, 0/0, 0/1 |
| | Category 1: mild | 1/0, 1/1, 1/2 |
| | Category 2: moderate | 2/1, 2/2, 2/3 |
| | Category 3: severe | 3/2, 3/3, 3+ |
| Shape/size | *Radiologic small, round opacities*: | |
| | P = diameter up to 1.5 mm | |
| | Q = diameters up to 1.5–3 mm | |
| | R = diameters 3–10 mm | |
| Shape/size | *Radiologic small, irregular opacities*: | |
| | S = opacities widths up to 1.5 mm | |
| | T = opacities widths 1.5–3 mm | |
| | U = opacities widths 3–10 mm | |
| | Two letters are used to record size and shape of observed opacities | |
| **Large opacities** | *Defined as having greatest dimension exceeding 10 mm*: | |
| Category A | One large opacity having longest dimension up to approximately 50 mm, or several large opacities with the sum of their longest dimension not exceeding about 50 mm | |
| Category B | One large opacity having longest dimension exceeding 50 mm, but not exceeding equivalent area of right upper zone, or several large opacities with sum of longest dimensions exceeding 50 mm but not exceeding equivalent area of right upper zone | |
| Category C | One large opacity which exceeds the equivalent area of the right upper zone, or several large opacities which when combined exceed the equivalent area of the right upper zone | |
| **II. Pleural abnormalities** | | |
| Pleural abnormalities are divided into plaques, costophrenic angle obliteration, and diffuse pleural thickening | | |
| 1. Plaques (localized pleural thickening): Presence or absence of calcification, site, and laterality are recorded, as is extent (recorded only for chest wall plaques) | | |
| Extent 1: total length up to ¼ of lateral chest wall | | |
| Extent 2: total length up to ¼ to ½ of chest wall | | |
| Extent 3: total length exceeding ½ of chest wall | | |
| 2. Costophrenic angle obliterations are recorded as present or absent, with designation as to side involved | | |
| **III. Additional descriptors/symbols** | | |
| Radiographic features of importance which may be relevant to dust exposure are also given | | |
| Examples include: | | |
| Es (eggshell calcification of hilar or mediastinal nodes) | | |
| Em (emphysema) | | |
| Cp (cor pulmonale) | | |
| Ho (honeycomb lung) | | |
| Ca (cancer, thoracic malignancies excluding mesothelioma) | | |

Modified from Ref. [78] with permission

exposure, attended by the above-described radiographic and physiologic derangements, seldom requires biopsy for histologic confirmation. The clinical evaluation for suspected asbestosis as well as other forms of diffuse interstitial lung disease often includes bronchoscopy with lavage and transbronchial lung biopsy as initial invasive diagnostic studies. The demonstration of asbestos bodies in sputum or bronchoalveolar lavage fluid correlates with heavy exposure and asbestosis [81] (see Chap. 9), but the presence of these in cytological preparations is not sufficient to establish the diagnosis of

asbestosis. The comparatively scant amount of alveolar tissue sampled at transbronchial biopsy is typically insufficient to diagnose asbestosis, and the limitations of this modality are discussed below. As those exposed to asbestos are not immune to other and potentially treatable forms of interstitial fibrosis, surgical biopsy is recommended in the instances where exposure history is not compelling and uncertainty exists on the basis of radiographic and/or clinical grounds [82, 83]. A discussion of the pathologic features of asbestosis found on autopsy or surgical biopsy material follows.

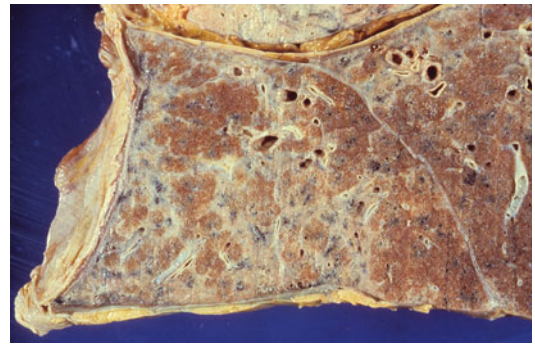

**Fig. 4.3** Coronal section of the lower lobe in an insulator with asbestosis. There is coarse linear interstitial fibrosis, but changes of advanced fibrosis or honeycombing are not present. Note accompanying visceral pleural fibrosis

## 4.6.2 Pathologic Features

### 4.6.2.1 Gross Morphology

For optimal visualization of the fibrosing process and concomitant pathologic processes such as emphysema, we recommend prosection of surgical or autopsy material following optimal distension with formalin and adequate tissue fixation of at least 2 days duration [14, 15]. In the earliest stages of asbestosis, the lungs may appear normal on gross examination. It is important not to misinterpret accompanying visceral pleural fibrosis as asbestosis, and the diagnosis must include the description of an appropriate pattern of diffuse parenchymal fibrosis. As the disease progresses, gray streaks of fibrous tissue become visible at the lung bases, accentuated at the periphery, with sparing of the central lung zones [14]. This pattern may be followed by coarse linear scarring with loss of lung volume (Fig. 4.3). Such areas are the counterpart to the reticular markings and small irregular opacities seen on plain chest films and contribute to the anatomic basis for the restrictive physiologic derangements observed in asbestosis. The excessive collagen deposition results in increased lung weight and firm consistency. Honeycomb changes are identified in advanced disease, most conspicuously in the subpleural areas of the lower lung zones. The honeycomb foci consist of cystic spaces, which measure up to 1.0 cm in areas of dense fibrosis (Fig. 4.4). In exceptional cases, and for uncertain reasons, the interstitial fibrosis may be most severe in the upper lung zones [84]. Progressive massive fibrosis has also been described in rare instances and

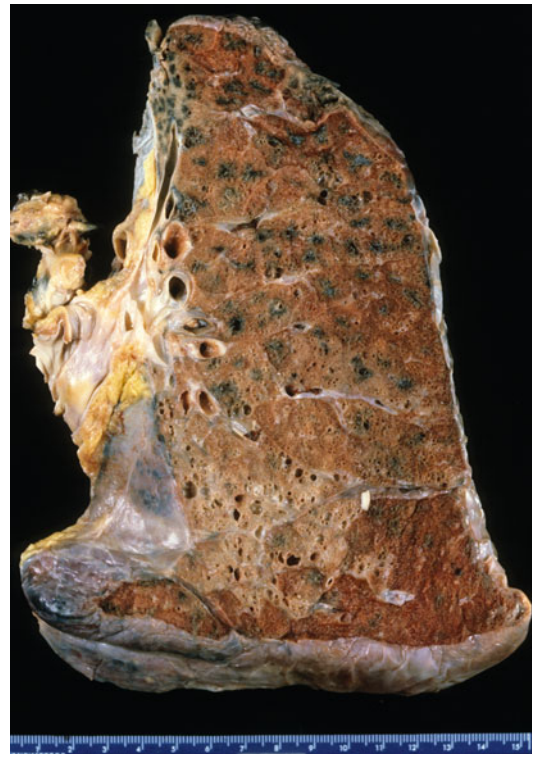

**Fig. 4.4** Coronal section of lung showing advanced fibrosis with traction bronchiectasis and basilar honeycomb cysts

is likely attributable to exposure to asbestos and silica. There are no typical asbestos-associated lesions in the airways, although traction bronchiectasis may develop in areas of dense scarring. Regional lymph nodes are generally unremarkable on gross inspection. The changes described

above are not specific for asbestosis and may be observed in a wide variety of chronic interstitial diseases and fibrosing disorders. One useful feature that may aid in the distinction of asbestosis from other fibrosing pneumonitides is the frequent association of pleural abnormalities with the former (see Chap. 6). Diffuse thickening of the visceral pleura is often an accompanying finding in asbestosis (Fig. 4.3), whereas fibrotic pleuropulmonary adhesions are variably observed. A finding yet more characteristic of asbestos exposure is the parietal pleural plaque, circumscribed areas of ivory-colored pleural thickening over the domes of the diaphragm or on the posterolateral chest wall running along the direction of the ribs [85, 86]. Pleural plaques may be smooth or nodular (the so-called "candle-wax dripping" appearance) when viewed grossly, have a cartilaginous consistency, and are often calcified. The demonstration of such pleural abnormalities do not constitute components of the diagnosis of asbestosis, but rather alert the pathologist to the possible presence of asbestos-related interstitial fibrosis and advise a search for asbestos bodies in histologic sections of lung tissue. Not all patients with asbestosis have pleural plaques, and certainly not all patients with pleural plaques have the interstitial fibrosis requisite for the diagnosis of asbestosis. The term asbestosis should not be applied to the benign pleural changes alone [9, 10, 86]. It has been suggested that there is no need to distinguish the clinically and epidemiologically disparate entities of pleural and parenchymal fibrosis as they both are causally related to asbestos exposure [87]. The weakness of this argument emerges when one considers that asbestos exposure may result in asbestosis, malignant mesothelioma, or both. Certainly, there is no rationale for including malignant mesotheliomas under the rubric of asbestosis, and there is no reason to include benign pleural fibrosis under that rubric either.

Due to the prevalence of cigarette smoking among asbestos workers, this group often exhibits parenchymal changes in the lung related to exposure to tobacco smoke. The pathologist must therefore take care to distinguish abnormalities resultant from the two different types of exposure. Centrilobular emphysema is frequently observed in the lungs of cigarette smokers [88] and may be of such a severe degree as to overshadow the fibrosis of asbestosis (Fig. 4.5). Emphysema must be distinguished from the honeycomb changes observed in advanced asbestosis. The gross distribution of the lesions is helpful in this regard, with emphysema tending to be most severe in the upper lobes, and the honeycomb changes most severe in the lower lung zones. The cystic changes of honeycomb lung are usually of uniform size (approximately 0.5 cm), with thickened fibrotic walls. The emphysematous spaces by contrast are of variable size, from barely visible to several centimeters in diameter. Representing areas of destroyed lung tissue, they have no "walls" and are not accompanied by visible fibrosis [88]. Another helpful gross feature is the presence of thin, delicate tissue strands that

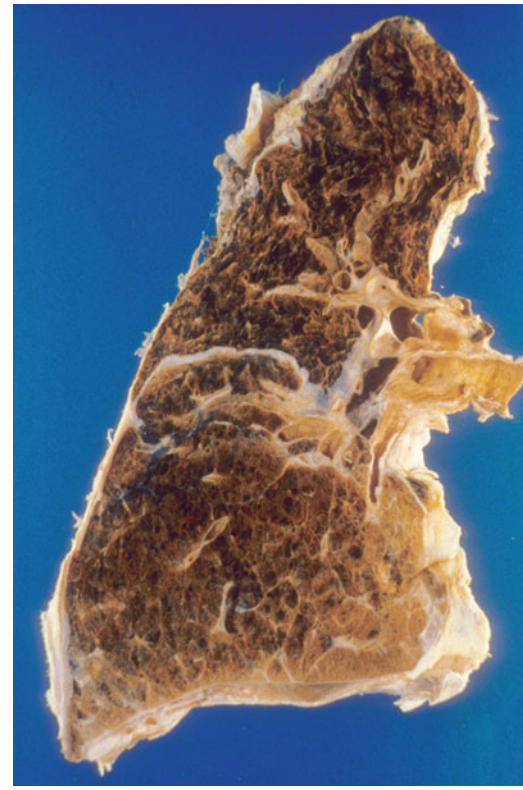

**Fig. 4.5** Coronal section of lung from a cigarette-smoking insulator showing advanced centrilobular emphysema, most severe in the mid- and upper lung zones. The fibrotic features of asbestosis are present in the lower lobe. There is also diffuse visceral pleural fibrosis encasing the lung and extending into interlobar fissures

traverse the emphysematous spaces. These represent vascular remnants that persist following destruction of alveolar tissue.

### 4.6.3 Microscopic Features

#### 4.6.3.1 Cytology/Role for Bronchoalveolar Lavage

As discussed above, clinical evaluation of patients with diffuse interstitial lung disease often begins with analysis of exfoliative cytological preparations obtained via fiberoptic bronchoscopy. Bronchoalveolar lavage is a useful technique to diagnose diseases involving the peripheral alveoli in the most distal anatomic regions of the lung. The technique involves the instillation of aliquots of sterile saline via a peripherally placed fiberoptic bronchoscope. Bronchoalveolar lavage fluid (BALF) is subsequently retrieved via the bronchoscope suction port and submitted for cytological analysis. Demonstration of asbestos bodies which can be found in BALF in greater than 95 % of patients with asbestosis using this methodology may alert to the presence of asbestos-related lung pathology. However, the diagnosis of asbestosis is not possible based solely on cytological grounds, and asbestos bodies are more properly considered markers of exposure rather than disease. Some studies have shown a direct relationship between concentration of asbestos bodies in BALF and the degree of exposure and lung tissue asbestos burden [89]. However, Schwartz studied the BALF asbestos body counts in a cohort of American construction workers and determined that in at least this cohort, predominantly exposed to chrysotile, concentrations of BALF asbestos bodies were valid measures neither of asbestos exposure nor of asbestos-related lung disease [90]. As of this writing, it seems reasonable to treat the asbestos bodies in BALF, when present, as a reproducible indicator of exposure to asbestos, and to use this piece of information as a single point in the patient's clinical database. A negative BALF examination for asbestos bodies does not exclude the possibility of asbestos-related pulmonary pathology. The examination of BALF to derive inflammatory cell counts and profiles as a means to diagnose or subclassify diffuse interstitial lung disease generally remains a research technique in this setting, and conclusions regarding the clinical utility of BAL in daily diagnostic practice appear unsubstantiated [91].

**Table 4.2** Published criteria for acceptable histopathologic definitions of asbestosis

| |
|---|
| *I. College of American Pathologists-National Institute for Occupational Safety and Health* (*CAP-NIOSH*): "demonstration of discrete foci of fibrosis in the walls of respiratory bronchioles associated with accumulations of asbestos bodies" |
| II. "*Helsinki criteria*": "diffuse interstitial fibrosis in well-inflated lung tissue remote from a lung cancer or mass lesion, plus the presence of either two or more AB in tissue with a section area of 1 $cm^2$, or a count of uncoated asbestos fibers recorded by the same laboratory for asbestosis" |
| *III. Asbestosis Committee of the College of American Pathologists and the Pulmonary Pathology Society*: acceptable pattern of alveolar septal (not bronchiolar) fibrosis and an average rate of asbestos bodies of at least 2/$cm^2$. Cases with diffuse interstitial fibrosis and an asbestos fiber burden, determined by an experienced laboratory using electron microscopy techniques, within the range of values observed for bona fide cases of asbestosis are also likely examples of asbestosis |

#### 4.6.3.2 Histopathology

Acceptable histopathologic definitions of asbestosis have been provided by the College of American Pathologists-National Institute for Occupational Safety and Health (CAP-NIOSH) [14] as well as by an expert group in the so-called Helsinki criteria [92] whose recommendations are echoed by the ATS (Table 4.2) [78]. These definitions were recently updated by the Asbestosis Committee of the College of American Pathologists and the Pulmonary Pathology Society [15]. In addition to an acceptable pattern of alveolar septal fibrosis, the histologic diagnosis of asbestosis requires the identification of asbestos bodies. These may be found in alveolar spaces, embedded in a fibrotic interstitium or within giant cells (Fig. 4.6). Iron stains should be routinely employed if asbestosis is suspected and asbestos bodies are not observed on routine-stained sections. The examination of multiple sections is recommended, if possible, as asbestos bodies may have an uneven distribution in lung tissue. The sine qua non for the histologic

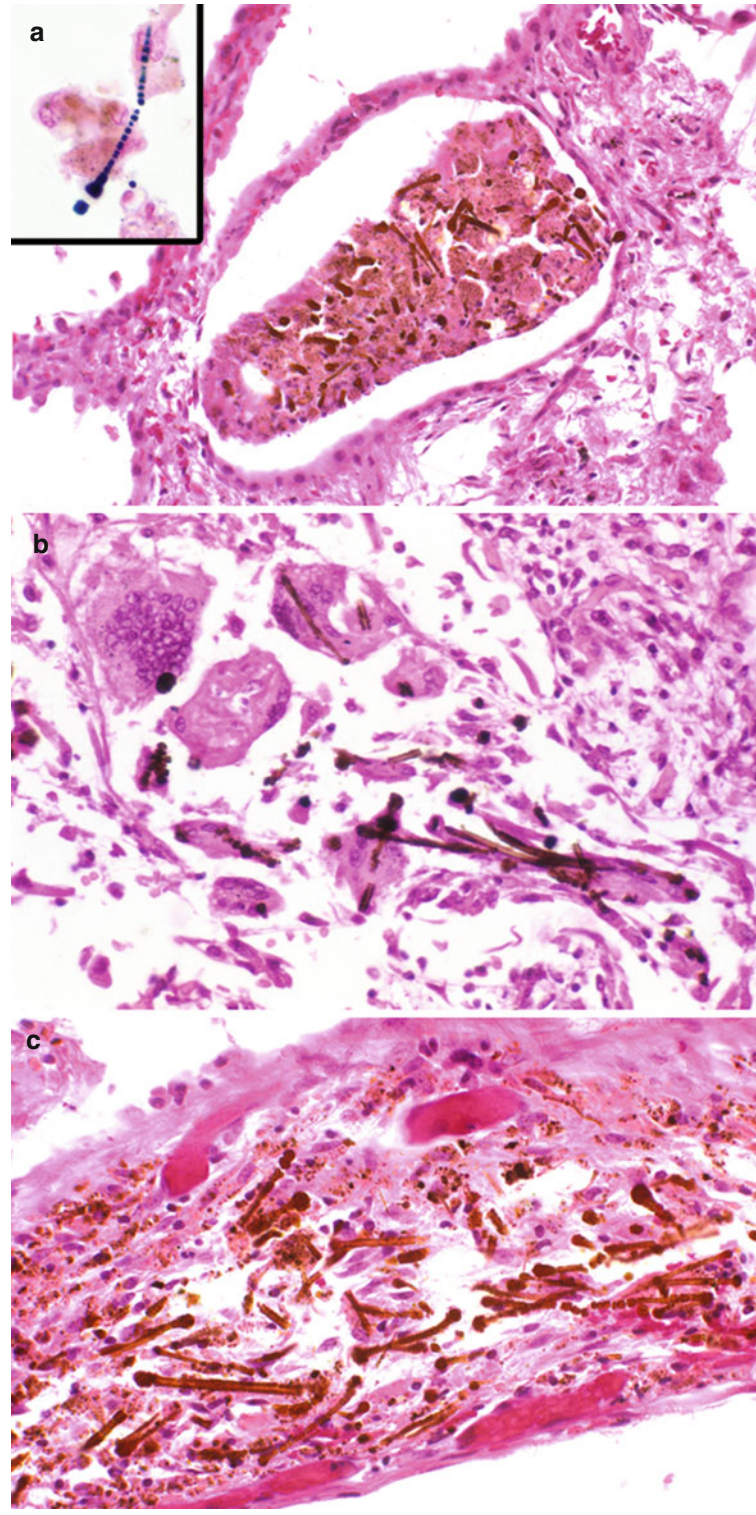


**Fig. 4.6** Composite showing peripheral lung from an insulator with asbestosis at intermediate to high magnification. (**a**) Routine-stained section showing delicate alveolar septal fibrosis with asbestos bodies present in airspaces, details of asbestos body on Perls iron stain (*inset*). (**b**) Asbestos bodies within macrophages in airspaces. (**c**) Hematoxylin-eosin, high magnification showing clusters of asbestos bodies within fibrotic interstitium of lung. (**a**, **b** and *inset* Reprinted from Ref. [129] with permission)

diagnosis of asbestosis is the demonstration of diffuse interstitial fibrosis and asbestos bodies in routine 5-μm sections [14, 15].

Observations of lung tissue obtained at autopsy from asbestos workers, as well as experimental models, have demonstrated that the earliest

microscopic abnormality is the presence of increased collagen in the walls of respiratory bronchioles [14, 93]. This has been termed peribronchiolar fibrosis, a misnomer owing to the fact that the fibrosis in such instances is not in the septa of adjacent tiers of peribronchiolar alveoli, but within the walls of the bronchioles themselves. Bronchiolar fibrosis may also be observed following inhalation of other metal and mineral dusts such as iron and silica, as well as following exposure to cigarette smoke [2, 15]. Accordingly, current recommendations are to term such lesions bronchiolar wall fibrosis, not asbestosis, and the appellation *asbestos airways disease* for bronchiolar fibrosis in association with asbestos bodies. With more advanced disease, fibrosis extends into the terminal bronchioles proximal to the respiratory bronchioles, as well as into distal alveolar ducts. Ultimately, the fibrotic process extends to involve the alveolar septa surrounding these structures (Fig. 4.6). The most extensive involvement is typically in the subpleural tiers of alveoli and in those alveoli in closest proximity to the bronchioles. In advanced cases, large zones of lung parenchyma consist of fibrotic alveolar walls, and honeycomb change may be present. The honeycomb areas consist of cystic spaces generally 1–15 mm in diameter, lined by cuboidal to low columnar epithelium. These spaces frequently contain mucus and inflammatory debris, and a lymphoplasmacellular inflammatory infiltrate may be observed within the fibrotic interstitium. The secondary interlobular septa may also be markedly thickened by fibrous tissue, and diffuse visceral pleural fibrosis may be observed as well. The fibrotic process may be patchy in the early stages, requiring the examination of multiple sections to find the diagnostic features [14, 15, 90]. Masson's trichrome stains can facilitate the assessment of the extent and distribution of the interstitial fibrosis. While it is apparent that asbestosis begins as, and propagates from, a peribronchiolar fibrosing process, Churg writes that this pattern may not always be obvious and notes in many acceptable cases a pattern indistinguishable from usual interstitial pneumonia except for the requisite presence of asbestos bodies [9].

The second component required for the histologic diagnosis of asbestosis is the identification of asbestos bodies in paraffin-embedded tissue sections [94]. The morphologic appearance of asbestos bodies and their distinction from other ferruginous bodies are described in detail in Chap. 3. Asbestos bodies may be observed within hilar or mediastinal lymph nodes, often associated with fibrosis of the lymph node parenchyma [95, 96]. This curious observation is largely confined to patients with a heavy pulmonary parenchymal fiber burden and is likely related to overloading of the clearance mechanisms [95]. In a study of 20 patients where asbestos bodies were observed in thoracic lymph nodes, 17 had histologically confirmed asbestosis [96].

**Table 4.3** Histologic findings in 100 cases of asbestosis

| Histologic feature | Percent |
|---|---|
| *Always present* | |
| Asbestos bodies | 100 % |
| Peribronchiolar fibrosis | 100 % |
| *Occasionally present* | |
| Honeycomb changes | 15 % |
| Foreign-body giant cells | 15 % |
| Bronchiolar metaplasia | 10 % |
| Cytoplasmic hyaline | 7 % |
| Desquamative interstitial pneumonitis-like areas | 6 % |
| *Rarely present* | |
| Osseous metaplasia (dendriform pulmonary ossification) | 2 % |
| Pulmonary blue bodies | 1 % |

Other less frequent histologic changes have been observed in asbestosis (Table 4.3). These include the presence of foreign-body-type giant cells within alveoli, or less commonly the fibrotic interstitium, in some 15 % of cases. Scarring and distortion of bronchioles with necrosis and disruption of the transitional zone from the respiratory bronchiole to the alveolar duct occasionally results in the lining of adjacent alveoli by cuboidal bronchiolar epithelium (Fig. 4.7). This process occurs in some 10 % of cases and has sometimes, and incorrectly, been termed "pulmonary adenomatosis," as this phenomenon is a proliferative response to injury, rather than a true neoplastic process. Hyperplastic type II alveolar pneumocytes may also line the fibrotic alveolar septa in asbestosis. These cells may in approximately 7 % of cases contain deposits of waxy, deeply eosinophilic material (Fig. 4.8). This

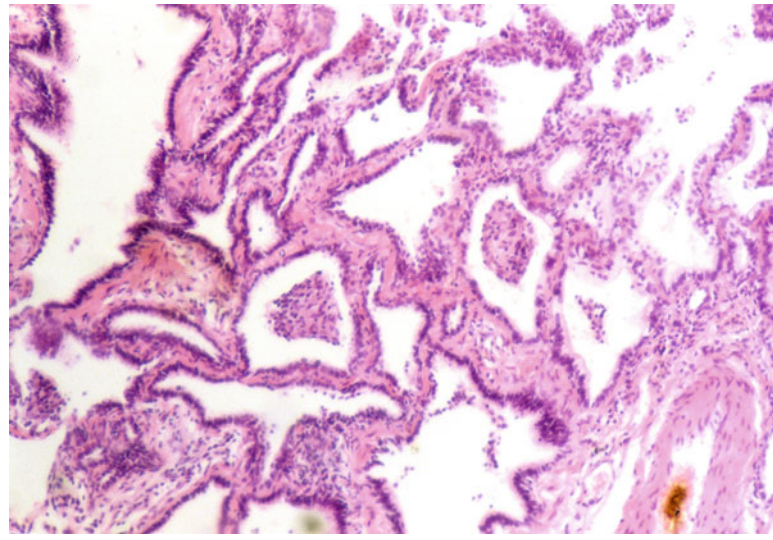

**Fig. 4.7** Hematoxylin-eosin, intermediate magnification of an area of peripheral bronchiolar metaplasia involving fibrotic alveolar septa. Septa are lined by low cuboidal bronchiolar epithelium

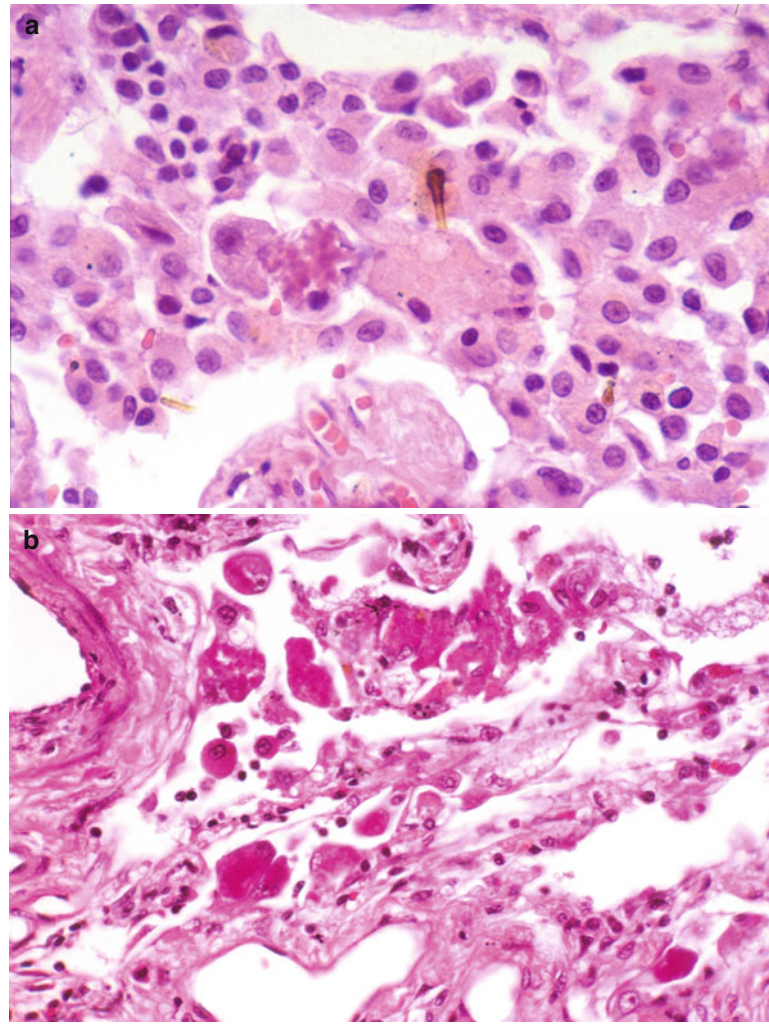


**Fig. 4.8** Hematoxylin-eosin, high magnification (**a**) hyperplastic type II pneumocytes in asbestosis, note presence of asbestos body. (**b**) Some pneumocytes contain cytoplasmic hyaline, with similar tinctorial characteristics to Mallory's hyaline within hepatocytes in cases of alcoholic liver disease

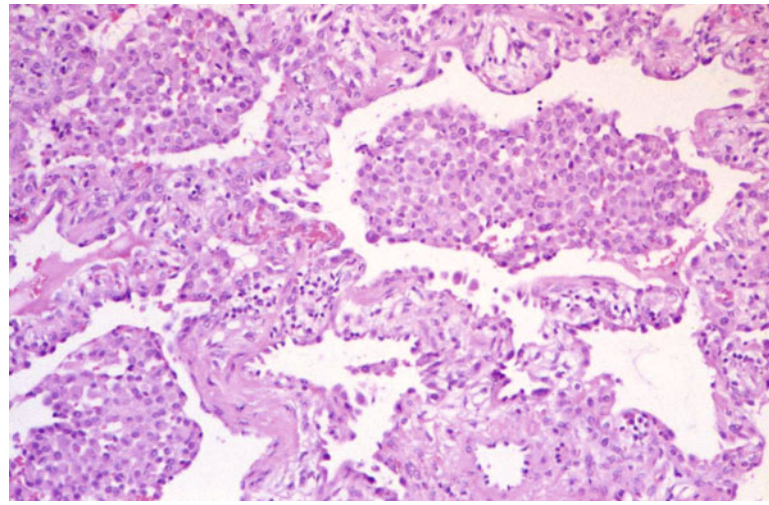

**Fig. 4.9** Hematoxylin-eosin, intermediate magnification. Fibrotic alveolar septa, alveolar spaces showing prominent accumulations of macrophages in a pattern resembling that seen in cases of desquamative interstitial pneumonia

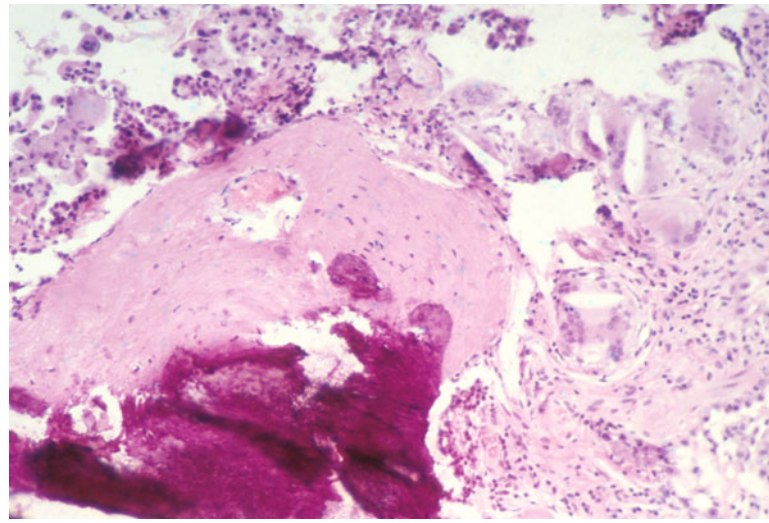

**Fig. 4.10** Hematoxylin-eosin, high magnification. Dendriform pulmonary ossification. Bony spicule with central calcification embedded in fibrotic interstitium

so-called cytoplasmic hyaline has tinctorial and ultrastructural characteristics identical to those observed in the Mallory's hyaline present within hepatocytes in cases of alcoholic hepatitis [97]. This unusual phenomenon is not specific for asbestosis, as once believed, and likely represents a nonspecific reaction to injury [98].

Alveolar macrophages are present in increased numbers in asbestosis and in a minority of cases may so densely pack the alveoli as to mimic the pattern of desquamative interstitial pneumonia (DIP) [8, 14, 19]. Such "DIP-like" reactions (Fig. 4.9) may be observed in other forms of diffuse pulmonary interstitial fibrosis. Dendriform pulmonary ossification is another unusual phenomenon, observed in 2 % of cases in one of the authors' series [99]. This process is characterized by branching spicules of bone, often containing hematopoietic elements, embedded within the pulmonary interstitium (Fig. 4.10). It is thought that this process represents osteoblastic metaplasia involving interstitial fibroblasts [100]. An additional unusual process observed in asbestosis is the occurrence of pulmonary "blue bodies." These basophilic laminated concretions consist primarily of calcium carbonate and are present

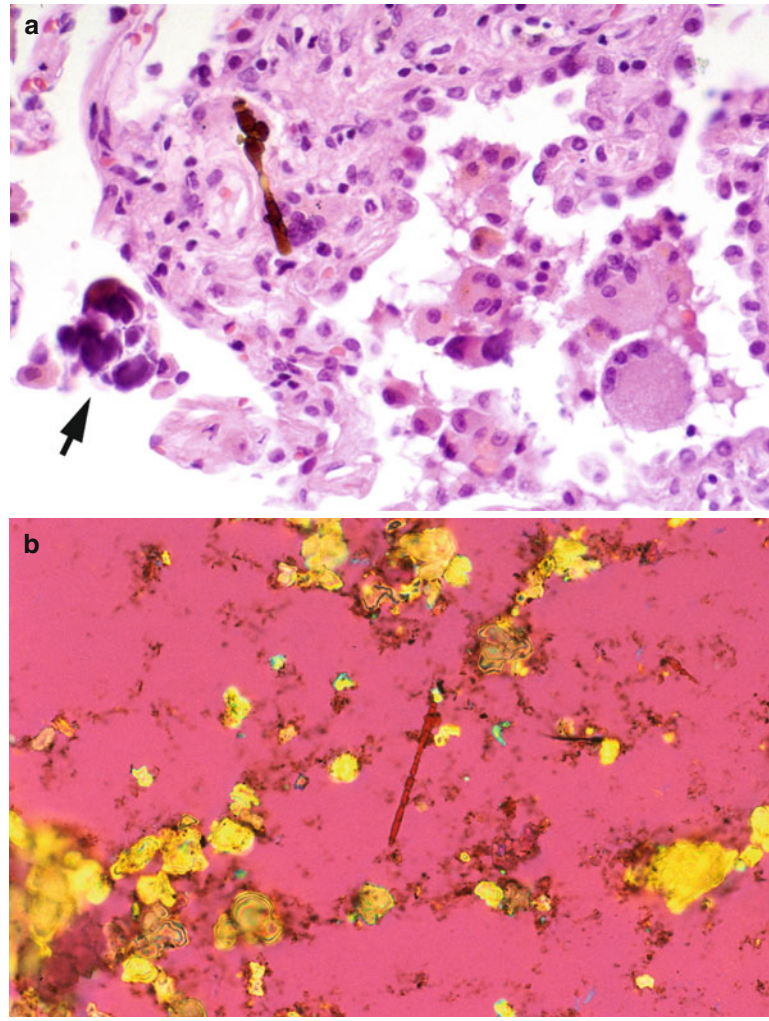


**Fig. 4.11** Hematoxylin-eosin, high magnification. (**a**) Pulmonary "blue bodies" (*arrow*) basophilic, laminated concretions typical of calcium carbonate. (**b**) Filter preparation of tissue digestion showing bright birefringence of "blue bodies" when viewed using polarized microscopy

within alveolar spaces in some 1 % of cases (see Table 4.3). They are not visualized with polarizing microscopy using hematoxylin-eosin-stained sections, but are brightly birefringent in unstained paraffin-embedded sections, or in filter preparations of tissue digests [8, 101] (Fig. 4.11). The mechanism of formation of blue bodies is unknown, but calcium salts have been observed to accumulate in the pulmonary interstitium of experimental animals exposed to aerosolized asbestos fibers (see Chap. 10) [102]. Pulmonary blue bodies are not specific for asbestos exposure and similar to cytoplasmic hyaline likely represent an unusual but nonspecific reaction to injury. These uncommon histologic abnormalities are generally observed in the more advanced cases of asbestosis.

Fungal infection with Aspergillus species is an unusual association shown in asbestosis, perhaps related to suppression of local cell-mediated immunity by asbestos [103]. Hillerdal and Hecksher reported four cases of this unusual association and suggested the infection may be related to anatomical alterations of the bronchial tree or lung parenchyma resultant from asbestos exposure [104]. One of the authors (VLR) has also observed five additional cases (Fig. 4.12), one of which was diagnosed by fine needle aspiration. No other opportunistic fungal infections associated with asbestosis have been reported or observed by the authors.

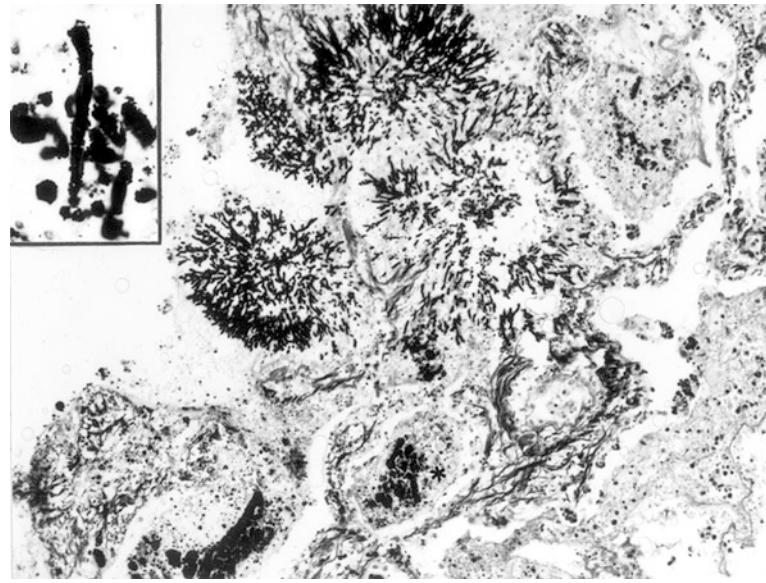

**Fig. 4.12** Methenamine silver, high magnification. Numerous acutely branching septate fungal hyphae present within fibrotic septa and airspaces, asterisk denotes clusters of asbestos bodies, (*inset*) details of clustered asbestos bodies

### 4.6.4 Ultrastructural Findings

Few ultrastructural studies of the lung have been reported in patients with asbestosis.

Shelburne et al. [105] observed that transmission electron microscopy (TEM) is an inefficient way to detect asbestos bodies, even in patients with heavy asbestos tissue burdens, due to the minute volume of tissue examined using this technique. Corrin et al. studied eight cases of asbestosis using TEM and observed a number of ultrastructural abnormalities, especially within the interstitium of the lung. Within the alveolar spaces, excess numbers of alveolar macrophages were observed. There was patchy loss of type I alveolar epithelium, and the thickened alveolar septa demonstrated interstitial edema and collagen deposition [106]. Changes were also observed in the capillary compartment, consisting of endothelial swelling, basement membrane thickening and reduplication. The changes observed were similar to those seen in seventeen cases of idiopathic pulmonary fibrosis, except for a paucity of interstitial inflammatory cells and the presence of asbestos fibers in the patients with asbestosis [106]. There was no ultrastructural evidence of immune complex deposition. From this work, it appears that the parenchymal fibrosis and hallmarks of epithelial and endothelial cell injury noted in asbestosis and the family of idiopathic fibrosing interstitial pneumonitides share common ultrastructural attributes.

## 4.7 Differential Diagnosis

The principal differential diagnostic considerations include common forms of diffuse pulmonary interstitial fibrosis, typically usual interstitial pneumonia (UIP) and the fibrosing variant of nonspecific interstitial pneumonia (NSIP). Similar to UIP, asbestosis typically features lower lung zone and subpleural accentuation, but is that of a temporally uniform and collagenous fibrosis, without the prominent fibroblast foci typical of UIP (Fig. 4.13). Honeycomb change is generally less marked in cases of asbestosis. The temporal and spatial uniformity of the interstitial fibrosis in asbestosis is similar to that of NSIP, but its distribution in the acinus of the lung is different. In contrast to asbestosis, NSIP features a more spatially uniform pattern of fibrosis and may also feature a variable degree of cellular interstitial inflammation [15] (Fig. 4.14).

In addition to the group of idiopathic fibrosing interstitial pneumonitides, asbestosis must also

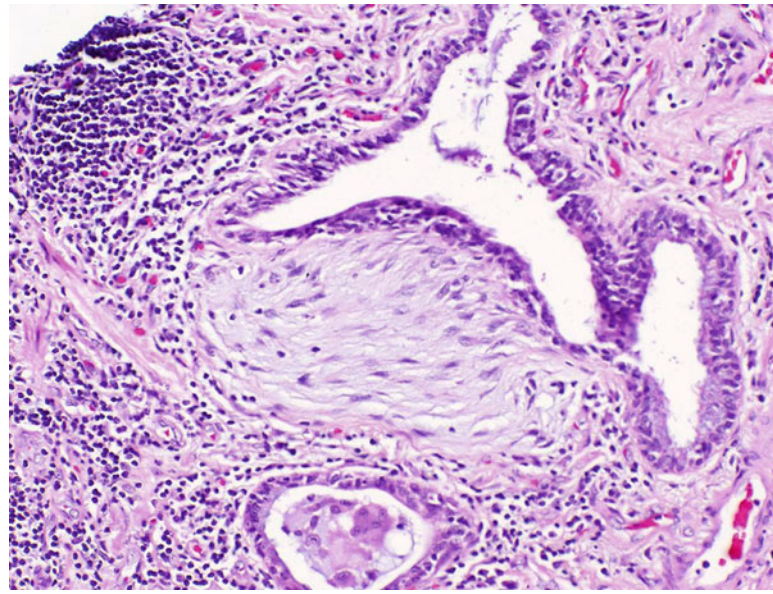

**Fig. 4.13** Hematoxylin-eosin, high magnification. Details of linear fibroblast array, the "fibroblast focus" typically observed in profusion in cases of usual interstitial pneumonia

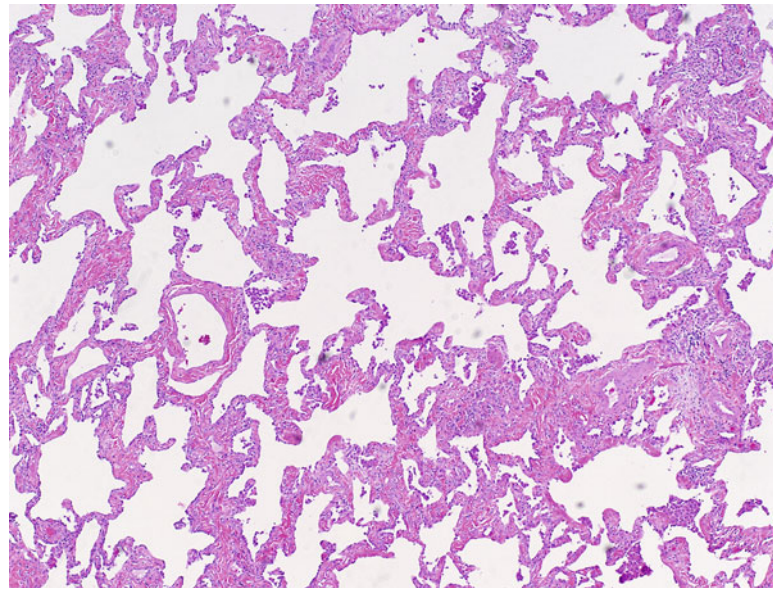

**Fig. 4.14** Hematoxylin-eosin, intermediate magnification. Temporally and spatially uniform septal fibrosis typical of the fibrosing variant of non-specific interstitial pneumonia

be distinguished from the forms of pulmonary injury resultant from inhalation of other dusts. Shipyard workers constitute a group of asbestos workers who may be exposed in the course of their occupation to substantial amounts of silica, talc, or welding fumes in addition to asbestos [2, 18]. Crystalline silica is used in sandblasting and also constitutes one component of the lining of steam boilers in ships. Individuals engaged in sandblasting, boiler scaling, or even working in the vicinity of these operations often exhibit silicotic nodules within the hilar lymph nodes or parenchyma of the lung, especially the upper lobes [2, 18]. Silicosis may be readily distinguished grossly from asbestosis, as it results in a circumscribed nodular pattern of fibrosis, as well as progressive massive fibrosis in complicated cases, as opposed to the irregular linear fibrosis

of asbestosis. The changes of silicosis are most severe in the upper lobes, whereas asbestosis typically involves the lower lobes. Furthermore, silicotic nodules are invariably present in the hilar lymph nodes, and pleural involvement, when present, consists of subpleural nodules up to several millimeters in size [2, 18, 107]. Similarly, shipyard welders are invariably exposed to some asbestos, so that the pathologist must take care to distinguish asbestosis from welder's pneumoconiosis. Welder's pneumoconiosis follows inhalation of metal fumes and is characterized by interstitial deposits of iron oxides, which appear as dark brown to black spherical particles, often featuring golden brown rims. Pseudoasbestos bodies with broad yellow or black cores are frequently seen (see Chap. 3). Welder's pneumoconiosis generally results in little collagen deposition [2, 18], and the presence of substantial amounts of interstitial fibrosis in a shipyard welder should alert the pathologist to the possibility of concomitant asbestosis.

Among 119 cases of welder's pneumoconiosis in shipyard workers from the consultation files of one of the authors (VLR), only 23 cases contained peribronchiolar and alveolar septal fibrosis and true asbestos bodies requisite for the diagnosis of asbestosis. In extreme examples, isolated instances of asbestosis, talcosis, silicosis, and berylliosis have been reported in a single individual [108], and diffuse interstitial fibrosis can follow exposure to a variety of inorganic particulates [2]. In such cases of fibrosis due to dusts other than asbestos, analysis of lung mineral content is invaluable to determine the composition of dust within the lung (see Chap. 11). Another useful feature to raise the index of suspicion for asbestosis is the frequent occurrence of visceral pleural fibrosis and parietal plaques in this group of patients. In the earliest stages of the disease, the diagnosis of asbestosis may be subtle and mimic other forms of mild alveolar septal fibrosis, distinguishable, though, from other entities by the characteristic presence of asbestos bodies in the former.

Asbestosis must also be distinguished from bronchiolitis obliterans organizing pneumonia (BOOP), also referred to as cryptogenic organizing pneumonia (COP). BOOP may appear radiographically as discrete pulmonary masses or infiltrates and mimic carcinoma [19]. BOOP is characterized by serpiginous plugs of loose, young edematous connective tissue within distal bronchioles, filling the alveolar ducts and sacs (Fig. 4.15). These plugs often incorporate clusters of chronic inflammatory cells. Pathologists may encounter this entity in asbestos workers, as have the authors, in view of the increased surveillance given this group related to its increased risk of lung cancer (see Chap. 7) or perhaps an increased predisposition toward the development of organizing pneumonia in this group. A useful diagnostic feature is the tendency for BOOP to occur as a localized process, whereas asbestosis is by definition bilateral and *diffuse*. BOOP is often associated with some degree of fibrotic thickening of alveolar septa and may be accompanied by alveolar type II pneumocyte hyperplasia, so that the diagnostic features of asbestosis may be overshadowed or obscured by superimposed BOOP.

Asbestosis must also be distinguished from iatrogenic pulmonary disease related to the treatment of pulmonary and non-pulmonary malignancies. External beam radiation and cytotoxic chemotherapy may both result in pulmonary interstitial fibrosis. Radiation pneumonitis is usually, but not invariably, confined to the irradiated lung, and may be suspected on the basis of its distribution in any given case [109]. Radiation pneumonitis may feature prominent changes in the vasculature, including thickening and fibrosis of vessel walls and endothelial vacuolization. Pathologic changes related to cytotoxic chemotherapeutic agents are generally more diffuse than is asbestosis and often show atypical alveolar type II pneumocyte hyperplasia as a prominent feature. When pulmonary fibrosis due to the administration of such chemotherapeutic agents is superimposed on asbestosis, it may be necessary to refer to pretreatment radiographs to confirm the diagnosis of asbestosis.

Perhaps the most difficult differential diagnosis is the separation of asbestosis from usual

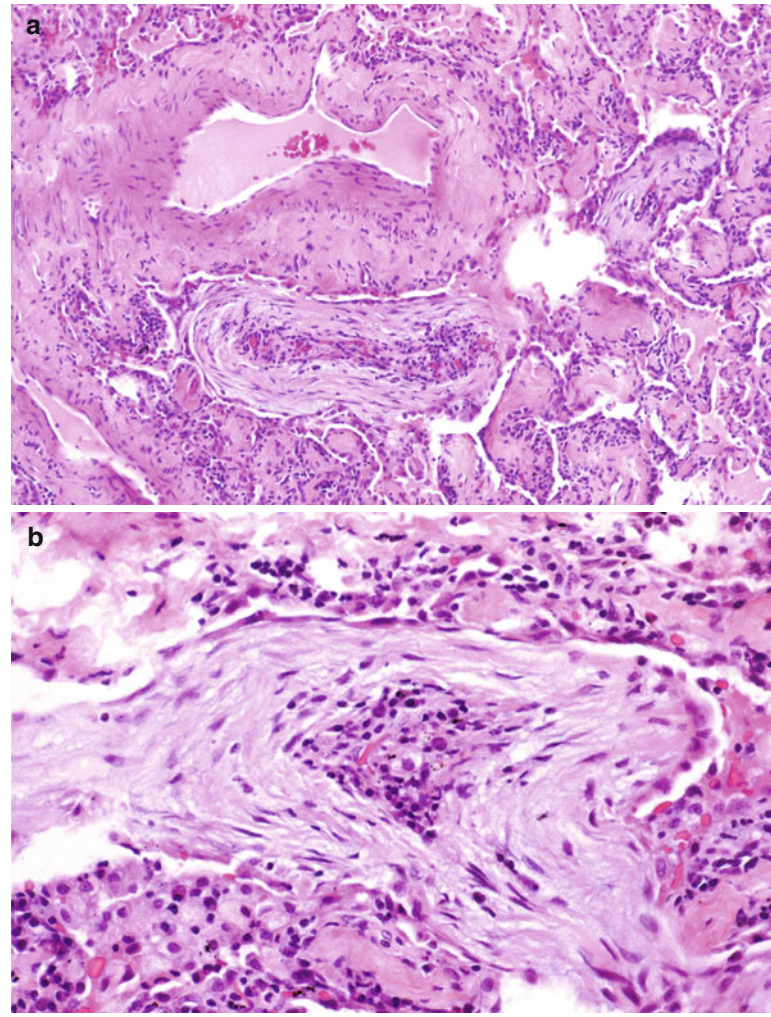


**Fig. 4.15** Hematoxylin-eosin, high magnification. (**a**) Bronchiolitis obliterans organizing pneumonia. Plugs of loose edematous connective tissue within distal bronchioles and alveolar sacs, (**b**) some plugs contain central cores of inflammatory cells

interstitial pneumonia in the patient with a history of asbestos exposure. As discussed in the foregoing sections, there are gross and microscopic findings common to both entities, particularly in late-stage disease. It seems only reasonable, though, in cases where the history of asbestos exposure is not compelling, and diagnostic material shows interstitial fibrosis but no asbestos bodies, to diagnose idiopathic fibrosing disease rather than asbestosis. The reasons for this are discussed in greater detail below.

Some investigators have suggested that transbronchial biopsy may be useful in the diagnosis of asbestosis [110, 111]. In general, transbronchial biopsies share the same profound limitations and inadequacies in the diagnosis of asbestosis as they do in cases of the diffuse and fibrosing interstitial pneumonitides. Rare transbronchial biopsies have demonstrated interstitial fibrosis and asbestos bodies, allowing the diagnosis to be made in concert with review of radiographic findings. Transbronchial biopsy is therefore viewed as an inadequate diagnostic study in the majority of cases [9, 14], and generous sampling of open or surgically obtained lung tissue, or autopsy material, is normally required to make the subtle and intricate histologic distinctions as outlined above.

## 4.8 Assessment of Diagnostic Criteria

The histologic diagnosis of asbestosis is of considerable importance, as it provides confirmation of the presence, or absence, of a fibrosing interstitial lung disease related to the inhalation of asbestos-containing dust. The Asbestosis Committee of the CAP and Pulmonary Pathology Society recommend that a diagnosis of asbestosis may be given only when there is an acceptable pattern of alveolar septal fibrosis and an average of two asbestos bodies/cm$^2$ of lung. The finding of asbestos bodies alone does not suffice for the histologic diagnosis and is only indicative of asbestos exposure. The requisite demonstration of fibrosis can be difficult when there is atelectasis, vascular congestion, or consolidation with pneumonia, and care should be taken not to overinterpret such sections as showing interstitial fibrosis [112]. The examination of Masson's trichrome-stained sections is indicated to evaluate presence and extent of fibrosis in those cases where its presence is not straightforward on routine sections. Ferruginous bodies in histologic sections should be examined carefully so that pseudoasbestos bodies, described above, are not mistaken for true asbestos bodies. In those cases where asbestosis is suspected, but asbestos bodies are not readily detected on routine sections, it is recommended that iron-stained sections be prepared and examined systematically and in their entirety at 200× magnification using a mechanical stage [113, 114]. Using this approach, several asbestos bodies should be observed in most 2×2-cm sections of lung parenchyma in cases of bona fide asbestosis (see Chap. 11). Since asbestos bodies are not always evenly distributed in histologic sections [14, 114], more than one iron-stained section should be examined when asbestos bodies are sparse.

The identification of asbestos bodies in histologic sections as a diagnostic prerequisite for asbestosis has not gone unchallenged. Arguments against such a requirement have included the observation that chrysotile forms asbestos bodies poorly in contrast to the amphiboles, and many asbestos workers are primarily exposed to chrysotile [115]. Secondly, there is great individual variability with regard to the efficiency in which inhaled fibers are coated to make asbestos bodies, with some individuals poorly capable of doing so [116, 117]. Holden and Churg have shown, however, that in at least one population exposed exclusively to chrysotile ore, i.e., chrysotile miners, individuals with asbestosis do have asbestos bodies in histologic sections, and these bodies contain chrysotile cores [118]. The second argument becomes problematic over time, as the number of cases with overt asbestosis decreases due to diminishing numbers of survivors of the heavy asbestos exposures of the past. Given the concomitant and necessary increase in the proportion of cases with idiopathic pulmonary fibrosis, any reduction in the diagnostic requirement for asbestos bodies in tissue sections would greatly reduce the specificity of the histologic criteria.

While it has been our observation that those patients lacking asbestos bodies in histologic sections typically have uncoated fiber levels well below those observed in cases of bona fide asbestosis, a common problem in referral clinical practice arises when asbestosis is suspected on the basis of exposure history or other clinical grounds, but no asbestos bodies are identified in sampled fibrotic lung tissue, even following performance of iron stains. To this end, specialty laboratories are often contacted to perform analytical testing of lung tissue to measure and quantify asbestos fiber burdens. Roggli et al. have shown in patients lacking asbestos bodies in histologic sections, an uncoated asbestos fiber burden well below that observed in cases with bona fide asbestosis is to be expected [119]. The fiber counts of these cases were compared with those measured from a series of autopsy cases with histologically confirmed asbestosis. Linear regression analysis showed that the fiber content of the cases of diffuse interstitial fibrosis of unknown cause fell below the 95 % confidence limit in every case, and the majority of fibers analyzed in these cases were not asbestos [119]. Gaensler et al. also showed when asbestos bodies

are identified in tissue sections in patients with interstitial lung disease and a history of asbestos exposure, high uncoated fiber burdens will be found on tissue analytical studies. In those cases where no asbestos bodies are identified, uncoated fibers are not elevated beyond those observed in control populations [83]. The authors also further evaluated cases of diffuse pulmonary interstitial fibrosis with asbestos exposure but whose biopsies did not meet established criteria for asbestosis and compared their respective fiber burdens with those of confirmed asbestosis cases [120]. Of 86 cases, seven had asbestos body counts within the 95 % predicted interval for asbestosis, but only rare cases had commercial amphibole fiber levels within the 95 % prediction interval. In view of these studies, we explain to the requestor that in the absence of asbestos bodies on histologic sections, the diagnosis of asbestosis and the detection of tissue levels of asbestos typical of asbestosis are both unlikely [120]. A negative review of multiple areas of iron-stained sections should obviate the need for tissue digestion studies; a positive review of such remains the most rapid and cost-effective means of confirming the diagnosis. This would indicate that, in the cases of diffuse pulmonary fibrosis studied, most cases did not contain asbestos fibers within the range typically observed for asbestosis. In addition, a history of asbestos exposure alone is not sufficient for a diagnosis of asbestosis in this setting [15]. Accordingly, in cases of pulmonary fibrosis where asbestosis is suspected on a historical basis, but no asbestos bodies are demonstrated, despite careful histologic examination of an adequate specimen, electron microscopy for asbestos fiber analysis is not recommended and unnecessary [15]. In such instances, the diagnosis of a non-asbestos-associated form of pulmonary fibrosis is quite permissible.

In common practice, fiber burden analysis cannot substitute for or overrule the histologic diagnosis of asbestosis [15]. Until uniform methodology of analysis of tissue mineral fiber burden is implemented, it is impractical to recommend a specific tissue asbestos fiber content as a diagnostic criterion for asbestosis [8]. Nonetheless, the analysis present in Chap. 11 indicates that clinically significant interstitial fibrosis is unlikely to be the result of asbestos exposure when there are fewer than one million fibers 5 μm or greater in length per gram of dry lung tissue. The fibrogenicity of fibers in this size range is well established [15, 121–124], whereas that of fibers less than 5 μm remains unproven [12] (see Chap. 10). Therefore, no tissue level of fibers in that range can be used at present for a diagnosis of asbestosis [8].

## 4.9 Grading Scheme

It is generally sufficient for pathologists to incorporate gross and microscopic features to estimate the extent of fibrosing or destructive processes within the lung and to term such extent as mild, moderate, or severe. It is also possible using proposed histologic grading systems to assess the extent of asbestosis in a semiquantitative manner and augment the qualitative descriptors [125–128]. One such system is that proposed by the College of American Pathologists (CAP) and the National Institute for Occupational Safety and Health (NIOSH) in 1982 [14]. The inter- and intra-observer variability for pathologists using this scheme was assessed, and the application of the criteria to a set of cases was found reasonably reproducible. Universal application of such a scheme would be highly desirable for epidemiologic studies, as well as for comparison with established radiologic schemes for classification of pneumoconiosis (see earlier section on Radiographic Findings). It should be noted that the diagnosis of asbestosis must be established using the criteria outlined in the previous section prior to any attempt at grading the disease. Accurate histologic grading depends on adequate tissue sampling, preferably the examination of sections obtained from the central and peripheral regions from each lobe of both lungs [14]. The limitations of sampling should be recognized: grading should be possible on thoracoscopically obtained lung tissue, whereas a transbronchial biopsy specimen is not sufficient. The grading

scheme of CAP-NIOSH includes scores for both severity and extent of disease [14]. A score for each of these parameters is determined for each slide examined, the two values multiplied to give a single value for each slide, and the individual values obtained for each slide averaged to give an average histologic grade for each case.

Grading of severity is as follows:

Grade 0 = No peribronchiolar fibrosis

Grade 1 = Fibrosis confined to the walls of respiratory bronchioles and the first adjacent tier of alveoli

Grade 2 = Fibrosis extending to involve alveolar ducts, or two or more tiers of alveoli adjacent to the bronchiole, with sparing of some alveoli between adjacent bronchioles

Grade 3 = Fibrotic thickening of the walls of all alveoli between at least two adjacent respiratory bronchioles

Grade 4 = Honeycomb changes (see earlier Sect. on 4.6.3.2)

Grading of the extent of disease is classified according to the percentage of bronchioles showing excessive peribronchiolar connective tissue:

Grade A = Only occasional bronchioles involved

Grade B = More than occasional involvement, but less than half

Grade C = More than half of all bronchioles involved by fibrosing process

This scheme allows 12 possible grades for each slide. However, practical application of this scheme by the authors indicates that certain combinations occur rarely or not at all. Virtually all cases with grades 3 or 4 severity show grade C profusion on the same slide, as do most cases with grade 2 severity. Furthermore, if one restricts the diagnosis in cases with grade 1 severity to those in which most of the bronchioles are involved (see earlier Sect. on 4.8), then one is left with only four grades of severity to consider. We recommend the adoption of this simplified version of the CAP-NIOSH grading scheme [14], summarized in Table 4.4 and illustrated in Fig. 4.16. Others have proposed a similar scheme and applied it to experimental models of asbestosis [126]. In view of reasonable inter- and intra-observer concordance obtained with the more extensive scheme [14], similar if not better concordance is expected with the modified and simpler version.

**Table 4.4** CAP-NIOSH asbestosis grading scheme*

| | |
|---|---|
| Grade 0 | No fibrosis associated with bronchioles |
| Grade 1 | Fibrosis involves wall of at least one respiratory bronchiole with or without extension into septa of first adjacent tiers of alveoli |
| Grade 2 | Fibrosis in grade 1 lesions, plus involvement of alveolar ducts and two or more tiers of alveoli adjacent to the bronchiole with some spared alveoli between adjacent bronchioles |
| Grade 3 | Fibrosis as in grade 2 lesions, plus fibrotic thickening of the septa of all alveoli between at least two adjacent bronchioles |
| Grade 4 | Fibrosis as in grade 3 lesions, plus the formation of honeycomb changes (formation of cyst-like spaces larger than an alveolus which may be epithelialized) |
| Grading of the *extent* of disease is classified according to the percentage of bronchioles showing excessive peribronchiolar fibrosis | |
| Grade A | Only occasional bronchioles involved |
| Grade B | More than occasional involvement, but less than half |
| Grade C | More than half of all bronchioles involved by fibrosing process |

Source: Modified from the scheme presented in Ref. [14]
*An average score is obtained for an individual case by adding the scores for each slide (0–4), then dividing by the number of slides examined

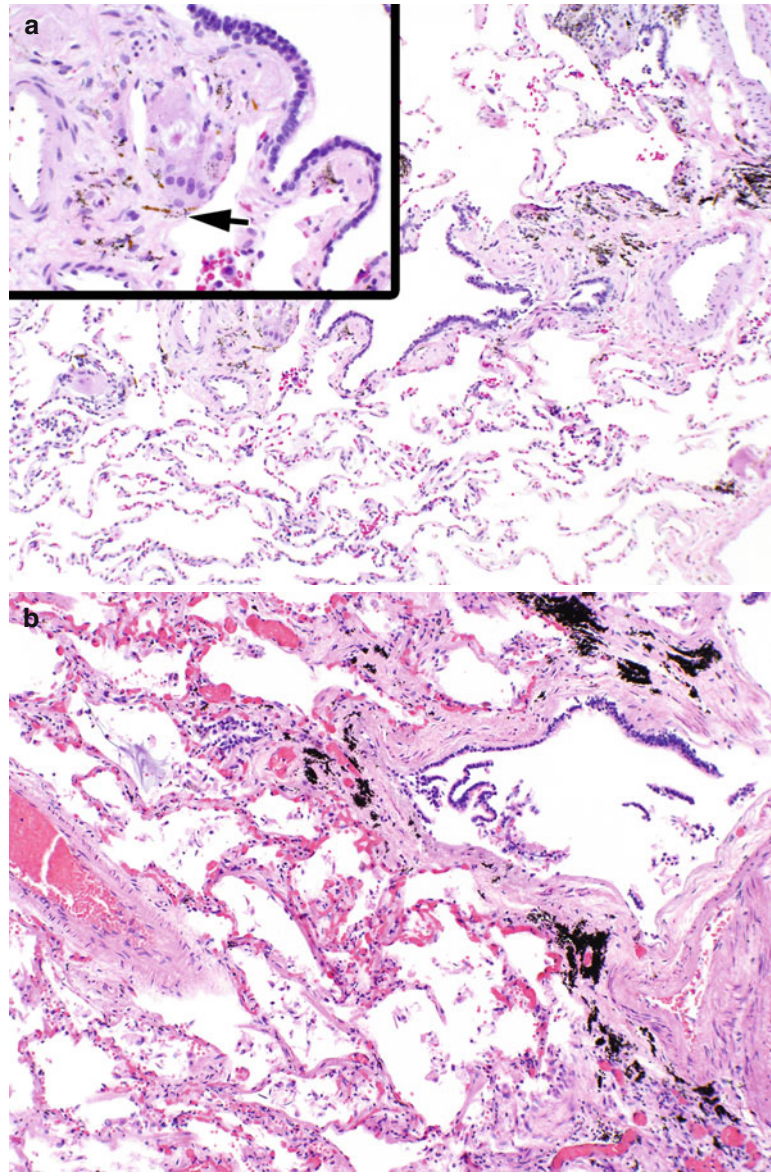


**Fig. 4.16** Series of photomicrographs illustrating proposed grading scheme for asbestosis, outlined in Table 4.4. (**a**) Grade 1 asbestosis with fibrosis involving bronchiolar wall and extending into first tier of peribronchiolar alveoli. Asbestos body at *inset*. (**b**) Grade 2 asbestosis with fibrosis involving more distant alveolar septa, but sparing some such alveoli. (**c**) Grade 3 asbestosis with fibrosis involving all alveoli between adjacent bronchioles. (**d**) Grade 4 asbestosis, honeycomb changes with cystic spaces lined by bronchiolar epithelium present in dense parenchymal fibrosis. Hematoxylin-eosin, intermediate-high magnification

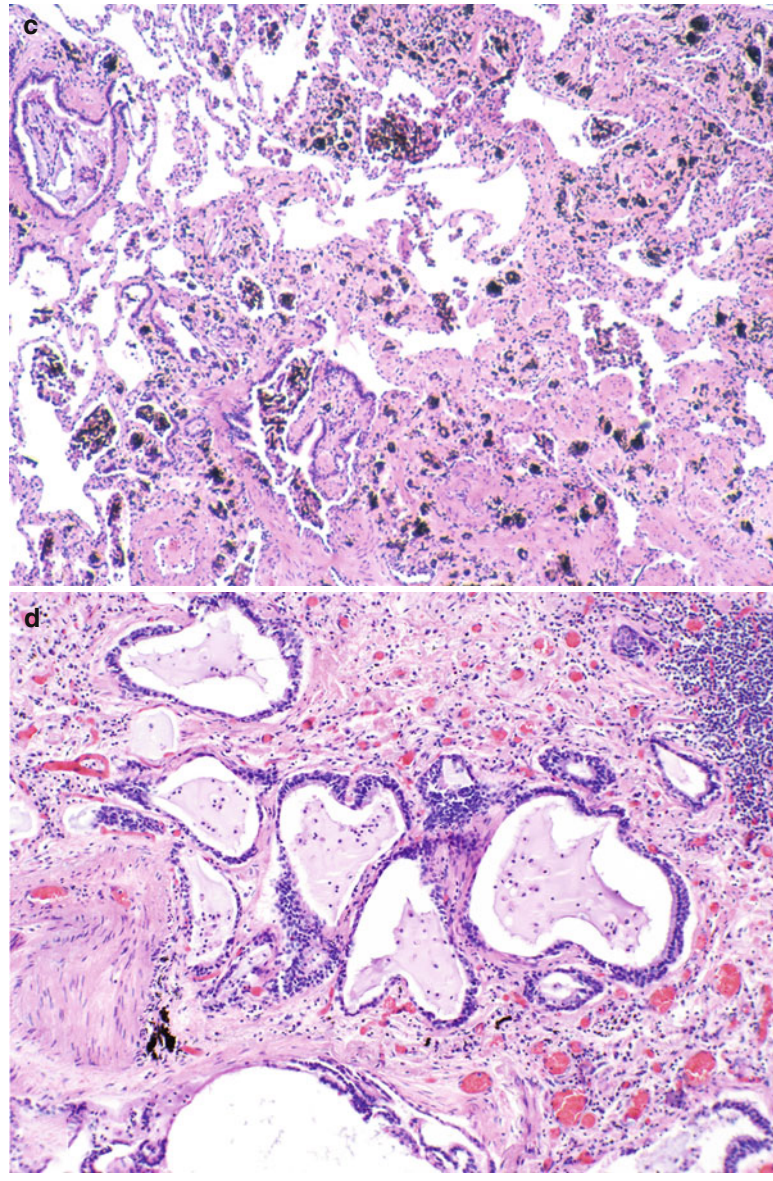


**Fig. 4.16** (continued)

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# Mesothelioma

# 5

Elizabeth N. Pavlisko and Thomas A. Sporn

## 5.1 Introduction

*Mesothelioma*, literally "tumor of the mesothelium," is a term often used synonymously with *malignant* (diffuse) *mesothelioma*, the malignant neoplasm arising from the serosal linings of the pleural, pericardial, or peritoneal cavities. These major body cavities are lined by a single layer of flattened to cuboidal cells of mesodermal origin that constitute the mesothelium proper [1]. This serosal membranous lining includes not only the mesothelium but also the underlying basement membrane, a matrix of elastic fibroconnective tissue containing lymphatic and vascular channels, and scattered mesenchymal cells as well. Mesothelial cells possess a complex cytoskeletal network of intermediate filaments, produce hyaluronic acid, and have distinctive ultrastructural features including numerous pinocytotic vesicles and long surface microvilli that project into the serous cavities (Fig. 5.1) [2, 3]. It remains uncertain whether mesothelioma results from the malignant transformation of the differentiated mesothelial cell or from more primitive progenitor cells, such as the submesothelial mesenchymal cell, or from both [4].

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Malignant (diffuse) mesotheliomas are rare neoplasms, with estimated incidence in North America of 15–20 cases per million persons per year for men, with a much lower incidence in women of two to three cases per million persons per year [5–10]. There is some suggestion that the incidence in North American men is decreasing following its peak in the 1990s [11]. In other regions, the experience is different and the incidence of mesothelioma continues to climb, for example, in Australia [12]. Malignant mesothelioma's rarity combined with its strong association with asbestos exposure makes it a signal malignancy, i.e., an epidemiologic marker for exposure to asbestos [7]. The mechanism whereby asbestos induces mesothelioma is not completely understood. This mechanism is reviewed in detail in Chap. 10, and the results of quantitative tissue analysis for asbestos content in cases with malignant mesotheliomas compared with those of other asbestos-related disorders and with normal controls in Chap. 11. The present chapter reviews the pathologic features of malignant mesothelioma, the means to distinguish mesothelioma from other conditions with which it may be confused, and the agents implicated in its etiology. A certain amount of confusion exists with regard to benign tumors of the serosal membranes, which have also been variously termed fibrous mesothelioma, localized fibrous tumor, and solitary fibrous tumor. These rare tumors have not been convincingly shown to be asbestos related and are reviewed elsewhere [2, 13].

T.D. Oury et al. (eds.), *Pathology of Asbestos-Associated Diseases*,
DOI 10.1007/978-3-642-41193-9_5, © Springer-Verlag Berlin Heidelberg 2014

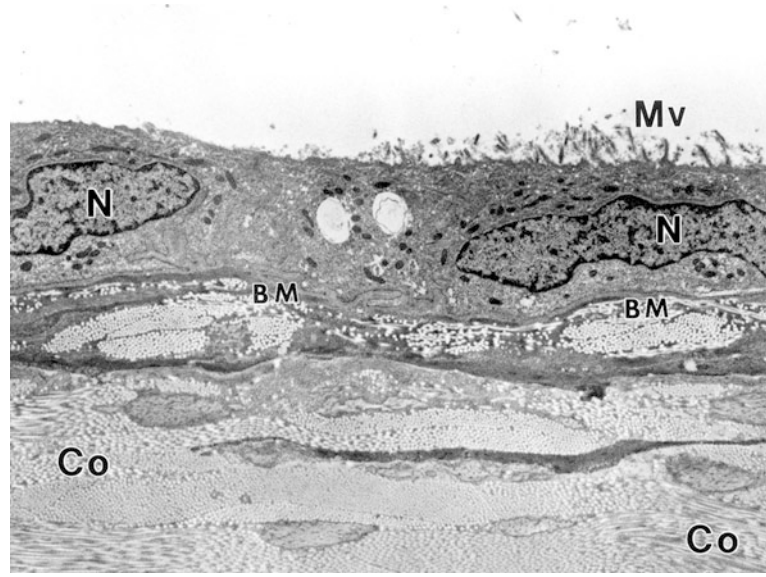


**Fig. 5.1** Transmission electron micrograph of normal mesothelium. Note flattened mesothelial cells, one of which has long surface microvilli (*Mv*). *BM* basement membrane, *N* nucleus, *Co* collagen. Magnified ×3,430 (Reprinted from Ref. [2], with permission)

## 5.2 Historical Background

Mesotheliomas are uncommon tumors, accounting for less than 1 % of cancer deaths worldwide [14], with few descriptions in the literature until the twentieth century. In 1767, Lieutand described two pleural tumors in a series of 3,000 autopsies that may have been mesotheliomas [15]. Wagner's detailed descriptions published in 1870 [16] leave little doubt that he was describing what we now recognize as malignant pleural mesothelioma [17, 18]. In 1924, Robertson reviewed earlier reports in the literature and concluded that only sarcomatous tumors could be regarded as primary pleural malignancies and that tumors with epithelial morphology represented metastases from other and possibly occult sites [19]. Klemperer and Rabin described in 1931 a series of five pleural tumors, four of which were localized and one diffuse [20]. These investigators separated the localized tumors of the pleura from the diffuse pleural malignancies and used the term *mesothelioma* to refer to the entire histologic spectrum of epithelial and spindle cell primary malignancies that diffusely involve the pleura [17, 18]. By the 1950s, growing numbers of diffuse primary peritoneal tumors were recognized, and malignant mesothelioma became generally accepted as a distinct clinicopathologic entity [21].

The British pathologist Gloyne is credited with the first description in 1933 of pleural malignancy in an individual occupationally exposed to asbestos [22]. This report referred to a case of "squamous carcinoma of the pleura," which the author did not believe was related to the patient's asbestosis [23]. Reports from Germany in 1943 by Wedler [24] and from the USA in 1947 by Mallory [25] describing further cases of pleural malignancy associated with asbestos exposure followed subsequently. Additional reports appeared in the 1940s and 1950s [22, 26], and by 1960 Keal had described the association between peritoneal mesotheliomas and asbestos exposure [27]. Any remaining doubt concerning the association between asbestos exposure and mesothelioma was dispelled by Wagner in 1960 [28]. This classic study described 33 cases of diffuse pleural mesothelioma occurring in the Northwestern Cape Province of South Africa, in which 32 cases had a documented exposure to asbestos. In some instances, the patient's only exposure was living in proximity to an asbestos mine. Since 1960, numerous studies have appeared in the literature confirming the association between asbestos exposure and malignant mesothelioma of the pleura, peritoneum, and pericardium [2].

## 5.3 Etiologic Considerations and Epidemiology

### 5.3.1 Asbestos

The association between mesothelioma and asbestos exposure is undisputed. Following Wagner's study of mesothelioma subsequent to environmental and occupational exposure to crocidolite [28], epidemiologic and case-control studies from many industrialized nations have documented rising rates of malignant mesothelioma following the heavy commercial use of asbestos [29–46]. A large proportion of cases in these studies have derived from shipyard workers [29, 35] and insulators [47, 48], where large numbers of workers had heavy exposures. Other occupational exposures to asbestos including those sustained by miners and millers [28, 36, 49], railroad machinists and steam locomotive repair workers [50, 51], and workers in asbestos factories have resulted in appreciable numbers of cases [52].

Two of the editors (VLR/TAS) undertook a study of 1,445 cases of mesothelioma with known exposure histories, 268 with tissue asbestos fiber burden analyses [53]. They found that these cases were classifiable into 23 predominant occupational or exposure categories and that 94 % of cases had exposures in one or more of 12 different industries, six occupational categories, or one nonoccupational exposure. The industry with the largest number of cases was shipbuilding, followed by service in the US Navy, the construction industry, and the insulation industry. The occupation with the largest number of cases was pipefitter (including welders), followed by boiler workers, maintenance workers, machinists, and electricians. As many individuals worked at more than one type of job, an exposure in some additional occupational setting was observed in 26 % of cases. The nonoccupational group with the largest number of cases was household contact of asbestos workers. Household contacts are known to sustain exposure by contact with contaminated clothing or personal effects of the asbestos worker, and epidemiologic studies have shown an increased risk of mesothelioma among this exposure group [5, 54–60]. Bourdès et al. reported an eightfold increase in relative risk (95 % CI, 5.8–12) of malignant mesothelioma among cases with household asbestos exposure [59], and Magnani et al., in a multicenter study, reported an odds ratio of 4.81 (95 % CI, 1.8–13.1) for malignant mesothelioma among cases with a moderate to high probability of domestic exposure [60].

Environmental or neighborhood exposures have also been described, sustained by those living in the vicinity of asbestos factories or mines [28]. A number of mesothelioma cases have been reported in Italy in neighborhoods of asbestos-cement plants among individuals who were never employed in the plant. The risk of developing mesothelioma was found to be inversely proportionate to the distance of residence from the cement plant [61, 62]. Barbieri et al. assessed lung tissue fiber burden in eight cases of mesothelioma (five men and three women) from such an area with no "definite" or "probable" asbestos exposure. Four of eight had history of possible asbestos exposure. In all cases, lung tissue fiber burden was elevated above reference range and did show some overlap with occupational exposed individuals [63]. Kurumatani and Kumagai set to map the risk of mesothelioma secondary to neighborhood exposure from a cement plant in Amagasaki City (Japan). There were 90 cases of mesothelioma with no occupational exposure to asbestos in which the affected individual lived within 1,500 m from the asbestos-cement plant. The greatest increase in standard mortality ratio (SMR) was among women who lived within a 300-m radius of the plant. Interestingly, a grid was mapped over the region surrounding the plant, and all 90 mesothelioma cases were placed according to the location of residence. Mesothelioma cases occurred more often in the south to southwest location which corresponded to the predominant direction of wind movement across the area [64]. Cases of environmental exposure have also been described in Libby, Montana, where vermiculite contaminated with the Libby amphibole was mined (vide infra), as well as in the town of Wittenoom, Australia, where crocidolite was mined [65–67].

Case and Abraham describe the free use of material containing asbestos in the Westbank region of Louisiana which was in close proximity to asbestos manufacturing plants. Materials were used residentially in substitute of concrete and with time became a friable health hazard. By the early twenty-first century, this region was reported to have the highest mortality rate among high-rate counties in the United States [68]. Pan et al. described mesothelioma risk correlating with proximity of residence to mineral deposits likely to contain asbestos in California [69].

Some unusual exposures to asbestos associated with the development of mesothelioma have been documented, including the manufacture of asbestos-containing cigarette filters [70] or the preparation of silver jewelry and ceremonial clothing by members of a Native American pueblo [71]. Mesothelioma has been reported among individuals exposed as children, whose diapers were made from cotton sacks previously used to transport asbestos insulation [72]. Marchevsky et al. set to establish evidence-based causation guidelines for cases of mesothelioma in individuals with nonoccupational asbestos exposure. The authors undertook an extensive search of the literature looking for cases of nonoccupational asbestos exposure where the source of exposure was identifiable and no additional exposures were confounding factors. They proposed four tiers of "evidence-based causation guidelines" based on (1) fiber analysis data, (2) the type of nonoccupational asbestos exposure (household contact of an asbestos worker, asbestos building occupant, environmental exposure, etc.), (3) duration of exposure and tumor location (pleural versus peritoneal), and (4) the frequency with which such exposures were described in the literature [73].

Mesothelioma is overwhelmingly a disease affecting men, reflecting the predominance of men in those occupations and industries most commonly associated with asbestos exposure. In North America, it has been reported that greater than 90 % of mesotheliomas in men are related to asbestos exposure while only 20 % are in women [74]. The rates of mesothelioma in men increased into the 1990s and may be starting to decline, the rates in women have remained relatively constant [6–11]. A geographic influence is also noted in North America, with the coastal areas housing the shipbuilding industries of the World War II era having the highest rates [75].

A prolonged *latent interval*, the period of time between initial exposure and the manifestation of disease, is typical of most asbestos-associated illnesses, and mesothelioma is not an exception. The latent interval for mesothelioma is measured in decades, peaks at 30–40 years postexposure, and may extend to 70 years postexposure [47, 76, 77]. The latent interval is virtually never less than 15 years [9] and, when claimed in any particular case, merits the search for evidence of more remote exposure [78]. Mesothelioma tends to be a disease of those in the seventh or eighth decades of life in keeping with the long latent interval. An inverse relationship between dose or level of exposure and latent interval is suggested, as we have observed the development of mesothelioma at a significantly younger age in insulators as compared to other asbestos workers [53].

The risk for the development of mesothelioma appears to increase dramatically with time from initial exposure. Peto et al. have examined this relationship mathematically and found that the available data are best explained by a model in which the mesothelioma risk increases with the third or fourth power of time from first exposure. These investigators also concluded that there is a linear dose-response relationship between the amount of asbestos to which an individual is exposed and the risk of developing mesothelioma [79]. A threshold level of exposure below which mesothelioma will not occur has not yet been identified [80]. Peritoneal mesotheliomas, historically comprising some 30 % of all cases, have fallen in proportion to approximately 10 % of cases as the incidence of pleural mesotheliomas has risen. Peritoneal mesotheliomas are associated on average with heavier and/or more prolonged exposure to asbestos [2, 47, 81], as evidenced by their frequency in the cohort of insulators [47, 49, 82, 83] who tend to have the highest tissue fiber burdens. However, a similar latency period for both pleural and peritoneal forms is observed [79]. The association between peritoneal mesothelioma and higher degrees of

exposure is supported by the observation of the clinical diagnosis of asbestosis in 50 % of male patients with peritoneal mesotheliomas but in only 20 % of patients with pleural mesothelioma [54]. More recent data from the WHO mortality database from 1994 to 2008 demonstrated mesothelioma of the peritoneum to be twice as common in females versus males [84] and Larson et al. reported it to be triple the incidence in men (14.8 % versus 5.4 %) [85]. Our fiber analysis data suggests that malignant peritoneal mesothelioma in women is less likely to be attributed to asbestos exposure.

There are marked differences in the potential for various types of asbestos fibers to produce mesothelioma. While amosite is the most common fiber type associated with mesothelioma among US workers [53, 86], crocidolite appears to pose the greatest risk among the commercially available species, followed by amosite [87–92]. Schneider and two of the editors (VLR/TAS) reviewed 299 fiber analysis cases where either crocidolite or amosite or both were present. From 1994 forward, there were an increasing number of cases in which crocidolite was present, likely secondary to the decrease in amosite usage since the 1970s and continued use of crocidolite up until the 1990s [93]. Whereas the epidemiologic association between exposure to commercial amphibole asbestos is indisputable, the mesotheliogenic potential of chrysotile has been much debated. The controversy surrounding chrysotile is multifaceted, influenced by the decreased biopersistence of the mineral in lung tissue and the frequent presence of its natural contaminant, the noncommercial amphibole form of asbestos tremolite [94–96].

It is sometimes difficult to gauge the degree and type of asbestos exposure for any given worker, and indeed mesotheliomas have developed in workers in some plants believed to utilize only chrysotile asbestos, who upon analysis have been shown to contain amphibole fibers in their lungs [97–99]. For example, Loomis and Dement report that in a cohort of North Carolina textile plant workers, there were four cases of mesothelioma, all of which worked in a plant that used only chrysotile asbestos [100]. We have performed fiber analysis on one of the four mesothelioma cases, a woman spinner/winder/weaver who developed pleural mesothelioma, and amphibole asbestos was present in her lung tissue. Interestingly, she was the wife of an insulator [5].

Furthermore, individuals with mesothelioma who are exposed to chrysotile through the milling and mining of asbestos have more tremolite than chrysotile in their lung tissue, even though the contaminant accounts for only a fraction of a percent of the chrysotile ore [96, 101]. This observation has led some investigators to suggest that it is the contaminating tremolite that is responsible for the increased risk of mesothelioma in miners and millers of chrysotile. Several studies have shown that environmental exposure to tremolite asbestos can result in an increased risk for developing mesothelioma, particularly in instances where the tremolite fibers have a high aspect ratio (i.e., ratio of length to diameter of fiber) [102]. In this regard, it is of interest to note that another noncommercial form of asbestos, anthophyllite, featuring broad fibers with low aspect ratio, has only rarely been implicated in the causation of human mesothelioma [55, 103]. Yarborough reports the number of mesothelioma cases in cohorts exposed to pure chrysotile asbestos (uncontaminated by amphibole asbestos) to be very low or zero. Of 14 cohorts which described exposure to chrysotile-only asbestos, there were seven cases of mesothelioma out of 32,000 subjects. While this is above the presumed background rate of mesothelioma, confounding factors could not be excluded [104]. No cases of mesothelioma have been reported with environmental exposure to South African chrysotile [105]. There is evidence in animal studies that chrysotile causes mesothelioma [106]; however, the chrysotile dose and life span of the animal do not translate to the effect of chrysotile in humans [104, 107, 108]. In our opinion, the evidence that pure chrysotile, uncontaminated by amphibole asbestos, results in the development of mesothelioma in humans is limited and low-level exposures are unlikely to increase such risk [104, 109].

Mesothelioma is thought to originate in the parietal pleura. Asbestos fibers may reach the

pleural surface via direct penetration following inhalational deposition in the respiratory bronchioles or via the lymphatics. Asbestos is a powerful mesothelial carcinogen, capable of inducing DNA damage alone or in concert with reactive oxygen species produced by inflammatory cells. Nonetheless, only a small fraction (10 % or less) of asbestos workers will develop mesothelioma [47, 54, 110]. In our own studies, approximately 16 % of mesotheliomas have a lung asbestos content indistinguishable from background (see Chap. 11 for more detail), and perhaps 10–20 % of cases are not due to asbestos exposure [111]. The size of the exposed population at risk for mesothelioma and the relative rarity of the disorder suggest variable individual susceptibilities, possibly genetically mediated. The observation that a substantial proportion of patients with malignant mesothelioma have no identifiable exposure to asbestos has led investigators to look for other potential etiologic or predisposing factors. These are reviewed in the following sections.

### 5.3.2 Zeolites

The discovery of an epidemic of malignant pleural mesothelioma in two villages in the Cappadocian region of Turkey [112] has prompted the current interest in the pathologic effects of zeolites [2]. The small villages of Karain and Tuzkoy are situated in a region whose caves and volcanic tuffs are rich in fibrous erionite, a hydrated aluminum silicate belonging to the family of zeolite minerals, and provide stones for dwellings there. In this area of Turkey where asbestos is ordinarily found in the volcanic terrain and construction materials containing tremolite asbestos are widely used, malignant mesothelioma attributable to environmental exposure to tremolite asbestos has been well documented [113, 114]. However, the high incidence of mesothelioma in these two villages could not be explained by environmental asbestos alone. The excess incidence of mesothelioma in these villages is believed to be attributable to erionite [115], whose fibers have been recovered from the lungs from some of the cases of mesothelioma in this area [116], although some asbestos fibers have been identified as well [117]. Erionite has physical characteristics and dimensions closely resembling amphiboles, and in experimental animal studies, a high rate of mesothelioma has been induced following intrapleural or intraperitoneal injection [118, 119]. Other studies have shown lower rates of mesothelioma induction following administration of erionite in a rodent model [120]. These variances are possibly attributable to different geographic sources of erionite used in the experimental studies. It is of interest that in the villages of Karain, Tuzkoy, and Sarihidir, a significant proportion of the villagers do not develop mesothelioma, and no other association with malignancy in this population has been demonstrated. A genetic predisposition toward the development of mesothelioma in families of these villages has been posited as in some families up to 50 % develop mesothelioma [121, 122]. Outside of the Cappadocian region in Turkey, erionite is also present in North America. North Dakota sits over geologic formations in the North Killdeer Mountains containing erionite which has been used for gravel. Carbone et al. studied erionite exposures in North Dakota in comparison to those in the Turkish villages mentioned above and demonstrated similar biological activity between erionite in Turkey and the erionite in North Dakota [123]. To date, there has been one case report of erionite-associated mesothelioma in North America—a 47-year-old male who worked in janitorial and maintenance services at a supermarket and had lived for many years in Mexico, where erionite deposits are also known to occur. In this case, the gross distribution of disease and presence of pleural plaque were reported to be similar to that of cases with amphibole asbestos-associated mesothelioma. Fiber analysis performed on lung tissue demonstrated ferruginous bodies and uncoated fibers with spectra characteristic of erionite by scanning electron microscopy [124].

### 5.3.3 The Libby Amphibole

Also in North America is the Libby amphibole. Vermiculite from Libby, Montana, was mined

from the 1920s until 1990 and contained amphiboles, including tremolite, actinolite, winchite, and richterite. The latter two are unregulated asbestos-like compounds which reside in the amphibole category of mineral classification. Significant exposure to amphiboles occurred during vermiculite mining/processing, as for much of the time that the mine was open, regulations limiting occupational exposure did not exist. There was a significant increase in the standardized mortality ratio (SMR) for both mesothelioma and asbestosis in workers exposed to vermiculite containing amphiboles [125]. Mortality following exposure to the Libby asbestiform amphibole was studied by McDonald et al. who described mortality from mesothelioma similar to that of South African crocidolite miners and miners in Australia, which they note to be ten times higher than the mortality from mesothelioma in Quebec chrysotile miners [126].

### 5.3.4 Radiation

There have been a number of case reports of the development of mesothelioma following thoracic or abdominal radiotherapy [127–129]. Radiation in these cases has been both internal and external beam, sometimes following the administration of intravascular thorium dioxide (Thorotrast) [130–131]. The latent interval following radiotherapy to the clinical development of mesothelioma is generally prolonged, ranging from 7 to 50 years following exposure [127]. Several cases have been reported of young adults developing mesothelioma following intensive chemoradiotherapy for Wilms' tumor [132–134], with radiation ports including the lower thorax. Lung tissue fiber burden analysis yielded values within the expected range of a reference population in the single case in which it was performed [134]. De Bruin et al. reviewed 2,567 patients with Hodgkin lymphoma and observed 13 cases of mesothelioma among patients treated with radiation therapy, 12 of which developed in the radiation field. The median time from treatment to mesothelioma was 27.7 years. They report a 26-fold increase in mesothelioma risk among patients treated with radiation and no cases of mesothelioma among those treated with chemotherapy only. Interestingly, 7/13 (53.8 %) of their cases of mesothelioma following radiation therapy had a history of asbestos exposure, most of which was occupational [135]. Henley et al. also report the development of pleural mesothelioma following radiation therapy for Hodgkin lymphoma in which the latency interval was 13 years [136]. A retrospective study by Neugut et al. reviewed 251,750 women registered with breast carcinoma, 24.8 % of whom had received radiation therapy, and 13,743 patients with Hodgkin disease, of which 50.6 % had received radiation therapy. Six cases of mesothelioma were discovered, all in the cohort of breast carcinoma patients. Four of the six had not received radiotherapy, thereby finding no association in a large controlled study with thoracic radiation and the development of mesothelioma. However, the follow-up period for patients in this retrospective study was only 20 years [137]. Teta et al. reviewed SEER data for patients with Hodgkin and non-Hodgkin lymphoma over a 30-year period. They report a statistically significant increase in mesothelioma in men treated with radiation for Hodgkin lymphoma and no significant increase in mesothelioma among men with non-Hodgkin lymphoma who received radiation [138]. Tward et al. reviewed 77,823 patients with non-Hodgkin lymphoma for the development of secondary malignancies and found that there were significantly more mesotheliomas in patients who were irradiated versus those who did not receive radiation therapy [139]. Travis et al. report an increased risk of mesothelioma following radiation treatment for testicular tumors [140]. There is a single case report of pleural mesothelioma following pneumonectomy and adjuvant radiation therapy for lung cancer. The latency interval in that case was 17 years [141]. Experimental animal studies support a role for radiation in the causation of mesothelioma [118, 119, 142]. The growing body of evidence appears to support an excess risk of mesothelioma following radiation therapy with a long latency interval; however, the risk appears minimal. There is no evidence that whole-body external radiation causes or

contributes to the development of mesothelioma. Reports of mesothelioma developing following chemotherapy do not allow for the frequently unrecognized exposure to asbestos [143].

### 5.3.5 SV40

Following a report by Carbone et al. in 1994 [144], there has been much interest and ongoing research regarding the role of simian virus 40 (SV40), a DNA tumor virus, as a carcinogen or cocarcinogen with asbestos in the induction of mesothelioma [110, 145, 146]. SV40 is capable of causing mesothelioma in animal models following intrapleural or intracardiac injection [147] and may result in the transformation of human cell lines in tissue culture. Human exposure to SV40 is believed to have occurred following administration of contaminated live and attenuated poliovirus vaccines, prepared from infected monkey kidney tissue culture cell lines [148, 149]. It is estimated that between 1954 and 1963, 96 million adults and children in the United States were potentially inoculated with contaminated vaccine, and some 32 million people may have been exposed to infectious SV40 [149–151]. Hundreds of millions of people worldwide were likely exposed to SV40 in this fashion.

The SV40 viral genome encodes several oncogenic proteins, most notably large T antigen (Tag). Tag is a potent carcinogen and mutagen and also serves to inhibit cellular tumor suppressor activity through inactivating p53 and p-retinoblastoma family proteins [152, 153]. It has been demonstrated that human mesothelial cells are particularly susceptible to SV40 infection and malignant transformation, much more so than other cell types, with synergy toward malignant transformation provided by asbestos [154]. Using the polymerase chain reaction (PCR) methodology, studies from multiple institutions in the United Sates have demonstrated the presence of SV40Tag in some 50 % of mesotheliomas [110, 155–158]. However, the trend in rate of mesothelioma in the United States following administration of contaminated poliovirus vaccines is not consistent with an exposure effect to the vaccine [159]. Additionally, Engels reported that following administration of contaminated poliovirus vaccines to children in Denmark from 1955 to 1961, there was no increase in the incidence of malignant mesothelioma [160]. Several studies have argued against the significance of SV40 and the development of mesothelioma as there was no detection of the SV40Tag DNA or SV40Tag protein in tissue of 69 mesotheliomas by Manfredi et al. in 2005 [161], and in a high mesothelioma incidence area of Sweden, Lundstig et al. failed to amplify SV40 DNA via real-time polymerase chain reaction (RT-PCR) in 18 biphasic malignant pleural mesothelioma cases [162].

The presence of SV40 sequences has been associated with a poor prognosis in non-epithelial mesothelioma. Studies have also shown selective expression of SV40 by mesothelioma cells but not in adjacent stromal cells or lung carcinomas [163]. Studies from Finland and Belgium have not shown the association between mesothelioma and SV40 [164–167].

The theory of SV40 as carcinogen and cocarcinogen is not without its detractors and skeptics, which stem from fundamental disputes about the infectivity of SV40 in humans and whether it is even possible to distinguish SV40 infection from infection by other viruses in humans. Furthermore, there have been inconsistencies in the ability of different laboratories to detect SV40 sequences in the same specimens [168]. Finally, epidemiologic studies have failed to document an increased risk for malignancy in those likely exposed to polio vaccine contaminated with SV40 [169]. The role of SV40 in causation of mesothelioma has yet to be established secondary to conflicting study results, and we remain unconvinced of its causal role at this time.

### 5.3.6 Familial Mesothelioma

In addition to the familial clustering of malignant mesothelioma in the Cappadocian region of Turkey discussed above, Bianchi et al. describe 40 cases of familial mesothelioma from the Trieste-Monfalcone region in Northeastern Italy. All were exposed to asbestos. The relationships between

affected individuals were as follows: eight parent-child, seven siblings, three conjugal/spouse, and three conjugal/in-laws [170]. Some believe that the familial aggregation of mesothelioma among blood-related individuals indicates potential host genetic factors which contribute to the development of malignant mesothelioma [171–174], while others suggest otherwise [170, 175]. Of note, in familial clustering an asbestos exposure is present in the majority of cases [176, 177].

The BAP1 (BRCA1-associated protein 1) gene located at 3p21.1 regulates the BRCA1 growth pathway by acting as a tumor cell growth suppressor through deubiquitination of DNA. Carbone et al. report that germline mutations of the BAP1 gene are associated with an increased incidence of mesothelioma [123], and Testa et al. linked BAP1 mutations to familial mesothelioma in two North American families from Wisconsin and Louisiana which lacked a clear source of occupational asbestos exposure, yet were exposed to asbestos in the home [178]. Testa as well as Bott et al. demonstrated BAP1 gene alterations in 22 and 23 % of sporadic cases of malignant mesothelioma, respectively [178, 179].

### 5.3.7 Other Factors

Additional factors implicated as contributing causes to the development of malignant mesothelioma are uncommon, but have included chronic empyema, peritonitis, and scarring of the serosa, or following the creation of therapeutic pneumothorax with intrapleural administration of leucite spheres for the treatment of pulmonary tuberculosis [180–182]. Malignant mesothelioma in such cases arises after several decades. The identification of malignant mesothelioma in oil refinery and petrochemical plant workers suggested a role for chemical cocarcinogens in the production of mesothelioma [182]. However, in the 30 years that have passed since this suggestion was made, no supporting evidence has been forthcoming. More recent studies have suggested that mesotheliomas arising in oil refinery and petrochemical plant workers occur among a subgroup of maintenance workers with asbestos exposure [183, 184]. Anecdotal reports have described malignant mesotheliomas developing following exposure to beryllium and nickel [185]. One epidemiologic study showed a slightly increased risk of malignant mesothelioma in fiberglass workers [30], although this has not been confirmed. Mesothelioma has been reported in sugarcane workers following inhalation of noncrystalline silica fibers with fiber dimensions similar to those of amphibole asbestos [186]. While important in the causation of bronchogenic carcinoma in patients with asbestosis (see Chap. 7), cigarette smoking has not been implicated as a risk factor for the development of malignant mesothelioma [54]. Finally, reports of mesothelioma developing in childhood or even in utero for which none of these risk factors could be identified indicate that there are probably as yet unknown factors involved in the pathogenesis of this rare malignancy [187, 188].

## 5.4 Pathologic Features

### 5.4.1 Gross Morphology

Malignant mesotheliomas are characteristically comprised of confluent, thick growth over the distribution of the serosal surface, usually with an associated effusion, leading to the obliteration of the serosal cavity and extensive involvement of the regional viscera, compressing and invading from without. The most common site of origin of malignant mesothelioma is the pleura. In a large series, the ratio of pleural to peritoneal locations is 10:1 [189]. The earliest lesions typically begin as small macules or nodules on the parietal pleura [190, 191], whose subsequent growth leads to coalescence of these nodules and finally fusion of the parietal and visceral pleura [192, 193]. Growth then follows the distribution of the pleural surface, with extension into fissures and interlobular septa (Fig. 5.2). Pleural mesotheliomas will invade the mediastinal structures, chest wall, diaphragm, and, in advanced cases, the contralateral pleural cavity and peritoneum. These gross features correlate well with the typical clinical symptoms of chest pain and dyspnea.

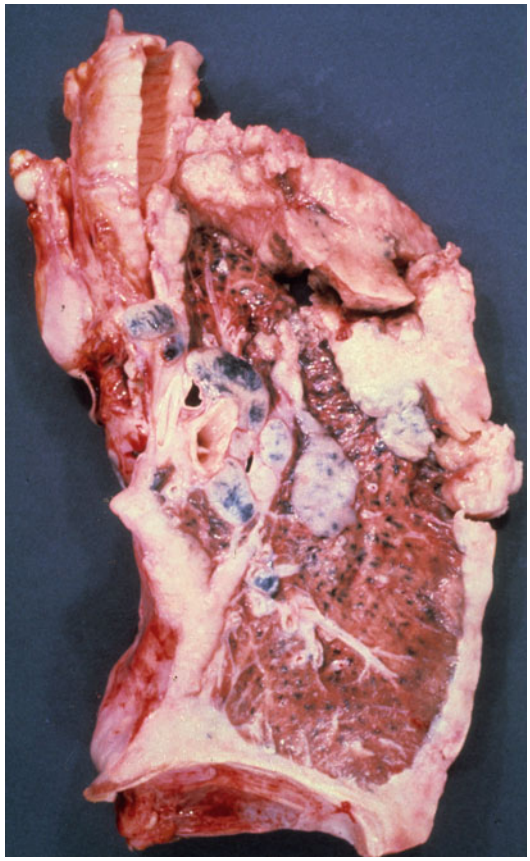

**Fig. 5.2** Coronal slice of the right lung in a patient with malignant (diffuse) pleural mesothelioma shows encasement of the lung by a rind of tumor. There is superficial invasion of underlying parenchyma (Reprinted from Ref. [2], with permission)

A large and often hemorrhagic pleural effusion is frequently present at the time of presentation [194] and may be responsible for some of the dyspnea observed. However, in late-stage disease, obliteration of the pleural space through tumoral fusion of the pleurae may not allow for significant fluid accumulation [2]. The presence of bulky disease may be accompanied by constitutional symptoms and weight loss. Parenchymal pulmonary masses are uncommon, except in late-stage disease, and dominant pulmonary masses should raise suspicions regarding the diagnosis of mesothelioma. In exceptional cases, malignant pleural mesotheliomas may present as large, localized pleural-based masses as seen in Fig. 5.3 [195–197].

The most common site of metastasis is via lymphatics to lymph nodes in the hilar areas of the lung or mediastinum, and this is commonly observed [181, 189]. In rare instances, extensive lymphangitic pulmonary spread may be present at the time of diagnosis [198]. The propensity for mesothelioma to grow in the subcutaneous tracks following needle biopsy or placement of ports for thoracoscopy [14] often necessitates the excision of these sites at the time of pleuropneumonectomy. Clinically evident metastatic disease outside the thorax at time of presentation is uncommon, but distant hematogenous metastases are frequently detected at autopsy, present in at least half of cases [182, 199–201].

Observations regarding gross distribution and morphologic features of the tumor are very important elements in the diagnosis of malignant mesothelioma. When this information is not available directly to the pathologist or prosector of autopsy or surgical material, it may be obtained through the observations of the surgeon at time of thoracoscopy, thoracotomy, or pleuropneumonectomy or from radiographic studies. Massive pleural effusions may obscure the details of tumor distribution on plain films, but additional radiographic studies may provide detailed information regarding salient gross pathologic features [202–204]. Computerized tomography (CT) can suggest the diagnosis by demonstrating effusions and nodular pleural thickening and may delineate invasion of local structures (Fig. 5.4). Magnetic resonance imaging (MRI) may provide additional detailed information regarding local invasion that is of potential utility in those patients considered for pleuropneumonectomy as well in assessing treatment effect [201, 202, 204, 205]. All of the above modalities may also demonstrate evidence of other concomitant intrathoracic asbestos-related pathology such as pleural plaques. Fluorodeoxyglucose-positron emission tomography (FDG-PET) imaging studies are useful in identifying location/extent of disease and sites of distant metastasis based on the differential usage of glucose by different tissues and may provide some assistance in the distinction between reactive/inflammatory lesions of the pleura and malignant neoplasms [206].

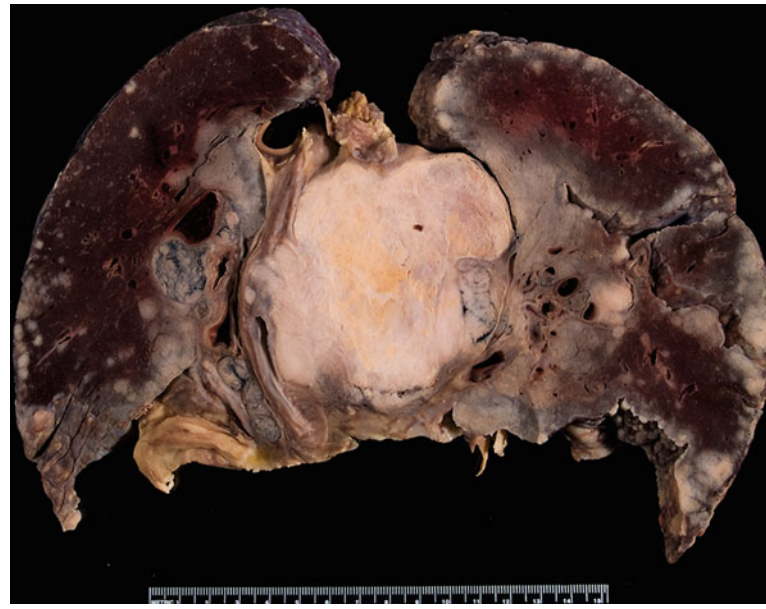

**Fig. 5.3** Gross image of large tumor mass situated between the lungs which was histologically confirmed to be a malignant mesothelioma

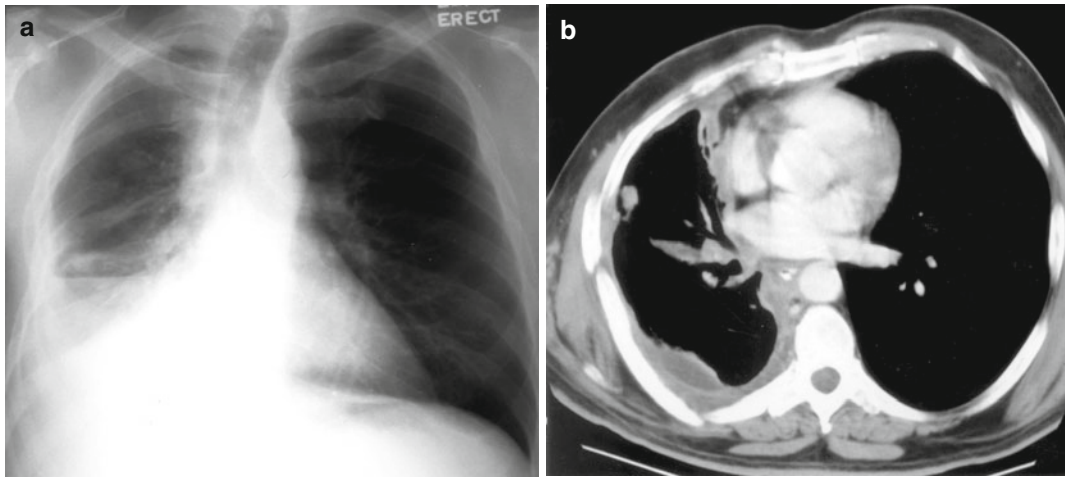


**Fig. 5.4** (**a**) Posteroanterior chest x-ray shows a unilateral pleural effusion. (**b**) Computed tomography of the thorax from the same patient shows irregular pleural thickening with encasement of the lung. These radiographic features are typical for malignant (diffuse) pleural mesothelioma (Courtesy of Dr. Caroline Chiles, Duke University Medical Center, Durham, NC)

Although the gross distribution of mesothelioma with circumferential encasement of the lung in a rind of tumor is characteristic, it is not pathognomonic of the entity. Other malignant tumors, either primary within the lung or metastatic from extrathoracic sites, may directly invade and diffusely involve the pleura. Small peripheral pulmonary adenocarcinomas may invade the pleura (Fig. 5.5) [207] and so closely mimic the gross appearance of mesothelioma that some investigators have termed such tumors "pseudomesotheliomatous adenocarcinomas" [208, 209]. Attanoos and Gibbs report a series of 53 pseudomesotheliomatous carcinomas (50 men and 3 women) in which 47 consisted of primary lung carcinoma with pleurotropic growth. The

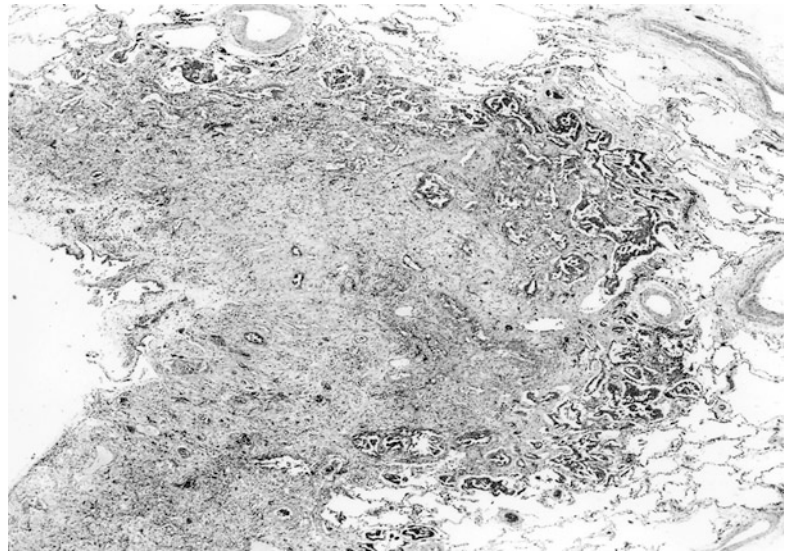

**Fig. 5.5** Low-power photomicrograph of a small peripheral adenocarcinoma found at autopsy that measured 5 mm in maximum dimension. Multiple pleural deposits of tumor histologically identical to the adenocarcinoma at the periphery of the central scar resulted in a pattern mimicking mesothelioma. H&E ×24

remaining six cases were metastatic carcinoma with diffuse pleural involvement. The metastatic tumors included transitional cell carcinoma of the bladder, pancreatic ductal adenocarcinoma, clear cell renal cell carcinoma, parotid squamous carcinoma, and prostatic adenocarcinoma. Of the primary lung carcinoma cases, tumor type included 34 adenocarcinomas, 5 pleomorphic carcinomas, 4 squamous cell carcinomas, 2 small cell carcinomas, 1 basaloid, and 1 carcinosarcoma. Immunohistochemistry proved to be most enlightening in distinguishing pseudomesotheliomatous carcinoma from malignant pleural mesothelioma [210]. Angiosarcomas and epithelioid hemangioendotheliomas, closely related mesenchymal malignancies of vascular origin, may mimic malignant pleural mesothelioma both in terms of gross distribution and in clinical behavior [211–214]. Therefore, the differential diagnosis of mesothelioma is extensive, including chiefly peripheral primary carcinomas of the lung, metastatic carcinomas of extrathoracic sites that may be clinically occult (e.g., kidney), thymic epithelial carcinoma, and primary pleural angiosarcomas. In view of this lengthy list, it is of critical importance to have an understanding of pertinent clinical and historical data, as well as radiographic findings, so as not to overlook primary malignancy elsewhere with secondary pleural involvement.

The diagnosis of malignant pleural mesothelioma depends not only on the presence of typical gross tumor distribution but also on the identification of a histologic, histochemical, immunophenotypic, or ultrastructural pattern compatible with mesothelioma and, moreover, exclusion of metastatic tumor [215]. These additional and ancillary studies to complement examination of routine stained sections will be reviewed subsequently. Studies describing the cytologic features of mesothelioma in effusion cytologies and aspiration biopsies are reviewed in detail in Chap. 9. The distinction of malignant mesothelioma, reactive mesothelium, and metastatic carcinoma may be difficult or impossible on limited material. Although with the now commonplace usage of immunocytochemistry in the evaluation of cytologic material, the pathologist may become highly suspicious of the diagnosis of mesothelioma, it remains our practice, and that of others [216], to treat exfoliative and aspiration biopsy specimens as screening tests and to rely on tissue specimens to secure the diagnosis [2, 17, 192–207].

### 5.4.2 Histopathology

Malignant mesothelioma is characterized by a broad range of microscopic appearances, both across the entire spectrum of the disease entity

**Table 5.1** Histologic classification of malignant mesothelioma

| |
|---|
| Epithelial |
| Tubulopapillary |
| Solid variant |
| Adenomatoid |
| Small cell |
| Deciduoid |
| Adenoid cystic |
| Pleomorphic |
| Well-differentiated papillary[a] |
| Sarcomatoid |
| Fibrosarcomatoid |
| Chondrosarcomatoid |
| Osteosarcomatoid |
| Malignant fibrous histiocytoma-like |
| Lymphohistiocytoid |
| Desmoplastic |
| Biphasic (mixed) |

[a]This variant is considered to be a tumor of low-grade malignant potential

and often within individual tumors themselves. This capability is likely a function of the potential for mesothelial cells to undergo varying pathways of differentiation. The World Health Organization recognizes four main histologic subtypes: epithelioid (epithelial) mesothelioma, sarcomatoid mesothelioma, desmoplastic mesothelioma, biphasic mesothelioma, and a separate category encompassing a variety of less common patterns, often featuring the presence of heterologous elements [11]. Epithelial, sarcomatoid, and biphasic mesotheliomas are the most common pleural forms, occurring in approximately 50, 20, and 30 % of cases, respectively [2, 182, 192]. The representation of these subtypes is different among the peritoneal mesotheliomas, Churg and Kannerstein reporting in a series of 82 cases, 75 % epithelial mesotheliomas, 24 % biphasic, and 1 % purely sarcomatoid [217]. Origin within the pleura or peritoneum does not confer any differences in the microscopic features of the mesothelioma subtypes themselves. The histologic classification of mesothelioma is summarized in Table 5.1.

The most common variant is the epithelial, defined as a tumor whose histologic pattern is that of tubulopapillary structures, trabeculae, acini, or sheets of atypical cells (Fig. 5.6a, b). Epithelial mesotheliomas are typically heterogeneous, and may assume different combinations of the above-described patterns throughout their expanse. The tubulopapillary pattern is most commonly observed, where the tumor consists of branching tubules and papillae lined by flat to cuboidal cells. Columnar tumor cells are uncommon, and while psammoma bodies may be observed in some 5–10 % of cases [176], their profusion suggests papillary carcinoma, especially in peritoneal malignancies.

Epithelioid cytologic features include cuboidal or polygonal shape, moderate to abundant cytoplasm, and paracentric nuclei, often with prominent nucleoli. Multinucleate forms and occasional mitoses may be observed, but anaplastic forms, extreme pleomorphism, and high-grade cytologic atypia are not common features of epithelial mesotheliomas. Atypical mitoses are distinctly uncommon [2, 218]. The epithelial variant is most likely to be confused with adenocarcinoma, and this distinction becomes more difficult as the epithelial tumor cells become more anaplastic [2]. A useful cytologic feature reported to be characteristic of the tumor cells of epithelial mesothelioma is a constant nuclear-to-cytoplasmic ratio.

Several histologic subtypes or patterns of epithelial mesothelioma are recognized. The *adenomatoid* subtype forms a microglandular pattern with lumina containing hyaluronic acid, mimicking adenomatoid tumors [176]. While typically comprised of large epithelial tumor cells, the *small cell* subtype demonstrates a diffuse growth of small tumor cells that may sometimes be confused with small cell carcinoma [176, 219, 220]. *Deciduoid* mesothelioma, originally described in young women as aggressive peritoneal tumors arising independent of exposure to asbestos [221], features sheets of large, round to polygonal cells with typically a single nucleolus and abundant eosinophilic cytoplasm, resembling cells of the decidual reaction (Fig. 5.6d). Puttagunta et al. have described focal rhabdoid differentiation associated with this tumor type as well [222]. As an aside, Matsukuma et al. and

Ordóñez described rhabdoid morphology in association with epithelial, biphasic, and sarcomatoid mesotheliomas [223, 224]. Deciduoid mesotheliomas have also been described in men and may occur in the pleura and pericardium as well as the peritoneum [225–228]. A history of asbestos exposure has been reported in a few cases [225, 227, 229]. Other unusual variants of epithelial mesothelioma include the *adenoid cystic* type, resembling tumors of salivary gland origin

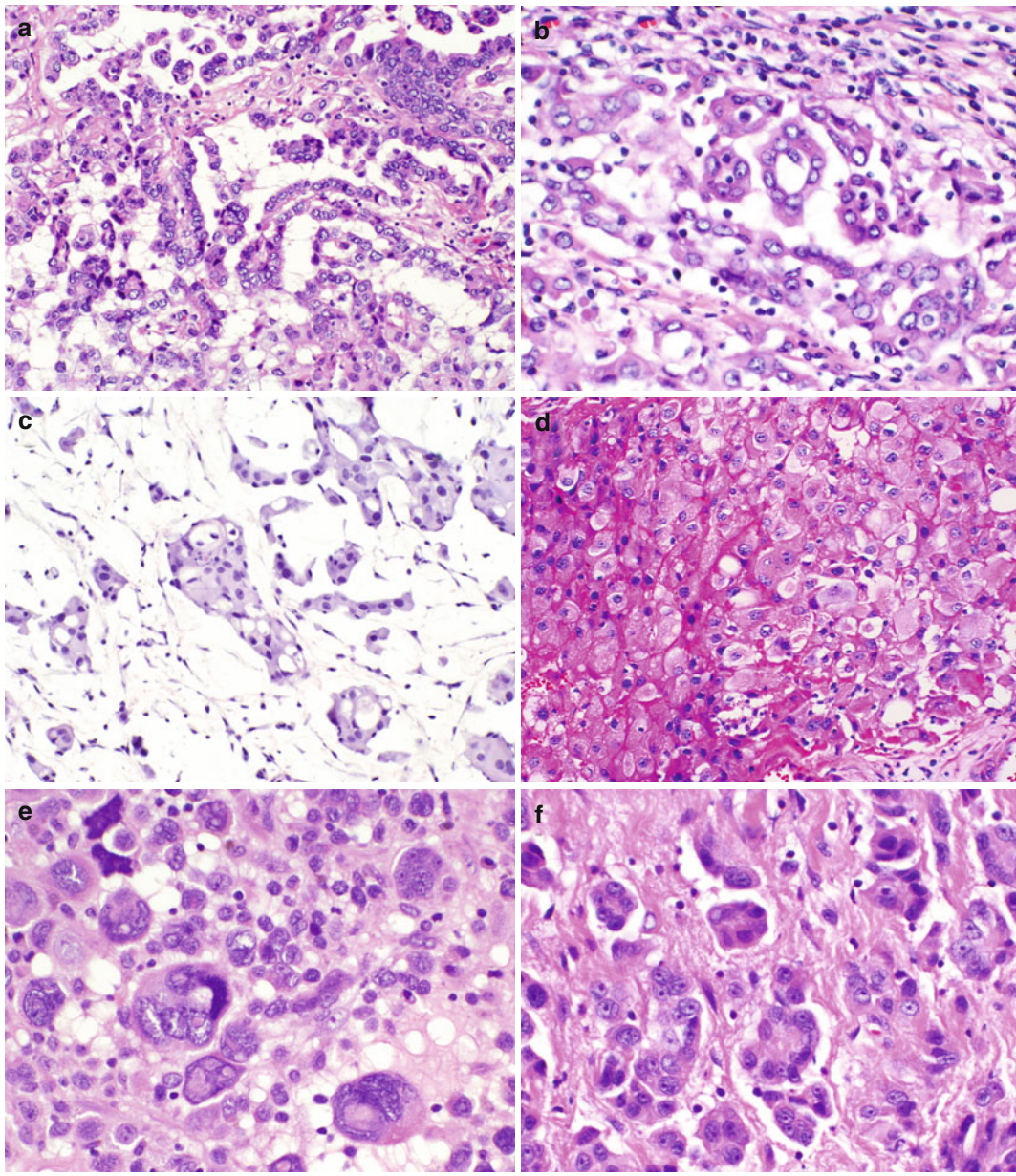


**Fig. 5.6** Histologic patterns of the epithelial variant of malignant (diffuse) mesothelioma: (**a**) papillary pattern, (**b**) tubular pattern, (**c**) adenomatoid (microcystic) pattern, (**d**) deciduoid pattern, (**e**) pleomorphic pattern of epithelial mesothelioma, (**f**) micropapillary, (**g**) well-differentiated papillary pattern. The latter pattern in pure form tends to have an indolent clinical behavior. H&E: Parts (**a–c**) ×200 (Part (**d**) reprinted with permission from Sporn [453]; Part (**e**) ×400; Part (**f**) ×400; Part (**g**) ×10)

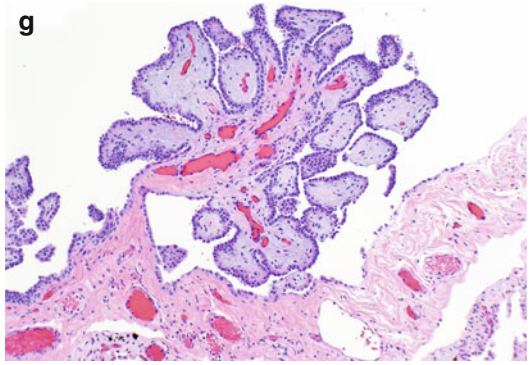


**Fig. 5.6** (continued)

(Fig. 5.6c) and the *pleomorphic* variant of epithelial mesothelioma (Fig. 5.6e), with a histologic resemblance to pleomorphic carcinoma of the lung [230]. Kadota et al. recently proposed that pleomorphic epithelial malignant mesothelioma be reclassified as either biphasic or sarcomatoid mesothelioma based on similar poor survival data [231]. However, the authors believe that a distinction should be made between the pleomorphic variant of epithelial mesothelioma and the pleomorphic variant (MFH-like) of sarcomatoid mesothelioma (Fig. 5.7c). The *micropapillary* variant, similar to the micropapillary variant of lung adenocarcinoma, confers a worse prognosis (Fig. 5.6e).

*Well-differentiated papillary mesothelioma* (WDPM) is an uncommon tumor tending to involve the peritoneal cavity of women in the

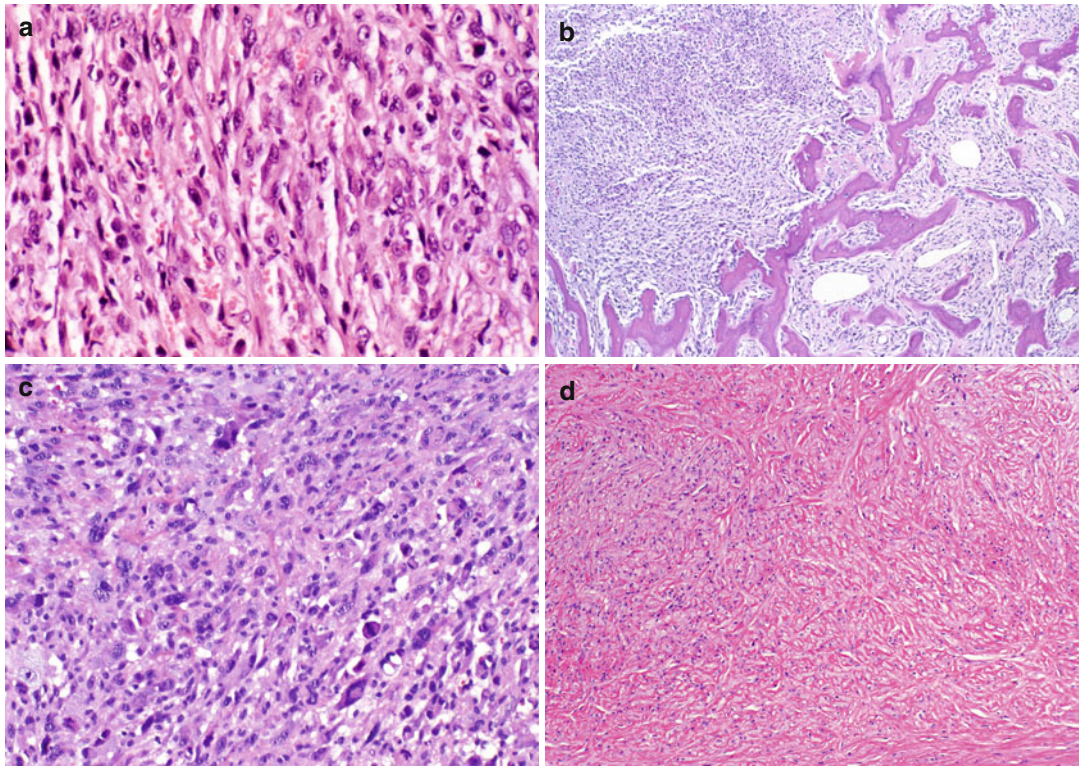


**Fig. 5.7** Histologic patterns of the sarcomatoid variant of malignant (diffuse) mesothelioma: (**a**) fibrosarcomatoid pattern; (**b**) osteosarcomatoid pattern. Note bony spicules surrounded by malignant spindle cells; (**c**) malignant fibrous histiocytoma-like pattern, with numerous tumor giant cells; (**d**) desmoplastic pattern, consisting of thick collagen bundles arranged in a storiform pattern with scattered inconspicuous tumor cells in spaces between the fiber bundles. H&E: Part (**a**) ×250; Part (**b**) ×70; Part (**c**) ×200; Part (**d**) ×100 (Parts (**a**) reprinted from Ref. [2]; part (**b**) courtesy of Dr. Tom Colby, Mayo Clinic, Scottsdale, AZ, and reprinted with permission from: Roggli and Cagle [454])

third and fourth decades of life but rarely reported to involve the other serosal membranes. Unlike typical pleural and peritoneal mesotheliomas, a history of asbestos exposure is not present in most cases [232, 233]. In a recent review, Butnor et al. described 14 cases of WDPM, including seven pleural tumors and one from the tunica vaginalis testis [234]. The histologic features of tumors in the pleural, pericardial, and peritoneal cavities and tunica vaginalis are similar, demonstrating prominent fibrovascular cores lined by a single layer of relatively uniform cuboidal cells with minimal nuclear atypia and no mitotic activity (Fig. 5.6g). Psammoma bodies were present in one of the cases reported by Butnor et al., and focal stromal invasion was identified in two cases. An asbestos exposure history may be present in some cases [234, 235]. WDPM typically has a good prognosis characterized by indolent clinical behavior but may pursue an aggressive course with death following the development of diffuse disease [234, 235]. Identification of invasion signals a poor prognosis, and such tumors should be classified as epithelial mesotheliomas.

The sarcomatoid variant is the least common of the three major histologic types. The tumor cells are elongated and spindled and may show considerable pleomorphism and mitotic activity [2]. Architecturally complex, the broad fascicular or storiform growth pattern resembles that of typical soft tissue sarcomas, including neurogenic sarcoma, leiomyosarcoma, chondrosarcoma, or osteogenic sarcoma (Fig. 5.7a, b) [189, 236, 237]. We have also observed cases in which the storiform fascicles of spindle cells contain intermixed bizarre tumor giant cells in a pattern reminiscent of malignant fibrous histiocytoma (Fig. 5.7c). True heterologous elements comprised of osteosarcomatous or chondrosarcomatous foci have been noted in otherwise typical mesotheliomas [236, 237]. In a review of 27 cases of malignant mesothelioma with heterologous elements by Klebe et al., only one case with heterologous elements was of epithelial type. The remaining consisted of 16 sarcomatoid and 10 biphasic [238]. The *lymphohistiocytoid* variant of sarcomatoid mesothelioma is an unusual form that may be misdiagnosed as an inflammatory pseudotumor or lymphoma [239]. The tumor consists of an admixture of large histiocytoid cells and dense lymphoplasmacellular infiltrate within a background of sarcomatoid tumor cells (Fig. 5.8). Mesothelial differentiation in these cases has been proven using cytokeratin immunostaining and electron microscopy. The authors accept the various patterns listed above as mesothelioma, provided the gross distribution of tumor is characteristic and there is no evidence of primary soft tissue sarcoma elsewhere in the patient [2]. Sarcomatoid mesothelioma is more common in men (96 % in our series versus 4 % in women) and most often originates from the pleura (97 % in our series versus 3 % in the peritoneum) [240].

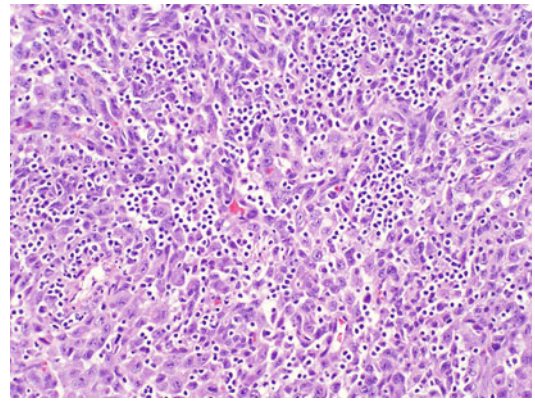

**Fig. 5.8** Lymphohistiocytoid malignant pleural mesothelioma, showing large pale neoplastic nuclei in a background of small lymphocytes. The pattern resembles that of a mixed small and large B-cell lymphoma. H&E ×200

The most distinctive pattern of malignant mesothelioma is the biphasic or mixed pattern. These tumors have areas that exhibit one of the epithelial patterns described above as well as areas with a spindle cell or sarcomatoid appearance. The transition from epithelial to sarcomatoid areas may be gradual, with transitional morphology (Fig. 5.9a), or abrupt, with sharp demarcation between the epithelial and sarcomatoid components (Fig. 5.9b). Metastases from biphasic mesotheliomas may contain either component alone, or both may be present together [2]. The frequency of the biphasic pattern ranges from 25 to 50 % in various series of pleural mesotheliomas and is somewhat dependent on the thoroughness of tumor sampling [182, 190].

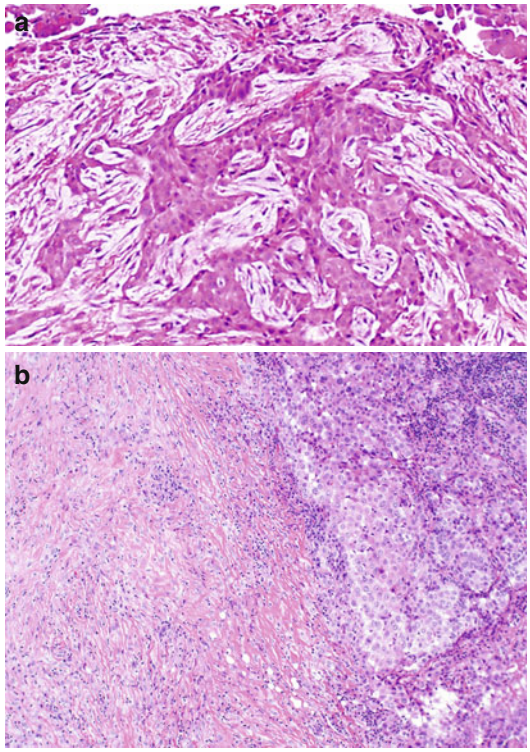


**Fig. 5.9** (**a**) Transitional morphology of malignant (diffuse) mesothelioma showing areas which appear more epithelial merging with those which appear more sarcomatoid. (**b**) Biphasic mesothelioma showing two distinct populations of epithelial and sarcomatoid cells. Part (**a**) ×300; Part (**b**) ×100

The pathologist must be careful to differentiate a true sarcomatoid component from a cellular fibroblastic stromal response.

A particularly deceptive pattern of sarcomatoid malignant mesothelioma is the *desmoplastic* variant [241, 242]. This uncommon form, constituting approximately 10 % of all malignant mesotheliomas, is typically less cellular than its counterparts and features largely hyalinized collagenous stroma. Desmoplastic mesothelioma mimics benign reactive processes, typically the fibrosing serositis that may occur in postoperative or postinflammatory conditions. Patients with malignant mesothelioma often have parietal pleural plaques, present in from 54 % to more than 70 % of cases with pleural mesothelioma [53, 111]. Plaques are a potential source of confusion as they too are generally acellular but demonstrate hyalinized collagen with a "basket weave" pattern, never in a storiform pattern. DMM was originally described by Kannerstein and Churg in 1980 [241]. Specific diagnostic criteria to more readily allow the distinction of this tumor from fibrous pleurisy have since been introduced by Mangano et al. [243]. The most striking feature of this tumor is a whorled and twisting paucicellular lesion, produced by distinct broad collagen fibers in a storiform array or in the "patternless pattern" of Stout (Fig. 5.7d) [244]. The storiform pattern can be focally found as a component of a large percentage of malignant mesotheliomas [245], so the term is restricted to cases in which this pattern predominates [241, 242]. Rarely, biphasic and epithelial mesotheliomas containing a predominant desmoplastic component have been described.

The diagnostic criteria for DMM include the presence of a tumor, more than 50 % of which consists of dense collagen bundles arranged in a storiform or patternless pattern, and at least one of the following additional findings. First is invasion of lung or chest wall by neoplastic spindle cells. This is often apparent on routine stained sections. The presence of more subtle invasion may be demonstrated with low-molecular-weight cytokeratin immunoperoxidase stains, showing keratin-positive spindle cells infiltrating alveolar septa or adipose tissue of the chest wall. The mere identification of cytokeratin-positive spindle cells within the lesion is not diagnostic, as keratin expression may be observed in reactive processes as well. The second criterion is the finding of bland necrosis, as evidenced by necrotic foci with minimal accompanying inflammatory infiltrate. Necrosis may be detected by subtle changes in the tinctorial quality of the fibrosis and nuclear fragmentation. The third criterion is that of frankly sarcomatoid areas, as defined by zones of transition from paucicellular fibrosis to areas of more abundant spindled cellularity. Accompanying increase in nuclear atypia may help distinguish sarcomatoid foci. Mitotic figures are not numerous in either fibrosing pleuritis or DMM. The fourth criterion is the presence of distant metastases [215, 243, 246].

In addition to these specific criteria, other findings may also aid in distinguishing DMM from a reactive process. Churg has noted that the most cellular areas in fibrosing pleuritis are oriented toward the luminal aspect of the pleura, whereas the process closest to the chest wall is less cellular [246]. This "top heavy" cellular pattern may also feature numerous perpendicular capillaries traversing the full thickness of pleura, favoring a reactive process [2, 246]. Thoracic imaging studies, particularly computed tomography or magnetic resonance imaging, are often of great value by demonstrating irregular pleural thickening, chest wall invasion, or bony involvement and may assist the pathologist in making a diagnosis of DMM in difficult cases [204, 243]. Immunohistochemistry for the p53 tumor suppressor gene protein has been suggested as a useful diagnostic adjunct for distinguishing reactive pleural processes from mesothelioma and metastatic carcinoma. Mutations of this gene are more likely to result in a stable protein as compared to the wild-type [247]. Staining of more than 10 % of nuclei is seen more frequently in DMM than fibrosing pleuritis, but the difference is not statistically significant in the small number of cases examined [243].

Metastases from malignant mesothelioma generally resemble the histologic appearance of the primary tumor, and a review of 42 pleural mesotheliomas showed no difference in the frequency of hematogenous or lymphatic metastases among the three major histologic variants [248], despite prior reports that sarcomatoid mesothelioma has more frequent distant metastases [249]. It is interesting to note that whereas regional lymph node metastases are commonly identified [181, 191], metastases to extrathoracic lymph nodes are infrequent, occurring in eight of 77 cases of pleural mesothelioma in a single series [250]. In exceptional cases, osseous metastases have been described as the initial clinical evidence of tumor dissemination in DMM [251]. Hematogenous metastases of DMM sometimes exhibit the curious phenomenon of central hyalinization with a peripheral cellular storiform pattern [2]. Finally, rare cases have been reported in which liver metastases from a malignant pleural mesothelioma underwent dystrophic calcification and presented as hepatic calcification initially detected radiographically.

### 5.4.3 Histochemistry

The distinction of malignant mesothelioma from its mimics, and adenocarcinoma in particular, is critical in regard to decisions for treatment, prognostication, and frequently for compensation in medicolegal cases involving allegations of exposure to asbestos. In view of these crucial decision points, studies adjunctive to the examination of hematoxylin and eosin-stained sections are required to secure the diagnosis of malignant mesothelioma in the vast majority of cases.

Histochemical staining for mucins and acid mucopolysaccharides provides one means for making this distinction. The histochemical basis for distinguishing malignant pleural mesothelioma from metastatic adenocarcinoma rests on the identification of hyaluronic acid production in the former instance and neutral mucin in the latter [2]. Neutral mucin may be identified by the periodic acid Schiff (PAS) stain. The specificity of the PAS stain is increased by prior digestion of the section with diastase in order to remove glycogen, which may be abundant in the cytoplasm of either mesothelioma or adenocarcinoma. The demonstration of PAS-positive, diastase-resistant droplets of neutral mucin as intraluminal secretions or cytoplasmic vacuoles within a tumor strongly supports a diagnosis of adenocarcinoma (Fig. 5.10a–c) [2, 14, 60, 176, 189, 190, 192, 193, 208]. A false-positive reaction with the PAS stain may occur if removal of glycogen by diastase is incomplete. Therefore, simultaneous controls should always be performed. In addition, basement membrane material, which may be prominent in epithelial mesotheliomas, will stain with the PAS reaction. Careful attention to the pattern of staining will usually prevent the confusion of residual glycogen or basement membrane material with positive staining for neutral mucin. The PAS stain is of no use for the diagnosis of sarcomatoid or desmoplastic mesotheliomas [2]. The mucicarmine stain may occasionally react with hyaluronic acid, giving a false-positive result. We therefore do not recommend mucicarmine for distinguishing mesothelioma from metastatic adenocarcinoma.

Identification of hyaluronic acid as the sole or major acid mucopolysaccharide in an epithelial

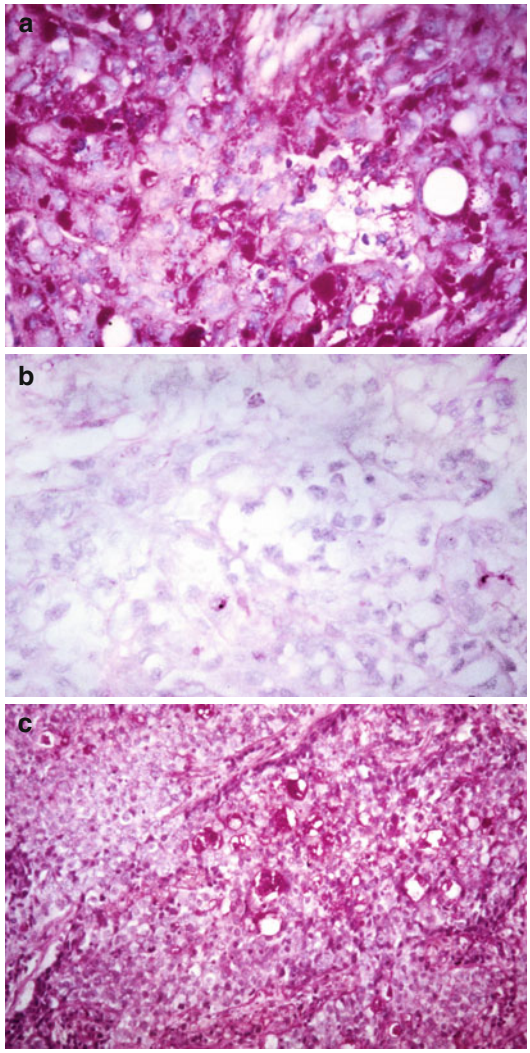


**Fig. 5.10** (**a**) In this epithelial type of mesothelioma, tumor cells contain finely granular, intracytoplasmic PAS-positive material. (**b**) Staining reaction in an adjacent section has been abolished by prior digestion with diastase. (**c**) In this adenocarcinoma metastatic to the pleura, PAS-positive material within glandular lumens remains following digestion by diastase, indicating the presence of neutral mucin (Reprinted from Ref. [2], with permission)

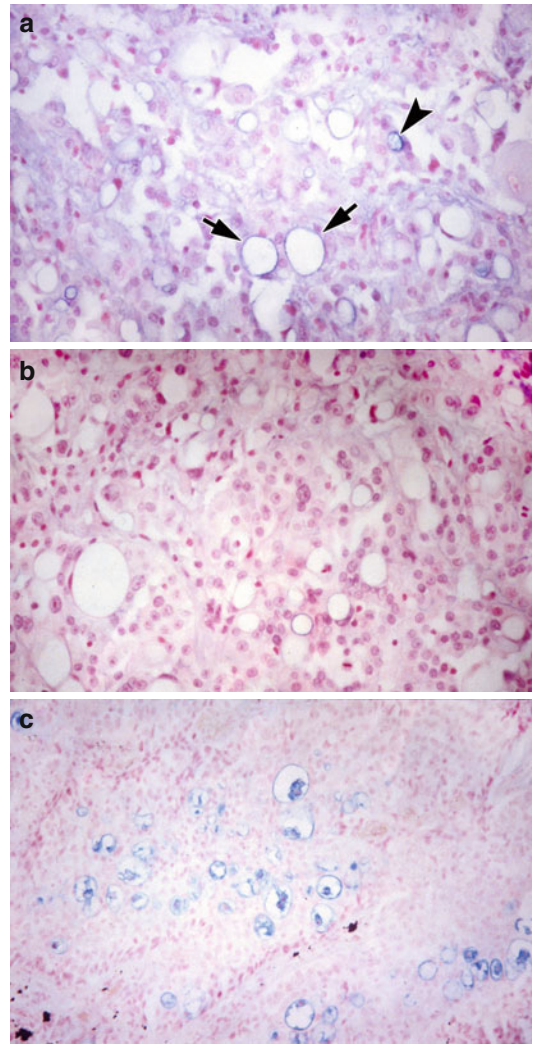


**Fig. 5.11** (**a**) In this epithelial pleural mesothelioma, tumor cells are forming nests with lumens that are filled with (*arrowhead*) or rimmed by (*arrows*) alcian-blue-positive material. (**b**) This material is completely removed from a serial section by prior digestion with hyaluronidase. (**c**) In this adenocarcinoma metastatic to the pleura, alcian-blue-positive material persists in the lumens following digestion with hyaluronidase (Reprinted from Ref. [2], with permission)

tumor has also been proposed as a useful histochemical marker for the diagnosis of malignant mesothelioma [2, 14, 78, 176, 189, 190, 192, 193, 208]. Such identification may be hampered by the tendency of water-soluble hyaluronic acid to leach out of tissue sections. Acid mucopolysaccharides, including hyaluronic acid, may be identified histochemically using alcian blue or colloidal iron stains. The specificity of the reaction for hyaluronic acid is determined by digestion of a serial section with hyaluronidase prior to staining (Fig. 5.11a–c). The results seen following enzyme predigestion depend to some degree on the actual type of hyaluronidase used in the test, since *Streptomyces* hyaluronidase is specific for hyaluronic acid, whereas testicular hyaluronidase digests chondroitin sulfate as well.

Only intracytoplasmic or intraluminal alcianophilia associated with epithelial cells should be considered diagnostic. Intracytoplasmic or intraluminal staining that is entirely eliminated by hyaluronidase strongly supports the diagnosis of malignant mesothelioma. Staining unaffected by hyaluronidase predigestion favors adenocarcinoma. Simultaneous controls should always be performed to ensure that the stains and enzyme are working properly.

The detection of glycosaminoglycans in pleural effusions using electrophoresis has been considered as a diagnostic adjunct [252], as the effusions associated with malignant mesothelioma may be rich in hyaluronic acid. In patients with malignant mesothelioma, hyaluronic acid is typically the sole or predominant mucopolysaccharide (glycosaminoglycan) identified electrophoretically, whereas malignant effusions associated with adenocarcinoma generally contain a mixture of glycosaminoglycans. However, rare cases of pleural mesothelioma have been reported in which chondroitin sulfate was the predominant glycosaminoglycan, and rare instances of pleural effusions complicating metastatic pancreatic carcinoma have been shown to contain predominantly hyaluronic acid.

It should be noted that the demonstration of PAS-positive, diastase-resistant, and alcian-blue-positive, hyaluronidase-resistant intracytoplasmic vacuoles has rarely been reported in mesothelioma. MacDougall et al. reported one such case of a pleural malignancy whose ultrastructural and immunohistochemical characteristics were otherwise typical for mesothelioma [253]. Cook et al. reported a similar case involving the peritoneum that mimicked gastric adenocarcinoma [254]. Hammar et al. reported a series of ten so-called mucin-positive epithelial mesotheliomas [255]. In this study, the intensity of PAS positivity was often eliminated or reduced following hyaluronidase treatment, suggesting that hyaluronic acid was responsible for the positive staining reaction. Ultrastructurally, the secretions had the appearance of crystalline hyaluronic acid or peptidoglycans. A diagnosis of "mucin-positive" mesothelioma should only be entertained in tumors that otherwise have clinical, gross, histologic, immunohistochemical, and ultrastructural features typical for mesothelioma.

In summary, histochemistry may be useful in discriminating between adenocarcinoma and mesothelioma. While offering generally lower cost and simpler methodology, the histochemical techniques described suffer from some limitations. A substantial proportion of adenocarcinomas fail to produce detectable amounts of neutral mucin, and only about half of epithelial mesotheliomas produce detectable quantities of hyaluronic acid. Therefore, negative histochemical studies provide no diagnostically useful information, and in great many cases, histochemistry will not discriminate between malignant mesothelioma and metastatic adenocarcinoma. In our practice, we reserve histochemical staining for tumors that have identifiable secretions on routine hematoxylin and eosin-stained sections.

### 5.4.4 Immunohistochemistry

Among the ancillary diagnostic studies employed in the diagnosis of mesothelioma, immunohistochemical studies currently play the dominant role in separating mesothelioma from its neoplastic mimics. Following Wang's report that expression of carcinoembryonic antigen (CEA) could be used to distinguish adenocarcinoma from mesothelioma [256], numerous different antibodies and panels of antibodies have been evaluated to strengthen this distinction. While immunohistochemistry has evolved to the point that there are arrays of immunophenotypes considered diagnostic or exclusionary of mesothelioma, several principles regarding its application remain axiomatic. First and foremost, there is no immunohistochemical marker that can distinguish a malignant cell, either mesothelioma or carcinoma, from a benign or hyperplastic cell with complete sensitivity and specificity. Second, there is no single marker completely sensitive and specific for the identification of mesothelial or carcinoma cells. Such limitations to the state of the art necessitate the employment of a panel of immunostains whereby one can demonstrate an immunophenotype generally considered

diagnostic or exclusionary of mesothelioma [213]. In the absence of mesothelium-specific antibodies, the major strength of immunohistochemistry in the diagnosis of mesothelioma historically has been one of exclusion. Recent advances have seen the development of antibodies with greater specificity for mesothelial differentiation, as well as markers with greater specificity for carcinoma.

#### 5.4.4.1 Cytokeratins

Cytokeratins consist of a family of some twenty 40–67-kDa polypeptides, forming one of five intermediate filaments. They are expressed to some degree in both benign and transformed epithelial cells as well as mesothelium. Pulmonary adenocarcinomas and mesotheliomas both demonstrate simple epithelial patterns of cytokeratin immunoreactivity with expression of cytokeratins 1, 8, 18, and 19 from the Moll's catalog [257]. Typical practice in diagnostic surgical pathology often employs a "cocktail" of low- and high-molecular-weight cytokeratins that will neither differentiate a reactive process from a neoplastic one nor reliably separate carcinoma from mesothelioma [245, 258–262]. However, such staining does permit the distinction of mesothelioma from the occasional lymphoma, melanoma, or epithelioid hemangioendothelioma that may involve the pleura.

The demonstration of cytokeratin expression will in most cases allow the exclusion of localized fibrous tumors or sarcomas involving the serosal membranes. Notable exceptions to this include synovial sarcomas and epithelioid sarcomas which often express cytokeratins, but are uncommonly encountered as primary tumors of the serosal membranes. Other mesenchymal malignancies such as some leiomyosarcomas and malignant fibrous histiocytomas may infrequently demonstrate aberrant expression of cytokeratins. Sarcomatoid mesotheliomas and the spindle cell component of biphasic mesotheliomas will generally stain positive for cytokeratins, and this remains a useful technique for distinguishing sarcomatoid mesotheliomas from other spindle cell malignancies (Fig. 5.12a, b) [263–265]. Reactive fibrous pleural lesions, including parietal pleural plaques and the desmoplastic stromal response induced by metastases, will also usually show positive staining of spindle cells for cytokeratins. Therefore, this technique does not aid in distinguishing reactive from neoplastic mesothelial proliferations [263, 265]. In addition, anti-cytokeratin antibodies will not discriminate between sarcomatoid mesotheliomas or the spindle cell component of biphasic mesotheliomas and sarcomatoid carcinomas, such as the sarcomatoid renal cell carcinoma or pleomorphic carcinoma of the lung [266].

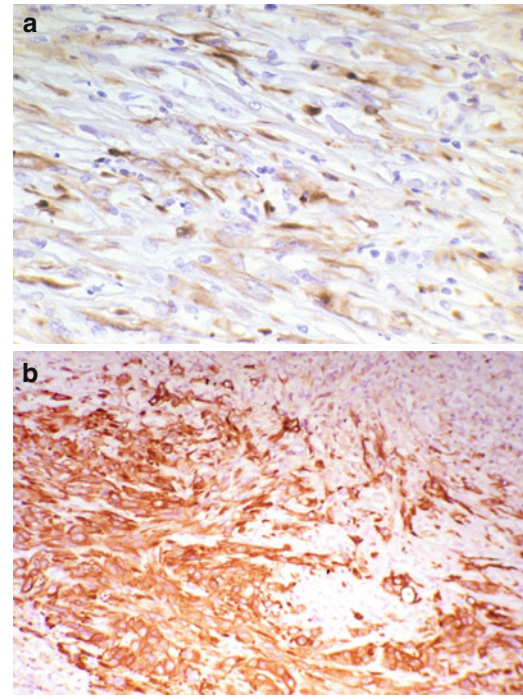


**Fig. 5.12** Immunohistochemical staining for cytokeratins using a cocktail of monoclonal antibodies to high- and low-molecular-weight cytokeratins. (**a**) Cytoplasmic staining of spindle cells in a sarcomatoid mesothelioma. (**b**) Staining of epithelial component (*middle*) and sarcomatoid component (*upper right corner*) of a biphasic mesothelioma

Recent investigation into the differential expression of the individual cytokeratins has proven useful to aid in the distinction of mesothelioma from carcinoma. Mesotheliomas selectively express cytokeratins 4, 5, 6, 14, and 17, which is not observed in adenocarcinomas (Fig. 5.13a) [267]. Ordóñez reported immunoreactivity for

cytokeratins 5/6 (CK 5/6) in each of 40 epithelial mesotheliomas but in none of 30 pulmonary adenocarcinomas. However, CK 5/6 was expressed in all 15 squamous cell carcinomas and in three of five large cell carcinomas of the lung, as well as 14 of 93 non-pulmonary adenocarcinomas [268]. Subsequent studies by Cury et al. report positive immunoreactivity for CK 5/6 in 56 of 61 epithelial mesotheliomas, compared with 9 of 63 cases of metastatic adenocarcinomas, including

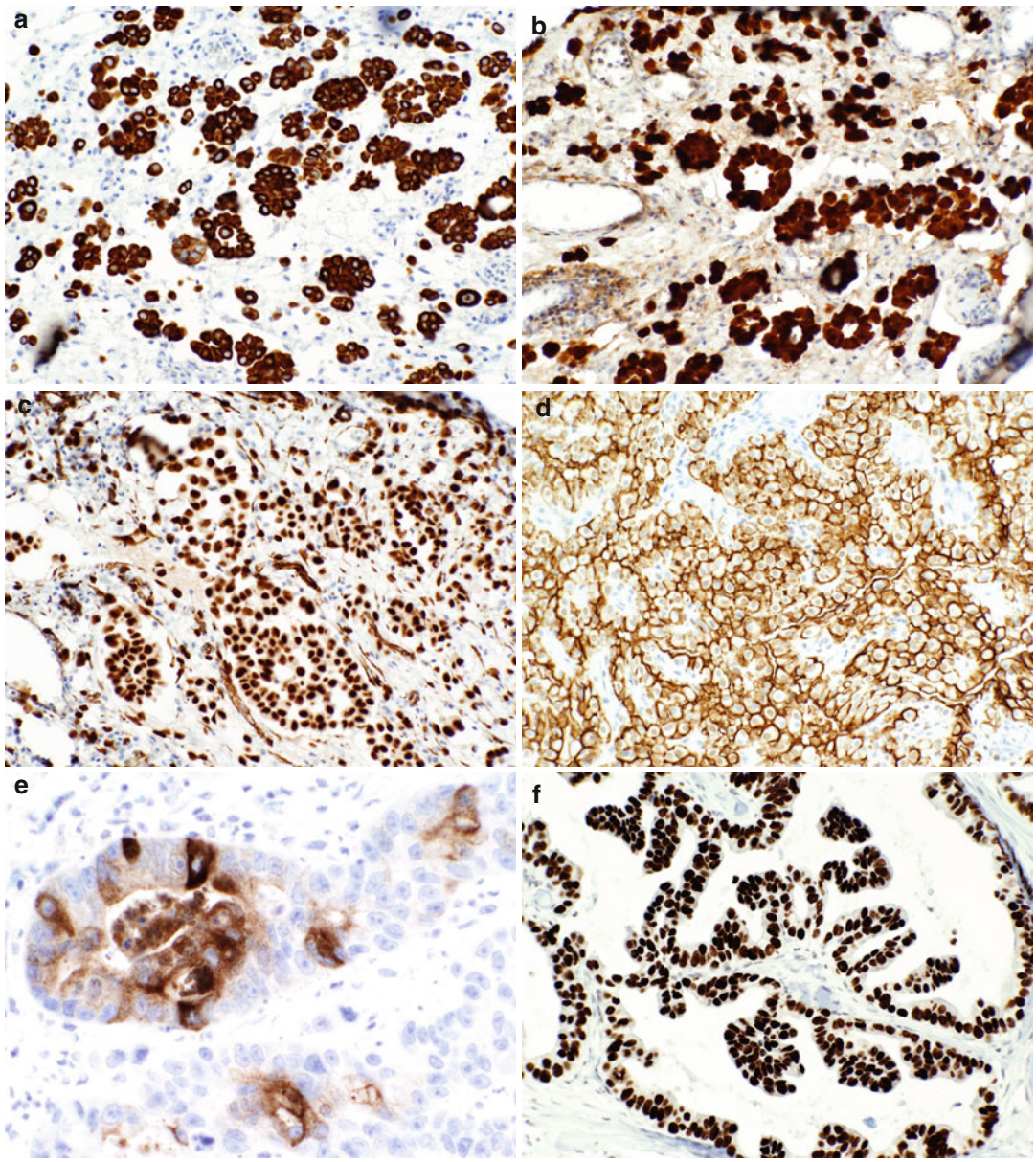


**Fig. 5.13** (**a**) Strong cytoplasmic staining of an epithelial mesothelioma for cytokeratins 5/6. (**b**) Strong nuclear staining of an epithelial mesothelioma for calretinin. (**c**) Strong nuclear staining of epithelial mesothelioma for WT-1. (**d**) Membranous staining of epithelial mesothelioma for D2-40. (**e**) In this adenocarcinoma metastatic to the pleura, the tumor cells are strongly positive for carcinoembryonic antigen (CEA). (**f**) Strong nuclear staining of a pulmonary adenocarcinoma for thyroid transcription factor-1 (TTF-1). (**g**) Cytoplasmic staining of pulmonary adenocarcinoma for Napsin-A. Parts (**a**–**c**) and (**d**) ×200; Part (**e**) ×400; Parts (**f**, **g**) ×200

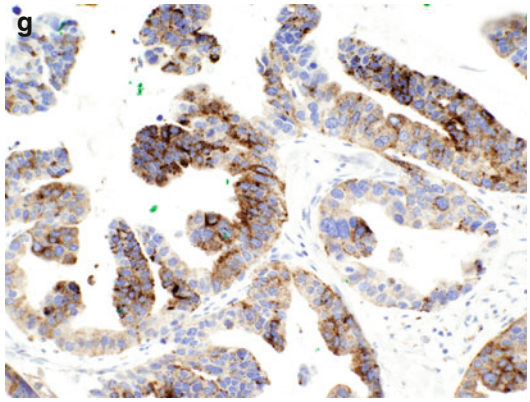


**Fig. 5.13** (continued)

one case of pulmonary adenocarcinoma [269]. Mesotheliomas also stain positive for cytokeratin 7 and usually stain negative for cytokeratin 20. We have observed occasional cases with focal moderate staining for the latter marker. This pattern is similar to that observed for primary adenocarcinomas of the lung and breast but differs from that of most adenocarcinomas of the gastrointestinal tract [270].

### 5.4.4.2 Glycoproteins

Numerous antibodies to cell surface glycoproteins have been evaluated for their pattern of differential expression in adenocarcinomas and mesothelioma. The antibodies considered for discussion are those routinely employed in the practice of diagnostic surgical pathology and include carcinoembryonic antigen (CEA), BerEP4, LeuM1 (CD15), B72.3, MOC-31, and epithelial membrane antigen (EMA). Excepting EMA, the diagnostic utility lies in that positive expression of these markers is a hallmark of most adenocarcinomas, and negative expression a feature of most mesotheliomas.

The oncofetal antigen CEA is a member of a large family of glycoproteins and the first immunohistochemical marker proven useful in the diagnostic evaluation of mesothelioma [256]. CEA is often expressed in adenocarcinomas of pulmonary and gastrointestinal origin (Fig. 5.13e), as well as those originating in the breast, liver, and pancreas [271]. Corson and Pinkus reported that some epithelial mesotheliomas may stain positively albeit weakly with CEA using polyclonal antibodies [272]. This is likely explained by the labeling of nonspecific, cross-reacting antigens and unrelated epitopes. Commercially available monoclonal CEA antibodies give cleaner backgrounds, less nonspecific cross-reactivity, and higher specificity for carcinoma with some reduction in sensitivity [273]. CEA is expressed in some 85–95 % of pulmonary adenocarcinomas and in at least 80 % of carcinomas from other sites [274–276]. Staining for CEA should be interpreted with some caution, as adenocarcinomas of the kidney, thyroid, and prostate do not generally express CEA. Moreover, papillary serous carcinomas of the ovary and peritoneum are usually CEA negative, which compromises the usage of this antibody in peritoneal mesotheliomas.

BerEP4 is a murine monoclonal antibody prepared from mice immunized with a breast carcinoma cell line [277]. Latza et al. first demonstrated its utility in the distinction between mesothelioma and adenocarcinoma, reporting immunoreactivity to the antibody by 99 % of adenocarcinomas from various sites, contrasted with no reactivity in 14 epithelial mesotheliomas [277]. Other studies indicated the potential staining for BerEP4 by some mesotheliomas. In view of the reported discrepancy, Ordóñez performed a review of the collective experience with this antibody [278]. This author observed BerEP4 immunoreactivity in 18/70 (26 %) mesotheliomas, compared with 20/20 (100 %) pulmonary adenocarcinomas and 55/59 (93 %) non-pulmonary adenocarcinomas. BerEP4 staining in mesothelioma, when present, was generally weak and restricted to limited numbers of cells, whereas focal, diffuse, or negative staining was observed in metastatic adenocarcinomas of unknown primary site. The primary utility of BerEP4 lies in its ability to separate adenocarcinoma of the lung from mesothelioma, with lesser ability to distinguish mesothelioma from metastatic carcinomas to the pleura originating from non-pulmonary sites.

The monoclonal antibody LeuM1 (also known as CD15) is a myelomonocytic antigen once believed a specific marker for Hodgkin disease,

owing to its ability to decorate Reed-Sternberg cells. Immunoreactivity to this antibody has since been shown in non-Hodgkin lymphomas, leukemias, as well as some carcinomas [279, 280]. Sheibani et al. were the first to report its application in the diagnosis of mesothelioma [273, 280]. These authors reported LeuM1 immunoreactivity in 105/179 (59 %) adenocarcinomas of various sites, in 47/50 (94 %) pulmonary adenocarcinomas, and in none of 28 mesotheliomas. LeuM1 appears to be a specific but rather insensitive marker for distinguishing adenocarcinoma from mesothelioma.

B72.3 is a murine monoclonal antibody generated against a membrane-enriched fraction derived from human breast cancer cells that recognizes tumor-associated glycoproteins present in a number of pulmonary and non-pulmonary adenocarcinomas. Lafebvre et al. were the first to report its utility in the diagnosis of mesothelioma, showing immunoreactivity in 17/20 (85 %) pulmonary adenocarcinomas, contrasted with weak staining in two of ten mesotheliomas [281]. Subsequent studies appear in concurrence with a high degree of B72.3 expression in adenocarcinomas, especially of pulmonary origin, with up to 5 % of mesotheliomas staining positive, albeit weakly and focally [282, 283].

Monoclonal antibody MOC-31, an anti-EP-CAM antibody, was formed using neuraminidase-treated cells from a small cell lung carcinoma cell line [284]. As a result, the antibody positively stains lung adenocarcinomas (90–100 %), lung squamous cell carcinomas (97 %), and serous carcinoma of the ovary/peritoneum (98 %) [285–288]. MOC-31 has value as a negative marker for mesothelioma; however, it has been reported to show focal staining in 2–10 % of mesothelioma cases [285, 286, 289–292].

Epithelial membrane antigens (EMA) are associated with human mucins produced in a broad array of glandular and lactating epithelia, and diagnostic immunohistochemical stains have been developed using monoclonal antibodies to human milk fat globules and carcinomas. Membranous staining of tumor cells by HMFG-2 has been reported to be a feature of mesothelioma, whereas adenocarcinomas generally show cytoplasmic immunoreactivity [293]. Other studies have discounted this observation, citing difficulties in consistency and interpretation of such staining patterns [294, 295]. Immunoreactivity to anti-EMA monoclonal antibodies is seen in both mesothelioma and carcinoma.

#### 5.4.4.3 Calretinin

Calretinin is a 29-kDa protein, which belongs to a large family of cytoplasmic calcium-binding proteins that also includes S-100 protein. Calretinin differs from the majority of the commonly employed diagnostic antibodies in that positive immunoreactivity is supportive of the diagnosis of mesothelioma. Doglioni et al. described positive calretinin staining in both a nuclear and cytoplasmic distribution in 44/44 (100 %) mesotheliomas, including sarcomatoid variants, compared to the focal staining observed in 28/294 (10 %) adenocarcinomas [296]. Subsequent studies demonstrated calretinin staining in 100 % of mesotheliomas, compared with weak and focal staining in 4/34 (12 %) adenocarcinomas [297, 298]. Ordóñez observed strong calretinin immunoreactivity in 100 % of 38 mesotheliomas as well as focal staining in 14/155 (9 %) adenocarcinomas and in 11/28 (39 %) squamous cell carcinomas using the Zymed monoclonal antibody [299]. Nuclear staining is far more specific than cytoplasmic staining (Fig. 5.13b) [269]. Staining in sarcomatoid mesotheliomas is less dependable and usually focal when present.

#### 5.4.4.4 WT-1

Wilms' tumor suppressor gene protein (WT-1) is located on chromosome 11p13. It has value as a positive marker of mesothelioma as seen by nuclear staining (Fig. 5.13c). It is primarily useful in distinguishing pleural mesotheliomas from lung adenocarcinomas. In 1995, Amin et al. reported positive staining in 20/21 (95 %) mesotheliomas and in 0/26 lung adenocarcinomas [300]. Kumar-Singh et al.'s findings were similar with positive staining in 39/41 (95 %) mesotheliomas as compared to negative staining in all 16 lung adenocarcinomas [301]. Ordóñez reported positive staining in 36/50 (72 %) epithelial

mesotheliomas [302]. It should be noted that WT-1 will positively stain renal cell carcinoma and papillary serous carcinoma of the ovary and thus has more limited use in securing a diagnosis of peritoneal mesothelioma.

#### 5.4.4.5 Podoplanin/D2-40

Podoplanin, a transmembrane mucoprotein present in lymphatic endothelial cells, and D2-40, a commercially available monoclonal anti-podoplanin antibody, demonstrate positive cytoplasmic membrane staining (Fig. 5.13d) in 96 % of malignant mesotheliomas and in only 7 % of lung adenocarcinomas [213]. While D2-40 is of value in discriminating between malignant mesothelioma and pulmonary adenocarcinoma, it should be further noted that D2-40 cannot distinguish mesothelioma from serous adenocarcinoma, seminoma, malignant peripheral nerve sheath tumors, or a subset of angiosarcomas [303]. Several studies have reported mixed results regarding D2-40 staining of sarcomatoid malignant mesothelioma. Takeshima et al. describe high background staining for D2-40 which can be compensated for by (1) seeking areas of higher cellularity as well as (2) gauging the intensity of staining. They report that using these two refining techniques can aid in discriminating sarcomatoid pleural mesothelioma from pulmonary sarcomatoid carcinoma. D2-40 positively stained 39/45 (87 %) cases of sarcomatoid pleural mesothelioma and 7/27 (26 %) cases of pulmonary sarcomatoid carcinoma [304]. Chu et al. reported less frequent staining of D2-40 in sarcomatoid and biphasic histologies when compared to epithelial. They demonstrated 100 % staining in epithelial mesothelioma, 62.5 % staining in biphasic mesothelioma, and 75 % staining in sarcomatoid mesothelioma cases [305]. Conversely, Ordóñez reported no staining in six cases of sarcomatoid mesothelioma and in five biphasic mesotheliomas [306]. Muller et al. had similar results with no staining observed in 18 cases of sarcomatoid mesothelioma [307].

#### 5.4.4.6 Thrombomodulin

Thrombomodulin is a surface glycoprotein involved in the regulation of intravascular coagulation and may be expressed in a variety of normal and neoplastic epithelia, as well as in the mesothelium and endothelium. Collins et al. first described its expression in mesothelioma, observing immunoreactivity in all 31 cases of mesothelioma studied, contrasted with 8 % of pulmonary adenocarcinomas [308]. Ordóñez observed immunoreactivity in 75–80 % of mesotheliomas, contrasted with 15 % of adenocarcinomas [309]. Since staining with this antibody is primarily surface membrane in distribution and since blood vessels also stain positive, interpretation can be difficult in some cases [269].

#### 5.4.4.7 HBME-1

HBME-1 is a monoclonal antibody developed from a suspension of cells from a well-differentiated epithelial mesothelioma, with the immunogen originally believed present on cell surface microvilli. The actual antigen remains unknown and this antibody is not specific for mesothelium, with positive immunoreactivity present in a number of adenocarcinomas. Initial experience with this antibody suggested that differences in staining patterns observed in mesothelioma (strong membranous staining) versus adenocarcinoma (cytoplasmic staining) were diagnostically useful [310, 311]. Subsequent studies have observed common staining patterns shared by the two classes of tumor [309]. Some investigators still use HBME-1 in their diagnostic armamentarium, but only at much higher dilutions (1:5,000 to 1:15,000) than that recommended by the manufacturer (1:50) (Doug Henderson, Sam Hammar, and Hector Battifora, personal communication).

#### 5.4.4.8 Cadherins

The cadherins constitute a family of glycoproteins involved in calcium-dependent intercellular adhesion [312]. E-cadherin is expressed in epithelial cells, whereas N-cadherin is present in the nerve, skeletal muscle, and mesothelium [313]. Soler et al. have reported positive immunostaining for E-cadherin in 13/14 (93 %) of pulmonary adenocarcinomas compared with negative staining in 13 mesotheliomas [314]. Similar findings have been reported by Leers et al. using the

monoclonal antibody HECD-1, wherein 20/21 (95 %) metastatic adenocarcinomas were positive, contrasted with 3/20 (15 %) mesotheliomas [297]. Ordóñez noted focal immunoreactivity for E-cadherin in 3/50 (6 %) mesotheliomas, compared to diffuse staining in 34/40 (85 %) pulmonary adenocarcinomas [314]. Soler et al. reported positive immunoreactivity for N-cadherin in 92 % of mesotheliomas, contrasted with 7 % of adenocarcinomas [313]. The utility of N-cadherin is somewhat limited by its common expression in ovarian adenocarcinomas [315].

#### 5.4.4.9 TTF-1

Thyroid transcription factor-1 (TTF-1) is a tissue-specific transcription factor expressed in the thyroid gland, in parts of the developing brain, and by type II pneumocytes and Clara cells in the lung, but not in the mesothelium. Studies by Bejarano et al. and Di Loreto et al. demonstrated high rates of TTF-1 expression in pulmonary adenocarcinomas, with no immunoreactivity observed in non-pulmonary adenocarcinomas or in epithelial mesotheliomas (Fig. 5.13f) [316, 317]. These observations were confirmed by Ordóñez, who detected TTF-1 immunoreactivity in 30/40 (75 %) pulmonary adenocarcinomas and 10/10 thyroid carcinomas, contrasted with negative expression observed in the remainder of the adenocarcinomas from various other sites and in all 50 mesotheliomas [233]. This antibody thus may serve the dual purpose of distinguishing pulmonary adenocarcinoma from mesothelioma and, if positive, demonstrate a high degree of specificity regarding the site of tumor origin.

#### 5.4.4.10 Napsin-A

Napsin-A, an aspartic proteinase expressed by type II pneumocytes which assists in the maturation of surfactant protein B, positively stains the cytoplasm of type II pneumocytes and alveolar macrophages (Fig. 5.13g) as well as renal epithelial cells. It has recently been identified as a marker for pulmonary adenocarcinomas [318]. Bishop et al. examined staining patterns of Napsin-A in a set of 95 adenocarcinomas, 45 squamous cell carcinomas, and 6 neuroendocrine neoplasms of the lung. Also included in this study were 38 cases of malignant mesothelioma. Napsin-A demonstrated positive staining in 81/95 (85 %) pulmonary adenocarcinomas, whereas negative staining was observed in all squamous carcinomas, lung neuroendocrine neoplasms, and malignant mesotheliomas [319]. Thus, although relatively few studies have examined the staining of Napsin-A in malignant mesothelioma, it may serve to differentiate adenocarcinoma of the lung from pleural mesotheliomas with epithelial histology.

### 5.4.5 Ultrastructural Features

While application of the above-described histochemical and immunohistochemical studies frequently is sufficient to diagnose mesothelioma, there are cases in which those studies are not adequate or equivocal. In such instances, the observation of characteristic ultrastructural attributes using electron microscopy may permit the diagnosis of mesothelioma. There is no single ultrastructural feature that is unique to malignant mesothelioma. Rather, there exists a constellation of ultrastructural features that are characteristic of the tumor. Such features include long surface microvilli, abundant intermediate filaments, and often prominent accumulations of intracytoplasmic glycogen [320–325]. The ultrastructural features common to mesothelioma may be demonstrated to varying degrees in individual cases, and the absence of a single feature (such as long surface microvilli) does not necessarily negate the diagnosis [326, 327].

One of the most conspicuous and useful ultrastructural feature observed in the epithelial variant of mesothelioma is the presence of long, slender, smooth surface microvilli that stand in contrast to the less abundant, shorter, blunt microvilli seen in adenocarcinomas (Fig. 5.14a, b). The microvilli of adenocarcinoma may also have a fuzzy appearance, due to the presence of ample surface glycocalyx [17, 327]. The microvilli of adenocarcinoma may also demonstrate glycocalyceal bodies and prominent rootlets [17]. As these differences in microvillus structure between mesothelioma

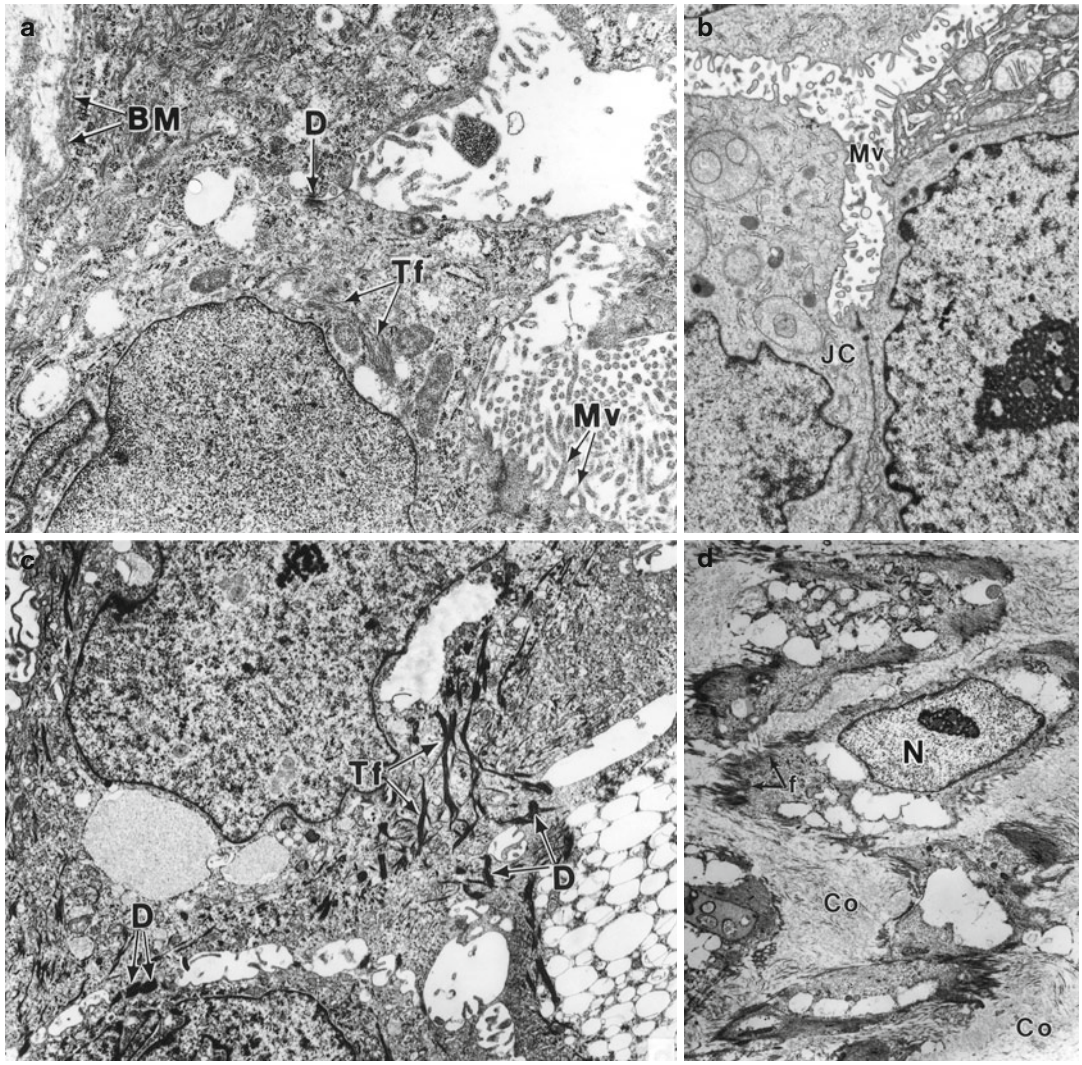


**Fig. 5.14** (**a**) This epithelial mesothelioma illustrates long, slender surface microvilli (*Mv*), tonofibrillar bundles (*Tf*), desmosomes (*D*), and basal lamina (*BM*). (**b**) Blunt surface microvilli (*Mv*) and a junctional complex (*JC*) are observed in this adenocarcinoma metastatic to the pleura. Intermediate filaments and tonofibrillar bundles are not identified. (**c**) Numerous tonofibrillar bundles (*Tf*) and prominent desmosomes (*D*) are present in this squamous cell carcinoma of the lung. (**d**) This sarcomatoid mesothelioma demonstrates spindle cells with cytoplasmic filaments (**f**) and abundant extracellular collagen (*Co*), *N* nucleus. Transmission electron micrographs, part (**a**) ×10,000, part (**b**) ×6,000, part (**c**) ×6,000, part (**d**) ×4,000 (Parts (**a**, **b**, and **d**) reprinted from Ref. [2], with permission)

and adenocarcinoma may be variable and to a degree subjective, some studies have sought to establish more objective criteria by examining the aspect (length to diameter) ratios of the microvilli in the two types of tumor cells [323–325]. Mesotheliomas have been found to have microvilli with a mean length-to-diameter ratio of approximately 16, compared to a value of approximately 9 for the microvilli of adenocarcinoma in most reported cases [2, 267, 323–325].

Another useful finding is the presence of such microvilli in mesothelioma not only at the luminal surface but also at the abluminal surface of the tumor cell. Dewar reported microvilli making direct contact with collagen through basal lamina defects in 10 of 12 mesotheliomas studied,

compared with 0 of 20 adenocarcinomas [328]. Although examination of ultrathin sections prepared from glutaraldehyde-fixed tissue is preferred, the presence of long surface microvilli can be detected using a formalin-fixed, paraffin-embedded material. Jandik et al. measured the aspect ratios of microvilli in seven mesotheliomas and seven adenocarcinomas using scanning electron microscopy, with results comparable to those performed using transmission electron microscopy on the same tumors [329].

Intercellular junctions of the macula adherens type (true desmosomes) are found with equal frequency in epithelial mesotheliomas and adenocarcinoma [2], although some qualitative differences have been reported. Burns et al. found that "giant desmosomes" (i.e., desmosomes greater than 1 μm in length) were more frequent in mesothelioma, although mean desmosomal length was not significantly different in the two groups [330]. Ghadially found giant desmosomes in two of ten epithelial mesotheliomas and in no adenocarcinomas studied [331]. Mukherjee et al. reported a freeze-fracture study of intercellular junctions in two cases of pleural mesothelioma obtained by biopsy and noted that both gap and tight junctions were less well developed and less numerous than those in exfoliated mesothelioma cells present in effusions or in benign mesothelial cells [332].

Mesotheliomas generally contain significantly more intermediate filaments, condensed as perinuclear tonofibrillar bundles, than do adenocarcinomas [2, 323]. These tonofilaments insert into the large desmosomes connecting the cells. Whereas studies by Warhol and Roggli have shown the increased tonofilament content of mesothelioma over adenocarcinomas of the lung, breast, ovary, and endometrium, these may be absent in some epithelial mesotheliomas [17, 323] and prominent in squamous or adenosquamous carcinomas of the lung (Fig. 5.14c). Hyaluronic acid, identified ultrastructurally as medium electron-dense material, or crystalline, scroll-like structures, may be found in tumor neolumina, in embedding microvilli, or in the extracellular space [17, 327]. Ordonez reported crystalloids in the cytoplasm of 9 of 59 consecutive epithelial mesotheliomas studied ultrastructurally [333].

Certain ultrastructural observations when present would tend to exclude a diagnosis of mesothelioma. These include the presence of mucous granules, dense core neuroendocrine granules, zymogen granules, and Clara cell granules. Adenocarcinomas derived from type II pneumocytes may contain multivesicular and lamellar bodies not seen in mesothelioma cells. Similarly, the presence of pinocytotic vesicles together with Weibel-Palade bodies is pathognomonic of vascular endothelial differentiation, which would suggest a diagnosis of epithelioid hemangioendothelioma or pleural angiosarcoma.

Sarcomatoid mesotheliomas generally have ultrastructural features that resemble those of soft tissue fibrosarcomas (Fig. 5.14d) [2, 17, 322, 334]. The features of these spindled tumor cells are elongated nuclei with prominent nucleoli, short cytoplasmic fragments of distended rough endoplasmic reticulum, occasionally prominent intermediate cytoplasmic filaments, and variable quantities of extracellular collagen. In some cases, the tumor cells resemble myofibroblasts, with peripherally located actin filaments, occasionally associated with dense bodies [17, 335]. Cells with transitional features intermediate between epithelial and mesenchymal cells have been described. These include the presence of intercellular junctions, occasional surface microvilli, partial or incomplete basal lamina, and even a few tonofibrillar bundles.

## 5.5 Differential Diagnosis

Malignant mesothelioma must be distinguished from benign, reactive mesothelial proliferations on the one hand and on the other from various primary and secondary malignancies involving the serosal membranes. The distinction between reactive and malignant mesothelial proliferations constitutes a major difficulty in diagnostic surgical pathology, especially when dealing with small specimens such as needle biopsies. In cases of both epithelial and spindle cell proliferations, the demonstration of true stromal invasion is the

most accurate hallmark of malignancy [246]. The demonstration of such invasion may not be possible in small, superficial biopsies, and caution is warranted to avoid the pitfall of over interpreting a tangentially cut section. Additionally, benign processes and organizing effusions may result in the entrapment of reactive mesothelium in organizing fibro-inflammatory and granulation tissues, mimicking stromal invasion. Linear, in situ proliferations of mesothelial cells projecting into the cavity lumen should not be diagnosed as malignant, except in the setting of unequivocal stromal invasion. Churg et al. recently described the phenomenon of "fake fat" in a series of nine cases for which the distinction between organizing/fibrinous pleuritis and desmoplastic mesothelioma was challenging. The nine cases had fibrotic, paucicellular thickened pleura with linear arrays of round to oblong and even slit-like spaces oriented parallel to the pleural surface. Keratin stains highlighted keratin-positive cells between the aforementioned spaces of "fake fat" which failed to have cell lining on vimentin stain. The authors also note that spindle cells in reactive processes are oriented parallel to the pleural surface versus the storiform and patternless pattern seen in desmoplastic mesothelioma. Of the nine cases, a majority were men between 60 and 70 years old, and in all cases survival was greater than that of sarcomatoid mesothelioma with the exception of one case which was lost to follow-up [336].

Densely packed, sheets of mesothelial cells within body cavities may actually be common in reactive conditions involving the serosal membranes, but such collections within the confines of stroma favor malignancy. The demonstration of cytologic atypia may not be helpful in the distinction of benign versus malignant, as reactive mesothelial hyperplasia may be accompanied by striking atypia, and some epithelial mesotheliomas may show bland and monotonous cytologic features [246]. Necrosis is typically associated with malignant processes but may be seen in benign conditions as well. Ordóñez has shown that lesions described as nodular mesothelial hyperplasia, while not readily confused with mesothelioma, are primarily histiocytic proliferations with positive immunoreactivity for CD68 and are cytokeratin negative [337]. In some cases, sampling may show obvious malignant mesothelioma at one site and atypical, reactive changes at another. With generous sampling and careful attention to histologic and cytologic detail, the distinction between malignant epithelial mesothelioma and reactive mesothelial hyperplasia is generally possible in an adequate biopsy specimen [2].

Similar difficulties may be encountered when attempting to distinguish reactive fibroblastic processes involving the serosal membranes, in particular the pleura, and the sarcomatoid and desmoplastic variants of malignant mesothelioma. Fibrous pleurisy often features a "top heavy" zonation phenomenon, with areas of greatest cellularity and accompanying atypia at the interface with the pleural space, and increasing maturation of fibrosis with reduction in cellularity proceeding toward the chest wall. Such a graded pattern of cellularity, atypia and fibrosis is not a feature of sarcomatoid or desmoplastic tumors. The presence of elongated, vertical capillaries perpendicular to the pleural surface is typical of organizing effusions and fibrous pleurisy (Fig. 5.15a, b) and not a typical feature of sarcomatoid mesotheliomas. The demonstration of invasion is an important indicator of malignancy. Immunoperoxidase stains for cytokeratins are useful in illustrating the distribution of mesothelial cells and may reinforce the demonstration of invasion by showing cytokeratin-positive cells in subpleural soft tissues. Immunoreactivity for anti-cytokeratins per se is of no utility, as both benign and malignant mesothelial proliferations will display this pattern [246].

Distinguishing between metastatic adenocarcinoma and the epithelial variant of malignant mesothelioma is the most common diagnostic problem confronting the surgical pathologist faced with a biopsy of an epithelioid pleural malignancy. This problem, too, is magnified in the small biopsy specimen. The diagnostic adjuncts to assist in this distinction, including histochemistry, immunohistochemistry, and electron microscopy, are discussed in detail in their respective sections. In addition, the expected

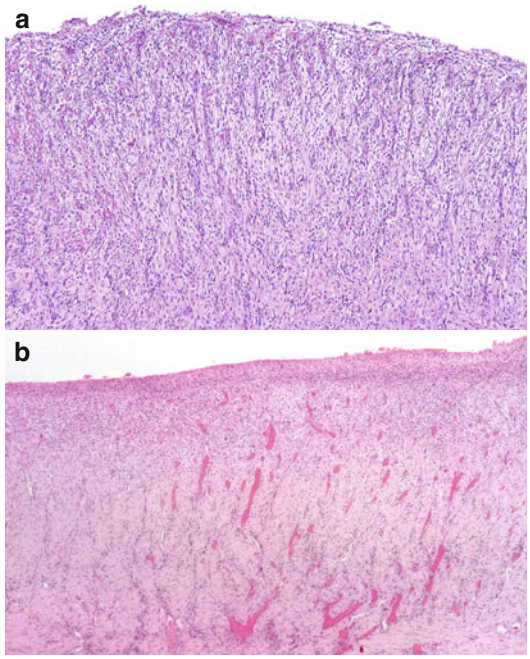


**Fig. 5.15** (**a**, **b**) Chronic organizing pleuritis delineates capillaries oriented perpendicular to the pleural surface almost completely traversing the fibrotic, thickened pleura. Parts (**a**, **b**) ×40

**Table 5.2** Expected immunohistochemical staining results for mesothelioma versus adenocarcinoma

| Antibody | Mesothelioma | Adenocarcinoma |
|---|---|---|
| Keratin cocktail | Pos. | Pos. |
| Cytokeratins 5/6 | Pos. | Neg.[a] |
| Cytokeratin 7 | Pos. | Pos/Neg[b] |
| WT-1 | Pos. (N) | Neg. |
| D2-40 | Pos. | Neg. |
| EMA | Pos. | Pos.[c] |
| HMFG-2 | Pos. | Pos.[c] |
| Calretinin | Pos. (N) | Neg. |
| Thrombomodulin | Pos. | Neg. |
| HBME-1 | Pos. | Neg. |
| N-cadherin | Pos. | Neg. |
| Cytokeratin 20 | Neg. | Pos/Neg[b] |
| CEA | Neg. | Pos. |
| BerEP4 | Neg. | Pos. |
| MOC-31 | Neg. | Pos |
| LeuM1 (CD15) | Neg. | Pos. |
| B72.3 | Neg. | Pos. |
| E-cadherin | Neg. | Pos. |
| TTF-1 | Neg. | Pos. (N) |
| Napsin-A | Neg. | Pos. |

*Pos*. positive staining, *Neg*. negative staining, *N* nuclear staining

[a]Many adenocarcinomas of extrapulmonary origin may stain positive. See Ref. [394]

[b]Varying combinations of CK7 and CK20 positivity are seen in adenocarcinomas, depending upon primary site. See Ref. [270]

[c]Distribution of staining is primarily membranous in mesothelioma, cytoplasmic in adenocarcinoma

results of common immunohistochemical stains used to distinguish mesothelioma from adenocarcinoma are listed in Table 5.2. Renal cell carcinoma metastatic to the pleura may be especially difficult to distinguish from pleural mesothelioma, as these tumors may feature sarcomatoid foci and exhibit an overlapping immunophenotype with coexpression of cytokeratins, EMA, vimentin, and occasionally CD10 and with negative CEA expression [338, 339]. Two of the editors (VLR and TAS) have observed ten such cases and believe that a diagnosis of pleural mesothelioma should be made with great caution in a patient with a solid renal mass that has not been sampled histologically.

Other neoplasms that may involve the pleura include localized fibrous tumors as well as leukemias and lymphomas, the latter requiring distinction from the lymphohistiocytoid variant of malignant mesothelioma [240]. Localized fibrous tumors are usually distinguished by the gross distribution as a pedunculated pleural tumor displaying the cytokeratin-negative, CD 34/bcl-2-positive immunophenotype. Soft tissue sarcomas most commonly involve the pleura via direct extension from the chest wall or as metastatic disease with hematogenous spread to the lung and thence to the pleura. This pattern of metastatic involvement has not been observed to result in the diffuse pleural thickening seen in advanced mesothelioma [21]. Metastatic sarcomas rarely pose a diagnostic problem, which may be further simplified by observing patterns of cytokeratin expression, which should be negative in the sarcoma and positive in sarcomatoid malignant mesotheliomas. However, some soft tissue sarcomas have been reported as primary pleural tumors. Those that seem to show a predilection for this include synovial sarcoma, angiosarcoma, and epithelioid hemangioendothelioma (EHE).

Primary vascular malignancies of the pleura are uncommon. The authors have observed seven cases, with an additional 29 reported in the literature. Six of our cases and ten of those reported in the literature were EHE. Weiss and Enzinger originally described EHE as a vascular malignancy of soft tissue with clinical behavior intermediate between hemangioma and angiosarcoma [340]. EHE has also been described in the lung, initially as intravascular bronchiolar and alveolar tumor (IVBAT) [341], subsequently recognized to be the pulmonary form of EHE [342]. In our experience, EHE of the pleura features a gross distribution identical to that of malignant (diffuse) pleural mesothelioma, forming a thick rind of tumor encasing the lung and spreading along fissures and secondary interlobular septa. Moreover, the clinical behavior of this tumor parallels that of malignant mesothelioma, with survival measured in months. Despite an epithelioid appearance, EHE is rather easily distinguished from mesothelioma using immunohistochemistry with positive staining observed in these cases for the vascular markers CD34, CD 31, and/or Factor VIII, combined with negative immunoreactivity for anti-cytokeratins [211–214]. Angiosarcomas of the pleura are exceedingly rare as well and consist of pleomorphic malignant endothelial cells lining the irregular and anastomosing vascular spaces. These tumors display an identical immunophenotype to that of EHE, with negative immunoreactivity to anti-cytokeratins and positive immunoreactivity to vascular markers. However, both EHE and epithelioid angiosarcomas may demonstrate keratin positivity in some cases. Both tumors may contain diagnostic Weibel-Palade bodies ultrastructurally, although these are less common in angiosarcoma.

Synovial sarcoma (SS) may under unusual circumstances provide a pitfall in the diagnosis of malignant pleural mesothelioma. Typically occurring in the thorax as metastatic tumor, SS has nonetheless been reported as a primary tumor of the lung, pleura, and mediastinum [343–347]. A typically biphasic tumor with epithelial and sarcomatous components with at least focal expression of cytokeratins and EMA, SS may mimic biphasic or sarcomatoid mesotheliomas

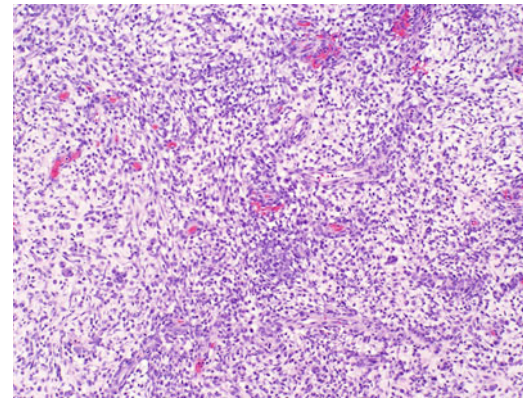

**Fig. 5.16** Biphasic mesothelioma mimicking biphasic synovial sarcoma. H&E ×100

(Fig. 5.16). Moreover, in a review of 103 cases of SS, Miettinen et al. found foci of calretinin expression in 29/41 (71 %) biphasic SS, particularly in the spindle cell component [343]. In contrast, epithelial mesotheliomas expressed calretinin diffusely while sarcomatoid mesotheliomas showed variable expression for this marker. BerEP4 and cytokeratins 5/6 were frequently expressed by SS. Differences in expression of cytokeratins by monophasic SS compared with sarcomatoid mesotheliomas have been found to be of help in distinguishing these two tumors [345]. TLE1 is a useful marker in the diagnosis of SS. However, this marker should be used cautiously as Matsuyama et al. demonstrated TLE1 expression in 28/29 mesothelioma (97 %) and staining was regardless of histologic type [346]. Finally, identification of the SYT/SSX transcript may be useful for confirming a diagnosis of SS of the pleura [345, 348].

### 5.5.1 Gross Distribution and Histologic Pattern of Disease

The accurate premortem diagnosis of mesothelioma involves a multi-tiered approach, beginning with information regarding the gross distribution of tumor. This information is seldom directly available to the pathologist when a small biopsy specimen is received for review in the laboratory. Information about the gross distribution can be

obtained from radiologic studies, such as chest roentgenograms, CT, or MRI of the thorax, or observations of the surgeon at time of thoracoscopy or thoracotomy for pleural tumors, and CT or MRI of the abdomen and pelvis or observations of the surgeon at time of laparoscopy or laparotomy for peritoneal or pelvic tumors. If the gross distribution is consistent with mesothelioma, then the next tier involves histologic assessment of the tumor for one or more of the patterns listed in Table 5.1. For tumors with visible secretions on routine histology, we then employ histochemical studies including PAS following diastase predigestion and alcian blue with and without hyaluronidase. If the secretions stain with DPAS and with alcian blue both before and after hyaluronidase, then adenocarcinoma is favored. If the secretions stain with alcian blue but are negative for DPAS and for alcian blue after hyaluronidase, then mesothelioma is favored (Figs. 5.10 and 5.11).

### 5.5.2 The Immunohistochemical Panel

The fourth tier involves immunohistochemical studies. As no single marker has sufficient sensitivity and specificity to distinguish between MM and other neoplasms, several authors have sought a limited/minimal panel of immunohistochemical stains for which MM can be accurately diagnosed. For epithelial pleural neoplasms, Kao et al. recommend calretinin, BG8, and CD15 with the addition of D2-40 in cases where the aforementioned three fail to be conclusive [349]. Klebe et al., in an analysis of 200 consecutive cases of 173 MM and 27 metastatic adenocarcinoma, also found positive staining for calretinin with negative staining for BG8 to be sufficient to distinguish between the two and also recommended CD15 in certain cases [350] and Yaziji et al. recommend calretinin, BG8, and MOC-31 [351]. The International Mesothelioma Interest Group does not recommend specific antibodies but does recommend both positive and negative markers with specificity of 80 % or greater [215]. The panel used in our laboratory differs somewhat by tumor type and location and is summarized in Table 5.3. For epithelial or biphasic tumors involving the pleura or the peritoneum, a cocktail of anti-cytokeratin antibodies that includes AE1/AE3, CAM 5.2, and MNF.116 is used to exclude lymphoma, melanoma, and epithelioid hemangioendothelioma. Epithelial mesotheliomas and most carcinomas stain strongly and diffusely with this antibody cocktail. CK 5/6, calretinin, WT-1, and D2-40 are also employed, as these stain a high percentage of epithelial mesotheliomas but a relatively low percentage of adenocarcinomas.

**Table 5.3** Suggested immunohistochemical panel for mesothelioma

| | Epithelial and/or biphasic | | |
|---|---|---|---|
| | Pleural | Peritoneal[a] | Sarcomatoid |
| First line | Cytokeratin cocktail<br>Cytokeratin 5/6<br>Calretinin<br>D2-40<br>WT-1<br>TTF-1<br>CEA | Cytokeratin cocktail<br>Cytokeratin 5/6<br>Calretinin<br>D2-40<br>WT-1<br>BerEP4<br>B72.3 | Cytokeratin cocktail<br>Vimentin<br>Calretinin<br>D2-40 |
| Second line | LeuM1<br>B72.3<br>BerEP4 | LeuM1<br>Thrombomodulin<br>HBME-1 | |

*CK* cytokeratin, *WT-1* Wilms' tumor -1, *CEA* carcinoembryonic antigen, *TTF-1* thyroid transcription factor-1, *ER* estrogen receptor, *PR* progesterone receptor. See text for details

[a]ER and PR added for peritoneal mesotheliomas in women

**Table 5.4** IHC staining results for 366 cases of epithelial or biphasic pleural mesothelioma

| | Cytokeratin | Calretinin | CK5/6 | WT-1 | D2-40 | TTF-1 | CEA |
|---|---|---|---|---|---|---|---|
| Epithelial | 193/193 | 194/198 | 198/216 | 203/224 | 195/213 | 1/212 | 21/206 |
| | 100 % | 98 % | 92 % | 91 % | 92 % | 0.50 % | 10 % |
| Biphasic | 76/76 | 73/79 | 56/72 | 64/80 | 60/72 | 0/70 | 3/69 |
| | 100 % | 92 % | 78 % | 80 % | 83 % | 0 % | 4 % |

*CK* cytokeratin, *WT-1* Wilms' tumor -1, *CEA* carcinoembryonic antigen, *TTF-1* thyroid transcription factor-1. See text for details

**Table 5.5** IHC staining results for 57 cases of epithelial or biphasic peritoneal mesothelioma

| | Cytokeratin | Calretinin | CK5/6 | WT-1 | D2-40 | BerEP4 | B72.3 |
|---|---|---|---|---|---|---|---|
| Epithelial | 40/40 | 39/40 | 40/45 | 40/44 | 43/47 | 4/44 | 0/48 |
| | 100 % | 98 % | 89 % | 91 % | 91 % | 9 % | 0 % |
| Biphasic | 6/6 | 4/5 | 3/4 | 2/4 | 3/5 | 0/3 | 0/3 |
| | 100 % | 80 % | 75 % | 50 % | 60 % | 0 % | 0 % |

*CK* cytokeratin, *WT-1* Wilms' tumor -1, *CEA* carcinoembryonic antigen, *TTF-1* thyroid transcription factor-1. See text for details

Nuclear staining for Zymed calretinin antibody is highly specific and sensitive for mesotheliomas. A cautionary note is that rare cases of thymic epithelial neoplasms have been reportedly positive for calretinin [352, 353]. For pleural tumors, the panel is rounded out with CEA and TTF-1, which stain a high percentage of pulmonary adenocarcinomas and a very low percentage of mesotheliomas. LeuM1, BerEP4, and B72.3 are held in reserve for cases with discordant immunohistochemical findings. For peritoneal tumors, CEA and TTF-1 are less effective at excluding adenocarcinomas, so BerEP4 and B72.3 are substituted. LeuM1, HBME-1, and thrombomodulin are second-line antibodies for cases with discordant results. Our experience with these antibodies is summarized in Tables 5.4 and 5.5 with the addition of staining intensity in Fig. 5.17.

Most of the antibodies that stain epithelial mesotheliomas have inconsistent or focal staining for sarcomatoid mesotheliomas. Therefore, the only antibodies we use for pure sarcomatoid malignancies involving the serosal membranes are vimentin and the cytokeratin cocktail. A high percentage of sarcomatoid mesotheliomas stain strongly and diffusely positive for low-molecular-weight cytokeratins, whereas most sarcomas are either negative or focally positive. Cytokeratin stains are also useful for detecting subtle invasion in desmoplastic mesotheliomas. Vimentin is a useful indicator of appropriate fixation, as a negative stain for vimentin in a sarcomatoid malignancy probably indicates poor fixation. Sarcomatoid mesotheliomas rarely are cytokeratin negative, and at least a portion of these are also vimentin negative. We have also found value in the use of calretinin and D2-40 with sarcomatoid mesotheliomas. Our experience with these antibodies is summarized in Table 5.6 with the addition of staining intensity in Fig. 5.18.

### 5.5.3 Electron Microscopy

The fifth tier of investigation is electron microscopy. An accurate diagnosis of mesothelioma can be made on an adequately sampled tumor in the vast majority of cases using the first four tiers of investigation. Therefore, we reserve ultrastructural studies for those cases in which the diagnosis remains in doubt. Examples include cases with discordant immunohistochemistry after the second-line antibodies have been used or very unusual variants such as localized malignant mesothelioma or the so-called "mucin-positive" mesothelioma.

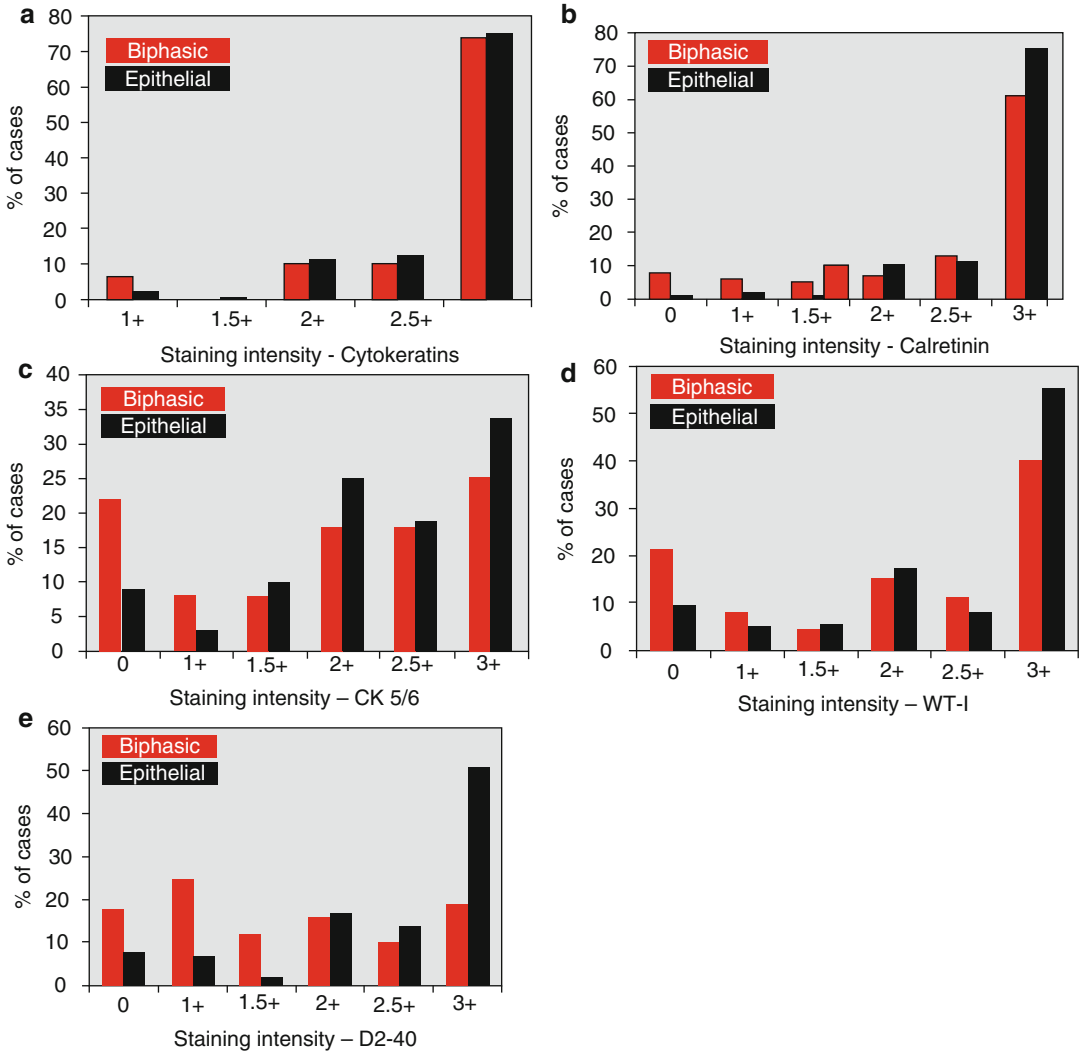


**Fig. 5.17** Epithelial and biphasic mesothelioma immunohistochemical data regarding intensity of immunopositivity for (**a**) cytokeratins; (**b**) calretinin; (**c**) CD5/6; (**d**) WT-1; (**e**) D2-40

**Table 5.6** IHC staining in 76 cases of sarcomatoid mesothelioma

| | Cytokeratin | Vimentin | D2-40 | Calretinin |
|---|---|---|---|---|
| Sarcomatoid | 69/75 | 57/60 | 11/20 | 17/36 |
| | 92 % | 95 % | 55 % | 47 % |

### 5.5.4 Biomarkers

As the distinction between benign/reactive and neoplastic mesothelia is often a challenge, an interesting area of development since the last publication of this text is the quest for molecular biomarkers which can signal a diagnosis of malignancy in the setting of reactive mesothelial proliferations (RM) versus malignant mesothelioma (MM) or even a non-mesothelial malignancy involving the serosa.

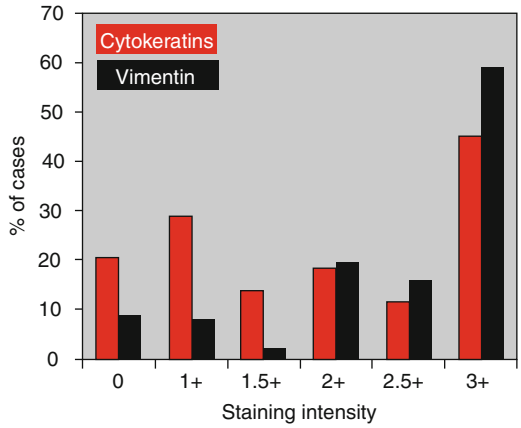


**Fig. 5.18** Sarcomatoid mesothelioma immunohistochemical data regarding intensity of immunopositivity for cytokeratins and vimentin

#### 5.5.4.1 EMA

Cury et al. assessed 31 consecutive cases of malignant mesothelioma for EMA expression as well as areas which they considered to be mesothelioma in situ. In all but one case, tissue was obtained via surgical biopsy with the remaining case being an extrapleural pneumonectomy. Strong and diffuse cytoplasmic membrane staining for EMA was seen in 30/31 (97 %) cases as well as in all areas considered to represent in situ disease. Additionally, 4/4 core biopsies previously classified as suspicious for mesothelioma were assessed and demonstrated strong staining for EMA. Weak to moderate staining was seen in 5/20 reactive mesothelial proliferations and 6/14 cases of reactive pleural fibrosis stained focally and weakly positive for EMA [354]. Saad et al. in 2003 investigated the use of two EMA clones, Mc5 and E29, in the distinction between reactive mesothelial proliferations and malignant mesothelioma. The E29 was demonstrated to have superior specificity in the distinction between reactive and neoplastic with positive staining in 15/20 (75 %) of mesotheliomas and 0/20 reactive processes. Saad concluded that the EMA M29 clone was a "reliable immunohistochemical marker to differentiate mesothelioma from reactive mesothelium" [355]. Attanoos et al. reviewed 40 cases of reactive mesothelial hyperplasia and 60 mesotheliomas using a panel of biomarkers which included EMA. Of the mesothelioma cases, 20 were from open biopsy, 22 from pleural biopsy, and 18 from autopsy. They found 48/60 (80 %) mesotheliomas and 8/40 (20 %) reactive lesions stained positively for EMA [356].

Shen et al. assessed EMA staining using cell blocks from body cavity fluids in 35 cases of histologically confirmed mesothelioma and in 38 benign effusions. Receiver operating characteristic (ROC) curves were created for several biomarkers including EMA, GLUT-1, and XIPA. They found EMA to perform better than other biomarkers assessed in distinguishing benign versus malignant [357]. Hasteh et al. also sought to determine the efficacy of a large panel of biomarkers including EMA, desmin, GLUT-1, p53, and Ki-67. They concluded that EMA-positive/desmin-negative staining favored mesothelioma while the opposite pattern favored reactive mesothelial hyperplasia [358]. Kuperman et al. in 2011 assessed staining for EMA, desmin, and polyclonal GLUT-1 in effusions in 25 cases of confirmed malignant mesothelioma versus benign and reactive pleural effusions. Of malignant mesothelioma cases, a greater proportion were epithelial ($n=19$). Immunohistochemical stains were graded based on percentage of cells staining positively and ROC curves were generated for each antibody. Kuperman found that combining staining results for EMA and GLUT-1 had the best discriminatory results [359].

#### 5.5.4.2 p53

p53 is a 53-kD protein which inhibits the entry of cells into the S-phase of the cell cycle. Ramel et al. in 1992 investigated p53 using three different antibodies (DO7, CM-1, and PAb240) to distinguish between mesothelioma and reactive mesothelium. They reported focal nuclear staining for p53 using DO7 and CM-1 antibodies in 9/36 (25 %) of mesothelioma cases. For these nine cases with positive staining, it should be noted that six (60 %) had only 1–5 % of cells stain positively [360]. Cury et al., in the aforementioned study, also examined staining

patterns of p53 among cases of malignant mesothelioma and found 30/31 (97 %) to have strong nuclear staining, although staining was often localized, with epithelial morphology performing better. Staining was also observed in areas of in situ disease and in three of four needle core biopsies previously classified as suspicious for mesothelioma. There was focal staining for reactive mesothelial fibrosis in 3/14 (21 %) cases and focal weak staining in 13/20 (65 %) cases with reactive mesothelial hyperplasia. Of note, 8 of the 20 reactive hyperplastic lesions demonstrated papillary morphology [354]. Attanoos et al. found no staining in 40 cases of reactive mesothelium and positive staining in 27/60 (45 %) mesotheliomas; however, they noted that in only 2 of the 27 cases with positive staining had staining in >75 % of the neoplastic cells [356]. Hasteh et al. found positive nuclear staining for p53 in 7/15 (47 %) mesotheliomas and 1/46 (2 %) reactive mesothelial hyperplasias [358].

#### 5.5.4.3 Glucose Transporter-1 (GLUT-1)

GLUT-1 is a transmembrane glucose transporter which has been identified in numerous malignant neoplasms. Hasteh et al. found 5/43 (12 %) reactive mesothelial hyperplasias and 7/15 (47 %) mesotheliomas to stain positively [358]. In the study by Shen et al., 14/38 (37 %) reactive mesothelial hyperplasias and 29/35 (83 %) mesotheliomas demonstrated positive cytoplasmic membrane staining for polyclonal GLUT-1. Their generated ROC curves for distinguishing benign versus malignant mesothelial processes showed polyclonal GLUT-1 to perform similar to EMA and better than monoclonal GLUT-1 and XIAP [357]. In a similar study, Kuperman et al., as described above, found utility in the combined results of EMA and GLUT-1 staining [359]. Kato et al. demonstrated positive staining for GLUT-1 in 0/40 reactive mesothelial hyperplasias and 48/48 (100 %) mesotheliomas [361]. Lastly, Monaco et al. found 5/70 (7 %) benign cases and 27/60 (40 %) cases of mesothelioma and noted GLUT-1 positivity to be more common in pleural versus peritoneal mesothelioma cases [362].

#### 5.5.4.4 Desmin

Desmin is an intermediate filament present in smooth and skeletal muscle and thus serves as a marker of myogenic differentiation. Several neoplasms including malignant mesothelioma have been reported to express desmin; however, it is unique in that it is one of the only biomarkers proposed to be preferentially expressed in non-malignant mesothelium. Attanoos et al. found desmin expression in 6/60 (10 %) mesotheliomas whereas it was present in 34/40 (85 %) reactive mesothelial cases [356], and similarly Hasteh reported positive staining in 54/64 (84 %) reactive mesothelial hyperplasias versus 3/52 (6 %) mesotheliomas. Hasteh also noted that when desmin and EMA were used together that 49/52 (98 %) mesotheliomas were EMA positive/desmin negative, and conversely, 55/64 (86 %) reactive mesothelial hyperplasias were EMA negative/desmin positive [358].

#### 5.5.4.5 Other biomarkers

X-linked inhibitor of apoptosis protein (XIAP) is present in various neoplasms as well as in normal tissue [357]. Wu et al. found 81 % of examined mesothelioma cases to stain positively for XIAP, while no staining was seen in benign cases and weak focal staining was seen in 1/3 (30 %) reactive cases [363]. Wu concluded that XIAP had value in distinguishing between benign and malignant mesothelioma, and Lyone-Bordeaux et al. had similar results with 4/5 (80 %) mesotheliomas staining positively for XIAP and 2/19 (11 %) benign cases stained positively [364]. However, Shen et al. found XIAP to have low specificity, with positive staining in 60 % benign cases and in 82 % mesotheliomas [357].

P-glycoprotein assists in cell membrane transport and has been associated with resistance to chemotherapy [356]. Ramael et al. found cytoplasmic staining for P-170 p-glycoprotein in 31/33 (93 %) mesotheliomas and in 0/27 benign cases [364]. However, Attanoos et al. found only 2/15 mesotheliomas to stain positively for p-glycoprotein, yet their results in reactive mesothelial cases were similar as no reactivity was seen. They concluded that there was no use for p-glycoprotein in distinguishing reactive

mesothelial hyperplasias from mesothelioma [365]. Bcl-2 is a proto-oncogene which promotes the survival of cells via the inhibition of apoptosis [365]. Attanoos, Segers, and Cury found no utility for bcl-2 in distinguishing reactive mesothelial hyperplasias from mesothelioma [354, 356, 365].

No single biomarker has thus far reliably demonstrated the ability to distinguish benign/reactive from malignant mesothelioma for which such a grave diagnosis should be made. Furthermore, while using a panel of biomarkers (EMA, desmin, GLUT-1, p53) may provide limited assistance in difficult cases, they should be used cautiously as Salman et al. reported a case of EMA-negative/desmin-positive malignant peritoneal mesothelioma which was diagnosed as such via omental biopsy and later confirmed in postmortem examination [366]. At this time, the histologic criteria with confirmatory immunohistochemistry, as outlined by Husain et al., as well as correlation of findings with radiographic studies and observations at time of tissue acquisition, remain the gold standard for diagnosis [215]. In view of the gravity of the diagnosis of malignant mesothelioma, a conservative approach toward the diagnosis is favored in equivocal cases.

Several serum/plasma biomarkers, including mesothelin, megakaryocyte potentiating factor, and osteopontin, have been studied for their value in detecting patients with mesothelioma over those with benign respiratory disease and normal individuals as well as in determining response to treatment or progression [367–373]. With the rarity of mesothelioma, those at high risk with an increased pretest probability would only likely benefit and the utility of these markers in diagnosis/detection of MM at an early stage remains to be determined.

## 5.6 Molecular Testing

*p16* is one of two proteins encoded by the cyclin-dependent kinase 2A (CDKN2A) gene located on chromosome 9p21. CDKN2A serves as a tumor suppressor gene and mutations in the p16 region lead to unregulated cell growth. It has been reported that homozygous deletions of 9p21 are the most common genetic aberration in malignant mesothelioma. Fluorescent in situ hybridization (FISH) techniques can be used to detect p16 deletions, and several have studied its use in distinguishing benign versus malignant mesothelial proliferations. Monaco et al. found 0/70 benign cases to carry the p16 deletion whereas the deletion was observed in 40/68 (59 %) mesotheliomas [362]. Flores-Staino et al. tested for homozygous p16 deletions using FISH in 68 pleural fluids consisting of 21 mesotheliomas, 29 metastatic carcinoma, and 15 benign effusions. They found that 12/21 mesothelioma and 2/20 metastatic carcinoma cases were homozygous for p16 deletion, while no benign effusions carried a homozygous or heterozygous p16 deletion [374]. Chiosea et al. analyzed malignant pleural and peritoneal mesothelioma cases for both a homozygous deletion in 9p21 using FISH as well as immunohistochemistry for loss of p16 expression. They found homozygous deletion of 9p21 in 35/52 pleural and 5/20 peritoneal mesotheliomas. None of the pleural reactive mesothelial cases (0/40) carried the deletion. The detection of lost p16 protein expression via immunohistochemistry was seen in 40 % pleural mesotheliomas, in 71 % peritoneal mesotheliomas, and in 15 % reactive mesothelial cases. Thus they concluded that loss of p16 protein expression did not correlate with FISH analysis for 9p21 deletion [375]. The usefulness of p16 FISH lies between malignancies that typically carry a p16 deletion (malignant mesothelioma; melanoma; pancreatic, gastric, and bladder carcinoma; etc.) and benign tissue. Immunohistochemical analysis for loss of p16 protein expression appears to have little value.

*DNA methylation* profiling of tumors and non-tumor tissue was proposed by Christensen et al. in 2009 as a method for distinguishing malignant pleural mesothelioma from pulmonary adenocarcinoma and benign lung tissue. Their work is based on the presence of methylated CpG in DNA loci which serve to control transcription. Methylation in regions of DNA containing tumor suppressor genes can potentially contribute to carcinogenesis. Christensen

analyzed benign and malignant (adenocarcinoma) lung tissue as well as benign and malignant (mesothelioma) pleura for aberrant DNA CpG methylation looking to reliably distinguish one from the other. There were 52 benign lung tissue cases of which 5 were misclassified as malignant, 4 as lung adenocarcinoma, and 1 as malignant mesothelioma, and of 18 benign pleura cases, 5 were misclassified as malignant mesothelioma. Of the malignant cases, one lung adenocarcinoma was misclassified as benign lung and two malignant pleural mesothelioma cases were misclassified as lung adenocarcinoma [376]. As the distinction between benign/reactive pleura and malignant mesothelioma is of paramount importance, more work is needed before DNA methylation profiles can be reliably used for such a purpose.

*microRNAs* are posttranscription regulators of genes. MicroRNA expression analysis has also been proposed as a molecular method for distinguishing malignant mesothelioma from other tumors as well as benign tissue [377–379]. Benjamin et al. analyzed 33 mesotheliomas and 210 carcinomas for microRNA expression signatures which could be used for such purpose. Three microRNA signatures were identified as candidates. Classification rules and a scoring system were established following analysis of a large training set. Lastly, a blinded validation set, including 14 histologically confirmed mesotheliomas and 49 carcinomas, was analyzed. 14/14 (100 %) MM and 38/49 (78 %) carcinomas were correctly classified as such via microRNA analysis. There were 11 carcinoma cases which by classification rules and scoring system fell into categories of mesothelioma ($n=3$) and non-mesothelioma/carcinoma ($n=8$) near cutoff range [379]. Molecular studies, particularly microRNA analysis, appear to be promising in the distinction between benign and malignant mesothelial proliferations as well as other malignancies and could be beneficial for times when the quantity of sampled tissue is limited. However, further studies are needed as they currently hold no proven value over and above histologic diagnosis with confirmatory immunohistochemical stains in routine practice.

## 5.7 Peritoneal Mesothelioma

The peritoneum is the second most common site of involvement by malignant (diffuse) mesothelioma, accounting for approximately 10 % of cases. Peritoneal mesotheliomas demonstrate spread over the peritoneal surface of abdominal viscera leading to encasement of the organs in a rind of tumor, a growth pattern similar to that encountered in the pleural form where growth over the visceral and parietal pleurae occurs. The tumor is typically firm and white, studding the peritoneal surface with numerous individual nodules, in a pattern indistinguishable grossly from peritoneal carcinomatosis (Fig. 5.19). Plaques of tumor or matted tumor masses may also be seen. The omentum is often thickened by an infiltrating tumor, and adhesions between the viscera and abdominal wall may be prominent. This gross distribution of tumor readily explains the typical clinical presenting complaints of abdominal pain, weight loss, obstruction, or abdominal mass. Increased abdominal girth may also be reported due to the accumulation of copious ascites or peritoneal fluid. This fluid may be watery and transudative or viscous due to the presence of hyaluronic acid, the latter feature suggestive of the diagnosis of mesothelioma but by no means specific.

As with pleural mesotheliomas, clinically evident distant metastases are seldom noted at

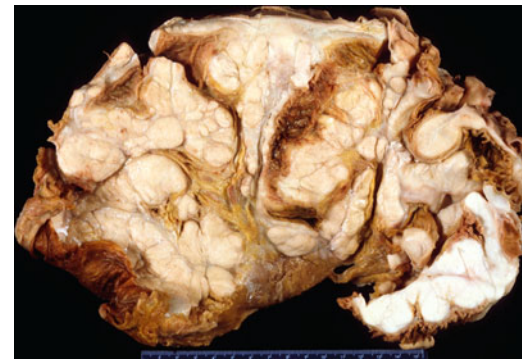

**Fig. 5.19** Coronal slice of abdominal viscera in patient with malignant (diffuse) peritoneal mesothelioma shows encasement and compression of bowel (*dark areas*) by confluent tumor nodules (Reprinted from Ref. [2], with permission)

initial presentation, although they are commonly detected at autopsy [180, 217]. Extension to involve one or both pleural cavities may occur [380], making it difficult to discern the exact site of origin [21]. At autopsy, careful inspection of the organs is necessary in order to exclude a primary malignancy with secondary peritoneal carcinomatosis, as may often occur with adenocarcinomas of the stomach, pancreas, and ovaries [2].

The diagnosis of malignant peritoneal mesothelioma depends on the findings of the typical gross features as described earlier, the identification of a histologic pattern compatible with mesothelioma, and the exclusion of metastatic disease involving the peritoneal cavity (peritoneal carcinomatosis). Information regarding the gross distribution of tumor may be obtained from computed tomography (CT) scans of the abdomen that may show mesenteric thickening, ascites, and peritoneal studding. Some patients with advanced peritoneal mesothelioma may only have modest abnormalities on CT scans, and magnetic resonance imaging may provide additional information in this regard [381, 382]. Surgical exploration may be required to inspect the abdominal viscera for the presence of primary tumors and to obtain sufficient tissue for pathologic diagnosis. The observations of the surgeon at time of laparoscopy/laparotomy are clearly useful for determining the gross distribution of tumor. In a report of 18 cases of peritoneal mesotheliomas diagnosed by laparoscopy and peritoneal biopsy, eight diagnoses were rejected following subsequent pathologic review [383].

Peritoneal mesotheliomas exhibit the same histologic spectrum as pleural mesotheliomas. In one of the editor's (VLR) series of 405 peritoneal mesotheliomas, 312 were epithelial, 77 were biphasic, and only 16 were purely sarcomatoid (Fig. 5.20). Cases of diffuse peritoneal mesothelioma have also been reported in which the tumor presented as innumerable cysts involving the visceral and parietal peritoneum [384–386]. It has been argued, however, that such cases do not represent mesotheliomas at all, but are examples of nonneoplastic reactive mesothelial proliferation [387]. In this regard, we have never seen an example of the so-called peritoneal cystic mesothelioma in over 400 peritoneal mesotheliomas reviewed for litigation purposes. The histochemical, immunohistochemical, and ultrastructural features of peritoneal mesothelioma are similar to those of pleural mesothelioma, although fewer cases have been studied [2, 21, 182, 255, 321, 325, 388–390].

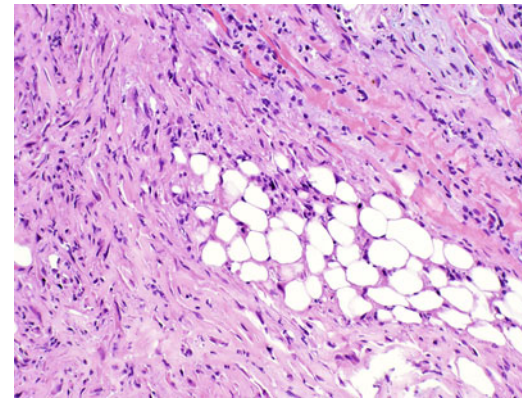

**Fig. 5.20** Sarcomatoid diffuse peritoneal mesothelioma shows invasion of peritoneal fat (*lower right*) and a focus of necrosis (*upper left*). Extensive sampling at autopsy showed that the tumor had a sarcomatoid appearance throughout. H&E ×200

Malignant peritoneal mesothelioma must be distinguished from other papillary peritoneal tumors in women, metastatic carcinoma with secondary involvement of the peritoneum, as well as reactive mesothelial hyperplasia. The peritoneal mesothelium has a remarkable capacity for undergoing marked hyperplastic changes, most notably in patients with cirrhosis and ascites. Florid hyperplastic changes include the formation of papillary structures, pseudoacini, and squamous nests [391]. Immunoperoxidase stains will not reliably permit the separation of reactive changes from neoplasia, but the demonstration of invasion, nuclear anaplasia, and focal necrosis may be diagnostic in this regard. Metastatic adenocarcinomas involving the peritoneum frequently express mucin (Fig. 5.21a), which may be detected using PAS stain following diastase predigestion, a histochemical finding distinctly unusual in mesothelioma.

A broad histologic spectrum of primary papillary serous tumors of the peritoneum may occur

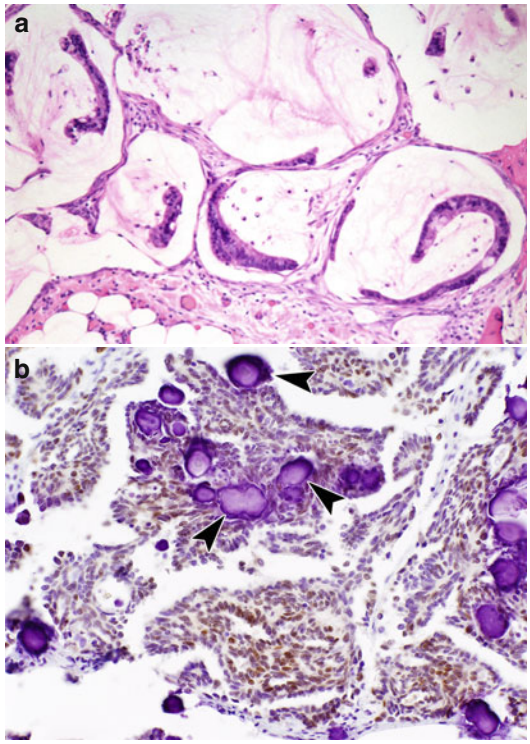


**Fig. 5.21** (**a**) Metastatic mucin-producing adenocarcinoma in the peritoneum consists of pools of mucin within spaces lined by delicate connective tissue stroma. Some of the spaces are partially lined by a layer of tall columnar tumor cells. (**b**) Serous papillary adenocarcinoma of the peritoneum contains numerous psammoma bodies (*arrowheads*) in this field. Tumor cells demonstrate positive nuclear staining for PAX-8. Parts (**a**, **b**) ×100 (Part (**a**) reprinted from Ref. [2], with permission)

in women. These tumors may show histologic similarities to both epithelial mesothelioma and papillary tumors of the ovary and presumably derive from extraovarian epithelium with Müllerian potential. Such serous papillary adenocarcinomas of the peritoneum often show considerable nuclear anaplasia and mitotic activity and may contain numerous psammoma bodies. Peritoneal epithelial mesotheliomas with papillary features may contain scattered psammoma bodies that are typically less prominent than those seen in papillary carcinomas [13, 21]. In some serous papillary carcinomas, psammoma bodies are so prominent that the term psammocarcinoma has been suggested (Fig. 5.21b) [392]. Papillary serous adenocarcinomas of the peritoneum will usually demonstrate intracytoplasmic mucin granules with the PAS stain, and display an immunophenotype typical of carcinoma. Some caution in the interpretation of immunohistochemical studies is warranted in peritoneal mesotheliomas, as a substantial proportion of ovarian adenocarcinomas may stain for a number of mesothelial markers [393]. In this regard, the use of immunohistochemical staining for estrogen and progesterone receptors is a useful differential diagnostic feature [215]. Similarly, a number of malignancies involving the peritoneal cavity will stain positive for CK 5/6, so such staining should be interpreted with caution [394]. PAX8, a relatively new antibody, has shown utility in distinguishing ovarian serous tumors from mesothelioma (Fig. 5.21b) [395]. In cases with conflicting or equivocal immunohistochemical data, examination of tumor cell ultrastructure will often demonstrate the long branching microvilli characteristic of mesothelioma [321, 325, 396] or the short stubby microvilli of carcinoma [324].

Peritoneal mesotheliomas account for between 17 and 32 % of the mesotheliomas affecting women in the United States [5, 217, 397]. In contrast to the generally dismal prognosis associated with pleural mesotheliomas, the prognosis for peritoneal mesotheliomas is less predictable, especially in women. Goldblum and Hart's review of 19 peritoneal mesotheliomas in women found considerable overlapping histologic features in the mesotheliomas displaying both indolent and aggressive behavior and found survivorship to be determined largely by gross distribution, with solitary tumors conveying a good prognosis, the opposite for diffuse tumors [233]. Kerrigan et al. reviewed a series of 25 peritoneal mesotheliomas in women that were exclusively diffuse. Controlling for age at time of diagnosis, presentation, and form of treatment, these investigators found that there were no morphologic attributes that could reliably predict the behavior of any given tumor. Survivorship in this population ranged from 1 month to 15 years. The authors concluded from these observations that the spectrum of diffuse epithelial peritoneal mesotheliomas includes forms with aggressive behavior akin to pleural mesotheliomas, as well as more

indolent forms, and that morphologic data alone do not appear predictive of clinical behavior in this population [397]. An indolent behavior is often observed for well-differentiated papillary mesotheliomas of the peritoneum in women. However, when invasion is present, these tumors may behave more aggressively [234].

Peritoneal mesotheliomas have been strongly associated with asbestos exposure, especially in men [398]. Asbestosis has been reported to be present in approximately 50 % of cases of peritoneal mesotheliomas, compared to approximately 20 % of pleural mesotheliomas [54]. Epidemiologic studies comparing the degree of asbestos exposure with occupation and ultimate site of mesothelioma development point to the peritoneal site as being associated with longer and more intense exposures to asbestos [53, 70, 72]. Peritoneal mesotheliomas follow exposure to commercial amphibole fibers (amosite or crocidolite), but have not convincingly been related to exposure to chrysotile asbestos [399]. The link of peritoneal mesothelioma with asbestos exposure in women is weak [5].

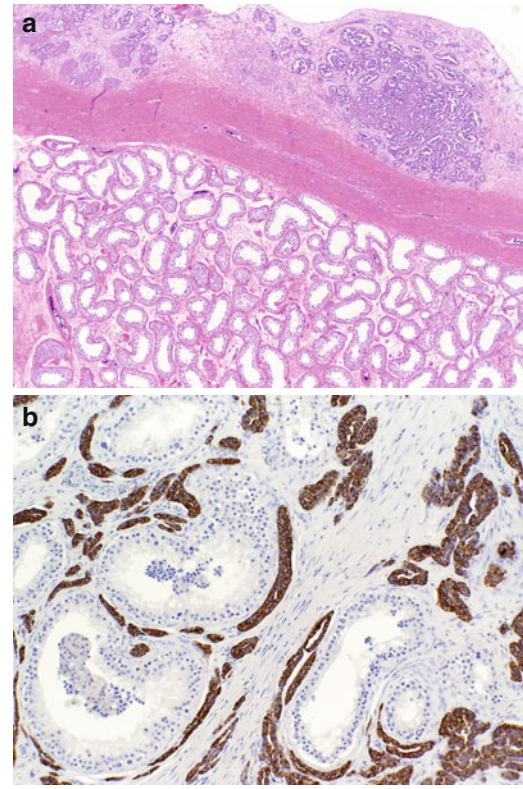


**Fig. 5.22** (**a**) Malignant mesothelioma arising from the tunica vaginalis. (**b**) Keratin stain showing invasion of seminiferous tubules. Part (**a**) H&E ×40; Part (**b**) Keratin ×100

## 5.8 Mesothelioma of the Tunica Vaginalis Testis

The tunica vaginalis testis is formed by an outpouching of the abdominal peritoneal membrane and is lined by a layer of mesothelial cells. Thus, it is an extension of the peritoneum. Infrequently, a malignant mesothelioma may originate in this location. Initially described by Barbera et al. in 1957 [400], this uncommon tumor has since been reported in single case and series form by others [401–404], including a case reported in a 6-year-old [405]. The collective experience with this tumor would indicate the reporting of approximately 100 cases worldwide. These tumors present clinically as hydroceles or paratesticular masses, whose malignant nature may not be suspected until pathologic evaluation of surgical material (Fig. 5.22). The tumor has the potential for local and regional spread, as well as distant and fatal metastases. An aggressive clinical course is typical, especially in those tumors not completely excised at the outset.

The histologic spectrum and immunophenotype are similar to that of malignant mesotheliomas from other sites and include papillary (epithelial), sarcomatoid, and biphasic variants [401, 403]. The ultrastructural features of the epithelial variants that have been examined are similar to those of epithelial mesotheliomas occurring elsewhere. A causative role for asbestos in some cases is favored. In Jones' report, the issue of asbestos exposure was specifically addressed in only 27 of 64 cases. In 11 of these cases (41 %), an occupational exposure to asbestos was reported [401]. Plas' review of the literature concerning mesothelioma of the tunica vaginalis testis indicates a positive history of asbestos exposure in 34.2 % [402]. The real prevalence of asbestos exposure in this patient population may be underestimated due to the lack of historical information. Causative roles

for radiation, testicular trauma, or viral infection have not been convincingly demonstrated [402].

The differential diagnosis chiefly includes carcinoma of the rete testis, which shares gross and histologic similarities with mesothelioma. Experience with the immunophenotype of rete testis carcinoma is limited, but potentially useful diagnostic information may be obtained on ultrastructural examination, as rete testis tumors tend to show microvillus features of length/width ratios more typical of carcinoma [400, 406]. The differential diagnosis also includes adenomatoid tumor. This tumor often involves the epididymis, displays ultrastructural evidence of mesothelial differentiation, but is usually non-infiltrative and sharply circumscribed [407]. Carcinomas of the lung and prostate are among the tumors that may commonly metastasize to the testis, but sharp differences in histology and immunophenotype should permit the distinction.

## 5.9 Pericardial Mesothelioma

Primary malignant mesotheliomas of the pericardium are distinctly uncommon, with approximately 180 cases reported in the literature involving the adult and pediatric population [2, 189, 408, 409]. These tumors invade the parietal and visceral pericardium, eventually encasing the heart in a rind of tumor (Fig. 5.23). Patients typically present with pericardial effusion or mediastinal mass that may be accompanied by dyspnea, arrhythmias, congestive heart failure, pericardial constriction, or cardiac tamponade [410–416]. Chest roentgenograms show cardiac enlargement or a mass, and low voltage may be demonstrable in anterior precordial leads on electrocardiogram. Magnetic resonance imaging may also provide detailed information regarding location and extent of tumor [417].

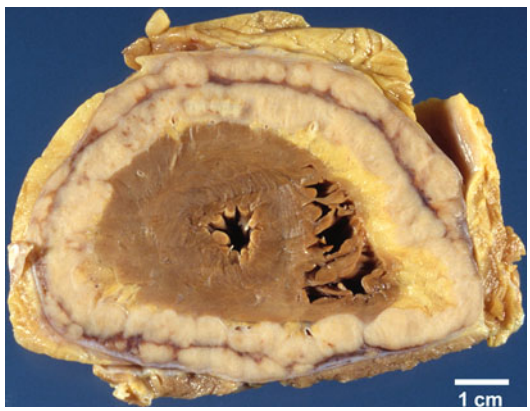


**Fig. 5.23** Transverse section of the heart, showing complete encasement by malignant pericardial mesothelioma

Microscopic examination of the pericardial tumors has shown a biphasic pattern in most cases, but epithelial and sarcomatoid variants have also been described [189]. Immunohistochemical and ultrastructural studies have only rarely been described [410, 411, 418], but these tumors appear morphologically identical to their pleural and peritoneal counterparts. Pericardial mesotheliomas must be distinguished from the much more common carcinoma directly extending into or metastatic to the epicardium or pericardium [419, 420]. In addition, pleural mesotheliomas may also directly extend into and invade the contiguous pericardium, further complicating the diagnosis of primary pericardial mesothelioma [21]. The so-called mesothelioma of the atrioventricular node is a benign tumor not derived from mesothelium at all, but from endoderm [2, 421–423]. An exposure to asbestos has been established in several patients suffering from pericardial mesothelioma [424–427].

## 5.10 Treatment and Prognosis

The prognosis of malignant (diffuse) mesothelioma is poor. In most series, a median survival between 4 and 18 months is expected for the pleural forms [231, 428–437]. Death typically results from respiratory failure or infection, but involvement of the heart and transdiaphragmatic involvement of abdominal viscera may also contribute to mortality [54, 438]. Physicians experienced in treating mesothelioma will report occasional patients with significantly greater longevity following treatment. Consequently, a limited set of prognostic factors has been derived to predict outcome and to identify those patients most likely to receive benefit from radical treatment regimens.

Numerous studies evaluating clinical prognostic factors have been reported over the past 20 years, identifying the importance of age, sex, performance status, weight loss, chest pain, and clinical stage [428]. Conflicting data have been reported due in part to variances in disease staging, therapies given, assessment of response, and enrollment eligibility.

The issue of clinical stage as a prognosticator is particularly problematic. Surprisingly, several studies found stage not to be an important prognostic factor [428]. This may be related to the necessity for exploratory and cytoreductive surgery to fulfill all staging descriptors. Such criteria have not been fulfilled for most patients with mesothelioma, even at centers with special expertise in the treatment and management of this disease. Thus most historical staging data is only approximate. The prognostic scoring systems of the Cancer and Leukemia Group B (CALGB) and European Organization for Research and Treatment of Cancer (EORTC) have been applied to large numbers of patients with mesothelioma [434, 435]. These distinct scoring systems have identified poor prognostic indicators which include non-epithelial subtype, male gender, poor performance status, and hematologic parameters of low hemoglobin, high leukocyte and platelet counts, and high serum lactic dehydrogenase (LDH) [435, 439, 440]. In a retrospective review of 121 cases of malignant pleural mesothelioma, univariate analysis demonstrated lower rates of survival in patients with poor performance status and non-epithelial histologic subtypes and found that any form of treatment beyond supportive care led to longer survival [441].

Many clinicians approach patients with malignant mesothelioma with a certain amount of therapeutic nihilism, mindful that single modality therapy has failed to change the natural history of the disease in a meaningful fashion [442]. Debate continues as to the selection of patients for cytoreductive surgery and adjuvant therapeutic modalities and the identification of patients likely to receive benefit from such an approach. This controversy is in part due to the lack of a universally accepted staging system for pleural mesothelioma. An ideal staging system should incorporate clinical and pathologic data to stratify survival and thereby tailor a therapeutic approach to the tumor. Several such staging systems have been offered, but none is universally accepted and most do not live up to the ideal. Butchart et al. proposed their staging system in 1976, and it remained popular despite its basis on only 29 patients and failure to stratify survival (Table 5.7) [443]. Other staging proposals include Sugarbaker's revised Brigham/Dana-Farber Cancer Institute system and the somewhat complex system proposed by the International Mesothelioma Interest Group (IMIG) (Tables 5.8 and 5.9) [444, 446]. Sugarbaker's system has the advantage of simplicity and results in survival stratification but has yet to be validated in an independent patient population. In the Brigham/Dana-Farber experience, survivals following rad-

**Table 5.7** The Butchart staging system

| Stage | Definition |
|---|---|
| I. | Tumor is confined to the capsule of the parietal pleura (i.e., involves only the ipsilateral lung, pericardium, and/or diaphragm) |
| II. | Tumor invades the chest wall or mediastinal structures (e.g., esophagus, heart, and/or contralateral pleura), or tumor involves intrathoracic lymph nodes |
| III. | Tumor penetrates the diaphragm to involve peritoneum, or tumor involves the contralateral pleura, or tumor involves extrathoracic lymph nodes |
| IV. | Distant blood-borne metastasis |

From Ref. [443] with permission

**Table 5.8** Revised staging system proposed by Sugarbaker et al. Brigham/Dana-Farber Cancer Institute

| Stage | Definition |
|---|---|
| I. | Disease completely resected within the capsule of the parietal pleura without adenopathy: ipsilateral pleura, lung, pericardium, diaphragm, or chest wall disease limited to previous biopsy sites |
| II. | All of stage I with positive resection margins and/or intrapleural adenopathy |
| III. | Local extension into the chest wall or mediastinum; into the heart or through the diaphragm or peritoneum; or with extrapleural lymph node involvement |
| IV. | Distant metastatic disease |

From Ref. [444] with permission

**Table 5.9** Staging system proposed by the International Mesothelioma Interest Group (IMIG)

| | |
|---|---|
| *Tumor (T) staging* | |
| Tla | Tumor limited to the ipsilateral parietal pleura, including the mediastinal and diaphragmatic pleura, without involvement of visceral pleura |
| Tlb | Tla + scattered foci of tumor involving the visceral pleura |
| T2 | Tumor involving each of the ipsilateral pleural surfaces (parietal, mediastinal, diaphragmatic, and visceral pleura)<br>Involvement of diaphragmatic muscle<br>Confluent visceral pleural tumor (including the fissures) or extension of tumor from the visceral pleura into the underlying pulmonary parenchyma |
| T3 | Locally advanced but potentially resectable tumor. The tumor involves all of the ipsilateral pleural surfaces with at least one of the following features:<br>Involvement of the endothoracic fascia<br>Extension into the mediastinal fat<br>A solitary, completely resectable focus of tumor extending into the soft tissues of the chest wall<br>Nontransmural involvement of the pericardium |
| T4 | Locally advanced, technically unresectable tumor. The tumor involves all of the ipsilateral pleural surfaces with at least one of the following features:<br>Diffuse extension or metastatic spread to the chest wall with or without rib destruction<br>Direct transdiaphragmatic extension to the peritoneum<br>Direct extension to the contralateral pleura<br>Direct extension to any mediastinal organ<br>Direct extension to the spine |
| *Lymph node (N) staging* | |
| Nx | Regional lymph nodes (LNs) cannot be assessed |
| N0 | No regional LN metastases |
| N1 | Involvement of ipsilateral bronchopulmonary or hilar LNs |
| N2 | Involvement of subcarinal or ipsilateral mediastinal LNs (including the internal mammary LNs) |
| N3 | Involvement of the contralateral mediastinal or internal mammary LNs or any supraclavicular LNs |
| *Metastases (M) staging* | |
| Mx | Presence of distant metastases cannot be assessed |
| M0 | No distant metastases |
| M1 | Distant metastases present |
| *Anatomic stage* | |
| Stage I | |
| Ia | T1a N0 M0 |
| Ib | T1b N0 M0 |
| Stage II | T2 N0 M0 |
| Stage III | Any T3 M0<br>Any N2 M0 |
| Stage IV | Any T4<br>Any N3<br>Any M1 |

From Ref. [445] with permission

ical pleural pneumonectomy and adjuvant chemoradiotherapy of 25, 20, and 16 months were reported for their stage I, II, and III cohorts, respectively [444].

Unimodality therapy employing radiation appears unfeasible due to the requirements of a large field imposed by diffuse tumor and the potential for injury to vital thoracic structures by

large doses of radiation. Low response rates, at the expense of radiation toxicity, limit the efficacy of this modality [444]. The surgical therapies for malignant pleural mesothelioma include pleurodesis, decortication and pleurectomy, and radical extrapleural pneumonectomy. To date, no randomized studies comparing the efficacy of these three procedures exist. Talc pleurodesis to palliate symptoms caused by pleural effusion has been shown to result in median survival similar to that of untreated patients. Decortication and pleurectomy and radical extrapleural pneumonectomy effect cytoreduction with curative intent. Neither results in significant prolongation of survival when offered as single modality treatment.

The largest studies of radical extrapleural pneumonectomy followed by chemoradiotherapy have been undertaken at Brigham and Women's Hospital. In a series of 183 patients undergoing this surgery, there were seven perioperative deaths. The remaining 176 patients underwent adjuvant chemoradiotherapy, resulting in 2- and 5-year survival rates of 38 and 15 %, respectively, the longest in any reported series. Of the patients with epithelial histologies, negative operative margins, and negative mediastinal nodal involvement, 2- and 5-year survivals of 68 and 46 %, respectively, were recorded. Prognostic indicators for this cohort of patients included histologic subtype, lymph node involvement, extrapleural extension, and integrity of operative margins. No 5-year survival was recorded in patients with sarcomatoid histology, mediastinal node involvement, or positive margins. This experience has yet to be duplicated, but earlier detection of disease combined with further developments of novel therapies, improved chemotherapy, and aggressive surgical approaches may result in improved outcomes in the future [444]. Yan et al. assessed 70 patients who underwent extrapleural pneumonectomy for treatment effect and prognostic factors. The authors found improved overall survival on univariate and multivariate analysis in patients with the following: (1) history of asbestos exposure, (2) negative lymph node involvement, (3) adjuvant radiation therapy, and (4) postoperative treatment with pemetrexed, an antifolate chemotherapeutic, and cisplatin or carboplatin. Interestingly, gender and histologic subtype were not found to be predictors of overall survival [446].

Some studies have suggested that survival for peritoneal mesotheliomas is worse than that for its pleural counterpart [430, 447], although this observation was not confirmed in a large study of 1,475 cases conducted by the Surveillance, Epidemiology and End Results (SEER ) program [432]. The prognosis for this tumor remains poor overall, with median survival of untreated peritoneal mesotheliomas ranging from 4 to 12 months in most series [448–451]. The optimism engendered by some reports of patients with early-stage peritoneal mesotheliomas experiencing disease-free intervals following combined modality therapy [449, 452] should be tempered by other reports of the not infrequently indolent nature of the this disease, especially in women [394].

Kadota et al. assessed survival data with respect to histologic subtype of epithelial pleural mesothelioma. They reported that the pleomorphic variant of epithelial mesothelioma prognosticates poor overall survival with survival time that was not statistically different from that of biphasic or sarcomatoid pleural mesothelioma [231]. Recently, Kadota et al. also reviewed survival data in patients with malignant (diffuse) pleural mesothelioma of epithelial variant in conjunction with a proposed nuclear grading system for epithelial mesothelioma which was constructed on two independent prognostic factors: (1) nuclear atypia and (2) mitotic activity. Their scoring system for nuclear atypia was from 1 to 3 (mild, moderate, and severe atypia). Mild nuclear atypia was defined as nuclei which were uniform in size and shape. Moderate atypia included nuclei which were slightly larger and allowed for some contour irregularity, and severe atypia included large and pleomorphic nuclei with some areas of two times variation in nuclear size. For mitotic rate, tumors were scored from 1 to 3 (low, intermediate, and high) which corresponded to zero to one mitosis per10 high-power fields (HPF), two to four mitoses per10 HPF, and five or greater mitoses per 10 HPF, respectively. Using this construct of nuclear atypia and mitotic activity, they were able to

stratify patients with epithelial pleural mesothelioma into three distinct survival groups: nuclear grade 1 with median overall survival of 28 months, nuclear grade 2 with median overall survival of 14 months, and nuclear grade 3 with median overall survival of 5 months [433]. With regard to survival in sarcomatoid mesothelioma, Klebe et al. reported a grim survival of 3.5 months [240].

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# Benign Asbestos-Related Pleural Disease

6

Michelle L. Manni and Tim D. Oury

## 6.1 Introduction

Benign asbestos-related pleural diseases are the most common pathologic and clinical abnormalities related to asbestos exposure, with a greater prevalence than asbestosis. Solomon et al. [1] emphasized that the pleural manifestations of asbestos exposure include four specific benign pleural reactions: (1) benign asbestos effusion, (2) parietal pleural plaques, (3) diffuse pleural fibrosis, and (4) rounded atelectasis, or an area of collapsed, airless lung adjacent to an area of visceral pleural fibrosis. Notably, there is considerable overlap among these four disease processes (Fig. 6.1), with various combinations manifesting simultaneously or sequentially in a single individual. For example, a patient with benign asbestos effusion may subsequently be found to have diffuse pleural fibrosis, or a patient with parietal pleural plaques may develop rounded atelectasis.

Benign asbestos-related pleural diseases may occur after low-level, indirect, or even environmental exposures to asbestos and the incidence of this disease increases with both time and frequency of exposure [2–4]. However, the prevalence of these abnormalities is clearly greatest in those who are exposed to asbestos in an occupational setting. Pleural plaques, which are characteristic of these diseases, usually develop 20–30 years after the initial exposure with calcifications manifesting greater than 30 years postexposure [5].

The pathogenesis of these disorders is poorly understood [6], but it undoubtedly involves the transport of asbestos fibers to the pleura. Asbestos fibers may migrate directly either through the lung parenchyma or through lymphatic

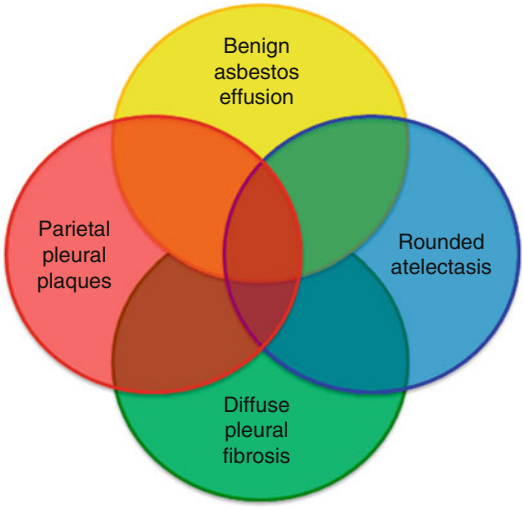


**Fig. 6.1** Venn diagram of benign asbestos-related pleural diseases showing the overlap among these four specific disorders

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T.D. Oury et al. (eds.), *Pathology of Asbestos-Associated Diseases*,
DOI 10.1007/978-3-642-41193-9_6, 

pathways and the bloodstream [7, 8]. In the former, asbestos fibers inhaled into the lung pass into the alveoli, where they eventually work their way to the visceral pleural surface. The mechanical theory suggests that this transport occurs when the needlelike fibers work their way through the lung tissue as a result of the lung's motion during inhalation and exhalation [9]. Alternatively, fibers reach the pulmonary interstitium through a process of translocation across the alveolar epithelium [10]. Within the interstitium, the fibers would have access to pulmonary lymphatics, which in the outer third of the lung drain centripetally to the pleura. Fibers reaching the visceral pleura can then penetrate this structure and hence reach the parietal pleura, which normally is directly apposed to the visceral pleura, separated only by a potential space. Asbestos fibers may also migrate passively or within macrophages to the pleural space. It is thought that inflammation induced by the presence of asbestos fibers in the lungs increases interstitial fluid accumulation and fluid movement to the pleural space, thereby facilitating fiber translocation [8].

In addition, the presence of fibers within the pleura elicits an inflammatory response, which may undergo organization or healing with subsequent fibrosis. In this regard, it is of interest that one study has shown that pleural mesothelial cells in culture release a chemotactic factor for neutrophils when stimulated with asbestos fibers [11]. Additionally, asbestos fibers have been shown to induce mesothelial release of chemoattractants for monocytes [12]. Clinical manifestations will then depend on the intensity of the initial inflammatory reaction and the degree and extent of any consequent pleural fibrosis.

## 6.2 Benign Asbestos Pleural Effusion

### 6.2.1 Clinical Criteria

Eisenstadt reported the first case of benign asbestos-related pleural effusion in 1964 [13]. This was a unilateral effusion in an asbestos worker. Dr. Eisenstadt stated that a diagnosis of benign asbestos pleural effusion should only be made after biopsies of the lung and pleura were performed to rule out other disease processes. More than 250 additional cases have subsequently been reported, and it is now recognized that asbestos pleural effusion (pleurisy) is the most common asbestos-related lesion during the first decade after exposure. However, it can occur at a later date [14]. It is usually a moderate-sized effusion of up to 2,000 ml that may be clear to hemorrhagic and of variable cellularity. Hillerdal lists three diagnostic criteria of an asbestos effusion: (1) tuberculosis, infection, or malignancy must be ruled out; (2) the individual must be followed for 2 years to verify the effusion is benign; and (3) there must be an occupational exposure to asbestos (Table 6.1). The asbestos effusion tends to recur and can last for months. Recurrence on the same or opposite side is common, and clinical symptoms are only mild to absent [15, 17]. In addition to this tendency to recur, another feature characteristic of benign asbestos effusion is the presence of either rounded atelectasis or converging pleural linear structures (so-called crow's feet) on the chest radiograph at the initial presentation [14].

**Table 6.1** Clinical criteria for benign asbestos pleural effusion

| |
|---|
| 1. Clinically documented pleural effusion |
| 2. History of exposure to asbestos |
| 3. Elimination of other causes of effusion (infection, collagen vascular disease, malignancy, etc.) |
| 4. Follow-up of 2 or 3 years to verify benign nature of process |

Source: Modified from Refs. [15, 16]

In 1982, Epler et al. reviewed chest x-rays of 1,135 employees in the asbestos industry. The prevalence of asbestos effusions was 7.0, 3.7, and 0.2 %, depending on whether the asbestos work exposure was, respectively, severe, indirect, or peripheral [18]. The latency for asbestos effusions was shorter than for asbestos plaques and was the only manifestation seen within 10 years of exposure. The incidence ranged from less than 1 to as many as 9 cases of asbestos effusion per 1,000 person-years of observation, depending on the degree of exposure. The recurrence rate was 29 %. In 66 % of the effusions, the workers were

asymptomatic. In a related article, Gaensler et al. reported on 68 patients with benign asbestos pleural effusions, the majority of whom had no symptoms [16]. These investigators stated that benign asbestos pleural effusions were the most common asbestos-related disorder during the first 20 years after initial exposure and were seen in approximately 5 % of all heavily exposed persons [16]. Robinson et al. reported on still another cohort of 22 asbestos workers with asbestos pleural effusion [19]. Their mean work exposure was 5 years, their time between work exposure and occurrence of pleurisy was 16 years, and the mean duration of the effusion was 4 months. The pleural fluid was blood tinged and rarely greater than 500 ml.

Hillerdal emphasized the benign course of these effusions even though they may be bloody and of large volume [20]. An exception was a very small group of more heavily exposed individuals who sometimes developed progressive pleural fibrosis after an initial effusion. This observation was confirmed by McLoud et al. [21] and, in some cases, may result in respiratory failure [22]. Lilis et al. described 20 patients in a series of 2,815 insulation workers (0.7 %) who had a history of symptomatic pleural effusion [23]. Sixteen of these 20 (80 %) had diffuse pleural fibrosis radiographically, whereas 5.0 % of the total group had diffuse pleural fibrosis. These observations suggest that diffuse pleural fibrosis in the patients without a history of benign asbestos effusion may be the residua of asymptomatic pleural effusion in at least some of these individuals [23]. In another article, Hillerdal made the observation that asbestos workers in Finland are exposed to anthophyllite asbestos, and there is a low incidence among them of both asbestos effusion and pleural mesothelioma [24].

### 6.2.2 Pathologic Findings

The pathologic features of benign asbestos effusion have not been well defined. Core needle biopsy of the pleura in a few of the cases in the series of Robinson and Musk showed pleural fibrosis with or without an inflammatory infiltrate [19]. Decortication in four of the cases of Mattson showed chronic nonspecific fibrotic pleurisy [25]. In one of these four cases, asbestos bodies and pulmonary fibrosis were observed in the adjacent lung parenchyma. The effusion itself is characteristically an exudate, with glucose and protein levels similar to that of plasma [26]. In more than half the cases, the fluid is grossly hemorrhagic. The cell count usually is less than 6,000/mm$^3$, with either a mononuclear or neutrophil predominance [27]. In about one-fourth of the cases, eosinophils are a prominent feature. In this regard, it should be noted that injection of asbestos fibers into the pleural cavities of experimental animals results in an exudative effusion [6]. Bilaterality of benign asbestos effusion is common, occurring in 11 of 60 patients studied by Hillerdal and Ozesmi [26]. In three cases the effusions were synchronous, whereas in the remaining eight cases they were metachronous, separated by an interval ranging from 1 to 15 years.

## 6.3 Parietal Pleural Plaques

### 6.3.1 Historical Background

Pleural plaques consist of circumscribed areas of dense, firm, gray-white fibrous tissue usually free of any inflammatory reaction. While most pleural plaques occur on the parietal pleura, they may occur on the visceral pleura as well [1]. They are most commonly located on the parietal pleural surface opposite the dependant portions of the lungs.

Cartilage-like plaques on the costal pleura have long been recognized by pathologists. They were considered to be remnants of inflammation, similar to "sugar icing" ("Zuckerguss"). The first description of pleural plaques in connection with asbestos workers was made by Sparks in 1931, who described irregular, small calcified plaques in the lower lung zones [28]. In 1938, Gloyne reported visceral pleural plaques that were horn-like and stiff [28]. The first description of pleural plaques in talc workers was made by Porro et al. in 1942 [29]. Siegal et al. reported the initial

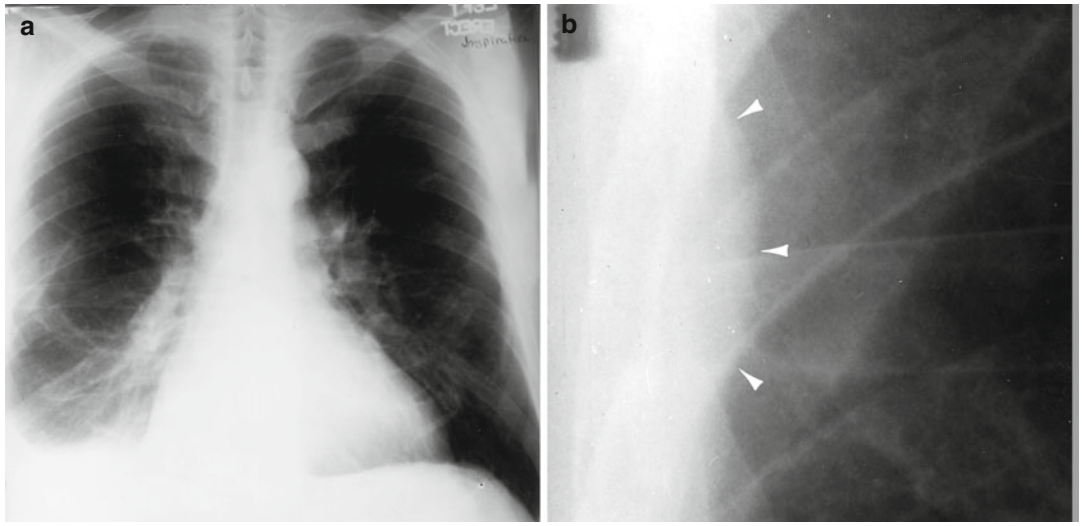


**Fig. 6.2** (**a**) Chest radiograph shows mild bilateral increase in interstitial markings most prominent in the lung bases, right pleural effusion, and pleural thickening with focal plaque formation. (**b**) The outline of this plaque viewed tangentially is seen to better advantage in this magnified view of the periphery of the right mid-lung field (*arrowheads*) (Reprinted from Ref. [39], with permission)

observation of pleural plaques in tremolite talc workers [30]. In the 1950s, several reports of pleural plaques in asbestos- and talc-exposed workers appeared [31, 32].

Talcosis is very similar clinically and roentgenologically to asbestosis, and it is probably the asbestos found in almost all types of talc that causes the pleural changes [33–35]. Indeed, some of the first studies of pleural plaques were made among talc workers [30, 32]. Animal experiments with "pure" talc (i.e., free of asbestos) have resulted in both pulmonary fibrosis and pleural reactions [36]. Also, talc particles have been found in pleural plaques. Talc also induces mesothelial cells to release several chemokines, which induce inflammation [37, 38]. Therefore, it is possible that talc itself may have some effect in the formation of pleural plaques.

### 6.3.2 Radiographic Features

Parietal pleural plaques appear on chest x-ray as discrete areas of pleural thickening, usually in the lower lung zones or on the diaphragms. They are best observed when viewed tangentially (Fig. 6.2) but may appear as a hazy density when viewed *en face*. The plaques often calcify, which usually does not occur until two to three decades after the initial exposure to asbestos [15, 40]. Calcification greatly enhances plaque detectability with routine chest films (Fig. 6.3). In addition, oblique views are useful for detecting plaques, especially noncalcified ones [41]. Plaques generally spare the costophrenic angles. When blunting is observed, one should suspect the presence of pleural effusions or adhesions. Pleural plaques are most often bilateral. Left-sided predominance of unilateral plaques on chest x-rays has been reported by some authors [42, 43], but has not been confirmed by thoracic CT studies [44, 45].

Radiographic surveys of populations have shown that 1–2 % of men and less than 1 % of women have pleural plaques. There is, however, a high rate of false negative results, since autopsy surveys have indicated that the postmortem prevalence of plaques ranges from 4 % to as high as 39 % (Table 6.2) [44]. In these autopsy studies, the percentage of plaques that were detected on premortem chest films ranged from 8 to 40 %. Noncalcified diaphragmatic plaques are particularly difficult to visualize on routine chest films and were observed in none of eight cases in the series of Wain et al. [44]. One must also use

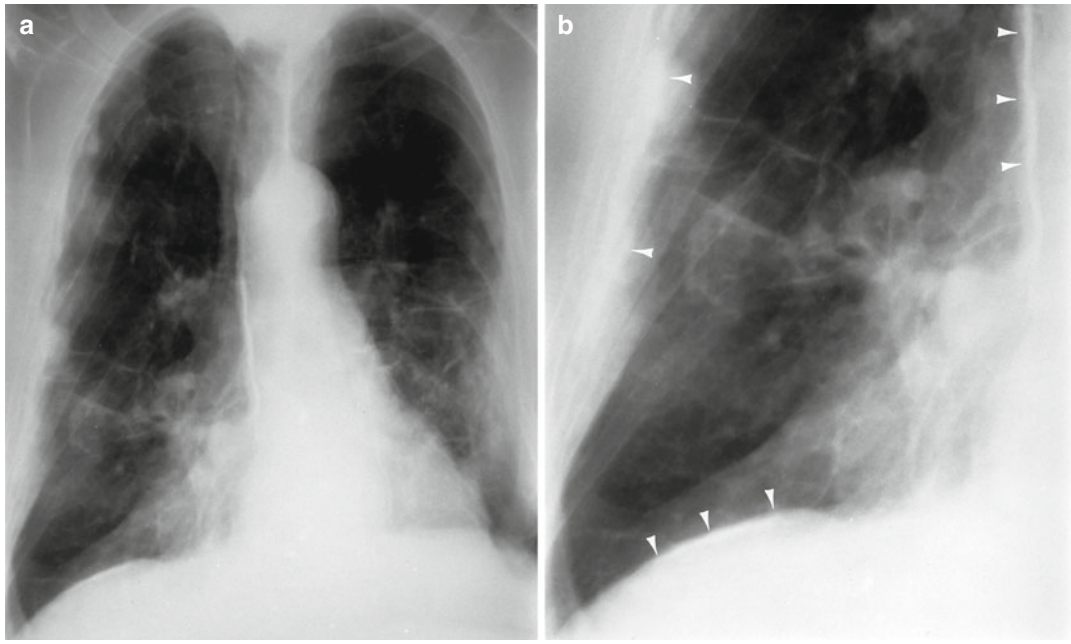


**Fig. 6.3** (**a**) Chest radiograph showing parietal pleural plaque formation with extensive bilateral pleural calcification. (**b**) The pleural calcification is seen to better advantage in this magnified view of the right hemithorax (*arrowheads*) (Courtesy Dr. William F. Foster, Department of Radiology, Durham VA Medical Center, Durham, NC)

**Table 6.2** Summary of previously reported pathologic x-ray correlation studies of patients with pleural plaques

| Author | Country | Population composition | No. of autopsies | % of pleural plaques[a] | % detected on chest films[a] |
|---|---|---|---|---|---|
| Rubino et al. [40] | Italy | General population in asbestos industrial region | 862 | 7.8 | 40.3 |
| Hourihane et al. [46] | England | General urban population | 381 | 4.1 | 13.7 |
| Hillerdal and Lindgren [33] | Sweden | General population screen | 437 | 6.8 | 12.5 |
| Meurman [120] | Finland | General population in coastal, urban, and asbestos mining region | 438 | 39.3 | 8.3 |
| Wain et al. [44] | United States | Male veterans | 434 | 5.8 | 28 |

Source: Reprinted from Ref. [44], with permission
[a]These values are calculated from published data

caution to avoid overinterpretation of films as showing pleural plaques (i.e., false positives), which can occur secondary to shadows produced by the serratus anterior in particularly muscular individuals or due to subpleural adipose tissue in the obese. Notably, a study by Miller and Zurlo suggests that even when plaques are radiographically evident, they are frequently overlooked or misdiagnosed and the patients are not followed as carefully as they should with proper recognition of this process [47].

Although pleural plaques are recognizable on chest radiography, computed tomography (CT) has been shown to improve both the specificity and sensitivity of routine chest films with respect to identification of asbestos-related pleural disease [48–51] (Fig. 6.4) and has recently been shown to be useful in quantifying plaque burden in patients as well [52]. One study comparing CT scanning to chest radiography found that CT was able to detect approximately 60 % more plaques than chest x-ray [50] and high-resolution CT may even be better [53].

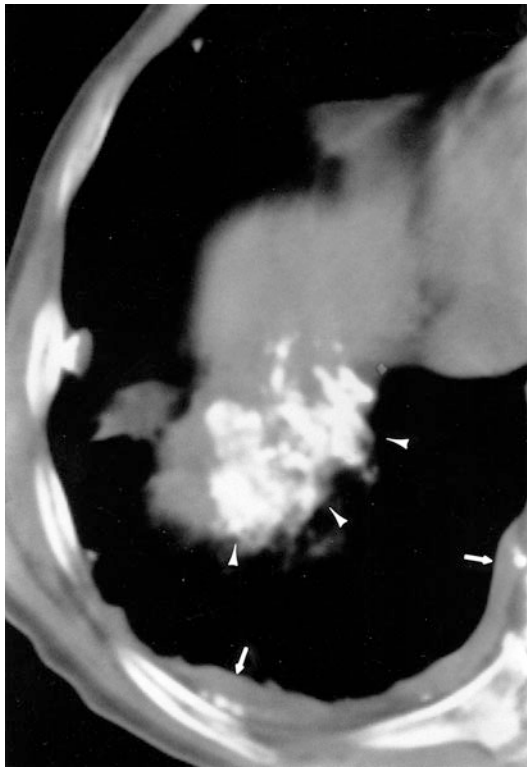

**Fig. 6.4** Computed tomographic view of the right hemithorax shows partially calcified parietal pleural plaques viewed tangentially (*arrows*) as well as an extensively calcified diaphragmatic plaque viewed en face (*arrowheads*) (Courtesy Dr. William F. Foster, Department of Radiology, Durham VA Medical Center, Durham, NC)

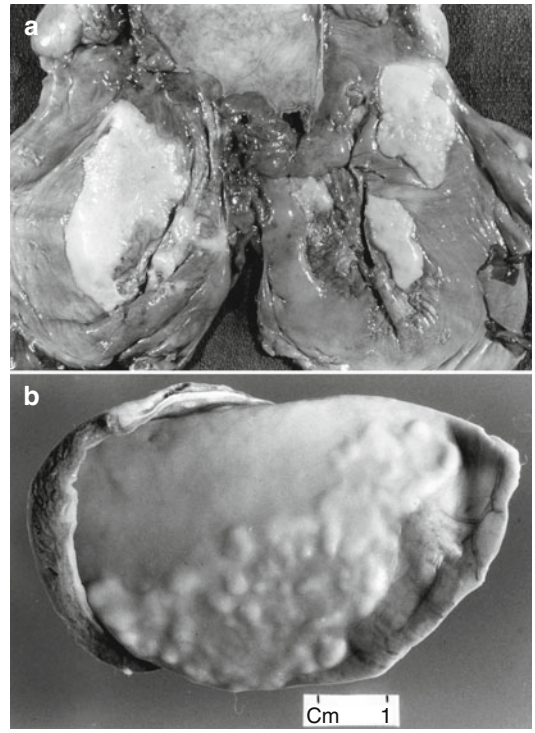


**Fig. 6.5** (**a**) Gross photograph from autopsy examination illustrates bilateral elevated white plaques on the diaphragmatic pleura. (**b**) Close view of a parietal diaphragmatic plaque showing smooth areas as well as knobby areas resembling "candle-wax drippings"

However, another study comparing conventional CT to high-resolution CT found that conventional CT was more sensitive for detecting plaques [54].

### 6.3.3 Pathologic Findings

Grossly, parietal pleural plaques are yellow-white, elevated, firm, and glistening and have sharply circumscribed borders [55, 56]. They are frequently bilateral and are usually seen within the costal pleura, where they lie parallel to the ribs. They are also seen on the domes of the diaphragm (Figs. 6.5 and 6.6). Pleural plaques vary in size from those that are just visible to the naked eye to structures that are 12 or more centimeters across [57]. They are frequently calcified. These ivory-colored structures may have either a smooth surface or a knobby appearance, consisting of multiple 5-mm nodules that create a

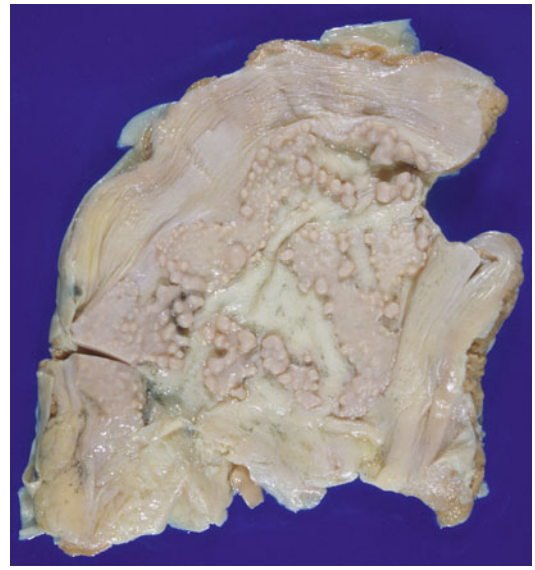

**Fig. 6.6** Gross appearance of diaphragm with parietal pleural plaque shows irregular, 10-cm plaque with smooth and nodular areas (Reprinted from Ref. [44], with permission)

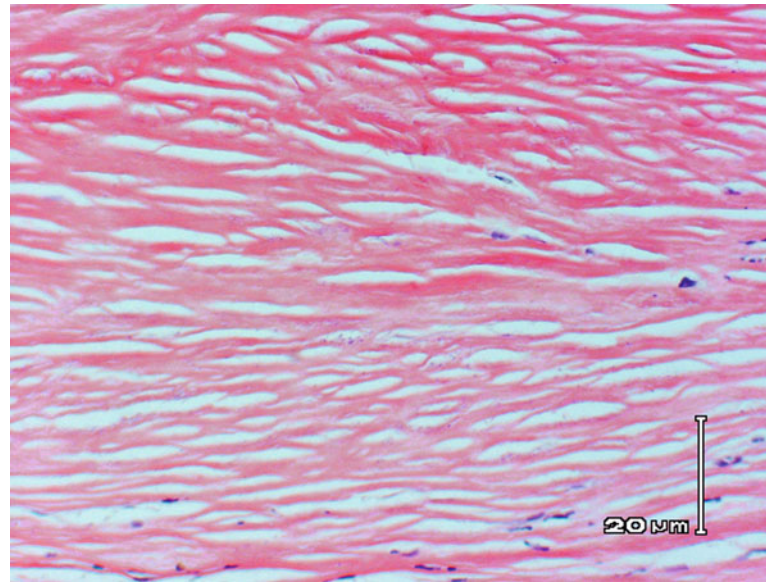


**Fig. 6.7** H&E photomicrograph of a parietal pleural plaque. Note the lack of cellularity and the "basket-weave" pattern of the collagen fibers

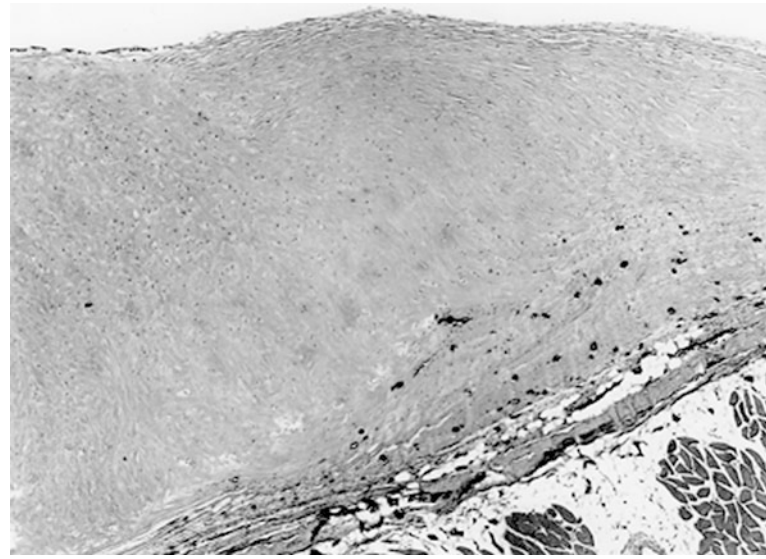

**Fig. 6.8** H&E photomicrograph of a parietal pleural plaque showing dense collagenous tissue without a "basket-weave" pattern (i.e., solid variant)

"candle-wax dripping" appearance [57]. The thickness of the plaques varies from a few millimeters to a centimeter or more. Visceral pleural plaques have been described as well, but are considerably less frequent [1]. Plaques have also been described within the peritoneum on the surface of the spleen or liver, and some of these are related to prior asbestos exposure [58, 59]. In rare instances, calcified plaques or asbestos-induced diffuse pericardial fibrosis may involve the pericardium [58, 60]. Adhesions between the surface of parietal pleural plaques and the adjacent visceral pleura are uncommon.

Microscopically, plaques are predominantly collagenous with scant cellularity (Figs. 6.7 and 6.8). This dense fibrous tissue often shows a "basket-weave" pattern [55, 56] (Fig. 6.8). However, plaques with a solid appearance lacking the "basket-weave" pattern may also be observed (Fig. 6.8). These solid-appearing plaques accounted for almost one-third of the plaques studied histologically by Wain et al. [44]. Rarely, a row of cuboidal mesothelial cells may be seen on the surface of the plaque. Although inflammatory cells are not observed within the plaque, small clusters of lymphocytes are

invariably found at the edge of the plaque or at the interface between the plaque and the subjacent chest wall [39]. Foci of dystrophic calcification are also commonly observed within the plaque. With light microscopy, neither asbestos bodies nor fibers are seen. With electron microscopy, asbestos fibers may be found [15].

Examination of histologic sections of lung parenchyma from patients with pleural plaques may show normal lung or a variety of pathologic features, including peribronchiolar fibrosis, visceral pleural thickening, organizing pneumonia, focal parenchymal scarring, paracicatricial emphysema, or asbestos bodies [61]. The presence of peribronchiolar and alveolar septal fibrosis with asbestos bodies in histologic sections is diagnostic of asbestosis (see Chap. 4). However, the term asbestosis, which refers to pulmonary interstitial fibrosis, should not be applied to parietal pleural plaques or any of the other benign asbestos-related pleural diseases.

### 6.3.4 Epidemiologic Considerations

Various epidemiologic studies have clearly established the role of inhaled asbestos fibers in the formation of parietal pleural plaques [15, 44, 46, 62–70]. By means of tissue digests, it has been shown that it is primarily amphibole asbestos fibers that are found in abnormal amounts in the lungs of patients with plaques [44, 64, 68–70]. The quantity of asbestos present in the lungs of patients with plaques who lack the histologic criteria for the diagnosis of asbestosis (see Chap. 4) is intermediate between that of the general population and that of individuals with asbestosis (see Chap. 11). These data agree well with the epidemiologic observations that pleural plaques often occur in individuals with brief, intermittent, or low-level asbestos exposure [15, 44, 57]. They also occur in individuals exposed to asbestos indirectly, such as family members exposed to dust brought home on an asbestos worker's clothes [65] or individuals living near an asbestos mine or production plant. Outbreaks of pleural plaques and calcification have also been observed in populations exposed to asbestos fibers from an environmental source. For example, a high prevalence of pleural plaques has been noted among Finnish immigrants, believed to be exposed to anthophyllite asbestos in rocks used to heat sauna baths or in insulation materials for the baths [71]. Similarly, a high prevalence of plaques has been described among inhabitants of the island of Cyprus [72] and in the Metsovo region of Greece [73], where tremolite occurs naturally. In the Metsovo region of Greece, long, thin tremolite fibers are found in the whitewash materials used inside and outside the homes of the inhabitants [73]. Other environmental exposures leading to plaque formation have been documented [74, 75], and additional epidemics of pleural disease due to environmental asbestos exposure undoubtedly await discovery.

Fibrous zeolites found in the soil and rocks in rural areas of Turkey are also causally associated with bilateral pleural plaques. Although not classified as asbestos, these zeolite fibers have length/width ratios that simulate those of asbestos fibers [76]. A detailed epidemiologic study of the fibrous zeolite (erionite) in Turkey was reported by Artvinli and Baris in 1982. In Tuzkoy, one of the villages with environmental zeolite exposure, the fibrous mineral was found in soil samples from roads and fields, as well as in building stones. Tissues of lung and pleura from the inhabitants of Tuzkoy also revealed the effects of zeolites, with 17 % showing calcified pleural plaques, 10 % showing fibrous pleural thickening, and 12 % revealing interstitial pulmonary fibrosis [76]. These Anatolian villages also have one of the highest rates of pleural mesothelioma yet identified anywhere in the world (see Chap. 5). In addition to Turkey, an extremely rare case of erionite-associated mesothelioma with pleural plaques was recently reported in the United States [77]. Microscopic analyses from a subject diagnosed with right pleural mesothelioma with metastasis to the lymph node revealed parietal pleural plaques with acellular hyalinized collagen in the classical "basket-weave" pattern [77]. Tissue digestion studies showed no elevation of asbestos fibers, but demonstrated markedly high levels of fibrous erionite suggesting that erionite was the

cause of the plaques and mesothelioma in this patient.

In addition to the observations of pleural plaques caused by asbestos and erionite, Hillerdal lists talc as another environmental mineral that may produce bilateral pleural plaques. However, talc is often contaminated with noncommercial amphiboles (anthophyllite and tremolite), so its exact role is unclear. It is of interest that cigarette smoking interacts with asbestos to greatly increase the risk for development of pleural plaques [69, 78, 79]. The mechanism is unknown, since smoking has no apparent effect on mesothelioma rates (See Chap. 5). Finally, whereas the vast majority of cases with bilateral parietal pleural plaques are due to asbestos exposure, unilateral plaques with or without calcification may be due to other causes, including trauma with organized hemothorax, old empyema, or tuberculous pleuritis [44, 64].

### 6.3.5 Clinical Implications

The clinical implications of parietal pleural plaques are twofold: (1) the implications of plaques with regard to functional disability and (2) the prognostic implications with regard to other asbestos-related diseases. The great majority of individuals with pleural plaques alone have no symptoms or physiologic changes [80–83]. In cases where either symptoms or clinical impairment is present, one must carefully consider contributions from diffuse pleural fibrosis (see below), cigarette smoking, or from radiographically inapparent parenchymal fibrosis. Impairment from cigarette smoking is most often due to emphysema, which can be recognized radiographically [84]. Pulmonary interstitial fibrosis (i.e., asbestosis) in the presence of a negative chest x-ray occurs in 10–18 % of cases [85, 86]. Although Schwartz et al. in a study of more than 1,200 sheet metal workers found a significant correlation between radiographically detected parietal pleural plaques and restrictive ventilatory defects [87], these authors concede that the most probable explanation is subclinical alveolitis or interstitial fibrosis not detected by routine chest radiographs [88]. A more recent study also suggests that parietal pleural plaques are unlikely to lead to relevant clinical effects [89].

The lack of symptoms or signs in the majority of patients with pleural plaques alone leads one to ask whether pleural plaques should be considered a disease. Stedman's Medical Dictionary defines a disease entity as characterized by at least two of the following criteria: (1) a recognized etiologic agent (or agents), (2) an identifiable group of signs or symptoms, and (3) consistent anatomical alterations [90]. Since pleural plaques clearly satisfy the first and third criteria, plaques, by this definition, constitute a disease entity. However, the asymptomatic nature of plaques is not necessarily applicable to other benign asbestos-related pleural disease (see later discussion).

The second issue regards the prognostic implications of plaques with respect to other potentially fatal asbestos-related diseases. Hourihane et al. state that pleural mesotheliomas are more common in patients with pleural plaques [46], an observation confirmed by others [91, 92]. Hillerdal found an 11-fold increased risk of mesothelioma among individuals with bilateral pleural plaques [92]. However, there is no evidence that pleural plaques are a precursor lesion of mesothelioma. Mollo et al. reported that patients with bilateral plaques are more likely to develop asbestosis than those without plaques [67]. Three different studies have independently shown a strong association between pleural plaques and laryngeal carcinoma [44, 67, 93]. Thus, plaques seem to be a predictor of increased risk for some asbestos-related disorders.

More controversial is the relationship between plaques and carcinoma of the lung. Studies from the United Kingdom have suggested that shipyard workers with pleural plaques are at increased risk for development of carcinoma of the lung [94, 95]. Others have found no increased risk of lung cancer associated with plaques alone [44, 67, 96], and Kiviluoto et al., in a study of 700 workers with pleural plaques, found an increased risk for bronchogenic carcinoma only when there was concomitant parenchymal fibrosis (i.e., asbestosis) [97]. Hillerdal reported a relative risk

for lung cancer of 1.43 for patients with bilateral pleural plaques, and this finding was statistically significant when controlled for asbestosis and cigarette smoking [94]. Another study by Roggli and Sanders showed that only 10 % of patients with pleural plaques in the absence of asbestosis had a fiber burden that has been associated with an increased lung cancer risk [98]. A consensus of experts from a meeting in Helsinki, Finland, in 1997 concluded that plaques alone are insufficient to relate lung cancer to prior asbestos exposure [99].

## 6.4 Diffuse Pleural Fibrosis

### 6.4.1 Radiographic Features

Diffuse thickening of the visceral pleura can be detected on routine chest films but is better identified by computed tomography [100]. It may occur as a consequence of a connective tissue disorder, such as rheumatoid arthritis or systemic lupus erythematosus [101]. However, in the absence of clinical evidence of a connective tissue disorder, the chest x-ray showing bilateral pleural fibrosis usually indicates prior asbestos exposure [62, 63]. Diffuse pleural fibrosis must be distinguished on the one hand from the more localized and often calcified parietal pleural plaque, and on the other hand from malignant pleural mesothelioma. Unlike pleural plaques, diffuse visceral pleural fibrosis is associated with blunting of the costophrenic angle(s) on plain films. Mesothelioma usually shows asymmetrical involvement of the hemithoraces, irregular thickening of the pleura, and invasion or destruction of portions of the chest wall. These features can often be seen to better advantage with computed tomography of the thorax [102, 103]. Diffuse pleural thickening may follow benign asbestos effusion [17] and is often unilateral (Fig. 6.9).

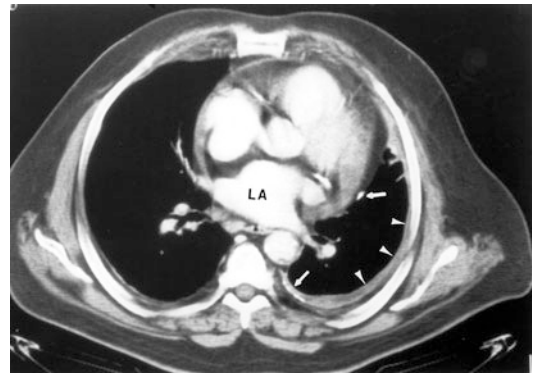


**Fig. 6.9** Computed tomography of the thorax at the level of the left atrium (*LA*) showing unilateral diffuse pleural thickening (*arrowheads*) in a 74-year-old manufacturer of asbestos cloth. Calcification is present posteriorly on the parietal pleural surface and also adjacent to the left heart border (*arrows*). No tumor was found at open thoracotomy and pleural biopsy (Courtesy of Dr. Caroline Chiles, Department of Radiology, Duke University Medical Center, Durham, NC)

### 6.4.2 Pathologic Findings

Diffuse pleural fibrosis is typically of varying and uneven thickness and can surround the entire lung [104]. The inferior and dorsal portions of the lung are the areas most frequently affected, and the process may extend into the major fissures (see Fig. 4.5). A constrictive pleuritis may occur and contribute to decreased vital capacity [17, 105]. With time, diffuse pleural fibrosis may progress [15]. Differential diagnosis of such lesions should include infectious pleuritis, rheumatoid arthritis, and systemic lupus erythematosus. The fibrous thickening of the visceral pleura is bland and nonspecific, consisting of dense collagenous tissue and varying numbers of chronic inflammatory cells (lymphocytes, macrophages, and plasma cells) (Fig. 6.10). Fibrin deposits may be observed on the surface of the collagenous tissue. Analysis of tissue asbestos content in the lung parenchyma of patients with diffuse pleura fibrosis who lack histologic features of asbestosis shows levels intermediate between those of the general population and those of individuals with asbestosis [104] (see Chap. 11). A dose-response relationship has been demonstrated between the degree of asbestos exposure and the extent of pleural thickening [66]. With light microscopy, neither asbestos bodies nor fibers are seen within the fibrotic visceral pleura. With electron microscopy, asbestos fibers may be found [15].

**Fig. 6.10** H&E photomicrograph showing diffuse fibrosis of the visceral pleura in an insulator with asbestosis. Asbestos bodies in adjacent lung parenchyma are just beyond resolution at this magnification

### 6.4.3 Clinical Implications

Diffuse pleural fibrosis may be asymptomatic, but in some cases may be of sufficient extent and severity as to result in functional impairment [17, 87, 105, 106]. This usually manifests as restrictive changes on pulmonary function tests, with a diminished vital capacity [107]. Picado et al. described six patients with extensive asbestos-related pleural disease that manifested diminished exercise tolerance [108]. These investigators felt that parenchymal fibrosis was unlikely, although lung parenchyma was not available for histologic examination in any of the cases. Although some of the patients were characterized as having parietal pleural plaques, it is likely that most or all had some degree of diffuse visceral pleural fibrosis. A study by Schwartz et al. found a correlation between the degree of pleural fibrosis detected by computed tomography and the restrictive lung function in the patients [109]. Surgical decortication is rarely indicated in these patients, because postoperative improvement is usually only marginal [15].

## 6.5 Rounded Atelectasis

### 6.5.1 Radiologic Features

Blesovsky originally described rounded atelectasis, also known as the folded lung syndrome, in 1966 [110]. It is characterized radiographically as a peripheral rounded mass, 2–7 cm in diameter, that is pleural based [111]. Pleural thickening that is greatest near the mass and interposition of lung parenchyma between the mass and the diaphragm are invariably present (Fig. 6.11). One of the most useful diagnostic features is the presence of curvilinear shadows extending from the mass toward the hilum [111, 113]. The intrapulmonary location of the mass is indicated by the acute angle formed between the pleura and mass. The intralobar fissure is frequently thickened. A recent case report suggests positron emission tomography may be useful in atypical cases [114]. When sequential films are available for review, the static nature of the lesion can be demonstrated. In cases where bronchography has been performed, bronchi have been demonstrated to curve toward the lower pole of the mass [111]. Computed tomography and high-resolution CT are much better at detecting this lesion [113] and may also detect other asbestos-related pleural changes, such as calcification [54, 115] (Fig. 6.12). Rounded atelectasis may be bilateral in some instances [116], and cases with spontaneous resolution have also been reported [117].

### 6.5.2 Pathologic Findings

Pathologists must be aware of the gross and microscopic features of rounded atelectasis, since they may be called upon to make the diagnosis at frozen section. The lesion is characterized by dense pleural fibrosis that is of greatest thickness overlying the mass (see Fig. 6.12). The pleura

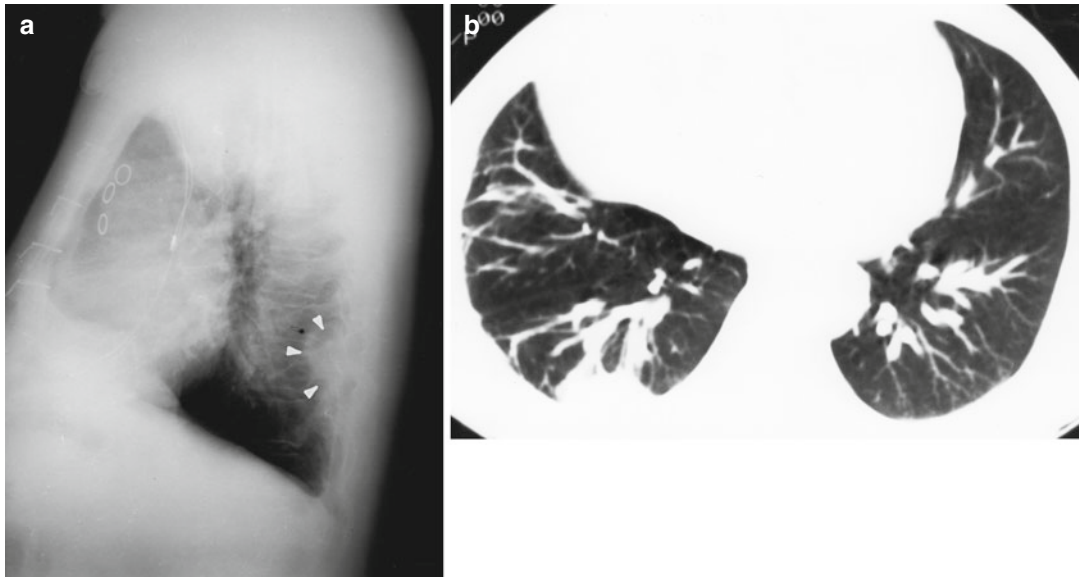


**Fig. 6.11** (**a**) Lateral chest radiograph showing a posterior, pleural-based mass (*arrowheads*). (**b**) Computed tomogram of the thorax shows the typical features of rounded atelectasis, with a pleural-based mass and curvilinear bronchovascular structures entering the mass (Reprinted from Ref. [112] with permission)

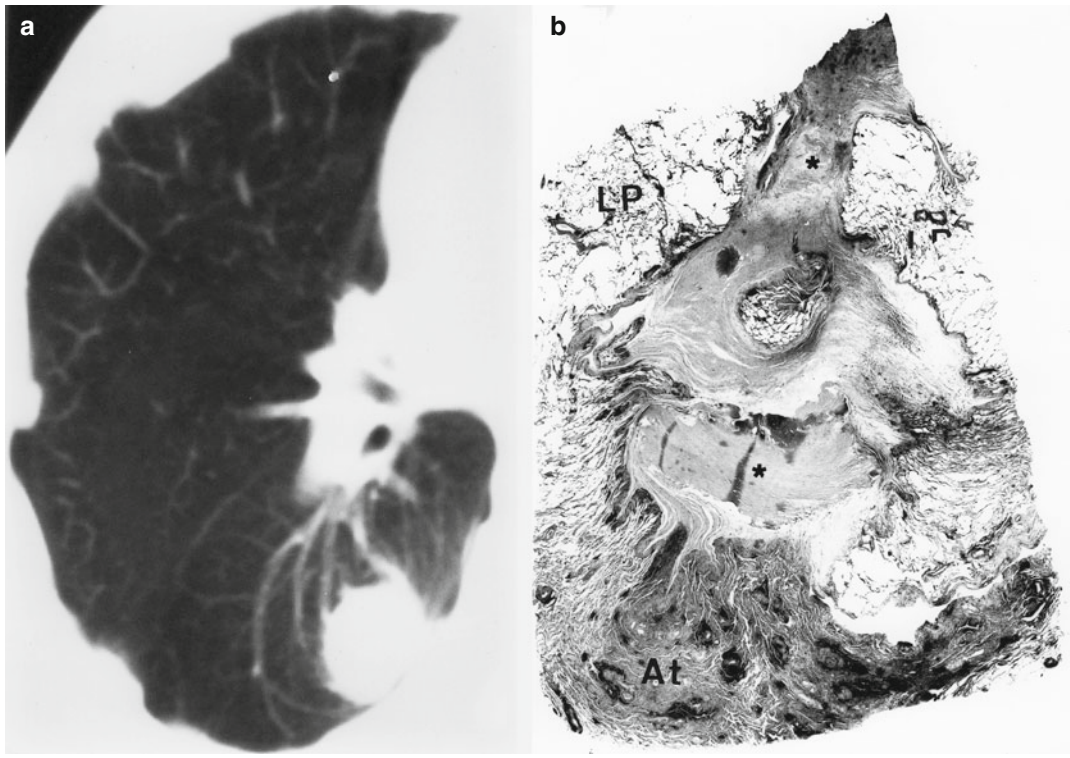


**Fig. 6.12** (**a**) Computed tomography of the left hemithorax in a patient with a left lower lobe mass on chest x-ray. Note the curvilinear bronchovascular structures entering the pleural-based mass, which is characteristic of rounded atelectasis. (**b**) Low-power H&E photomicrograph of the resected lesion shown in (a), with pleural surface toward the top. There is localized thickening and fibrosis of the visceral pleura, which has buckled inwards (*). Adjacent lung parenchyma is atelectatic (*At*). Elsewhere, the lung parenchyma (*LP*) is normally expanded

may be buckled or puckered and thus drawn into the underlying lung parenchyma. The lung itself may contain some fibrosis but is largely atelectatic. Because of the frequent association with asbestos exposure [111], asbestos bodies should be searched for within the lung parenchyma [112]. However, care must be taken not to diagnose asbestosis based on changes secondary to the pleural lesion. Blesovsky believed that the mechanism of formation of rounded atelectasis involved localized visceral pleural thickening and fibrosis in which adhesion between visceral and parietal pleura was prevented from forming because of an associated pleural effusion. Contraction and buckling of the fibrotic visceral pleura then led to atelectasis and folding of the immediately adjacent lung parenchyma. In support of this pathogenetic concept, it was observed at thoracotomy that the collapsed lung re-expanded when the thickened pleura was dissected away [110].

### 6.5.3 Clinical Implications

The strong association between rounded atelectasis and prior asbestos exposure has been emphasized [111]. Indeed, all three of the original cases described by Blesovsky had been occupationally exposed to asbestos [111], but can occur in other conditions as well [118]. Because of this association and the increased risk of lung cancer in asbestos workers (see Chap. 7), rounded atelectasis may be confused with lung cancer clinically and radiographially [119]. Recognition of the clinical and radiographic features of rounded atelectasis is important, since the rendering of the correct diagnosis can spare the patient a thoracotomy [16].

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# Carcinoma of the Lung

7

Victor L. Roggli

## 7.1 Introduction

During the past 50 years, the USA and other industrialized nations have witnessed a remarkable increase in mortality from carcinoma of the lung. Today, this disease is the number one cause of cancer mortality in the USA, accounting for more than 180,000 deaths annually [1]. Unraveling the various causes of this increased risk has required painstaking epidemiologic studies, but it has become apparent that cigarette smoking is the single largest preventable cause of lung cancer in the world today [2]. It has been estimated that between 85 and 95 % of deaths from lung cancer are directly attributable to smoking [1, 2]. Cigarettes are the leading offenders, but pipe and cigar smokers are also at risk, though only if they inhale the smoke [1–3]. Asbestos workers are also at increased risk for lung cancer, particularly those who smoke tobacco products [4, 5]. It is the purpose of this chapter to review the characteristics of asbestos-associated lung cancers and to discuss the role of the pathologist in recognizing asbestos as a causative factor. The historical context in which asbestos was recognized to be a carcinogen for the lower respiratory tract will first be reviewed, followed by a discussion of the epidemiologic features of asbestos-related lung cancer, including the role of asbestosis, synergism with cigarette smoking, and asbestos fiber type. The role of cytopathology in the diagnosis of lung cancer in asbestos workers is discussed in Chap. 9, experimental models of pulmonary carcinogenesis in Chap. 10, and lung fiber burdens in asbestos workers with lung cancer in Chap. 11.

## 7.2 Historical Background

The first report of carcinoma of the lung in an asbestos worker was that of Lynch and Smith in 1935, a squamous carcinoma in a patient with asbestosis [6]. In 1943, Homburger reported three additional cases of bronchogenic carcinoma associated with asbestosis, bringing the world total reported to that date to 19 cases [7]. In his annual report for 1947 as chief inspector of factories in England and Wales, Merewether noted that among 235 deaths attributed at autopsy to asbestosis, 13 % had a lung or pleural cancer [8]. During the 20-year period following Lynch and Smith's initial case report, some 26 reports were published covering approximately 90 cases of carcinoma of the lung found at autopsy in asbestos workers [9]. Then in 1955, Sir Richard Doll published his classic study, which was the first systematic combined epidemiologic and pathologic study of lung cancer among asbestos workers [10]. Doll concluded that carcinoma of the lung was a specific industrial hazard of asbestos workers. Also in 1955, Breslow published a

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T.D. Oury et al. (eds.), *Pathology of Asbestos-Associated Diseases*,
DOI 10.1007/978-3-642-41193-9_7, 

case-control study of asbestosis and lung cancer from California hospitals [11]. In 1968, Selikoff published data from a cohort of asbestos insulation workers which showed that insulators who smoked had a 92-fold increased risk of carcinoma of the lung over non-asbestos-exposed, nonsmoking individuals [12]. This was also the first study to suggest that there is a multiplicative, or synergistic, effect between cigarette smoking and asbestos exposure in the production of pulmonary carcinomas. Buchanan noted that more than half of all patients with asbestosis would eventually die of respiratory tract cancer [13]. Since these pioneering studies, there have been numerous reports confirming the association between asbestos exposure and carcinoma of the lung [14–23].

## 7.3 Epidemiology

### 7.3.1 Asbestos or Asbestosis?

Epidemiologic studies have demonstrated a dose-response relationship between asbestos exposure and lung cancer risk, and there is a long latency period between initial exposure and manifestation of disease, usually beginning more than 15 years after initial exposure [4, 5, 9, 19]. There are three primary hypotheses that have been put forward to describe the relationship between asbestos exposure and lung cancer risk [24]. The first hypothesis [H1] is that there is only an increased risk of lung cancer in asbestos workers who also have asbestosis. The second hypothesis [H2] is that it is the dose of asbestos rather than the occurrence of fibrosis that is the determinant of lung cancer risk. The third hypothesis [H3] is that there is a no threshold, linear dose-response relationship between asbestos exposure and subsequent lung cancer risk, with any level of exposure potentially increasing one's risk of disease. Whether or not there is a threshold for asbestos-induced carcinoma of the lung and whether or not asbestosis is a prerequisite precursor lesion are issues of more than academic importance [25], since the number of individuals exposed to low levels of asbestos greatly exceeds the numbers of individuals with asbestosis.

All investigators are in agreement that there is a dose-response relationship between asbestos exposure and lung cancer risk [26, 27] and that the highest risk occurs among those workers who also have asbestosis. Proponents of [H1] believe that only those with asbestosis have an increased lung cancer risk [28–32]. In the original study by Doll [10], all 11 of the asbestos workers dying of carcinoma of the lung had pathologically confirmed asbestosis. Furthermore, in the review by An and Koprowska of asbestos-associated carcinoma of the lung reported from 1935 to 1962, all 41 cases occurred in individuals with asbestosis [33]. Published mortality data reveal a close correlation between relative risks of death from lung cancer and from asbestosis [14, 16, 34–39]. In addition, a longitudinal study of Quebec chrysotile miners indicated that most of the observed cancers have occurred in subgroups of workers with prior radiographic evidence of asbestosis [40].

Further support for this hypothesis includes studies of Louisiana asbestos-cement workers, South African asbestos miners, insulators, and individuals with non-asbestos-related interstitial lung disease. The Hughes-Weill study of 839 asbestos-cement workers found a statistically significant increased risk of lung cancer among workers with radiographic evidence of asbestosis (International Labor Organization score $\geq$1/0), but not among those with pleural disease only or with no radiographic abnormality [19]. Sluis-Cremer and Bezuidenout reported on an autopsy study of 399 amphibole miners, in which increased lung cancer rates were observed in cases with pathologic asbestosis, but not in those lacking asbestosis [41]. Kipen et al. studied 138 insulators with lung cancer and tissue samples available for histologic review and found evidence for asbestosis in all 138 cases [42]. In addition, there is an increased risk of lung cancer among patients with interstitial lung disease other than asbestosis [43–45].

There are a number of weaknesses in the hypothesis that asbestosis is a prerequisite for asbestos-induced lung cancer. First of all, the Hughes et al. [19] study lacks the statistical power to detect an increased risk of lung cancer among patients without radiographic

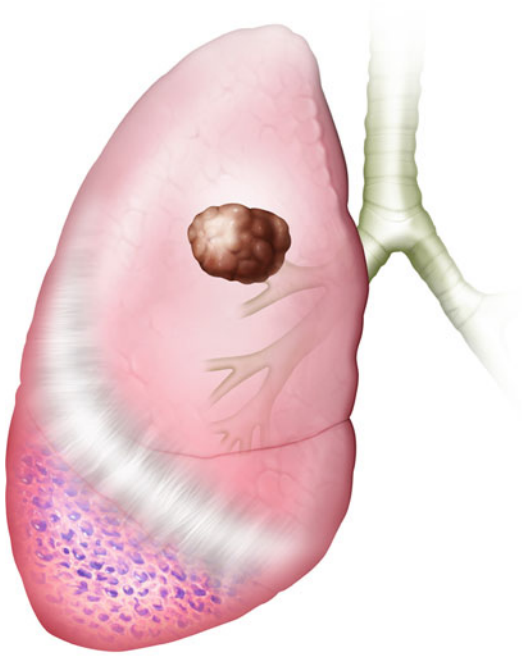

**Fig. 7.1** Artist's rendering of the location of the typical lung cancer in an asbestos worker (central and upper lobe) versus the location of fibrosis in most cases of asbestosis (peripheral lower lobe). This distribution of disease is difficult to reconcile with the hypothesis that fibrosis is the precursor of asbestos-induced lung cancers

evidence of asbestosis [46]. Second, the studies by Sluis-Cremer and Bezuidenout [41] and Kipen et al. [42] have unconventional definitions for the histologic diagnosis of asbestosis [47, 48]. For example, the Kipen study diagnosed asbestosis in eight cases lacking asbestos bodies in histologic sections [42]. Third, the rates of lung cancer among individuals with asbestosis (from 40 % to more than 50 %) are much higher than the rates in cases with idiopathic pulmonary fibrosis (pooled estimate from 14 studies of 17 %) [45]. Notably, patients with idiopathic pulmonary fibrosis usually die of their disease in 3–5 years from the time of diagnosis as compared to patients with asbestosis who frequently live decades. Thus, it is not too surprising that patients with idiopathic pulmonary fibrosis develop less cancer as they have much less time to develop cancer than patients with asbestosis. Fourth, the vast majority of lung cancers among asbestos workers are bronchogenic carcinomas not distinguishable on the basis of their morphology or histologic features from those occurring in nonexposed smokers and not the peripheral adenocarcinomas typically associated with diffuse interstitial fibrosis. It is difficult to reconcile the requirement for the peripheral fibrosis of asbestosis with the proximal bronchogenic carcinomas seen in the majority of asbestos workers (including those with asbestosis) (Fig. 7.1) [49]. Finally, it is difficult to explain the synergistic effect between asbestos exposure and cigarette smoking in lung cancer induction on the basis of [H1] [25].

Proponents of [H2] believe that lung cancer and asbestosis are independent manifestations of asbestos exposure, each following a dose-response relationship with exposure. Hence, both diseases are likely to occur among individuals with the heaviest exposures. Accordingly, it is the dose of asbestos rather than the development of fibrosis per se that is the determining factor. Asbestosis is not invariably present in cohorts of asbestos workers with a demonstrable excess risk of lung cancer [25, 40, 50, 51]. In addition, studies with greater statistical power than that of the Hughes et al. study [19] have shown an increased risk of lung cancer among asbestos workers without radiographic evidence of asbestosis [52–55]. Furthermore, studies have shown an increased risk of lung cancer based on the fiber burden within the lung, independent of asbestosis and cigarette smoking [56, 57]. For example, Karjalainen et al. studied 113 surgically treated male lung cancer patients versus 297 autopsy cases on males as referents [57]. For subjects with amphibole fiber counts exceeding one million/g of dry lung, the adjusted odds ratio was 4.0 for adenocarcinoma and 1.6 for squamous cell carcinoma. The odds ratio for a lower-lobe carcinoma was 2.8 for patients with a fiber count between one and five million and 8.0 for those with fiber concentrations greater than or equal to five million/g of dry lung.

There are several weaknesses to the hypothesis that fiber burden rather than asbestosis is the primary determinant of lung cancer risk among asbestos workers. First, studies with an increased lung cancer risk but no radiographic evidence of asbestosis do not exclude the possibility that the patients actually had subclinical asbestosis that would have been detected histologically. Second,

it is difficult to reconcile the preferential association between fiber burden and a specific histologic type (i.e., adenocarcinoma) and location (i.e., lower-lobe tumors), when studies have not consistently shown an association between any histologic pattern or tumor location and asbestos exposure (see below). Third, there are few epidemiologic studies that have examined the relationship between fiber burden and lung cancer risk [58–60]. Finally, the fiber burden levels in patients without asbestosis did not have a statistically significant odds ratio for lung cancer in the Karjalainen study [57]. However, the study did show a trend from a low to a higher odds ratio with transition from an intermediate- to a higher-level fiber count. Furthermore, the odds ratio for adenocarcinoma did show a statistically significant elevation with fiber burden greater than one million, even when all cases with any fibrosis were excluded [46].

Proponents of [H3] believe that asbestos exposure rather than asbestosis is the key element in lung cancer induction by asbestos and that any level of exposure increases one's risk for cancer. Hence there is no threshold for asbestos exposure and increased lung cancer risk according to this hypothesis. In published cohorts with the steepest dose-response relationship, excess lung cancers were detected even in the groups with the very lowest level of exposure [34, 50]. Although some investigators have suggested that there is a threshold level of exposure to asbestos below which no excess deaths from carcinoma of the lung will occur [17, 61], investigation of the consequences of low-level exposures is the Achilles' heel of epidemiologic studies because it requires large cohorts followed for extended periods of time in order to detect statistically significant associations [62, 63]. Nonetheless, the consensus based on a number of cohort mortality studies as well as studies of populations with environmental asbestos exposure is that there is some level of exposure below which no statistical excess of lung cancers can be demonstrated [25, 64–72].

Experimental animal studies also bear on the issue of the mechanism of asbestos-induced carcinogenesis [25], and this subject is reviewed in detail in Chap. 10. It is the author's view that the literature in this regard indicates that fibrogenesis and carcinogenesis are separate and distinct effects of asbestos pathobiology, which have as a common denominator a dose-response relationship with respect to asbestos exposure and a dependence upon fiber length.

In summary, the weight of the evidence at this time seems to favor [H2]: asbestos-induced lung cancer is a function of fiber dose (and hence fiber burden) with a threshold for increased lung cancer risk [73, 74]. Therefore, in order to attribute a substantial contributing role for asbestos in the causation of lung cancer, asbestosis must be present clinically or histologically, or there should be a tissue asbestos burden within the range of values observed in patients with asbestosis [75] (see Chap. 11). The mere presence of parietal pleural plaques is not sufficient to establish causation (see Chap. 6) [76, 77]. Furthermore, studies have shown a very close correlation between fiber burden levels associated with an increased lung cancer risk and the presence of histologically confirmed asbestosis. A fiber burden in the range determined by Karjalainen et al. [57] to be associated with an increased lung cancer risk was found in 82 % of 70 cases with histologic asbestosis but in only 6 % of 164 cases without asbestosis [75]. Hence it is unlikely that the distinction between [H1] and [H2] can be resolved by epidemiologic studies [78].

### 7.3.2 Cigarette Smoking and Synergism

Epidemiologic studies have indicated that there is a synergistic effect between cigarette smoking and asbestos exposure in the production of lung cancer [5, 12, 79, 80]. This concept is well illustrated in the study by Hammond et al. [5] in which cancer mortality in 17,800 asbestos insulators was compared with cancer death rates in the general population. In this study, it was noted that cigarette smoking increases one's risk of lung cancer approximately 11-fold, whereas asbestos exposure increases the risk about fivefold, when compared to a nonsmoking, nonexposed reference population. If these two effects were merely

additive, one would expect an approximately 16-fold increase in lung cancer risk among cigarette-smoking asbestos insulators. Instead, what is actually observed is a 55-fold increased risk, indicating that the two effects are multiplicative rather than additive [5]. Other investigators have also indicated that the interaction between asbestos and cigarette smoke in increasing the lung cancer risk is a synergistic or multiplicative effect [14, 81–93]. Some studies have reported an additive effect [94, 95] or an effect that was intermediate between additive and multiplicative [80, 95, 96]. More recent studies favor a model that is more than additive and less than multiplicative [97–99]. Possible mechanisms for synergism are discussed in Chap. 10.

The US Surgeon General's report on the effects of smoking cessation on the risk of developing carcinoma of the lung indicates that ex-smokers have a risk which is intermediate between that of current smokers and nonsmokers [100]. The magnitude of the decrease in risk is related to a number of factors, including the age when the patient started smoking, total duration and intensity of smoking, the age at cessation of smoking, and the time elapsed since the individual quit smoking. In this regard, studies have indicated that the risk of developing lung cancer in an ex-smoker is still greater than that of a lifelong nonsmoker even 20 or more years after cessation of smoking [99, 100]. These factors must be considered in the evaluation of the role of asbestos exposure in the development of carcinoma of the lung in an ex-smoker.

Since most lung cancers among asbestos-exposed individuals occur in workers who also smoke, it is difficult to obtain information regarding the lung cancer risk among nonsmoking asbestos workers. Hammond et al. [5] reported four such cases among their asbestos insulators, with an expected value of 0.8, hence their calculation of a fivefold increase in risk among nonsmoking asbestos workers. Berry et al. [95]. reported four additional cases of lung cancer among nonsmoking asbestos factory workers. They concluded that after allowance had been made for the effect of smoking on lung cancer, the relative risk due to asbestos was highest for those who had never smoked, lowest for current smokers, and intermediate for ex-smokers ($p < 0.05$). More recently Berry and Liddell report that the relative risk due to asbestos was higher for light smokers than for heavy smokers [101]. Lemen [102] reported four more cases of lung cancer among nonsmoking women in a predominantly chrysotile asbestos textile plant.

The author has also observed 23 additional cases of lung cancer in nonsmokers with some history of asbestos exposure in which fiber burden analyses had been performed. Sixteen of these cases have been reported previously [75, 103]. Twenty of the twenty-three were adenocarcinomas, including 3 bronchioloalveolar carcinomas, 1 pseudomesotheliomatous carcinoma, and 1 adenosquamous carcinoma. The other three were large cell carcinoma, squamous cell carcinoma, and pleomorphic carcinoma. Two cases occurred in the setting of idiopathic pulmonary fibrosis (usual interstitial pneumonia), including one bronchioloalveolar carcinoma. Six of the patients had pleural plaques, including one pseudomesotheliomatous carcinoma. One patient with adenocarcinoma had asbestosis. Only the latter case of the 23 had a fiber burden within the range described by Karjalainen et al. as being associated with an increased odds ratio for lung cancer [57]. In a review of lung cancer in nonsmokers, no evidence for a role of asbestos was identified [104]. Carcinoma of the lung is quite rare among nonsmokers [105]. In such cases, one must consider other possible factors such as the effects of passive smoking [1, 106] and of household radon gas exposure [1, 107].

### 7.3.3 Role of Fiber Type and Fiber Dimensions

Epidemiologic data indicate that carcinoma of the lung may develop in response to exposure to any of the types of asbestos [4, 9, 14, 34, 85, 108]. However, there is considerable controversy regarding the relative potency of the various fiber types for the production of pulmonary neoplasms [25]. Individuals who believe that chrysotile is less potent as a lung carcinogen than the

amphiboles amosite and crocidolite cite as evidence the relatively low rate of carcinoma of the lung among chrysotile miners and millers [64, 109, 110], asbestos-cement workers [17, 111], and friction-product manufacturers [65, 66]. On the other hand, some chrysotile asbestos textile plants have reported extremely high lung cancer rates, with exceptionally steep dose-response curves [34, 36, 112]. Although it has been suggested that contamination of the asbestos fibers with mineral oil might explain the high rate of carcinoma of the lung among asbestos textile workers [9], the steep dose-response relationship among these workers also holds for asbestosis, which is difficult to explain on the basis of contaminating oil. One major difficulty for studies trying to assess the relative potency of asbestos fiber types is the inaccuracy of historical estimates of asbestos exposure [25, 113]. In this regard, Newhouse [114] noted that chrysotile textile plants were particularly dusty when compared with other types of occupational exposure to chrysotile. Furthermore, in comparing the cancer mortality for two different asbestos textile plants, Finkelstein concluded that the risk of death from asbestos-associated cancer in factories manufacturing similar products is unrelated to the type of asbestos fiber used [36, 112, 113].

The author suspects that much of the variation in lung cancer rates among chrysotile workers can be explained on the basis of dose and relative fiber size, with longer fibers being more potent. For example, the low rate of lung cancer among automotive maintenance and brake repair workers [115] can be explained on the basis of relatively low dust levels, the low proportion of asbestos in the dust generated, and the preponderance of very short chrysotile fibers in brake dust [116, 117]. The relative ability of fibers to penetrate the bronchial mucosa may also be an important factor. Churg and Stevens in a study of smokers and nonsmokers with similar exposure histories and similar fiber burdens in the lung parenchyma examined this question [118]. These investigators found that the amosite content was six times greater in the bronchial mucosa of smokers as compared to nonsmokers and the chrysotile content was 50 times greater. Thus there is evidence that cigarette smoking increases the penetration of fibers into the bronchial mucosa, and this effect appears to be greater for chrysotile than for the amphiboles.

The issue of relative potency of chrysotile versus amphiboles in lung cancer production has been addressed in great detail by Hodgson and Darnton [119] and Berman and Crump [120, 121]. The former concluded that the relative potency of amphibole fibers (amosite and/or crocidolite) as compared to chrysotile for lung cancer was between 10:1 and 50:1. The latter proposed a model for predicting risk of lung cancer based on fiber dimensions and fiber type, with amphiboles more potent than chrysotile and long fibers more potent than short. Recent studies of lung cancer in asbestos textile workers lend support to the importance of fiber length in this regard [122, 123]. Differences in relative potency of fiber types is reflected in levels of exposure associated with a doubling of the risk of lung cancer: 25 fiber/cc-yrs for amphibole exposure versus 40 fiber/cc-yrs for mixed exposures [73, 74].

## 7.4 Pathology of Asbestos-Related Carcinoma of the Lung

### 7.4.1 Gross Morphology

Lung carcinomas have been classically divided into the proximal bronchogenic carcinomas, which arise from a mainstem, segmental, or subsegmental bronchus and typically present as a hilar mass, and peripheral carcinomas, arising from small airways (i.e., bronchioles or peripheral bronchi) and presenting as a "coin" lesion on chest roentgenogram [124]. Asbestos-related lung cancers can assume either of these gross appearances. In fact, there are no discernible differences between the macroscopic appearance of carcinomas of the lung among asbestos workers and those in individuals not exposed to asbestos [29, 49, 124, 125]. One possible exception to this observation is the lobar distribution, with carcinomas among cigarette smokers from the general population occurring about twice as often in the upper as compared to the lower lobes,

**Table 7.1** Tumor location in 312 lung cancer cases with and without asbestosis

| | Asbestosis | PPP[a] | Others[b] |
|---|---|---|---|
| Upper lobe | 26 | 45 | 78 |
| Lower lobe | 18 | 23 | 33 |
| Right lung | 36 | 52 | 91 |
| Left lung | 24 | 43 | 69 |

[a]*PPP* parietal pleural plaques, no evidence of asbestosis
[b]No evidence of asbestosis or plaques or uninformative cases

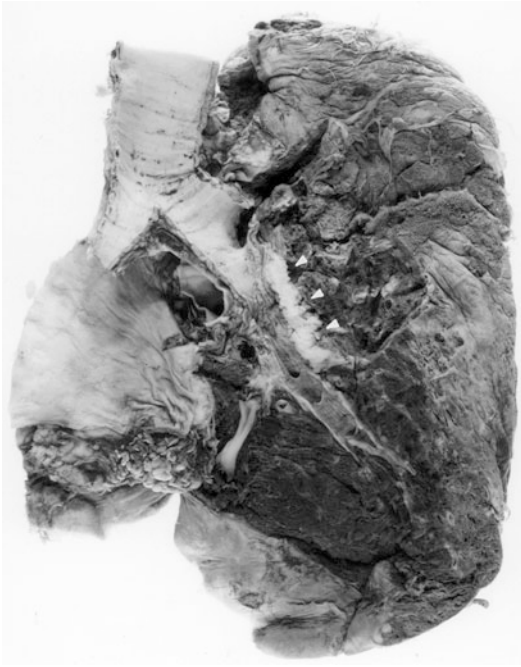

**Fig. 7.2** Gross photograph showing infiltrating carcinoma involving the bronchus intermedius of the right lung (*arrowheads*). The patient was a guard in a plant which manufactured amosite pipe insulation for 7 years (Reprinted from Ref. [128], with permission)

whereas the reverse is true for carcinomas among asbestos workers [56, 57, 126]. However, more recent studies have failed to confirm this observation and have found instead that lung cancers in asbestos workers occur more commonly in the upper lobe (Table 7.1) [75, 127]. At any rate, the overlap is great enough that the lobar distribution is hardly sufficient to assign attribution to asbestos exposure in the individual case [75, 126].

Typical examples of carcinoma of the lung in asbestosis patients are illustrated in Figs. 7.2, 7.3, and 7.4. One shows a proximal bronchogenic

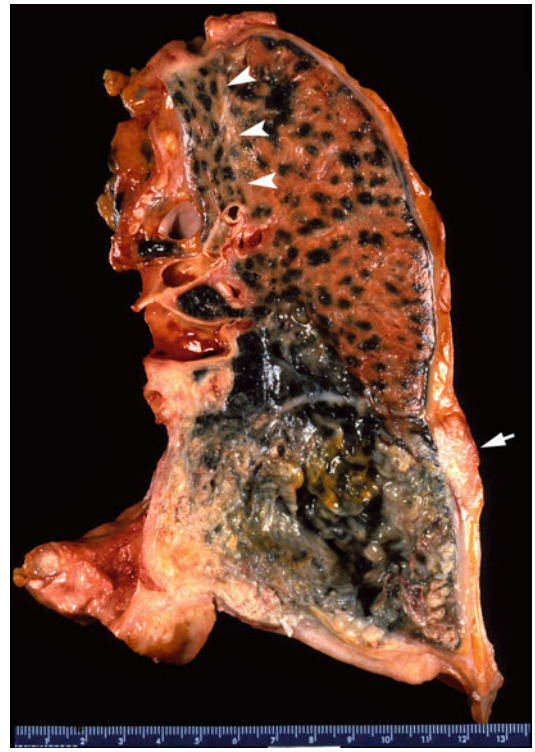

**Fig. 7.3** Gross photograph showing a cavitating carcinoma of the right lower lobe (*arrow*). The patient was an asbestos insulator in a shipyard for 30 years (same case as Fig. 4.4). Radiation fibrosis is present in the medial aspect of the right upper lobe (*arrowheads*), and a few scattered silicotic nodules were also palpable in the right upper lobe

carcinoma (Fig. 7.2) from a Tyler asbestos plant worker who was a guard at the Tyler plant for 7 years and developed the neoplasm 21 years after initial exposure. This plant made pipe insulation material from amosite asbestos [128, 129]. The second example is a lower-lobe cavitating cancer (Fig. 7.3) from a shipyard insulator and boiler scaler for 30 years. The third example shows a massively enlarged hilar lymph node secondary to metastatic bronchogenic carcinoma (primary tumor not visible in the section). Very fine interstitial fibrosis was just visible to the unaided eye in the lower lobes (Fig. 7.4). This patient was admitted comatose and died shortly thereafter, without providing any occupational history; asbestosis was confirmed upon histologic examination. All three examples are squamous cell carcinomas (Fig. 7.5), and two of the individuals also smoked cigarettes (180 and 50 pack-years, respectively). The smoking history of the third is unknown.

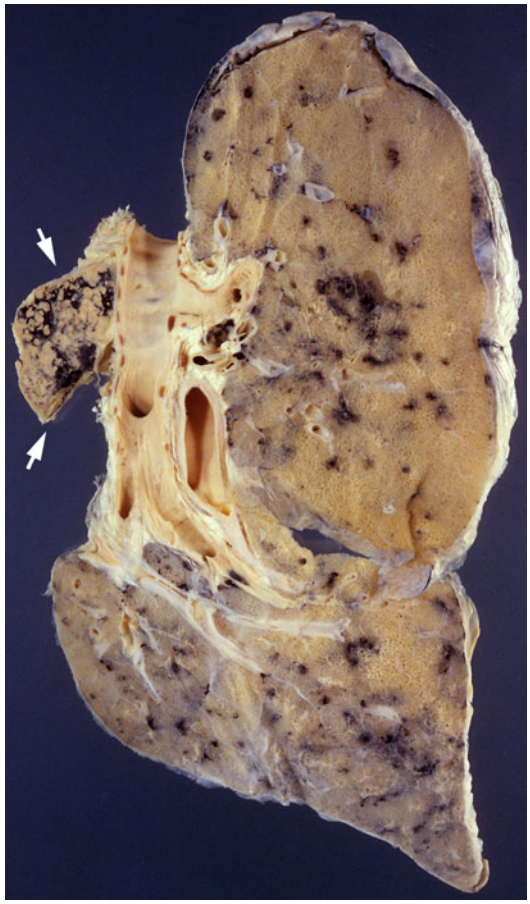

**Fig. 7.4** Metastatic bronchogenic carcinoma in a hilar lymph node (*arrows*). Asbestosis was present in histologic sections

### 7.4.2 Histopathology

Carcinomas of the lung have conventionally been categorized into four histologic patterns: squamous cell carcinoma, small cell carcinoma, adenocarcinoma, and large cell carcinoma [124, 130–132]. These patterns are illustrated in Figs. 7.6 and 7.7. The most recently revised WHO classification for the more common lung cancer types is summarized in Table 7.2 [133]. Squamous cell carcinomas are characterized by keratinization or intercellular bridges. In well-differentiated tumors, keratinization manifests in the form of keratin pearls and, in more poorly differentiated tumors, as individual cell keratinization (Fig. 7.6a). Squamous cell carcinomas account for about 30 % of primary lung carcinomas and usually present as proximal hilar masses. Small cell carcinomas have scant amounts of cytoplasm with high nuclear-to-cytoplasmic ratios. The nuclei are often hyperchromatic or else have finely stippled chromatin with inconspicuous nucleoli (Fig. 7.6b). Small cell carcinomas account for about 10–15 % of primary lung carcinomas and also present as proximal tumors. Large cell carcinomas consist of sheets or nests of tumor cells with moderately abundant cytoplasm, anaplastic nuclei, and prominent nucleoli (Fig. 7.6c). They do not keratinize, form glandular or papillary structures, or

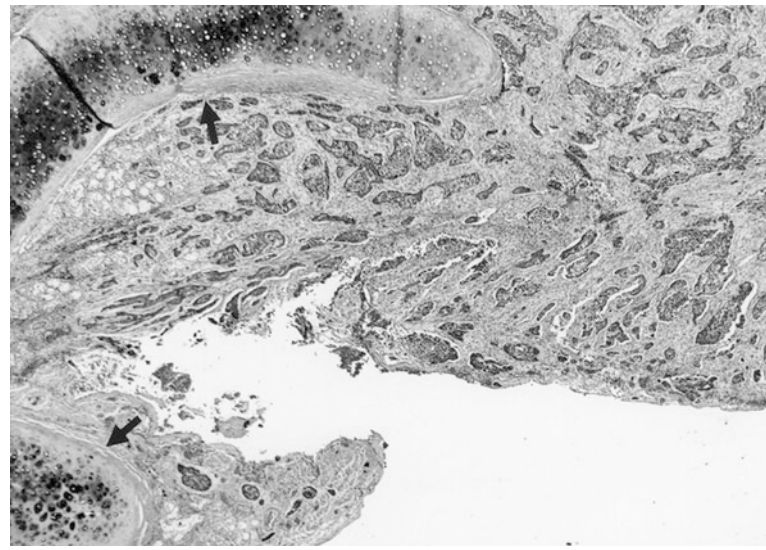

**Fig. 7.5** Squamous cell carcinoma of the right lung invading the wall of the bronchus intermedius in close proximity to the bronchial cartilages (*arrows*). Same case as Figure 7.2. Hematoxylin and eosin, ×39 (Reprinted from Ref. [129], with permission)

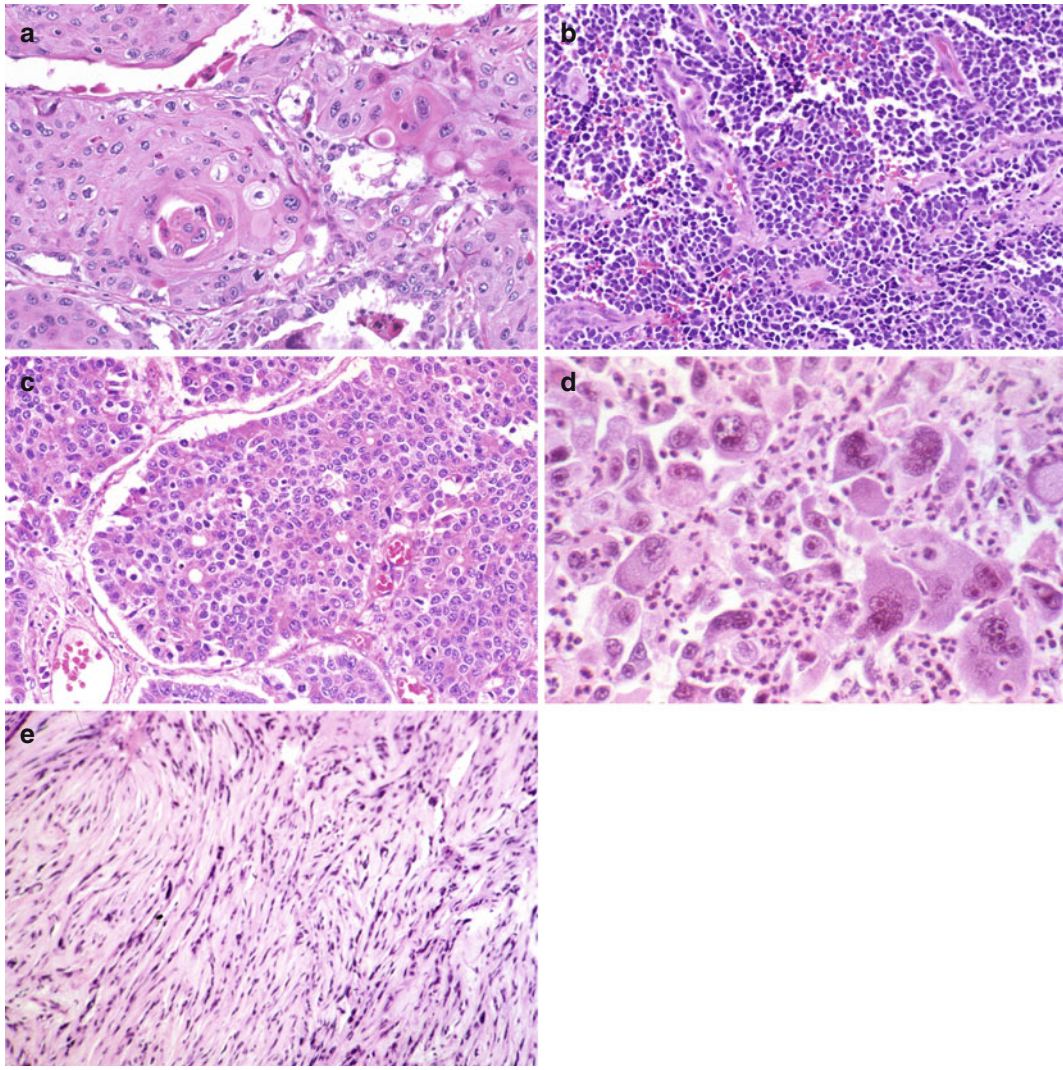


**Fig. 7.6** High-magnification photo micrographs illustrating major cell types of carcinoma of the lung—(**a**) squamous carcinoma, (**b**) small cell carcinoma, (**c**) large cell neuroendocrine carcinoma, (**d**) giant cell carcinoma, and (**e**) sarcomatoid carcinoma. Hematoxylin and eosin, ×600

produce mucosubstances. Large cell carcinomas account for about 15 % of primary lung carcinomas and more often present as a peripheral mass.

The classification of adenocarcinomas has undergone extensive revision in recent years [134]. These tumors are recognized by their tendency to form glandular, acinar, or papillary structures (Fig. 7.7). In some cases, the tumor cells form solid sheets and can only be distinguished from large cell carcinoma by means of special stains for mucosubstances or by immunohistochemistry [134]. Adenocarcinomas account for about 40 % of primary lung carcinomas and usually present as peripheral nodules or masses. An uncommon variant of adenocarcinoma, formerly known as bronchioloalveolar cell carcinoma, consists of tall columnar tumor cells which tend to grow along intact alveolar septa (Fig. 7.7e). These tumors are now referred to as mucinous adenocarcinoma and are nearly always accompanied by focal areas of invasion [134]. This variant accounts for about 1–2 % of

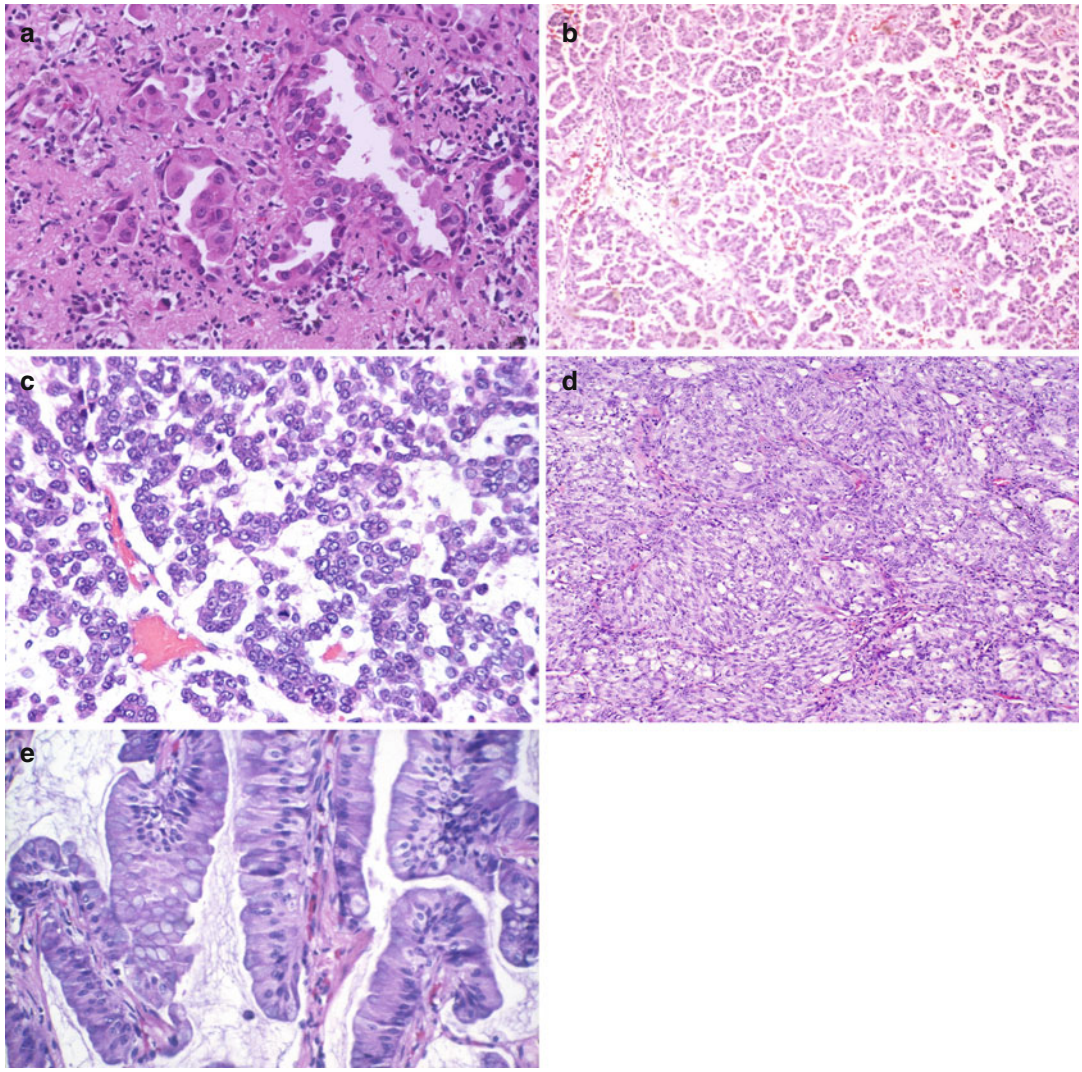


**Fig. 7.7** High-magnification photomicrographs illustrating the most common variants of adenocarcinoma—(**a**) acinar or glandular type, (**b**) papillary type, (**c**) micropapillary type, (**d**) solid type, and (**e**) mucinous adenocarcinoma (formerly bronchioloalveolar cell carcinoma). Hematoxylin and eosin, ×130 (**a**–**d**), ×600 (**e**)

lung cancers. All of the major lung cancer histologic types are associated with cigarette smoking, although adenocarcinoma is the type most likely to occur in a nonsmoker (Fig. 7.8) [105].

Some pulmonary carcinomas may have a pleomorphic or sarcomatoid appearance (Fig. 7.6e) [135, 136]. We have seen examples of such carcinomas in asbestos workers presenting as superior sulcus (Pancoast) tumors (Fig. 7.9) or as proximal hilar masses (Fig. 7.10). These tumors may invade the pleura or chest wall and thus must be distinguished from sarcomatoid or biphasic malignant mesotheliomas (see below). Mixtures of the major histologic cell types may also occur, resulting in a heterogeneous histologic appearance of many primary carcinomas of the lung. With thorough sampling, various combinations of the four major histologic patterns can be found in almost half of the cases [137]. In addition, the authors have encountered examples of asbestos workers with synchronous primary lung neoplasms of differing histologic type (e.g., a patient

with asbestosis and adenosquamous carcinoma and small cell carcinoma in the same lung) [75].

All of the histologic patterns of lung cancer described above may occur in asbestos workers [29, 75, 124, 125, 138, 139]. However, there is some confusion in the literature regarding the distribution of histologic types in asbestos workers as compared to nonexposed individuals. A number of studies described an excess of adenocarcinomas among asbestos workers with carcinoma of the lung [13, 57, 140–143]. Other investigators have reported that the distribution of histologic types of lung cancer was similar for asbestos workers and members of the general population [75, 127, 144–148]. Possible reasons for these discrepancies include selection bias for surgical resection (with patients with peripheral adenocarcinomas more likely to be surgical candidates) or referral bias. In the author's opinion, the histologic features of a lung tumor are of no particular value in deciding whether or not it is an asbestos-related malignancy [75, 125].

**Table 7.2** Histologic typing of lung cancer

| |
|---|
| I. Squamous cell carcinoma |
| II. Small cell carcinoma |
| III. Adenocarcinoma |
| A. Acinar type |
| B. Papillary type |
| C. Micropapillary type |
| D. Solid adenocarcinoma |
| E. Mucinous adenocarcinoma (formerly bronchioloalveolar cell carcinoma) |
| IV. Large cell carcinoma |
| A. Large cell neuroendocrine carcinoma |
| B. Basaloid carcinoma |
| C. Lymphoepithelioma-like carcinoma |
| D. Clear cell carcinoma |
| E. Rhabdoid phenotype |
| V. Adenosquamous carcinoma |
| VI. Sarcomatoid carcinoma |
| A. Pleomorphic carcinoma |
| B. Spindle cell carcinoma |
| C. Giant cell carcinoma |
| D. Carcinosarcoma |
| E. Pulmonary blastoma |

Modified after WHO classification of lung tumors [133]

The distribution of histologic types of lung cancer in 1,258 patients from the author's series is shown in Table 7.3. The first column includes patients with carcinoma of the lung in which asbestosis was confirmed histologically, whereas the second column includes patients with parietal pleural plaques but without asbestosis. The third column includes cases with no histologic evidence of asbestosis or cases for which only a biopsy of the tumor was available (no lung tissue sampled). The fourth column includes 100 consecutive lung cancer resections or autopsies

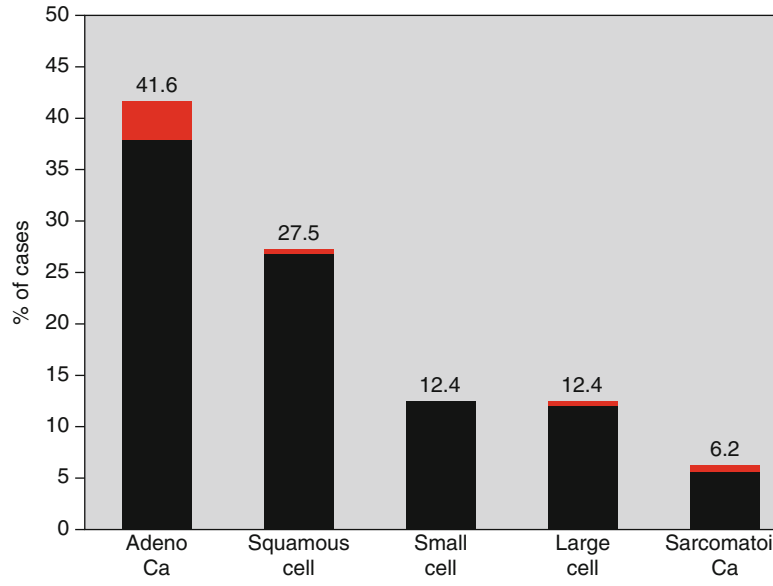


**Fig. 7.8** Histogram showing the percentage distribution of histologic types and percentage of lifetime nonsmokers in a series of 1,051 lung cancers for which smoking status was available. *Red bars* indicate the percentage of cases by histologic types that were reportedly nonsmokers. Adenocarcinoma group includes adenosquamous carcinoma and mucinous adenocarcinomas (formerly known as mucinous bronchioloalveolar cell carcinomas). Large cell carcinoma group includes cases categorized as non-small cell carcinoma

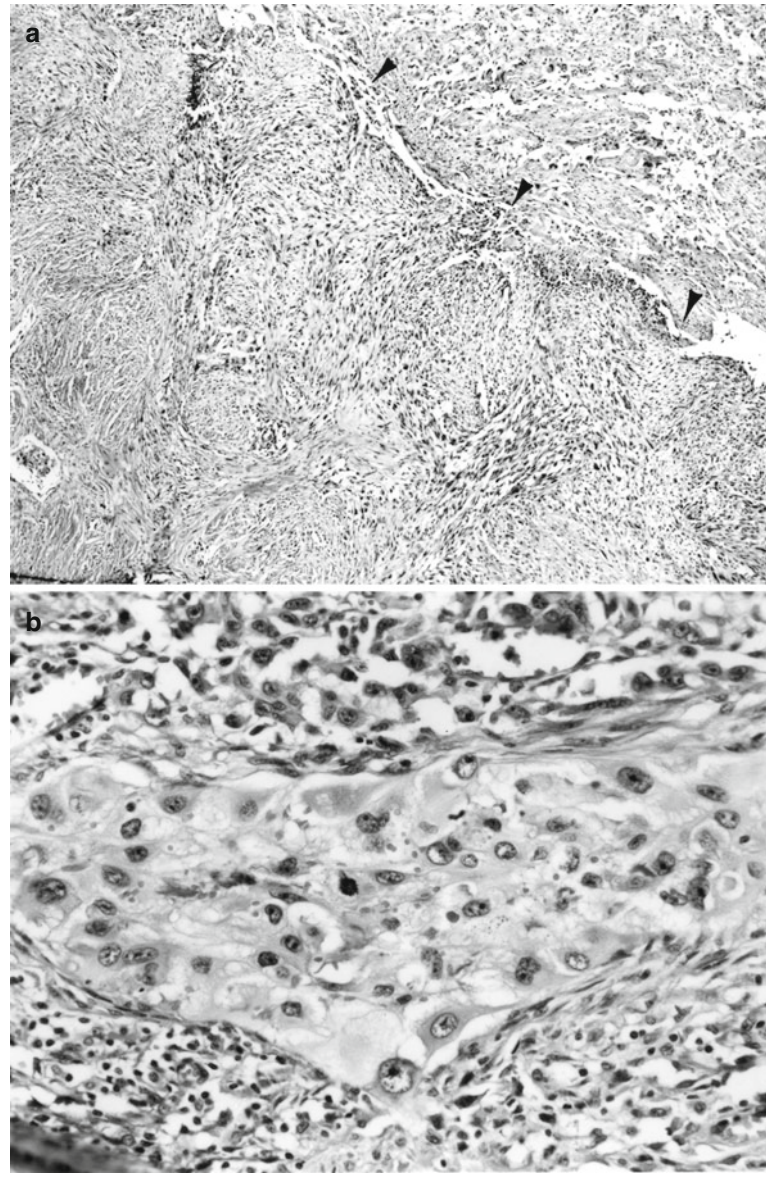


**Fig. 7.9** (**a**) Predominantly spindle cell carcinoma of right upper lobe of an asbestos worker, presenting as a superior sulcus tumor. The margin of tumor invading the underlying lung parenchyma can be discerned (*arrowheads*). (**b**) Higher magnification elsewhere in the tumor shows epithelial component composed of large anaplastic cells with abundant cytoplasm. Hematoxylin and eosin, (**a**) ×40, (**b**) ×250

collected at Baylor Affiliated Hospitals, Houston, TX, from 1979 to 1980 [137]. The percentage of adenocarcinoma cases is similar across all four groups (39–43 %). The data in Table 7.2 are consistent with the proposition that most carcinomas of the lung occurring in asbestos workers are histologically similar to those occurring in nonexposed cigarette smokers. Adenocarcinomas derived from the scarring process account for only a small proportion of cases, resulting in a statistically insignificant increase in the percentage of adenocarcinomas.

### 7.4.3 Differential Diagnosis

Primary lung carcinomas must be distinguished from pulmonary metastases and from other primary intrathoracic malignancies. Knowledge of the clinical information and radiographic

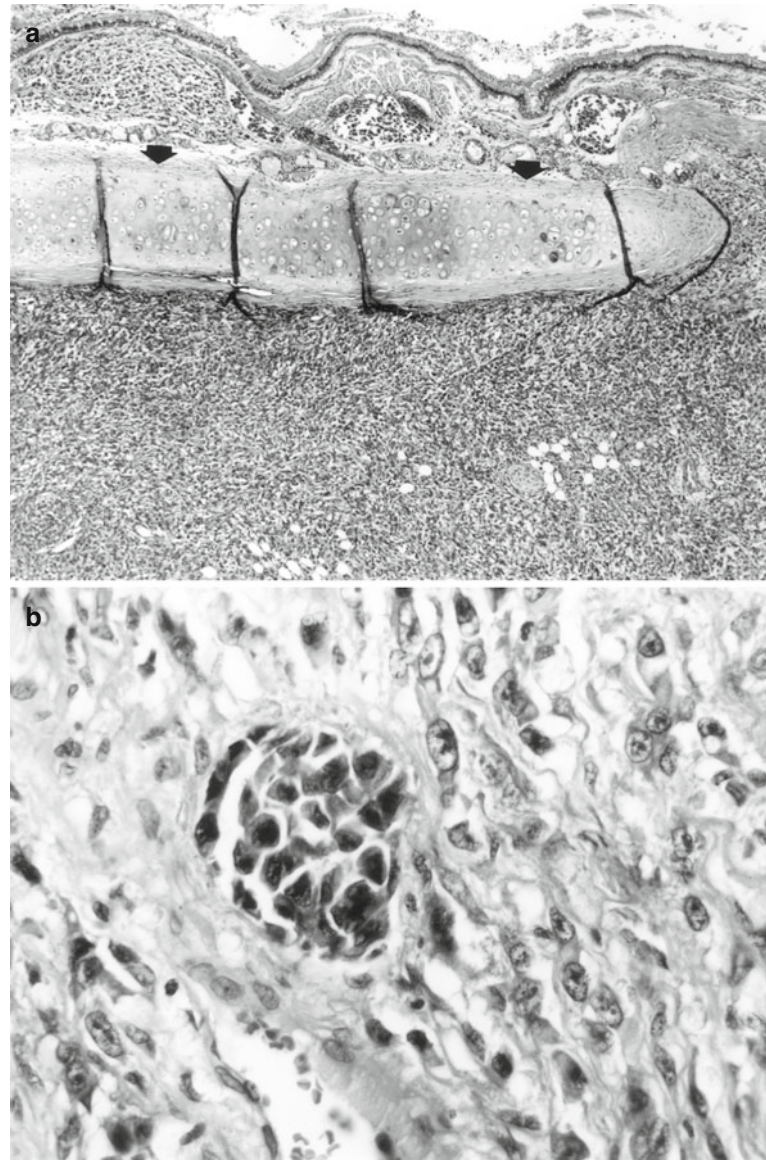


**Fig. 7.10** (**a**) Predominately spindle cell carcinoma invading the right mainstem bronchus in close proximity to the bronchial cartilages (*arrows*). Asbestosis was confirmed histologically in the pneumonectomy specimen. (**b**) Higher magnification elsewhere in the tumor shows epithelial component composed of a nest of loosely cohesive polygonal-shaped tumor cells which were strongly positive for cytokeratins. Hematoxylin and eosin, (**a**) ×40, (**b**) ×400

**Table 7.3** Distribution of histologic types in 1,258 lung cancer cases with and without asbestosis

| | Asbestosis | PPP[a] | Others[b] | Ref. pop.[c] |
|---|---|---|---|---|
| Squamous cell carcinoma | 64 (30 %) | 74 (31 %) | 197 (28 %) | 31 (31 %) |
| Small cell carcinoma | 28 (13) | 28 (12) | 76 (11) | 11 (11) |
| Adenocarcinoma | 85 (40) | 103 (43) | 303 (43) | 39 (39) |
| Large cell carcinoma | 27 (13) | 30 (12) | 116 (16) | 19 (19) |
| Adenosquamous carcinoma | 9 (4) | 7 (3) | 11 (2) | – |
| Total | 213 | 242 | 703 | 100 |

[a]*PPP* parietal pleural plaques, no evidence of asbestosis
[b]No histologic evidence of asbestosis or biopsy of tumor only (no lung tissue sampled)
[c]100 consecutive lung cancer cases collected at Baylor Affiliated Hospitals, 1979–1980 [137]

findings is often useful in this regard. Primary lung carcinomas usually present as a solitary pulmonary mass or nodule, whereas metastatic disease most often manifests as multiple and bilateral nodules of similar size, most numerous in the lower lobes. A history of a primary malignancy in an extrapulmonary location is of obvious significance in this regard. The histologic appearance of the tumor is of limited use in determining whether a lung neoplasm is primary or metastatic. Most small cell carcinomas are primary to the lung, whereas adenocarcinomas are common histologic patterns in a number of primary sites, and histologic features alone (especially on a small biopsy) usually are not indicative of a primary site of origin. Immunohistochemistry can be useful in sorting out primary versus metastatic adenocarcinomas. For example, primary lung adenocarcinomas typically stain positive for TTF-1 and cytokeratin 7 but negative for cytokeratin 20 [134]. In contrast, metastatic colon cancer typically stains positive for cytokeratin 20 and CDX2 but negative for TTF-1 and cytokeratin 7. For tumors with a prominent clear cell component, a renal primary source needs to be excluded. Here again, immunohistochemistry may be of assistance.

Primary lung carcinomas must also be distinguished from other pulmonary neoplasms, most of which are distinctly uncommon [149]. Peripheral carcinomas which invade the pleura must be distinguished from malignant mesothelioma (see Chap. 5). The gross features of the tumor may be of limited utility in this regard [150, 151], and the pathologist must rely on histologic, histochemical, immunohistochemical, or ultrastructural features of the tumor to make this distinction. Uncommonly, a pulmonary carcinoma with a prominent spindle cell component may occur in the lung periphery and invade the pleura, mimicking a biphasic or sarcomatoid pleural mesothelioma (Figs. 7.9 and 7.10). The localized nature of the tumor with a prominent pulmonary parenchymal component, or the presence of a hilar mass with prominent involvement of a proximal bronchus, are useful differentiating features in this regard. Immunohistochemistry plays a rather limited role in making this distinction [152, 153].

## 7.5 The Pathologist's Role in Identification of Asbestos-Associated Carcinomas of the Lung

It has been estimated that in the 25-year period from 1985 to 2009, 76,700 deaths from asbestos-related carcinomas of the lung would occur in the USA alone [154]. In contrast, there are 180,000 lung cancer deaths annually (or 4.5 million over the above time period), the great majority of which are related to cigarette smoking [1, 2]. These observations are consistent with other estimates indicating that 2–3 % of lung cancers are asbestos related [155–157]. Thus it is clear that a major challenge for the medical profession and society in general will be to determine which lung cancers are related to asbestos exposure in order that appropriate compensation may be provided where indicated. This will require careful consideration of clinical, radiographic, and pathologic data in the individual case, as well as epidemiologic and relevant experimental animal studies. The challenge is all the greater considering that the percentage of asbestos-related lung cancers appears to be decreasing and modification of workplace conditions has resulted in lower exposures with decreasing rates of asbestosis [103, 158, 159].

As noted in the previous discussion, there are no pathologic features of carcinoma of the lung in asbestos workers that permit their distinction in the individual case from the much more common tobacco-related cancers in non-asbestos-exposed individuals. Therefore, the primary role of the pathologist is to render an accurate and precise diagnosis of carcinoma of the lung based on available pathologic materials and to help exclude other differential diagnostic considerations. Another important aspect of the pathologists' role has been referred to as the "second diagnosis" [160], that is, the identification of other abnormalities that are related to inhalation of asbestos fibers. These include the

identification of benign asbestos-related pleural diseases, such as parietal pleural plaques or diffuse pleural fibrosis (Chap. 6), asbestosis (Chap. 4), and asbestos bodies in histologic sections [161]. Similarly, the pathologist should search for evidence of tissue injury related to inhalation of tobacco smoke, including centrilobular emphysema, chronic bronchitis, and small airways disease [162, 163]. This requires adequate sampling of lung parenchyma at a distance well removed from the primary tumor and its effects on immediately adjacent tissues [125, 164]. These changes are best observed with lungs that have been fixed by intratracheal instillation of formalin [47, 162], which procedure should be employed when feasible on lobectomy or pneumonectomy specimens. In addition, lung cancer cases for which a role for asbestos is suspected should have portions of formalin-fixed lung tissue uninvolved by tumor preserved for possible tissue asbestos analysis at some subsequent time if indicated (Chap. 11). Such analyses should preferably be performed at specialized centers with experience with these procedures, since proper interpretation of results requires determination of a normal range of expected values.

It has been suggested that in the future, molecular genetic markers may be found that specifically link a lung cancer to asbestos exposure [165]. Since asbestos acts primarily as a promoter for cigarette smoke carcinogens, it is likely that molecular changes in patients with asbestos-related cancers would be the same as those in tobacco-induced cancers but accumulate at a higher rate following exposure to a cocarcinogen such as asbestos [166]. There is evidence that asbestos causes specific molecular changes that could accelerate the progression of lung cancer. For example, loss of 3p21 and EGFR activation are more common in asbestos-exposed patients. Asbestos-exposed workers with lung cancer can have mutations in the k-ras gene at codon 12 in the absence of radiographic evidence of asbestosis, indicating that these two events are not necessarily linked [167]. In addition, a variety of asbestos-related microRNAs are either overexpressed or under-expressed in asbestos-induced lung cancers, and DNA copy number alterations correlated with the deregulated microRNAs [168]. More work is required in this area, both to improve our understanding of the mechanisms by which asbestos induces malignancy and to identify markers that are specific for asbestos carcinogenesis.

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# Other Neoplasia

8

Faye F. Gao and Tim D. Oury

## 8.1 Introduction

While the carcinogenic effect of asbestos in the etiology of mesothelioma and lung cancer is widely accepted today (see Chaps. 5 and 7), conflicting opinion exists for cancer of other sites. This is partly because of the fact that methodological limitations and weaknesses may be found in most of the studies reported in the literature. Some epidemiologic studies have suggested a relationship between asbestos exposure and malignancies of the gastrointestinal tract, pharynx/larynx, kidney, liver, pancreas, female reproductive system, and hematopoietic systems [1, 2]. However, one must bear in mind that in any population studied for asbestos exposure, cigarette smoking, alcohol consumption, and diet remain confounding variables. Also, the route of asbestos exposure, inhalation versus ingestion, has its own implications. Despite evidence both for and against, one cannot ignore the possibility that the carcinogenicity of asbestos may extend to sites of the body other than the lung and pleura to which the carcinogenic fibers can gain access. The pathologic features of these malignant neoplasms do not differ from those occurring in individuals not exposed to asbestos. Therefore, the role of the pathologist includes the accurate diagnosis of these diseases and also the examination of the lungs and pleural cavities for evidence of other asbestos-related tissue injury (see Chaps. 4 and 6).

This chapter reviews the evidence for the association of these various malignancies with exposure to asbestos, including relevant experimental studies where the data are available. Pathologic features will also be noted when they are of relevance to the interpretation of the available epidemiologic studies. Possible mechanisms of asbestos-induced carcinogenesis (reviewed in Chap. 10) are not included in this chapter.

## 8.2 Cancers of the Digestive Tract

### 8.2.1 Historical Background

In 1964, Selikoff et al. [3] were the first to suggest that there was an excess of cancers of the digestive tract among individuals exposed to asbestos. This observation was based on an epidemiologic study of asbestos insulation workers, and the association was maintained in subsequent follow-up studies involving larger numbers of workers followed for longer periods of time [1]. The rationale for this observation relates to the fact that many asbestos fibers deposited in the

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T.D. Oury et al. (eds.), *Pathology of Asbestos-Associated Diseases*,
DOI 10.1007/978-3-642-41193-9_8, © Springer-Verlag Berlin Heidelberg 2014

airways are removed by the mucociliary escalator and can be recovered from the sputum of exposed workers (see Chap. 9). As sputum can be swallowed, the fibers may be transported to various sites in the gastrointestinal tract. Direct contact of asbestos with epithelial cells of the gastrointestinal tract could then result in malignant transformation, similar to that believed to occur from interaction of asbestos fibers with bronchial epithelial cells or mesothelial cells. These considerations have generated concern regarding possible risks not only among asbestos workers but also among populations exposed to asbestos in food, beverages, and drinking water [4].

### 8.2.2 Animal Studies

Relatively few studies have examined the occurrence of gastrointestinal neoplasms in experimental animals exposed to asbestos. In the classic inhalation studies of Wagner et al. [5] and Davis et al. [6], no excess numbers of neoplasms were observed at sites other than the lung and serous cavities. Animal studies involving the ingestion of chrysotile [7, 8] or amosite [8] asbestos in Fisher 344 rats resulted in a trend toward increased incidence of intestinal tumors, but the increase did not reach statistical significance. Asbestos fibers were recovered from ashed colon specimens, and the colonic tissue level of cyclic AMP was significantly decreased in animals fed asbestos as compared to control diets, but there was no increase in colon cancer [7]. In another study, animals were exposed to both radiation of the colon followed by asbestos ingestion [9] to determine if asbestos would augment colon cancer after radiation injury. This study also failed to demonstrate increased colon cancers in animals exposed to asbestos. In a study of hamsters fed amosite asbestos in their drinking water, two squamous cell carcinomas of the forestomach were identified, though these could not be specifically attributed to asbestos [10]. In addition, there are several NIH publications that have evaluated the potential of ingested asbestos in pelleted food over the lifetime of the animal to induce colon cancer. These studies found no increase in colon cancer compared to controls in rats fed crocidolite [11] over their lifetime or for hamsters fed amosite [12] or chrysotile [13] for their entire life. In contrast, a study by Huff et al. [14] found a fivefold increased incidence in adenomatous polyps of the large intestine in male rats exposed to asbestos (intermediate-range chrysotile) compared to controls. In addition, a study by Corpet et al. [15] demonstrated increased aberrant crypts, thought to be a precursor of cancer, in the colon, but no increase in colon carcinoma in rats fed chrysotile or crocidolite. It is of interest that studies have demonstrated penetration of the intestinal mucosa by asbestos fibers, and this migration of fibers to the peritoneal serosal tissues may be relevant to the pathogenesis of peritoneal mesothelioma [16–18]. However, in a study of a baboon gavaged with chrysotile and crocidolite asbestos, no significant intestinal penetration or migration of fibers to other tissue sites was demonstrated [19]. All in all, experimental animal studies do not support a role for inhaled or ingested asbestos fibers in the production of gastrointestinal neoplasms [20].

### 8.2.3 Epidemiologic Studies

The American Cancer Society has estimated that there were 274,330 new cases and 139,580 deaths from digestive system cancers in 2010. It ranked in the top three of estimated new cases and estimated deaths in the USA for many years. Although family history, obesity, physical inactivity, a diet high in red or processed meat, heavy alcohol consumption, long-term smoking, and possible inadequate intake of fruits and vegetables are considered the major risk factors, other environmental exposure may also contribute to the high incidence of gastrointestinal cancer. The association between asbestos exposure and cancer of the gastrointestinal system has been examined in many cohort and case-control studies. The major strengths of the occupational cohort studies are that the magnitudes and durations of asbestos exposure tend to be substantially higher and the exposure information better documented than in case-control studies of the general population.

A number of studies have demonstrated an excess of gastrointestinal carcinomas among asbestos workers [1, 3, 21–24]. However, a number of investigators have challenged the hypothesis that asbestos exposure is causally related to gastrointestinal carcinomas [25–28]. In a review of 32 independent cohorts of asbestos workers, Edelman [27] found no consistent evidence to indicate that exposure to asbestos increases the risk of gastrointestinal cancer. Furthermore, there was no apparent dose-response relationship between accumulated asbestos dose and the risk of gastrointestinal cancer [27]. On the other hand, not all of the cohorts in Edelman's review showed an increased standardized mortality ratio (SMR) for lung cancer, which is universally accepted as causally related to asbestos exposure (see Chap. 7). In this regard, a review of 18 studies by Doll and Peto [2] showed a statistically strong correlation between SMRs for lung cancer and SMRs for gastrointestinal cancer. These results imply that when sufficient asbestos exposure in a population has occurred to result in a detectable increase in lung cancer risk, then it is likely that that population will also demonstrate an increased risk of gastrointestinal cancers.

Goodman et al. [29], applying meta-analysis to existing asbestos-exposed occupational cohorts, found no evidence of association between gastrointestinal cancer and asbestos exposure and no evidence of a dose-response effect. Tsai et al. [30] studied 2,504 maintenance employees who had a minimum of 1 year of potential exposure to asbestos-containing material, especially thermal insulation, and found that the total population had a decreased SMR for all causes and for all cancers. The only statistically significant excess of mortality found was a fourfold increase in mesothelioma. There was decreased mortality from cancers of the esophagus, stomach, large intestine, rectum, and pancreas. In addition, an epidemiologic study by Garabrant et al. [31] in which they controlled a large number of confounding variables, which were lacking in most other studies, demonstrated that there was no increase in colon cancer in asbestos-exposed individuals. In fact they found there was a trend for decreased colon cancer in the most heavily exposed individuals. This study by Garabrant et al. emphasizes the importance of controlling for the many confounding variables that have been shown to be important risk factors in this disease.

Browne et al. [32] published their study results regarding cancer incidence and asbestos in drinking water in the Town of Woodstock, Ulster County, New York, from 1980 to1998. The study was based on the discovery of asbestos contamination in the public water supply that resulted from asbestos-cement pipes installed in the water system in the mid- to late 1950s and the corrosiveness of the local water. The New York State (NYS) Department of Health established the Woodstock Asbestos Exposure Registry (WAER) in 1986 to monitor rates of cancer among individuals who lived on the water supply between 1960 and 1985. Demographic, health, and residential information were collected on 2,936 registrants. The follow-up period for observation of cancer was 1980–1998, consistent with the expected lag of 20–30+ years for the development of asbestos-related cancers. In this study, the general pattern of results did not demonstrate a likely link between exposure to asbestos in drinking water and cancer occurrence among participants in the WAER.

In contrast, Szeszenia-Dabrowska et al. [33] reported a statistically significant increase in mortality for carcinoma of the large intestine as well as for pleural mesothelioma in male workers occupationally exposed to asbestos (chrysotile and crocidolite). In addition, Raffin et al. [34] reviewed cancer incidence and mortality in workers in the Danish asbestos-cement industry and found a significantly increased risk among men for cancer of the lung, pleura, mediastinum, and stomach. Kishimoto [35] in a study aimed at determining the relationship between malignancy and asbestos exposure, by estimating the number of asbestos bodies in wet lung tissue, reported that 37 % of gastric cancers in his patient population occurred among patients exposed to asbestos.

In addition to the lack of controls for confounding variables in many of the prior studies, another difficulty in these epidemiologic studies

is the uncertainty regarding the diagnosis of gastrointestinal carcinomas [2, 36–38]. Doll and Peto [2] believe that the excess risk of gastrointestinal carcinomas reported in some cohorts of asbestos workers could be explained on the basis of carcinomas of the lung or pleural or peritoneal mesotheliomas being misdiagnosed as gastrointestinal carcinomas. Although this could be the explanation for excess cases of carcinoma of the stomach, colon, or rectum, it seems considerably less likely to be the case for esophageal carcinomas. Indeed, these authors state that the evidence relating esophageal cancer to asbestos exposure is suggestive of a causal relationship, but not conclusive [2]. This conclusion is tempered by the observation of Acheson and Gardner [38] that social factors are particularly important in regard to cancers of the upper alimentary tract, and differences between the workforces studied and the standard population with which they have been compared must be taken into account.

Despite a number of epidemiologic studies investigating the association between asbestos ingestion and gastrointestinal cancer [39–43], the existence of such an association has not been definitely established [4]. Only one study, which involved the population in the San Francisco Bay area, suggested a positive correlation, and that was weak [39]. The types of fibers present in drinking water are generally short, ultramicroscopic fibers of questionable carcinogenic potential (see Chap. 10). Overall, the evidence fails to indicate any increased risk of alimentary tract tumors following the direct ingestion of asbestos [44].

In an attempt to help clarify the association of asbestos exposure with cancers other than mesothelioma, the Institute of Medicine's (IOM) Board on Population Health and Public Health Practices oversaw a study that comprehensively reviewed, evaluated, and summarized the peer-reviewed scientific and medical literature regarding the association between asbestos and colorectal, esophageal, stomach, and pharyngeal/laryngeal cancers. A multidisciplinary committee was appointed by IOM that included experts in biostatistics, epidemiology, mineralogy, oncology, toxicology, and cancer biology. In 2006, the committee reviewed both case-control and cohort studies of esophageal cancer and found that although some showed an association between asbestos exposures and esophageal cancer, the overall results of epidemiologic studies are mixed. In addition, animal experiments concerning the carcinogenic potential of asbestos specifically on esophageal tissues do not support biological activity at the site. The committee concluded that the evidence is *inadequate* to infer the presence or absence of a causal relationship between asbestos exposure and esophageal cancer (Fig. 8.1) [45].

Evidence of asbestos exposure and esophageal cancer is still mounting after the release of the committee's 2006 report. A multicentric hospital-based case-control study published in 2008 was conducted in two Mediterranean provinces of Spain. Occupational, sociodemographic, and lifestyle information was collected from 185 newly diagnosed male esophageal cancer patients and 285 frequency-matched controls. For all histologic types of esophageal cancer combined, a threefold increase in risk was found with a significant trend for asbestos exposure (OR 3.46, 95 % CI 0.99–12.10) [46].

The National Academies committee (IOM appointed) final review covered 34 occupational cohort studies, including a total of 42 cohorts and 5 population-based case-control studies that provided data on stomach cancer risk. The occupational cohort studies were the most informative source of evidence to reveal a generally consistent pattern of fairly modest risk increase. However, the limited available evidence from experimental research does not indicate that asbestos is carcinogenic to the stomach. In the end, the committee concludes that the evidence is *suggestive but not sufficient* to infer a causal relationship between asbestos exposure and stomach cancer (Fig. 8.2). A similar conclusion was made for colorectal cancer (Fig. 8.3) after reviewing 41 occupational cohort populations and 11 case-control studies of colorectal cancer epidemiologic data [45].

A more recent review [47] summarizes the weight of epidemiologic evidence to evaluate the hypothesis that asbestos exposure is causally associated with increased risk of gastrointestinal

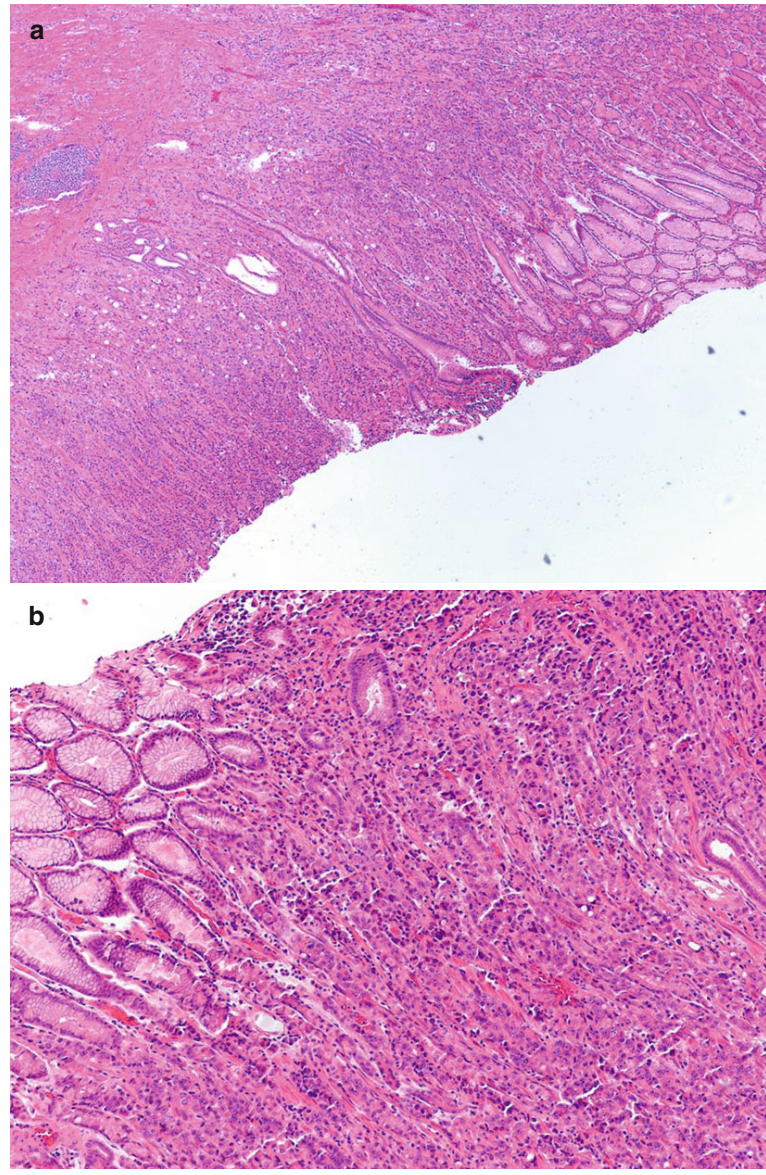


**Fig. 8.1** (**a**) Low-power view of gastroesophageal junction carcinoma in an 85-year-old man with remote history of asbestos exposure, showing ulcerated gastroesophageal junction mucosa with infiltrating nests of carcinoma. (**b**) Medium-power view showing detail of infiltrating carcinoma. H&E, (**a**) ×40, (**b**) ×100

(GI) cancers, namely, stomach, colon, and rectal, as suggested by Selikoff et al. [1] in an early study of insulation workers. Guidelines for assessing causality are strength of association, biological gradient, and consistency of the associations. Exposure-response (E-R) was evaluated using three methods to estimate exposure. Rate ratios (RRs) for lung cancer and percent of mesothelioma are used as surrogate measures of asbestos exposure for all the cohorts of exposed workers. Quantitative or semiquantitative estimates of cumulative exposure to asbestos were also used to assess E-R trends and were compared to E-R trends for lung cancer and mesothelioma in individual studies. Surrogate measures are important since there are few individual studies that have assessed E-R. None of the various methods to estimate asbestos exposure yielded consistent E-R trends, and the strength of the associations was consistently weak or nonexistent for the four types of GI cancers. The epidemiologic evidence detracts from the hypothesis that occupational

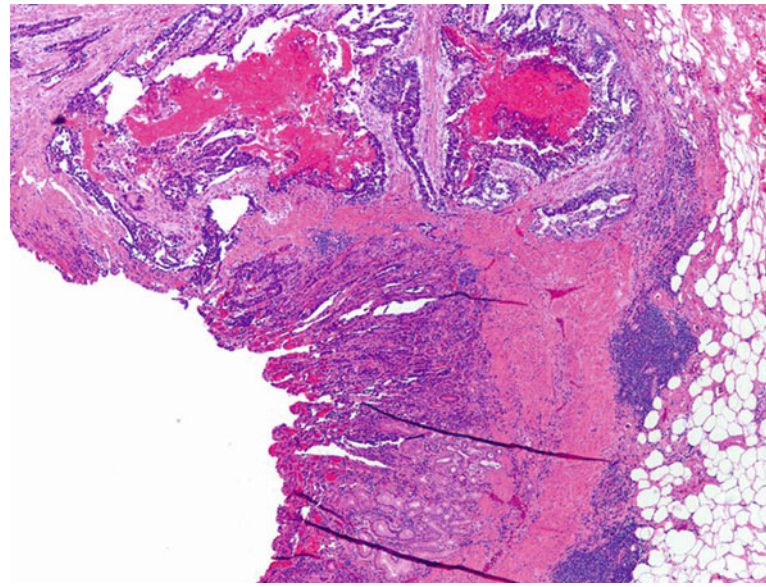

**Fig. 8.2** Low-power view of gastric carcinoma in a 60-year-old female, showing gastric carcinoma (*above*) *with a few remaining normal-appearing gastric glands* (*below*). H&E ×40

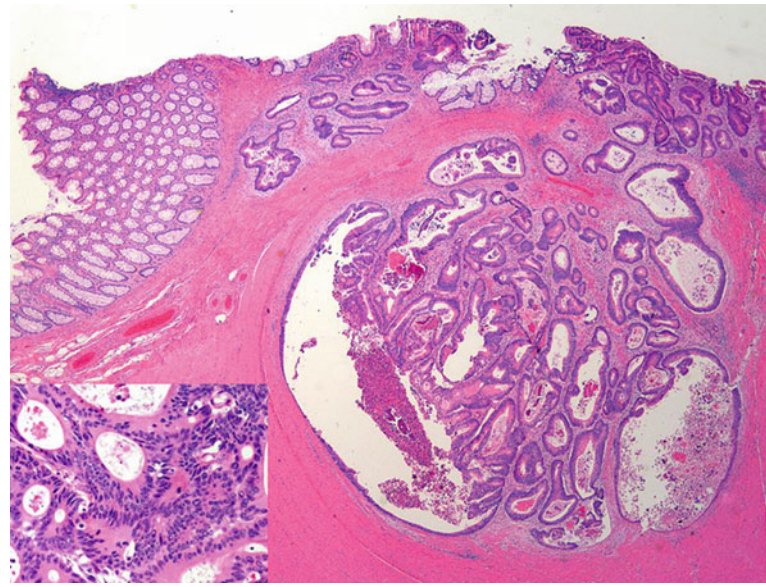

**Fig. 8.3** Low-power view of colorectal carcinoma in a 55-year-old man, showing normal colorectal mucosa in the left upper corner and infiltrating nests of carcinoma *invading the muscularis propria* (H&E ×40). The infiltrating carcinoma forms glandular structures with dirty necrotic debris (*inset* H&E ×100)

asbestos exposure increases the risk of stomach, colon, and rectal cancers [47]. On the other hand, in 2009, Harding et al. reported a cohort study of 98,117 Great Britain asbestos workers that were followed for 1,779,580 person-years. This study found a statistically significant elevation in the SMR for stomach cancer [48].

In summary, there is a lack of definitive epidemiologic studies with adequate controls for the many confounding variables associated with malignancies of the digestive track that demonstrate a conclusive role for asbestos in these diseases. In addition, there is a lack of biological plausibility for asbestos in causing these cancers in animal studies. Thus, it is the opinion of the authors that there is insufficient evidence to link asbestos exposure to cancers of the digestive tract at this time.

### 8.2.4 Pancreatic Cancer

Selikoff et al. [1] initially reported an excess of pancreatic carcinoma among asbestos insulation workers. However, their subsequent review of death certificate diagnoses of pancreatic cancer based on the best medical evidence available in individual cases led to the reclassification of 26 of 49 cases as peritoneal mesothelioma, metastatic lung cancer, metastatic colon cancer, or peritoneal carcinomatosis with unknown primary site [49]. This left 23 cases of pancreatic cancer with an expected number of 17.5, a difference that was not statistically significant. A meta-analysis was performed on publications (1969–1998) surveyed concerning occupational exposures and pancreatic cancer. No definitive association of asbestos exposure and pancreatic cancer was found [50]. Other available information also does not support an association between pancreatic carcinoma and occupational exposure to asbestos [30, 51–53].

A recent retrospective cross-sectional, case-only study published by Yeo et al. [54] included cases of familial pancreatic cancer (FPC, $n=569$) and sporadic pancreatic cancer (SPC, $n=689$) from the Johns Hopkins National Familial Pancreas Tumor Registry (NFPTR) enrolled between 1994 and 2005. They found that occupational and environmental exposures may act synergistically with inherited or acquired genetic polymorphisms, resulting in earlier occurrence of pancreatic cancer. Exposure to cigarette smoking and environmental tobacco smoke exposure in nonsmokers when younger than 21 years of age are associated with a younger mean age of diagnosis in FPC and SPC cases and Ashkenazi Jewish smokers, when compared to nonexposed cases. Risk prediction models in which environmental exposures as well as family history were taken into account may more accurately predict the risk of pancreatic cancer.

Overall, in the authors' opinion, the balance of the evidence available at present does not support an association between asbestos exposure and cancers of the pancreas.

### 8.2.5 Laryngeal/Pharyngeal Cancer

The American Cancer Society has estimated that there were about 12,660 new cases/2,410 deaths from pharyngeal cancer and 12,720 new cases/3,600 deaths from laryngeal cancer in the USA in 2010. The association between asbestos exposure and cancer of the larynx has been examined in many cohort and case-control studies. Some but not all the studies include pharyngeal cancer due to the close relationship between the larynx and pharynx. In the past, a number of investigators have reported an association between asbestos exposure and pharyngeal/laryngeal cancers [34, 55–60]. The rationale for such an association involves contact of the pharyngeal and laryngeal mucosa both with aerosolized fibers breathed into the lung and with fibers in sputum cleared from the lung by the mucociliary escalator. Digestion studies of laryngeal tissues obtained from asbestos workers have demonstrated the presence of asbestos bodies [61] as well as uncoated asbestos fibers [62].

Some investigators have challenged the relationship between asbestos exposure and laryngeal carcinoma [25, 63–65]. Battista et al. [53] studied asbestos-related mortality in railway carriage construction and repair workers and could not establish a causal relationship between asbestos and laryngeal cancer. Experimental studies with rats exposed to aerosolized asbestos have not shown an excess of laryngeal tumors [5, 6]. In a review of 13 cohort and 8 case-control studies, Edelman [65] concluded that an increased risk of laryngeal cancer for asbestos workers has not been established. Other risk factors, such as cigarette smoking and alcohol consumption, have not been adequately accounted for in most of the reported studies. On the other hand, not all of the studies included in Edelman's review showed an increased SMR for lung cancer, either. Furthermore, the SMRs for laryngeal carcinoma exceeded 1.0 in at least some subgroups of workers examined in 10 of 13 cohort studies [65]. Edelman's review does not include references to three separate pathologic studies showing a strong relationship between laryngeal carcinoma and parietal pleural plaques [66–68]. Finally,

Doll and Peto [2] and Smith et al. [69] reviewing essentially the same cohort and case-control studies, concluded that asbestos should be regarded as one of the causes of laryngeal cancer. However, the relative risk is less than that for lung cancer and the absolute risk is much less [2]. Saric and Vujovic [70] studied a Croatian population in an area with an asbestos-processing plant where the number of primary malignant tumors of the pharynx and peritoneum was fewer than that of Croatia as a whole. Also, the incidence of lung cancer in this population was half that in Croatia. In contrast, they found the incidence of primary tumors of the pleura to be more than five times as high and of laryngeal tumors more than twice as high in this group as in the country as a whole. Further studies [71–73] have also reported an increased risk of laryngeal/hypopharyngeal cancer related to asbestos exposure. The study by Murai and Kitagawa [73] compared autopsy cases with histologic evidence of asbestosis to those without asbestosis and found an increased risk of laryngeal cancer in patients with asbestosis. The authors agree with the position that asbestos, along with cigarette smoking and alcohol consumption, is a risk factor for laryngeal carcinoma, particularly in individuals with a substantial exposure to asbestos and evidence of other asbestos-related tissue injury.

In the report of "asbestos: selected health effects" published by The National Academies committee appointed by IOM, the committee identified and included in its analyses 35 cohort populations and 18 case-control studies of asbestos exposure and laryngeal cancer. Subjects in the studies had been exposed to asbestos in a wide array of industries and occupations in many different countries. The committee also reviewed four experimental studies in which rodents were exposed over much of their lifetime to high concentrations of asbestos through inhalation. After considering all lines of evidence, the committee placed greater weight on the consistency of the epidemiologic studies and the biological plausibility of the hypothesis than on the lack of confirmatory evidence from animal studies or documentation of fiber deposition in the larynx and concluded that the evidence is *sufficient* to infer a causal relationship between asbestos exposure and laryngeal cancer [45] (Fig. 8.4). The same committee also reviewed 16 cohort populations and 6 case-control studies of pharyngeal cancer, of which 4 included high-quality exposure assessment or adjustment for possible confounding by smoking and alcohol consumption. Several cohort studies suggest an association between pharyngeal cancer and asbestos. The contrast with the abundance and consistency of data available in the larynx, the absence of information on a dose-response relationship, and the lack of supportive data from animal studies reduce the overall degree of evidence for causality. Overall, the committee concludes that the evidence is *suggestive but not sufficient* to infer a causal relationship between asbestos exposure and pharyngeal cancer [45].

Most recent studies published after the release of the committee's report in 2006 still have controversial results regarding the association of asbestos exposure and pharyngeal and laryngeal carcinoma. Purdue et al. studied altogether 510 squamous cell carcinomas of the head and neck (171 in the oral cavity, 112 in the pharynx, 227 in the larynx) identified during 1971–2001 among 307,799 male workers in the Swedish construction industry. Their results show that asbestos exposure was related to an increased laryngeal cancer incidence (RR 1.9, 95 % CI 1.2–3.1). Excesses of pharyngeal cancer were observed among workers exposed to cement dust (RR 1.9, 95 % CI 1.2–3.1). No occupational exposures were associated with oral cavity cancer [74]. Magnani et al. studied mortality for asbestos-related diseases and the incidence of mesothelioma in a cohort of Italian asbestos-cement workers after cessation of asbestos exposure. The cohort included 3,434 subjects active in 1950 or hired in 1950–1986, ascertained from company records, without selections. Their results show mortality was

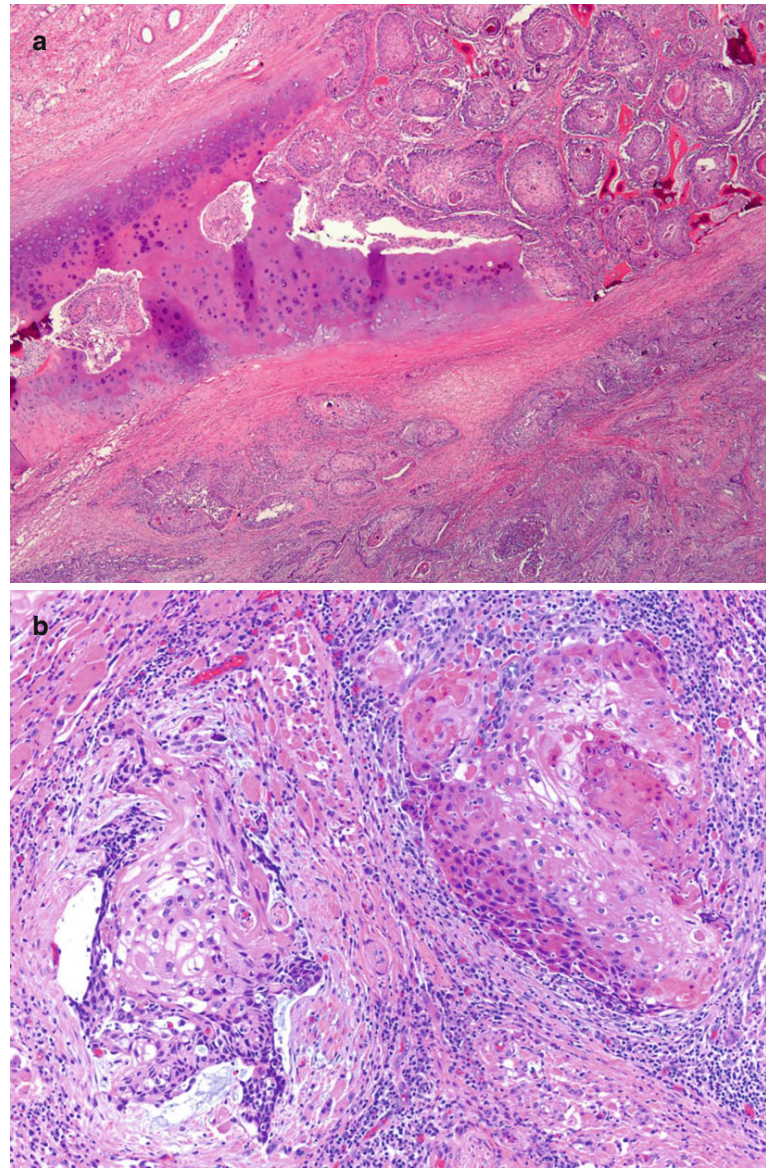


**Fig. 8.4** (**a**) Low-power view of laryngeal carcinoma in an 83-year-old man showing invasive squamous cell carcinoma invading laryngeal cartilage (H&E ×40). (**b**) High-power view showing detail of squamous cell carcinoma with keratin pearls. H&E, (**a**) ×40, (**b**) ×200

increased in both sexes for all causes, for pleural and peritoneal malignancies and lung cancer. In women, ovarian and uterine malignancies were also in excess in the same study. However, no statistically significant increase was found for laryngeal cancer [75].

In their 2005 publication, Pira et al. reported a cohort of 889 men and 1,077 women employed for at least 1 month between 1946 and 1984 by a former Italian leading asbestos (mainly textile) company, characterized by extremely heavy exposures often for short durations, followed up to 1996, for

a total of 53,024 person-years of observation. They observed an increased SMR for pleural and peritoneal mesotheliomas, as well as lung cancer. However, no significantly increased SMR was found for ovarian, laryngeal, and oropharyngeal cancers [76]. In 2009, the same group of authors reported on another cohort study (Balangero cohort) of chrysotile asbestos miners. Their cohort of 1,056 men, for a total of 34,432 man-years of observation, showed a significant excess mortality from pleural cancer only (SMR 4.67) and pleural and peritoneal cancers combined (SMR 3.16). The SMRs were 1.27 for lung cancer, 1.82 for laryngeal cancer, and 1.12 for all cancers [77].

In a case-control study conducted by Medicina et al., detailed data on smoking, alcohol consumption, and occupational history were collected for 122 laryngeal cancers and 187 controls matched by frequency (according to sex and age). Laryngeal cancer was associated with exposure to respirable free crystalline silica (OR = 1.83; 95 % CI: 1.00–3.36) [78].

The pathologic features of laryngeal carcinomas in asbestos workers are not different from those occurring in individuals with no known exposure to asbestos. Among the 45 cases reviewed by one of the editors (VLR), 43 have been squamous cell carcinomas (Fig. 8.4), one pleomorphic spindle cell carcinoma, and one verrucous carcinoma. Asbestos bodies have never been described in histologic sections of laryngeal tissues, and normal ranges of asbestos fiber content have not been established for larynges from the general population. Thus, there is at present no indication for performing digestion analysis of laryngeal tissues in an individual case. In the presence of neoplastic processes, uncontrolled replication of malignant cells will result in a dilutional effect on whatever fibers may have been present in the tissues prior to the initiation of the malignant process.

Overall, in the authors' opinion, the balance of the evidence available at present supports an association between asbestos exposure and laryngeal cancer and is suggestive of an association with pharyngeal cancer.

### 8.2.6 Renal Cell Carcinoma

Selikoff et al. [1] were the first to report an association between asbestos exposure and renal cell carcinoma. This observation was supported by the cohort study of Enterline et al. and others [24, 79, 80]. Smith et al. [80] reviewed the cohort study of Enterline et al. [24] and two other large cohort studies of asbestos workers, including the study of Selikoff et al. [1] and concluded that the available evidence supports a causal association between asbestos exposure and renal cell carcinoma. The rationale for this association involves the penetration of asbestos fibers into the lumen of capillaries, where they may then be transported to other organs, such as the kidneys [81, 82]. Studies have identified the presence of asbestos fibers in human urine samples [83, 84], and both amphibole and chrysotile fibers have been observed.

On the other hand, Acheson et al. [85] and Peto et al. [86] were unable to confirm an association between asbestos exposure and renal carcinoma in humans. In this regard, Smith et al. [80] argued that with the exception of the three large cohort studies noted earlier, none of the other studies in the literature had sufficient statistical power to detect an excess mortality from kidney cancer among workers exposed to asbestos. Experimental animal studies in which rats were chronically exposed to aerosolized asbestos fibers have failed to produce an excess of renal tumors [5, 6], although one study, in which rats were fed 50 mg/kg body weight/day of a powdered filter material composed of 53 % chrysotile, reported a statistically significant excess of renal malignancies [87]. Studies of urine samples from populations drinking water contaminated with asbestos [88] or from chrysotile asbestos-cement workers [89] failed to show a significant elevation of urinary asbestos fibers. In the latter study [89], considerable precautions were taken to avoid sample contamination, a problem that has plagued some of the earlier studies [84]. In a study of a baboon gavaged with chrysotile and crocidolite asbestos, none of the urine samples

from the test animal exceeded the level of background contamination for chrysotile, and only one crocidolite bundle was observed in a test sample [19]. Furthermore, virtually all fibers found in urine samples are shorter than 2.0–2.5 μm [89], and the carcinogenic potential of such fibers is questionable (see Chap. 10). Overall, although cases of renal cell carcinoma with malignant pleural mesothelioma after asbestos exposure have been reported [90] and some larger cohort or case-control studies show an increasing risk, they do not provide solid evidence to prove the association between asbestos and renal cell carcinoma [91–94].

Recently, the Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, DHHS, and Occupational and Environmental Epidemiology Branch investigated whether asbestos, as well as 20 other occupational dust exposures, was associated with an increased risk for renal cell carcinoma in a large European, multicenter, hospital-based renal case-control study. They reported that no association between renal cell carcinoma risk and asbestos exposure was observed [95].

Overall, in the authors' opinion, the balance of the evidence available at present does not support an association between asbestos exposure and renal cell carcinoma.

### 8.2.7 Lymphoma/Leukemia

Ross et al. [96] reported in 1982 an excess of large cell lymphomas primary to the gastrointestinal tract and oral cavity in a case-control study of male patients with a substantial exposure to asbestos. Kagan and Jacobson [97] reported six cases of multiple myeloma, six cases of chronic lymphocytic leukemia, and one case of primary large cell lymphoma of the lung in patients with a history of asbestos exposure ranging from 3 to 37 years. Roggli et al. [98] referred to three patients with hematopoietic malignancies and parietal pleural plaques, including one patient with nodular poorly differentiated lymphocytic lymphoma, one with chronic granulocytic leukemia, and one with acute myelomonocytic leukemia. None had asbestosis histologically. Kishimoto et al. [99] reported two additional cases of acute myelocytic leukemia in individuals with a long history of exposure to asbestos. Asbestos bodies and crocidolite asbestos fibers were recovered from the bone marrow in both instances. In a study aimed at determining the relationship between malignancy and asbestos exposure by estimating the number of asbestos bodies in wet lung tissue, the same author reported that five out of ten cases of leukemia were related to asbestos exposure [35]. The rationale for the association between asbestos exposure and lymphoid neoplasm relates to the occurrence of asbestos bodies and fibers in the lymph nodes [100] and to the variety of perturbations of the immune system observed in patients with exposure to asbestos [101].

Other studies have failed to identify an increased incidence of leukemia or lymphoma among asbestos workers. These include two reports from Sweden [102, 103] as well as the long-term follow-up of a large cohort of US and Canadian insulation workers by Selikoff et al. [1]. In a study of 412 tumors other than lung tumors or mesotheliomas occurring in rats exposed to aerosolized asbestos or room air (controls), Wagner et al. [5] observed eight lymphomas/leukemias in asbestos-exposed rats versus two in controls. Davis et al. [6] also noted a single example of lymphoma/leukemia in a rat exposed to aerosolized chrysotile asbestos. None of these observations were found to be statistically significant [5, 6].

Seidler et al. [104] analyzed the relationship between asbestos exposure and malignant lymphoma in a multicenter case-control study conducted in Germany and Italy according to a common core protocol. Their study did not support an association between asbestos exposure and risk of malignant lymphoma. A more recent study by Treggiari and Weiss [105] also showed no support for the hypothesis that occupational asbestos exposure is related to the subsequent

incidence of non-Hodgkin lymphoma of the gastrointestinal tract.

Overall, in the authors' opinion, the balance of the evidence available at present does not support an association between asbestos exposure and lymphoma or leukemia.

## 8.3 Cancers of Female Reproductive System

Mortality studies among women exposed occupationally to various types of asbestos have reported increased risks for ovarian [75, 76, 106, 107] and cervical [34, 107] cancers. Excess mortality has also been reported for uterine cancer, wherein corpus and cervix were not differentiated [36, 108, 109]. In a case-control study of ovarian cancer, Cramer et al. [110] reported that women with ovarian cancer were about three times more likely to have used talcum powder for perineal dusting or sanitary napkins containing talc than matched control patients without ovarian neoplasm. Cosmetic talc is known to be contaminated with the noncommercial amphibole fibers, tremolite and anthophyllite (see Chap. 1). Wagner et al. [5] reported ten examples of ovarian cancer among more than 350 rats at risk that were exposed to aerosolized asbestos and none in controls. However, this difference did not reach statistical significance. Germani et al. [108] studied the cause-specific mortality of women compensated for asbestosis and reported significantly increased mortality for ovarian cancer as well as for lung and uterine cancer.

Asbestos fibers have been found in the ovaries of women whose household contacts worked with asbestos and among Norwegian paper and pulp workers [109, 111]. The mechanism of transportation of asbestos fibers to the ovary is not clearly understood. Some suggest passive transfer of fibers via the vaginal canal [109] because the transfer of pathogens from the lower to the upper genital tract has been shown to occur this way [112]. This route may also explain any association between asbestos exposure and cancer of the cervix and uterus. Experimental studies have shown that injection of asbestos fibers (tremolite) into the peritoneal cavity produced epithelial changes in the ovaries of guinea pigs and rabbits, similar to those seen in early ovarian cancer patients [113].

As noted by Doll and Peto [2], peritoneal mesothelioma and ovarian carcinoma may have similar clinical presentations, and there is some overlap in histologic appearances as well (see Chap. 5). The difficulty in distinguishing between peritoneal mesothelioma and serous carcinoma of the ovary is still difficult today [114]. It seems at least as likely that the epidemiologic association of asbestos exposure and ovarian cancer is due to the misdiagnosis of peritoneal mesothelioma as that occupational asbestos exposure actually causes ovarian cancer [2]. More recently, Camargo et al. performed a meta-analysis on 18 cohort studies of women occupationally exposed to asbestos and concluded there was an increased risk of ovarian cancer associated with prior asbestos exposure [115]. However, this is again limited by the diagnostic uncertainties in these prior publications for ovarian cancer versus peritoneal mesothelioma. Notably, Reid et al. examined the incidence and exposure-response relationships of these cancers among 2,968 women and girls exposed to blue asbestos at Wittenoom, Western Australia [116]. They found that there was no consistent evidence of increased risk for gynecologic cancers in the asbestos-exposed women from Wittenoom when compared with the nonexposed Western Australian population. Ovarian cancers (Fig. 8.5) and peritoneal mesotheliomas were not misclassified in this cohort.

Overall, in the authors' opinion, the balance of the evidence available at present does not support an association between asbestos exposure and cancers of the female reproductive system.

Additional associations between asbestos exposure and cancer that have been reported include an association with cancer of the eye [24] and with cancer of the penis [117]. More information is needed before definitive conclusions regarding these sites can be made.

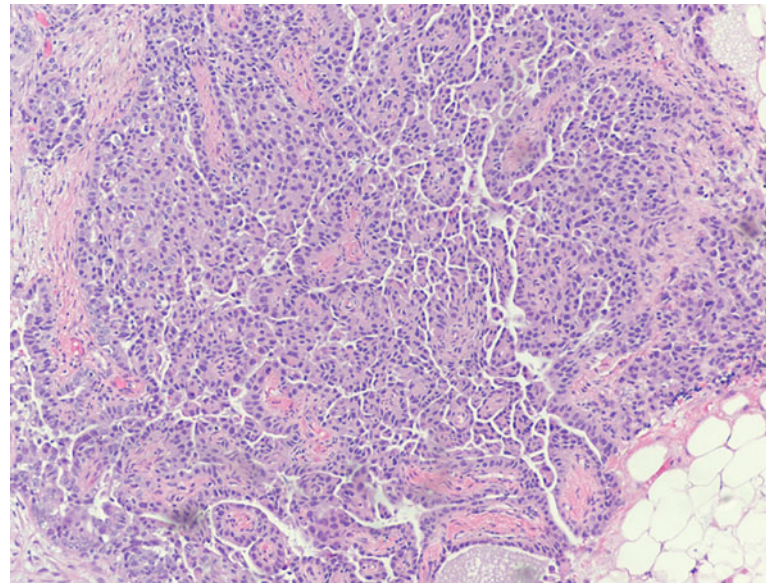

**Fig. 8.5** Papillary serous carcinoma of the ovary invading the surrounding soft tissue. H&E ×40

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# Cytopathology of Asbestos-Associated Diseases

9

Frank Schneider and Thomas A. Sporn

## 9.1 Introduction

Asbestos is the generic term typically used for six naturally occurring fibrous silicates that are or have been exploited commercially: the serpentine chrysotile and the amphiboles amosite, crocidolite, anthophyllite, tremolite, and actinolite [1].

Following its distinction in 1971 as the first material to be regulated by the Occupational Safety and Health Administration (OSHA), asbestos has earned notoriety among commonly encountered compounds matched only by its ubiquity and industrial utility. A versatile industrial product owing to its thermal and chemical stability, high flexibility, tensile strength, and low electrical conductivity, asbestos has been employed as insulation material in applications from heavy industry to hairdryers. Asbestos has not been mined in the USA since 2002; therefore, all asbestos used in manufacturing is imported. In the year 2010, the USA imported 820 metric tons of asbestos, a 95 % decrease since the year 2000 [2]. An estimated 72 % of imported asbestos was used for roofing products, while all other materials, including gaskets and friction products, accounted for the remaining 28 %. Millions of people have been exposed to asbestos in the occupational and para-occupational setting, with millions more exposed resulting from contamination of ambient air, albeit at a much lower intensity. The health hazards associated with asbestos led to regulatory steps restricting the use of asbestos. While amosite usage ended around the mid-1970s, crocidolite was imported and remained in use until the mid-1990s [3, 4].

As asbestos fibers enter the lung, they may undergo phagocytosis and become coated with iron by alveolar macrophages, resulting in the formation of asbestos bodies (Fig. 9.1) [5]. Animal models indicate that asbestos bodies may form as early as 2 months after exposure, and a similar time course is believed to be true for humans [6, 7].

Other fibers may escape such coating and become injurious to the lungs and serosal membranes, resulting in effusions, interstitial fibrosis (i.e., asbestosis; see Chap. 4), carcinoma of the lung (Chap. 7), and malignant mesothelioma (Chap. 5). Inhaled asbestos fibers are of varying lengths and widths with length-to-diameter ratios ranging from 20 to greater than 1,000. Deposition into the tracheobronchial tree is largely a function of fiber diameter rather than length. Those fibers longer than 100 μm are for the most part trapped within the nasal vibrissae and do not usually enter the tracheobronchial tree. Those fibers longer than 40 μm tend to impinge upon the walls of the trachea and larger bronchi and do not usually enter the peripheral airways or alveoli. Thus, the

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T.D. Oury et al. (eds.), *Pathology of Asbestos-Associated Diseases*,
DOI 10.1007/978-3-642-41193-9_9, © Springer-Verlag Berlin Heidelberg 2014

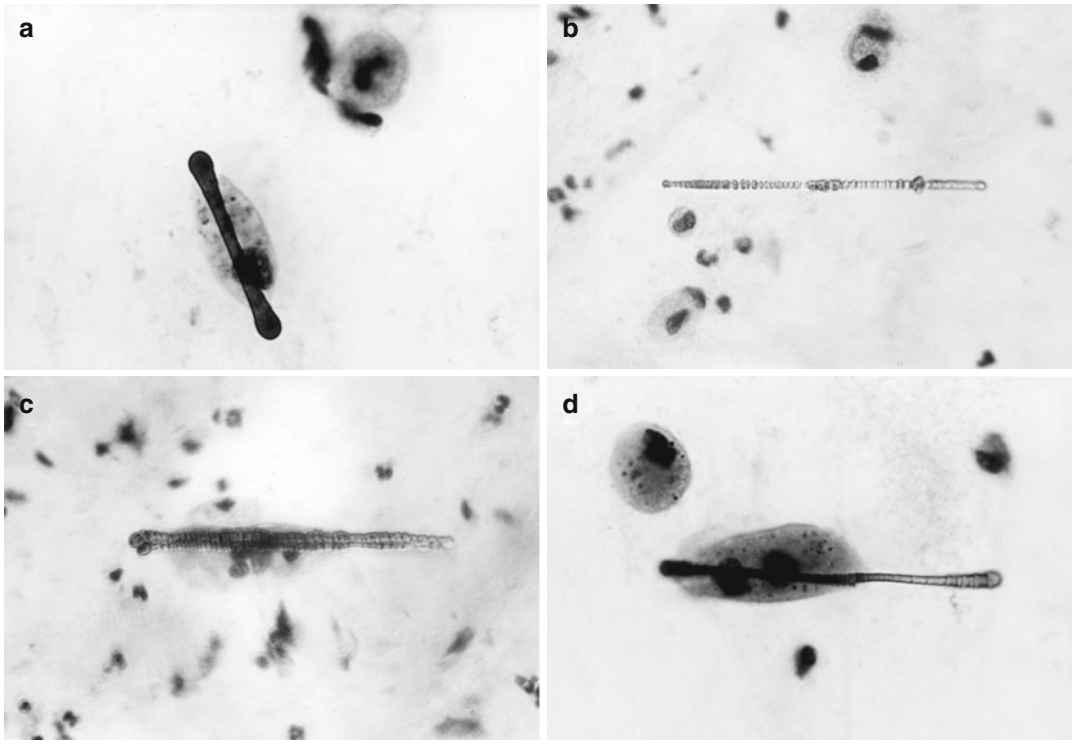


**Fig. 9.1** (**a**–**d**) Close view of single asbestos bodies in sputum. Several are partly within alveolar macrophages. Papanicolaou, ×700 (Reprinted from Ref. [5], with permission)

mean length of asbestos bodies is approximately 35 μm with a 2–5-μm diameter (Fig. 9.2) [9, 10]. However, as the respirability of a particular fiber is chiefly determined by its diameter, some very long fibers may reach the peripheral lung.

Cytopathology may be quite useful in the evaluation of patients with asbestos-related diseases. Its utility includes the diagnosis of malignancy in this setting as well as detection of excess tissue levels of asbestos. The advent of fine-needle aspiration cytology and immunohistochemical staining procedures has aided the clinician in this regard while decreasing the need for more invasive procedures. This chapter discusses in detail the uses and limitations of cytopathology in the evaluation of asbestos-associated diseases.

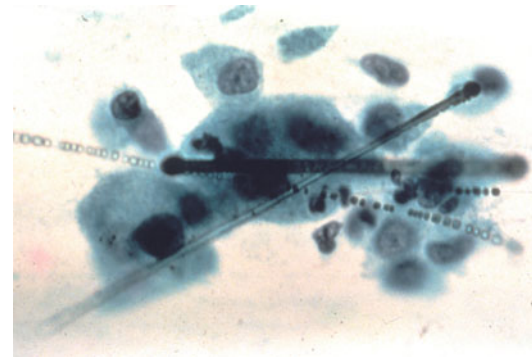

**Fig. 9.2** Photomicrograph showing numerous asbestos bodies within a thick covering of alveolar macrophages. Papanicolaou, ×600 (Reprinted from Ref. [8], with permission)

## 9.2 Historical Background

Stewart reported the presence of asbestos bodies, termed "curious bodies" in the sputa of asbestos miners in 1929 [11]. The cytologic examination of specimens in those with asbestos-associated disease as a diagnostic tool was then largely ignored until the report of An and Koprowska in 1962. These authors described the first case of a concurrent cytologic diagnosis of asbestos bodies and squamous cell carcinoma of the lung in a cigarette smoker [12]. In 1978, Huuskonen examined the

sputum cytology of asbestos workers, 114 with asbestosis, 59 % of whom were chronic cigarette smokers. Although a range of squamous metaplasia, cytologic atypia, and dysplastic changes were detected, the study did not conclusively determine a role for the routine examination of sputum cytology in the early detection of bronchogenic carcinoma in this population [13]. In 1981, Gupta and Frost reported that sputum and bronchoscopy cytologies in those with asbestos exposure may demonstrate one or more of the following abnormalities: asbestos bodies, chronic inflammation, and epithelial atypia. They noted that those with known exposure to asbestos may not demonstrate asbestos bodies in cytologic preparations, while those without known exposure may show asbestos bodies (the latter likely with unrecognized exposure) [14]. In 1982, Kotin and Paul reported on the results of a lung cancer detection program in an asbestos industry. They concluded that there was no evidence that early diagnosis will significantly improve the prognosis of lung cancer [15].

Dodson et al. in 1983 reported on the ultrastructural study of sputa from former asbestos workers [16]. Their results were the first to confirm the presence of uncoated asbestos fibers, diatomaceous earth, and aluminum silicates in sputum. In 1984, Kobusch et al. found that sputum cellular atypia increased with age and asbestos exposure in a cohort of 867 Canadian chrysotile workers [17]. Unfortunately their study did not mention asbestos bodies, which could have provided insight as to the potential for chrysotile to form asbestos bodies. In 2002, Paris et al. found asbestos bodies in the sputa of 37 % of chrysotile workers. However, fiber analysis was unable to prove that their asbestos bodies were formed on chrysotile fibers [18].

The remainder of this chapter will discuss the findings of exfoliative and aspiration cytopathology in asbestos-associated disease.

## 9.3 Bronchial Epithelial Atypia

The most crucial role for respiratory cytology in those with asbestos-associated pulmonary disease is in the detection and classification of lower respiratory tract malignancies alleged to be caused by an exposure to asbestos. Any histologic type of lung carcinoma can occur in this setting, and the classification of epithelial malignancies in this patient population is the same as that used for those not exposed to asbestos [19, 20]. Some have found a statistically significant predominance of squamous cell carcinomas in patients exposed to crocidolite, while others described a predominance of adenocarcinomas in asbestos workers [21, 22]. The true incidence of lung carcinoma due to asbestos is difficult to determine because of the disease's multiple causes. A recent meta-analysis combining multiple cohorts concluded that asbestos kills at least twice as many people through lung cancer than through mesothelioma [23].

Exfoliative cytology samples of the tracheobronchial tree include expectorated sputum, bronchial brushings, washings or lavages obtained during bronchoscopy, and post-bronchoscopy sputum [24]. Diagnostic sensitivity and specificity are affected by multiple factors, including the gross distribution (mostly central versus peripheral) and degree of differentiation of any particular tumor, as well as the technique of specimen procurement and processing. Bocking et al. report that three satisfactory sputum specimens may detect up to 60 % of lung cancers [25]. Diagnostic yield is affected by location, size, and degree of differentiation of the tumor, with larger, central, and higher-grade lesions detected more readily. Well-differentiated squamous cell carcinomas and small cell carcinomas are the most accurately classified using sputum cytologies. Bronchoscopic specimens in general are more cellular with better preservation of cytologic features, and their procurement will aid in the detection of more peripheral tumors. Recent navigational systems have increased the diagnostic yield of bronchoscopic biopsies of small peripheral lesions [26]. Malignant mesothelioma does not produce exfoliated cells in sputum except possibly in those rare cases that are complicated by direct extension of tumor into the lung parenchyma [27].

A major pitfall in the interpretation of cytology specimens is the false-positive diagnosis of malignancy in the presence of inflammatory changes. One must also keep in mind that sputum

cytology may detect not only cancers of the lower but also the upper respiratory tract [28]. Ancillary studies that may enhance sensitivity and specificity of cytology samples include copy number alterations or detection of mutations commonly seen in lung cancers. Copy number alterations have been found to be slightly more sensitive than sputum cytology to detect carcinoma, but there appeared to be no significant difference among individuals exposed to asbestos and those who were not [29]. It is unclear whether K-RAS mutations, which are commonly seen in lung cancers of smokers, are more prevalent in those exposed to asbestos [30]. There is no reported association between EGFR mutations and asbestos-related lung cancers.

A comprehensive review of pulmonary cytopathology is beyond the scope of this chapter. The following briefly reviews cytologic features of lung cancers seen in patients believed to be exposed to asbestos. However, there are no specific cytologic features to suggest or prove asbestos exposure.

### 9.3.1 Squamous Cell Carcinoma

Epithelial cells may undergo a series of reactive and metaplastic changes in response to injury and irritation. Reactive changes include nuclear enlargement and hyperchromasia with the formation of visible nucleoli, but preservation of nuclear-to-cytoplasmic ratios. Squamous metaplasia, while not necessarily a preinvasive phenomenon, is associated with the development of invasive carcinoma. Squamous metaplastic cells are generally uniform in both size and shape, with abundant cyanophilic cytoplasm. These cells may develop small nucleoli if inflamed. Following squamous metaplasia, injured or irritated epithelium may undergo the spectrum of dysplastic changes leading to carcinoma. Mild dysplasia is evidenced by nuclear hyperchromasia and slight increase in nuclear-to-cytoplasmic ratio. Moderate to severe dysplasia shows progression in the nuclear abnormalities, with increase in granular and dispersed nuclear chromatin, nuclear membrane abnormalities, and further increase in nuclear-to-cytoplasmic ratio. Squamous cell carcinoma shows cellular pleomorphism with abnormal or bizarre shapes and strikingly hyperchromatic "ink drop" nuclei (Fig. 9.3). Cytoplasmic orangeophilia in Papanicolaou-stained preparations signals keratinization in better-differentiated tumors but can also be artifactual due to air-drying. The detection of single cells with malignant features is an important observation in the diagnosis of malignancy [31].

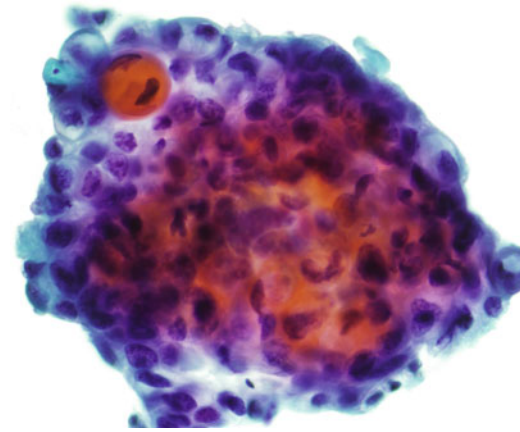

**Fig. 9.3** Cytologic specimen showing attempted keratin pearl formation characteristic of squamous cell carcinoma. Orangeophilia constitutes a sign of keratinization. Papanicolaou stain, ×400 (Courtesy of Dr. M. Zarka, Scottsdale, AZ)

### 9.3.2 Adenocarcinoma

Adenocarcinoma is the most common histologic subtype of bronchogenic carcinoma and is less strongly associated with cigarette smoking than squamous or small cell carcinoma. The typically peripheral location of adenocarcinoma makes it more difficult to diagnose in sputum specimens, but bronchial exfoliative cytologies and directed aspiration biopsies of peripheral lesions allow its detection in most cases. The cells may be arranged in abortive papillae, acinar units, or crowded into three-dimensional clusters. Cell size is typically large, with appreciable cytoplasm that may contain mucin vacuoles, large nuclei with polar orientation, vesicular chromatin, and prominent nucleoli (Fig. 9.4). Reactive respiratory epithelial cells can be abnormally

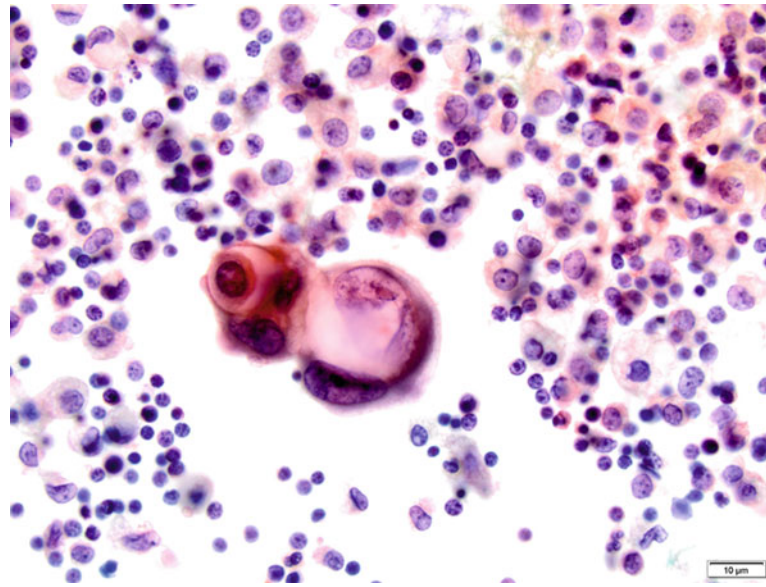

**Fig. 9.4** Cytologic specimen showing cluster of pleomorphic cells with high nuclear-to-cytoplasmic ratio and macronucleoli in a somewhat glandular arrangement, characteristic of adenocarcinoma. Papanicolaou stain, ×400

large, multinucleated, and apparently devoid of surface differentiation, constituting a pitfall in the interpretation.

### 9.3.3 Small Cell Carcinoma

The gross distribution and cytologic features of small cell carcinoma of the lung stand in contrast to non-small cell carcinoma variants and provide additional challenges to the cytopathologist. While typically arising in the major bronchi, small cell carcinoma often presents a submucosal infiltrative pattern and may demonstrate only extrinsic compression of the airway to the bronchoscopist. This stands in contrast to the obstructing endobronchial tumor mass characteristic of squamous cell carcinoma. The exfoliated cells are generally small, 1.5–4 times the size of a small lymphocyte, with scant cytoplasm, hyperchromatic nuclei, and inconspicuous nucleoli (Fig. 9.5). The nuclei of neighboring cells appear to "mold" one another's shape, and the cells often appear in a diathesis of cellular debris and necrosis. If the necrosis is undersampled in the specimen, small cell carcinoma can be mistaken for lymphoma, especially if a mediastinal lymph node was sampled. Immunohistochemistry using a cytokeratin antibody such as Cam5.2 usually suffices to confirm the diagnosis with its characteristic delicate, perinuclear staining pattern.

### 9.3.4 Large Cell Carcinoma

Large cell carcinomas comprise a heterogeneous group of malignant epithelial tumors that lack the cytologic features of squamous or glandular differentiation or the features of small cell carcinoma. In cytologic preparations, large cell carcinoma shows cell clusters as well as dispersed single cells with clearly malignant features. Cytoplasm is usually abundant but lacks evidence of keratinization or mucin production (Fig. 9.6). The nuclear features are also striking, with demonstration of thickened, irregular nuclear contours, coarse chromatin, and large, often multiple, nucleoli. Ultrastructural examination of these cells may occasionally show evidence of rudimentary squamous or glandular differentiation, suggesting that large cell carcinoma may be related to squamous cell or adenocarcinoma [32]. The use of the diagnosis large cell carcinoma is not encouraged for cytologic preparations, as it requires more extensive sampling of the tumor to ascertain the absence of glandular and squamous

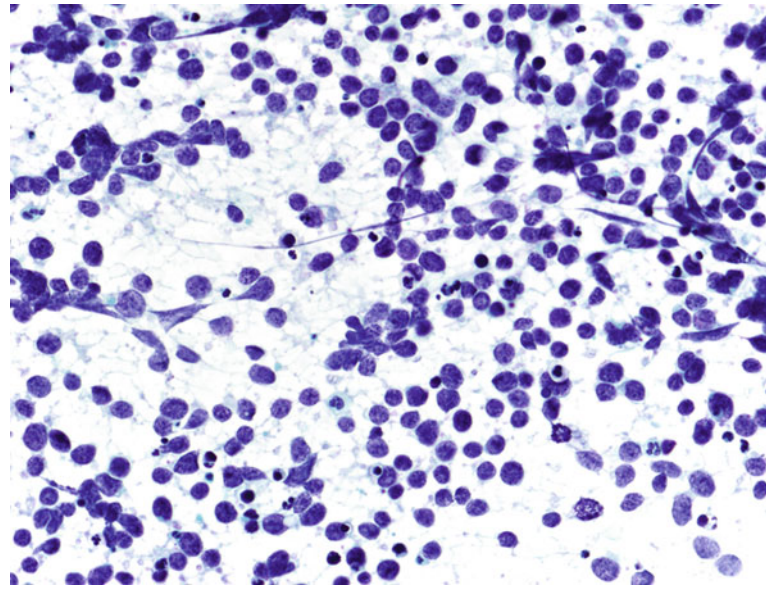

**Fig. 9.5** Cytologic specimen showing tumor cells with high nuclear-to-cytoplasmic ratio, salt and pepper chromatin, inconspicuous nucleoli, and nuclear molding, characteristic of small cell carcinoma. Papanicolaou stain, ×400 (Courtesy of Dr. M. Zarka, Scottsdale, AZ)

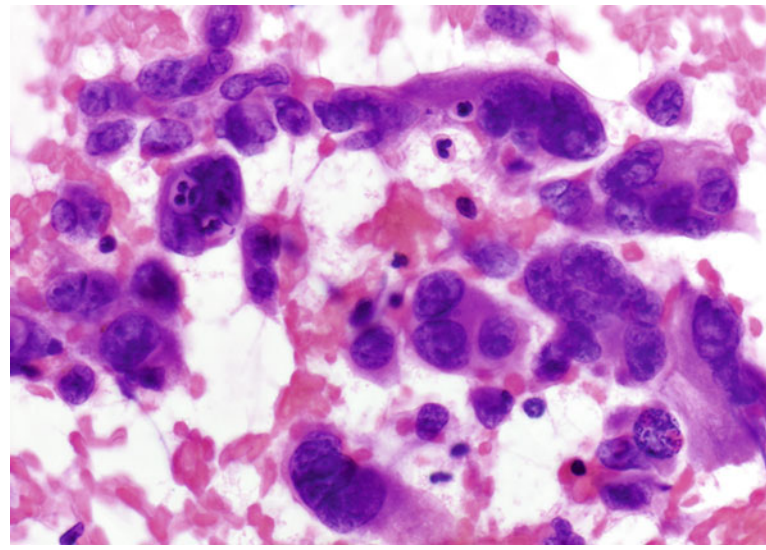

**Fig. 9.6** Cytologic preparation of large cell carcinoma, showing malignant nuclear features and nondescript cytoplasmic detail, characteristic of large cell carcinoma. Papanicolaou stain, ×400

differentiation. Tumors fulfilling these latter criteria are best classified as non-small cell carcinomas and considered for molecular testing [19]. The differential diagnosis of large cell carcinoma includes metastatic melanoma, epithelioid sarcoma, and germ cell neoplasms. Cytologic atypia in benign cells following chemotherapy and/or radiation is often marked, and failure to distinguish such from large cell carcinoma may also constitute a diagnostic pitfall.

## 9.4 Effusion Cytologies

Effusions in the pleural or peritoneal spaces generally require significant accumulation before becoming clinically evident (300 ml in the pleural cavity, 1,000 ml in the peritoneal cavity), although much smaller effusions may be detected on radiologic studies [33]. The development of pleural effusions in particular is an important clinical sequela of exposure to asbestos. In addition to the typical

non-asbestos-related pathologies such as heart failure or parapneumonic effusions, those exposed to asbestos may develop benign pleural effusions (see Chap. 6) or malignant effusions complicating asbestos-associated pleuropulmonary malignancy. Cytopathologic examination of exfoliated cells plays an important role in the evaluation of the broad differential diagnosis these effusions pose. Some 40–80 % of pleural effusions are malignant, most commonly due to metastatic adenocarcinoma of the lung, followed by metastatic adenocarcinoma of the breast [34]. Mesothelioma commonly results in malignant pleural effusions that present a special set of challenges to the cytopathologist. Ascites commonly results from hyponatremia, portal venous hypertension, or malignancy. Malignant ascites most commonly complicates ovarian or gastrointestinal malignancy but may be associated with hepatobiliary carcinoma and peritoneal mesothelioma as well.

### 9.4.1 Benign Effusions

Benign asbestos pleural effusions (BAPE) are a common and dose-related phenomenon affecting those exposed to asbestos, with the shortest latency time of any of the common asbestos-associated diseases [35]. Often asymptomatic, BAPE may be attended by dyspnea and pleurisy and may also accumulate in the pericardial and peritoneal spaces as well [14]. The effusions are typically exudative and may be serous or serosanguinous. An inflammatory pleocytosis is usual, often with a conspicuous population of eosinophils. Asbestos bodies have not been identified within the effusion specimen. Benign effusions may result in the exfoliation of mesothelial cells with striking cytologic atypia, including large size and nuclear abnormalities such as multinucleation. Misinterpretation of reactive changes in the mesothelium as malignant mesothelioma or carcinoma constitutes a major pitfall in exfoliative cytology and will be discussed in more detail below.

### 9.4.2 Malignant Effusions

Malignant pleural effusions are most commonly caused by involvement with adenocarcinoma, while only less than 1 % are related to mesothelioma [36]. On the other hand, up to 90 % of malignant mesotheliomas may present with serous effusions [37]. Direct extension of tumor or studding of the pleural surface usually leads to malignant cells in the fluid, while lymphovascular obstruction alone may result in paramalignant effusions without malignant cells. Evaluating a malignant pleural effusion is a two-step process. Firstly, malignancy must be established and distinguished from reactive changes. This is often solely based on morphology, but the cytologic distinction between mesothelioma and reactive mesothelial hyperplasia is often problematic (Fig. 9.7). Secondly, once malignancy has been established, lineage and origin need to be investigated. On cytologic grounds alone, it may be difficult or impossible to distinguish metastatic adenocarcinoma from malignant mesothelioma. Therefore, this step often benefits from ancillary studies such as immunocytochemical phenotyping.

Benign proliferations contain only mesothelial cells admixed with varying numbers of macrophages and inflammatory cells. They can exfoliate as monolayers, small groups, or single cells. A key feature of the benign effusion is the absence of a second, morphologically different, abnormal cell population. The demonstration of mucin in such cells is strongly suggestive of malignancy, especially carcinoma. Once malignancy is established or suspected, a uniform population of exfoliated cells favors mesothelioma over adenocarcinoma [38, 39].

Carcinomatous pleural effusions and ascites are characterized by tight, three-dimensional clusters of cells with high nuclear-to-cytoplasmic ratios, nuclear membrane irregularities, pleomorphism, hyperchromasia, and prominent nucleoli. The general cytologic criteria for malignancy, namely, large cells with large and hyperchromatic nuclei, clumped chromatin and nuclear membrane irregularities, macronucleoli, and high nuclear-to-cytoplasmic ratios, also hold true for malignant mesothelioma although no single cytologic feature is diagnostic. At low power, mesothelioma may be suggested by the presence of cell aggregates, consisting of “more and bigger cells” in “more and bigger clusters” [33, 40–42]. Other

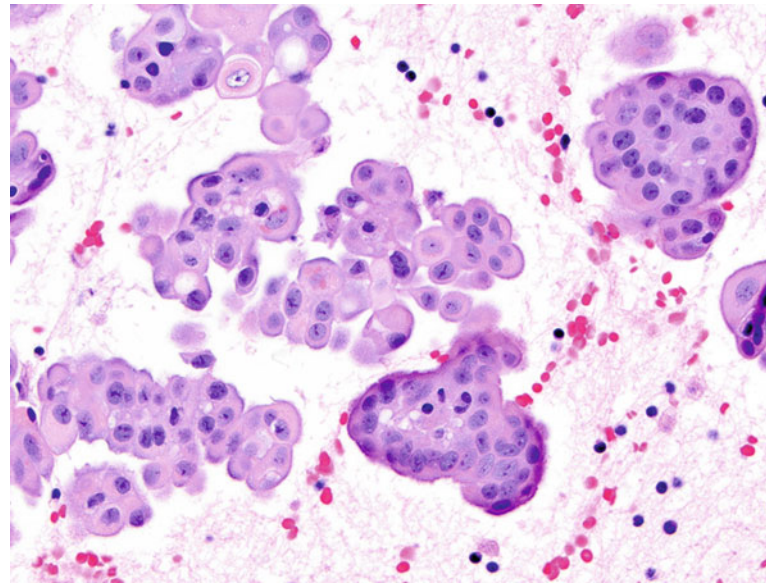

**Fig. 9.7** Cytology cell block of a fine-needle aspirate from a patient with malignant mesothelioma. Such specimens are difficult to distinguish from metastatic adenocarcinoma on the one hand and atypical reactive mesothelium on the other. Hematoxylin and eosin, ×200

findings suggestive of mesothelioma include peripheral cytoplasmic blebbing, cell-to-cell apposition with formation of intercellular windows, and cell cannibalism. Cell groups exhibiting scalloped or "knobby" borders are thought to be more typical of mesothelioma, while adenocarcinoma groups often show smooth, rounded borders. Papillary aggregates with fibrovascular cores or micropapillary clusters may be seen in both pleural mesotheliomas and adenocarcinomas. Although such papillary aggregates are rare in benign pleural effusions, they have been described in benign effusions of the pericardial and peritoneal spaces [38]. Nuclear features suggestive of mesothelioma include paracentral location, macronucleoli, and multinucleation with atypia. Bizarre or anaplastic forms favor an alternative diagnosis. The number of diagnostically useful cells in effusions depends on the histologic subtype of mesothelioma, with sarcomatoid and desmoplastic subtypes tending to produce more paucicellular effusions [41]. The fibrotic component of the tumor in these cases likely retards tumor cell exfoliation into the effusion.

Exceptions to these general observations exist and warrant caution in distinguishing malignant from reactive mesothelial processes. Zakowski reported that the cytologic features of benign mesothelial cells exfoliated into pericardial effusions of patients with the acquired immunodeficiency syndrome (AIDS) may have striking atypia beyond that normally encountered in pericardial effusions, and particular caution is warranted in the examination of fluids from this population [43]. Another pitfall constitutes glycogen or degenerative cytoplasmic vacuoles in mesothelial cells that should not be interpreted as proof of glandular differentiation.

Ancillary studies may be of some use in this regard. Chief among such studies is immunocytochemistry. An extensive review of the histochemical and immunohistochemical profiles of mesothelioma is presented in Chap. 5. In brief, establishment of mesothelial differentiation has historically been based on absence of staining for mucin or adenocarcinoma-associated epitopes [44]. The development of antibodies with varying degrees of specificity for mesothelium has advanced the ability to distinguish mesothelioma from adenocarcinoma in histologic preparations. These antibodies include calretinin, CK5/6, D2-40 (podoplanin), WT-1, and HBME-1 used in concert with antibodies directed against carcinoma-associated antigens such as CD15 (Leu M-1), Ber-EP4, B72.3 (TAG72), carcinoembryonic antigen (CEA),

blood group 8 (BG8), estrogen receptor (ER), paired box proteins 2 and 8 (Pax-2, Pax-8), caudal type homeobox transcription factor 2 (CDX-2), and thyroid transcription factor 1 (TTF-1) [45–48]. Antibodies directed against ER, Pax-2, Pax-8, and CDX-2 have found use particularly in distinguishing peritoneal mesotheliomas from papillary serous carcinomas (ER), renal cell carcinomas (Pax-2, Pax-8), and intestinal carcinomas (CDX-2) [49].

The utility of such antibodies in cytologic preparations in distinguishing mesothelial differentiation among the constituent cells is less well established. Immunostains may be performed on formalin-fixed, paraffin-embedded cell block specimens of exfoliative material as well as material obtained by aspiration biopsy, including direct smears, spin, and liquid-based preparations [50, 51]. Immunophenotyping by flow cytometry using various antibodies is another method shown to be of potential use [52].

The monoclonal antibody HBME-1 was among the first developed with enhanced mesothelial specificity. However, calretinin has emerged as the antibody with the greatest specificity, especially with respect to nuclear staining [53]. Calretinin is expressed in normal, reactive, and neoplastic mesothelium as well as some neural tissues. The distinction of mesothelioma from adenocarcinoma is typically undertaken using a panel of the above antibodies. The International Mesothelioma Panel recommends that at least two mesothelioma markers and two markers specific for the tumor in the differential diagnosis be used for such a panel [49]. If these stains are conclusive, the diagnosis of mesothelioma may be considered established. In equivocal cases or suboptimal staining, a second, more expansive, round of immunohistochemical stains should be utilized. Strongly positive staining for one or more of the carcinoma-associated antibodies renders the diagnosis of mesothelioma unlikely. Ber-EP4 has been reported to be a very useful epitope for identifying neoplastic epithelial cells in effusions [54]. It is most useful in discriminating between metastatic carcinoma and mesothelioma since there is usually no positive staining detected in the latter. Another sensitive marker to detect carcinoma in effusions is MOC-31 [55]. Specificity for this marker increases if only a membranous, but not cytoplasmic, staining pattern is interpreted as positive [56].

It is crucial to note that while antibodies such as calretinin are sensitive and specific markers for mesothelial differentiation, they are unable to discriminate between mesothelioma and reactive mesothelial proliferations. While reactive mesothelium more commonly expresses desmin, mesotheliomas are more commonly found to react with antibodies against epithelial membrane antigen (EMA), insulin-like growth factor-II mRNA-binding protein 3 (IMP3), and glucose transporter-1 (GLUT-1) [57, 58]. Especially oncofetal protein IMP3 may be a promising marker. It was shown to be positive in 33 of 45 MMs (73 %) and negative in all 64 reactive mesothelial lesions tested [59]. Other studies have evaluated the usefulness of E- and N-cadherin with similarly equivocal results [60, 61]. While progress has been made in this area, the separation of reactive from neoplastic mesothelium on cytologic grounds remains problematic, and consensus regarding a sensitive and reproducible immunophenotype to distinguish the two for usage in routine clinical practice has not yet been reached.

Homozygous deletion of 9p21 has been found in approximately two-thirds of pleural mesotheliomas, although the frequency in peritoneal mesotheliomas may be lower [62]. The 9p21 region harbors the p16 gene, a cyclin-dependent kinase inhibitor, and may be more prone to damage by asbestos [63]. Detection of homozygous deletion by fluorescence in situ hybridization (FISH) has been shown to be useful in distinguishing reactive from neoplastic mesothelial cells in effusion specimens [64] (Fig. 9.8). To date no reactive mesothelial proliferations have been reported to show this deletion; therefore, demonstrating this abnormality in a specimen appears to be specific for neoplasia. Since p16 deletions have been found in various other neoplasms, including lung, breast, and urogenital cancers, its use in the differential diagnosis with adenocarcinoma cannot be recommended. Somatic mutations of the BAP1 gene, a tumor

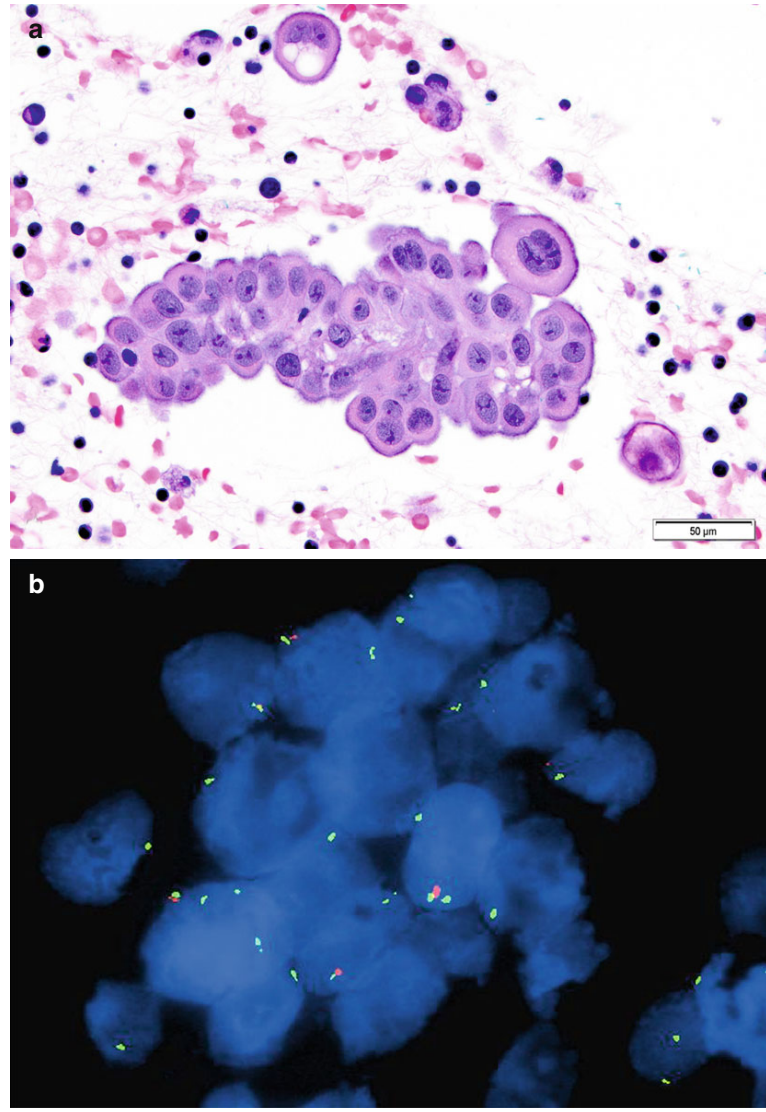


**Fig. 9.8** (**a**) Cytology cell block from a patient with malignant mesothelioma. Numerous papillary structures and single cells are present. (**b**) Fluorescence in situ hybridization detected a homozygous 9p21 deletion, an abnormality seen in about two-thirds of mesotheliomas but also in other epithelial neoplasms (*green*, chromosome 9 centromere probe; *red*, 9p21 probe) ((**b**) Courtesy of Kathleen Cieply, MSL, Pittsburgh, PA)

suppressor involved in BRCA1 regulation, constitute another genetic factor implied in the pathogenesis of mesothelioma [65]. It is detected in about one-quarter of mesotheliomas, and its role in the diagnosis of mesothelioma remains to be established. Detection of chromosomal abnormalities by FISH as well as DNA ploidy analyses has also been exploited to distinguish mesothelioma from reactive mesothelial cells in effusions [66–68].

Sakuma et al. examined the utility of electron microscopy in the diagnosis of malignant effusions. Their observations concerning exfoliated mesothelioma cells join the body of ultrastructural literature which holds that mesothelioma is distinguishable from adenocarcinoma in tissue sections on the basis of long slender surface microvilli that characterize mesothelial cells. Such cells also have more abundant intermediate filaments and fewer free ribosomes. Reactive mesothelial cells, by contrast, contain fewer mitochondria than mesothelioma cells [69].

In summary, cytologic differences among adenocarcinoma, epithelial and biphasic subtypes of malignant mesothelioma, and reactive mesothelial proliferations are subtle with overlapping features in some cases [46, 53, 54, 70–75].

The broad application of immunohistochemistry has rendered obsolete views expressed in older literature that the diagnosis of mesothelioma could not be made with certainty in the absence of an autopsy. Nonetheless, the diagnosis of mesothelioma based solely on examination of cytologic specimens, even with ancillary studies, remains fraught with hazards. While some advocate never to make a diagnosis of mesothelioma based on a cytology specimen alone, others would consider it if the disease distribution is characteristic and the clinical scenario compatible [39, 76]. It is our practice to utilize the cytologic examination of pleural or ascites fluid as a screening test. Such cytologic examination, augmented by the application of immunohistochemical studies as described above, can allow one to become extremely suspicious of the diagnosis of mesothelioma. However, we advocate additional biopsy directed by CT, or guided by thoracoscopy or laparoscopy, to secure the diagnosis. Fine-needle aspiration (FNA) has also been employed in the evaluation of mesothelioma, but in our opinion the same caveats hold true for cytologic preparations obtained by aspiration biopsy as for exfoliative specimens [77–81]. Core tissue specimens obtained by FNA in some cases may be sufficient for diagnosis. The confirmation of positive cytologic findings with surgical biopsy is also advocated by other centers with extensive experience in the care of mesothelioma patients as well as the International Mesothelioma Interest Group [45, 49, 82, 83]. Although biopsy tract seeding appears to be five times as common in surgical biopsies as compared to core needle biopsies, the increase in diagnostic certainty should be worth it in view of the dire prognostic, therapeutic, and medicolegal ramifications following the diagnosis of mesothelioma [84].

## 9.5 Occurrence and Significance of Asbestos Bodies in Cytologic Preparations

Inhaled asbestos fibers are physical and mechanical irritants, injurious to the lung. They are deposited in alveolar ducts, and their sharp ends allow penetration across the alveolar walls and into adjacent units. Inhaled fibers undergo phagocytosis by alveolar macrophages, where they are coated by ferritin and glycoproteins, forming the asbestos body [85]. Asbestos body maturation has been studied using scanning electron microscopy, demonstrating progression from a membrane-bound, smoothly coated fiber to the characteristic beaded form [86]. Changes in the morphology of the asbestos body may reflect physical forces imparted on the fibers during the inspiratory and expiratory phases of breathing [86]. Studies of macrophage viability following incubation with asbestos bodies confirm the minimal potential for cytotoxicity of these coated fibers [87]. In histologic sections, asbestos bodies may be observed embedded within fibrotic pulmonary interstitium or free within alveolar spaces. The latter may be mobilized onto the mucociliary apparatus, expectorated or swallowed, and otherwise rendered accessible to removal through the techniques of bronchoalveolar lavage or fine-needle aspiration. A similar fate also applies, obviously, to uncoated fibers within the lung.

Some degree of caution is advised in the interpretation of ferruginous bodies/asbestos bodies. Coated fibers resembling asbestos bodies, so-called pseudoasbestos bodies, have been described in end users of refractory ceramic fibers (RCF). Dumortier found such bodies in the lavage fluid of 9 of 1,800 such end users (0.5 %). Seventy percent of core fibers analyzed proved to be aluminum silicates typical of RCF, and 30 % were asbestos fibers [88]. The presence of asbestos bodies in lavage fluids thus is a valid marker for asbestos exposure and fiber retention. However, the possibility of pseudoasbestos bodies at least in this population merits consideration.

Not all asbestos types have the same capacity to form asbestos bodies, and one should be aware that even with high exposure, the absence of ferruginous bodies in sputum or bronchoalveolar lavage fluid (BALF) may be a false-negative finding. Alexopoulos et al. found asbestos bodies in only one-fifth of workers exposed to chrysotile [89]. Inhabitants of a community near a stone quarry in Sicily were found to have fluoro-edenite amphibole fibers in their sputum and BALF by fiber analysis; however, no ferruginous bodies were seen in any of the samples [90].

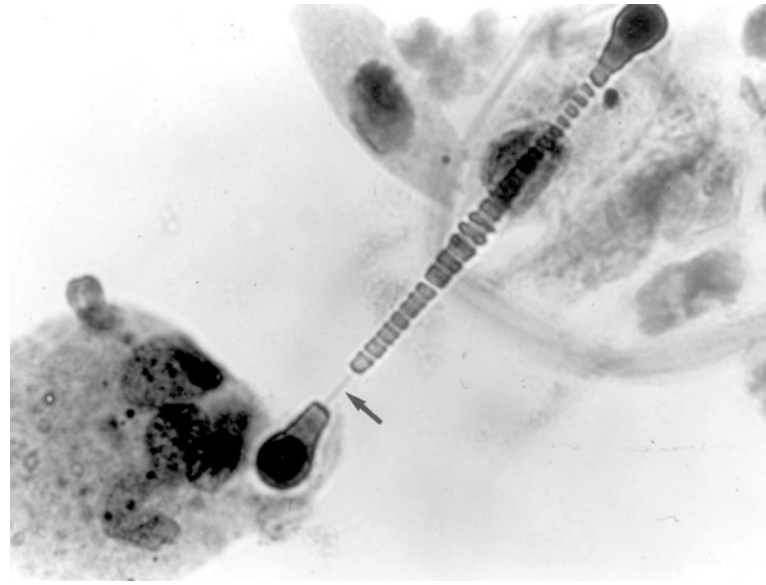

**Fig. 9.9** Asbestos body with an incomplete coating of iron and protein, found in the sputum of a Tyler asbestos worker. *Arrow* points to the fiber forming the core of the body. Papanicolaou, ×700 (Reprinted from Ref. [92], with permission)

### 9.5.1 Sputum

Although asbestos bodies, the hallmark of asbestos exposure, are commonly identified using digestion techniques in the lung tissue of the general population, their presence in the sputa of nonexposed individuals has not been reported. Alderisio et al., for example, found no asbestos bodies in the sputa of 119 inhabitants of rural areas and only 1 asbestos body in a single sputum from a cohort of 164 traffic police officers [91]. The one affected officer was involved in inspecting illegal building construction.

On the other hand, asbestos workers may frequently have asbestos bodies in sputum specimens (Fig. 9.9). A study of asbestos workers in Tyler, Texas, demonstrated asbestos bodies in their sputa, statistically related to the age and duration of the worker's exposure (Fig. 9.10). None of the control subjects studied showed asbestos bodies in their sputum [95]. Correlative studies of sputum and lung asbestos body content show that asbestos bodies do not appear in sputum until there is a substantial parenchymal asbestos fiber burden. Bignon et al. showed that the presence of sputum asbestos bodies correlated with a lung asbestos burden of 1,000 bodies or more per cubic cm of lung [93]. Roggli et al. showed that asbestos bodies appear in sputum when the lung asbestos burden is 900 or more asbestos bodies per gram of wet lung tissue (Table 9.1) [8]. Lack of better correlation between sputum and lung tissue digest may

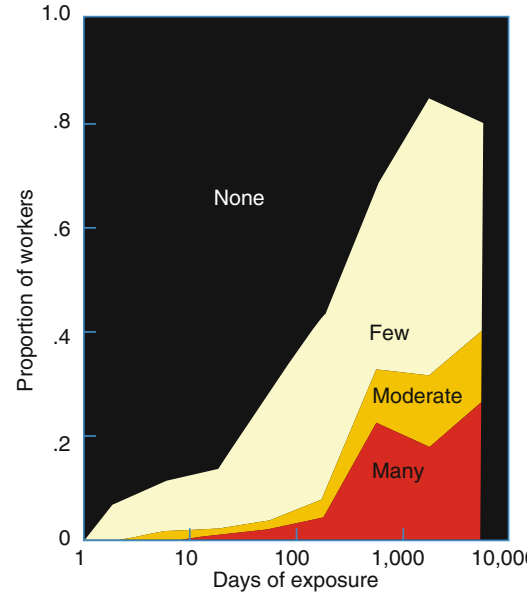


**Fig. 9.10** Graph demonstrating the proportion of asbestos workers with asbestos bodies in their sputum compared to the length of their employment (in days) (Reprinted from Refs. [93, 94], with permission)

**Table 9.1** Correlation between asbestos bodies in cytologic specimens and lung tissue digests

| Source | Cytologic specimens | Lung tissue |
|---|---|---|
| *Sputum* | | |
| Bignon et al. [93] | >1 AB | >1,000 AB/cm$^3$ |
| Roggli et al. [8] | >1 AB | >900 AB/g |
| *Bronchoalveolar lavage fluid* | | |
| De Vuyst et al. [96] | 1 AB/ml | 1,800 AB/g |
| Sebastien et al. [97] | 1 AB/ml | 2,200 AB/g |
| Karjalainen et al. [98] | 1 AB/ml | 2,500 AB/g |
| Teschler et al. [99] | 1 AB/ml | >1,000 AB/cm$^3$ |

Sputum studies are reported as AB/cm$^3$ or AB/g of wet lung. BALF studies are reported as AB/g of dry lung or AB/cm$^3$. 1 AB/cm$^3$ ≅ 1 AB/g of wet lung ≅ 10 AB/g of dry lung

reflect a diminution in sputum clearance of asbestos bodies in those with high fiber burdens, with severely fibrotic lungs trapping fibers within the interstitium. Paris et al. found asbestos bodies in about half of the sputa collected from a cohort of textile and friction materials workers [18]. The presence of asbestos bodies correlated with the exposure history, showing risk ratios of 1.7 and 2.3 for cumulative exposures of 200–400 and >400 fibers·ml$^{-1}$ × years, respectively. Interestingly, asbestos bodies were also found in those exposed exclusively to chrysotile. No lung tissue digestion studies were performed; however, therefore contamination of the chrysotile by amphibole fibers could not be excluded.

The demonstration of asbestos bodies in the sputum of occupationally exposed individuals may antedate radiographic changes. The Tyler Asbestos Workers Program examined the relationship between asbestos bodies in sputum and clinical findings in 674 former asbestos workers. Over a 5-year study period, statistical analysis showed that asbestos bodies in the sputum were significantly related to radiographic findings of interstitial lung disease, pleural fibrosis, and restrictive ventilatory defects, suggesting that it is a sensitive marker for pulmonary impairment [92]. It appears that the detection of asbestos bodies in sputum is not only a highly specific, albeit somewhat insensitive, marker of occupational exposure, but also a predictor of parenchymal lung disease.

### 9.5.2 Bronchoalveolar Lavage

Recovery of asbestos bodies from BALF may be influenced by areas sampled, with lavages of lower lung zones more likely to yield asbestos bodies in the exposed subject [100]. Unlike sputum, asbestos bodies may be detected in the lavage fluid of populations with no historical exposure to asbestos, although infrequently and in low concentration [94, 101, 102]. Modin et al. reviewed 31,353 sputa and BALF specimens over a 5-year period, finding asbestos bodies in five cases (3 sputa, 2 BALFs). Further investigation determined that all five cases had significant exposure to asbestos and asbestosis was identified in four of the five cases [94]. From these studies it was concluded that asbestos bodies in sputum and bronchial washing specimens are highly specific markers for past asbestos exposure and reflect a significant asbestos fiber burden within the lung. This has led some entities to make sputum examinations part of occupational screening protocols for exposed individuals [103].

The presence of asbestos bodies in lavage fluid is best considered a marker of exposure to asbestos but may also predict disease. Vathesatogit et al. found a higher prevalence of parenchymal disease as well as reduced pulmonary function and diffusion capacities in subjects who had asbestos bodies in BALF compared with those who did not [104].

Roggli et al. provided qualitative and quantitative assessment of asbestos fibers, coated and uncoated, in BALF obtained from patients with asbestosis, those exposed to asbestos but without parenchymal lung disease, those with idiopathic pulmonary fibrosis, and nonexposed controls (Figs. 9.11 and 9.12). They observed excessive numbers of asbestos bodies only in highly exposed individuals (Figs. 9.13 and 9.14) but noted that the uncoated fiber burden as determined by scanning electron microscopy (SEM) was similar in all

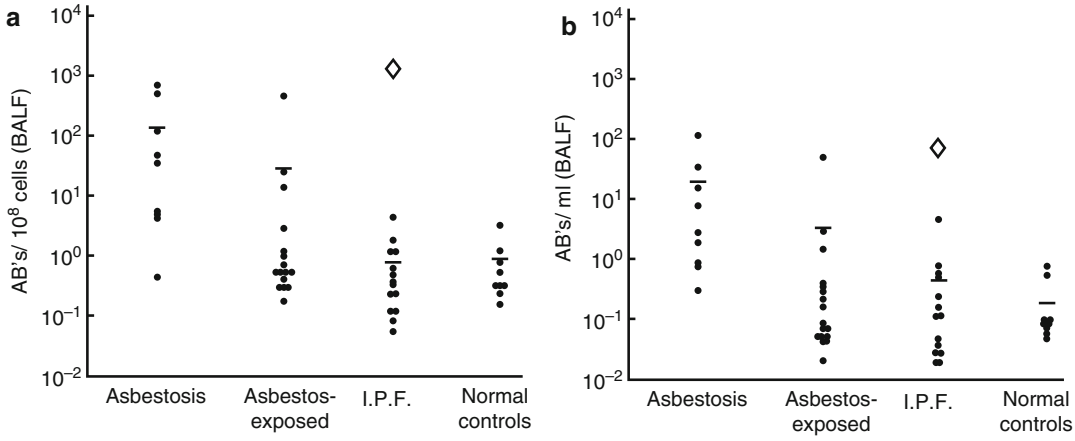


**Fig. 9.11** Distribution of asbestos body content per million cells recovered (**a**) or per ml BALF (**b**) for 50 cases as determined by light microscopy. Each *dot* represents one case, and a *horizontal line* indicates the median value for each group. *Open diamond* represents black-cored pseudoasbestos bodies isolated from one case that was excluded from the median calculation. Note the logarithmic scale (Reprinted from Ref. [105], with permission)

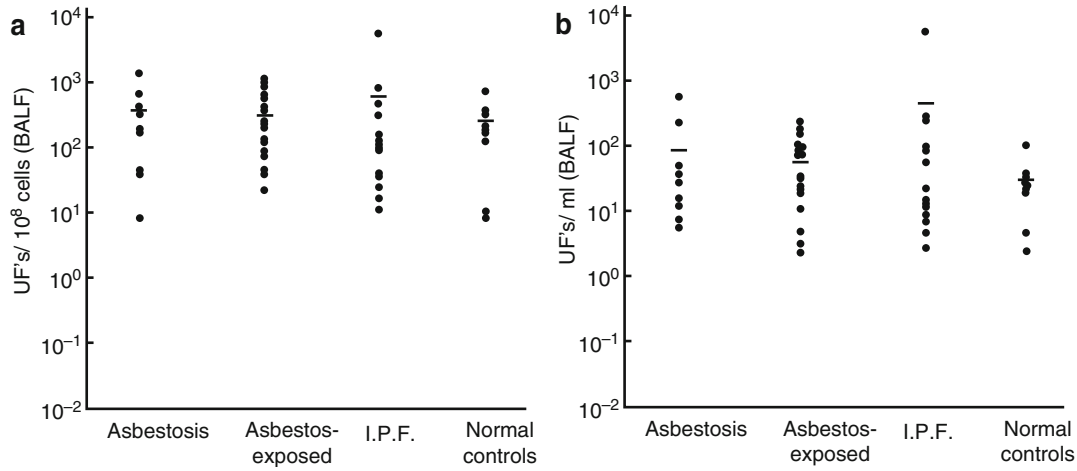


**Fig. 9.12** Distribution of uncoated fibers ≥5 μm in length per million cells recovered (**a**) or per ml BALF (**b**) for 50 cases as determined by scanning electron microscopy. Each *dot* represents one case, and a *horizontal line* indicates the median value for each group. Note the logarithmic scale (Reprinted from Ref. [105], with permission)

groups. Commercial amphibole asbestos (amosite and crocidolite) fibers were detected more frequently in lavage fluid from patients with asbestosis than those from other groups. They concluded that the findings of >1 asbestos body per $10^6$ cells, 1 asbestos body per ml of lavage fluid by light microscopy, or commercial amphibole fibers by SEM all were indicative of considerable exposure to asbestos in the majority of cases [105]. The concentration of one asbestos body per ml of lavage fluid has been corroborated by Karjalainen et al. as a threshold concentration, excesses of which are indicative of significant exposure in the majority of cases [106].

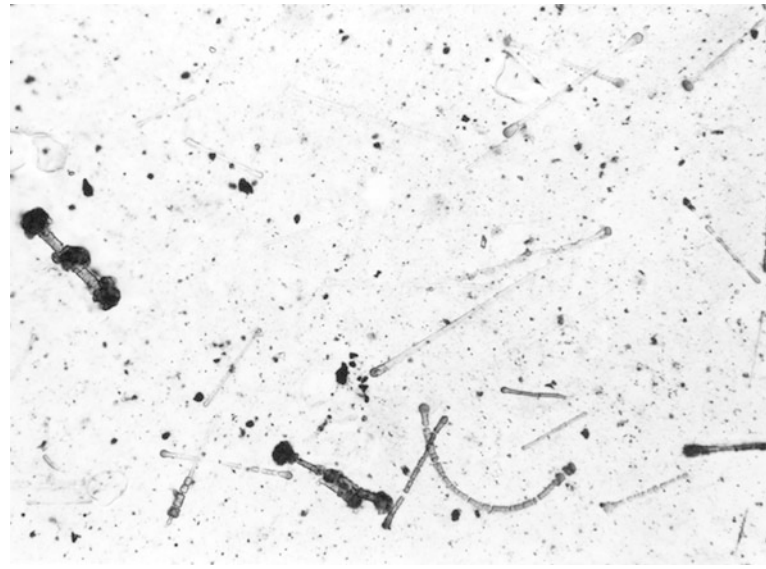

**Fig. 9.13** Asbestos bodies on Nuclepore filter isolated by digesting bronchoalveolar lavage fluid pellet in hypochlorite solution. Unstained, ×400 (Reprinted from Ref. [102], with permission)

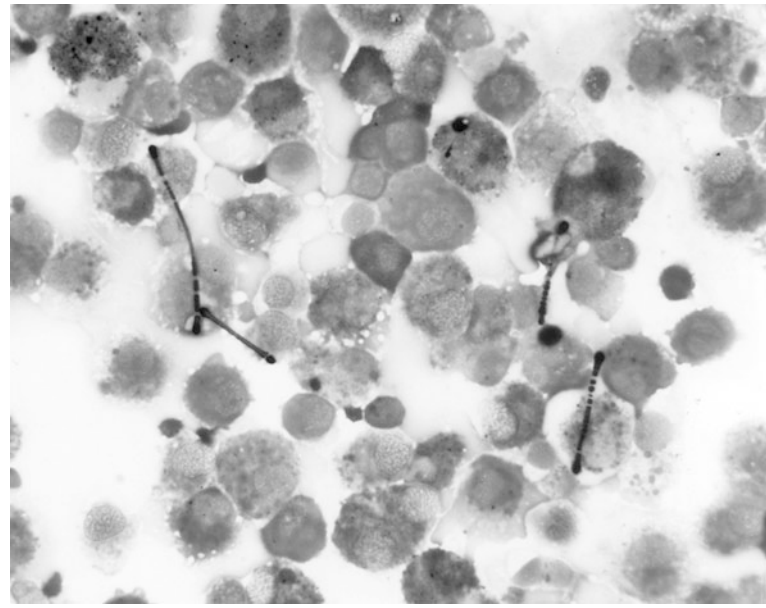

**Fig. 9.14** Cytocentrifuge preparation of bronchoalveolar lavage fluid in an individual with asbestosis. Typical asbestos bodies are present. Wright stain, ×400 (Reprinted from Ref. [102], with permission)

A subsequent study by Karjalainen found significant correlations between the concentrations of asbestos bodies in lavage fluid and in lung tissue, the concentrations of asbestos bodies and amphibole asbestos fibers in lung tissue, and the concentration of asbestos bodies in lavage fluid and amphibole asbestos fibers in lung tissue (Table 9.1) [98]. Unlike other industrialized nations, commercial amphibole usage in Finland, the origin of this study, has historically included anthophyllite. In those patients who had been exposed to predominantly commercial anthophyllite, significantly higher concentrations of asbestos bodies were observed relative to the

total pulmonary amphibole burden. This observation is probably related to the greater likelihood of commercial anthophyllite fibers becoming coated. This study also supports prior observations that a low number or absence of asbestos bodies in BALF does not exclude heavy exposure and that bronchoalveolar lavage is an insensitive indicator of cumulative chrysotile exposure [96, 97, 107, 108].

Teschler et al. evaluated the lavage fluid profiles of 64 patients with diverse asbestos exposure histories and compared these to a control population of nonexposed patients. Ninety-nine percent of controls had less than 0.5 asbestos bodies per ml BALF. In this study, the demonstration of greater than one AB/ml BALF was associated with the high probability of tissue levels of more than 1,000 asbestos bodies per $cm^3$ of lung tissue (Table 9.1) [99]. In a subsequent study, the same group analyzed asbestos fiber counts in 23 individual sample pairs of BALF and lung tissue samples from patients with occupational asbestos exposure, using transmission electron microscopy [109]. Fiber type, size, and aspect ratio were compared. The study concluded that concentrations of both coated and uncoated amphibole asbestos fibers in lavage fluid correlate with the degree of concentrations in lung tissue. The authors of this study had three cases in which no asbestos bodies were detected in lavage fluid, yet were present in lung parenchyma, further supporting the notion that absence of asbestos bodies does not exclude exposure. This study found no significant correlation between lavage fluid and lung tissue specimens for chrysotile, in keeping with the findings that bronchoalveolar lavage is not a reliable indicator of parenchymal chrysotile burden or exposure [97, 107].

Bronchoalveolar lavage has also been used to assess environmental asbestos exposure in areas of Turkey with a high incidence of disease attributable to asbestos in the soil. Compared to control populations in Belgium and Turkey without environmental exposure, BALF tremolite burdens were demonstrated in this study in the same range as commercial amphiboles in subjects occupationally exposed in Belgium [110, 111]. In a study of American construction workers largely exposed to chrysotile, Schwartz et al. found that the concentration of asbestos bodies in BALF correlated poorly with measures both of exposure and clinical/radiographic hallmarks of asbestos-associated pulmonary disease, likely at least due in part to chrysotile's diminished biopersistence or propensity to form asbestos bodies [107].

The reasonable conclusion from the balance of these numerous studies is that asbestos bodies, when present in BALF, are an accurate and reproducible predictor of asbestos exposure. However, sensitivity is limited, and their absence does not exclude significant exposure or necessarily exonerate asbestos in the causation of disease, particularly in those patients exposed to chrysotile asbestos.

### 9.5.3 Fine-Needle Aspiration Biopsy

Aspiration biopsies of the lung are typically undertaken as part of the diagnostic work-up of peripheral nodular lesions and largely for this reason are not likely to yield asbestos bodies as these are much less numerous in tumors than in adjacent sections of lung. Roggli et al. reported the first two instances of asbestos bodies identified in aspiration biopsies [112]. In one case, aspiration biopsy of a peripheral infiltrate yielded asbestos bodies and fungal hyphae typical of aspergillus infections, highlighting the reported association between asbestosis and aspergillus infection (Fig. 9.15). In the second case, aspiration biopsy of a peripheral adenocarcinoma also yielded asbestos bodies (Figs. 9.16 and 9.17). Tissue asbestos analysis was performed in each case, confirming markedly elevated amphibole asbestos concentrations. Only one case had histologic evidence of asbestosis. Roggli et al. also reported a case of two asbestos bodies detected on the aspiration biopsy of a cavitary lesion. This patient was shown to have asbestos body counts within the normal range for that laboratory [112]. Such a chance occurrence is quite uncommon, and we have not encountered asbestos bodies in the several thousand aspiration biopsies that have followed.

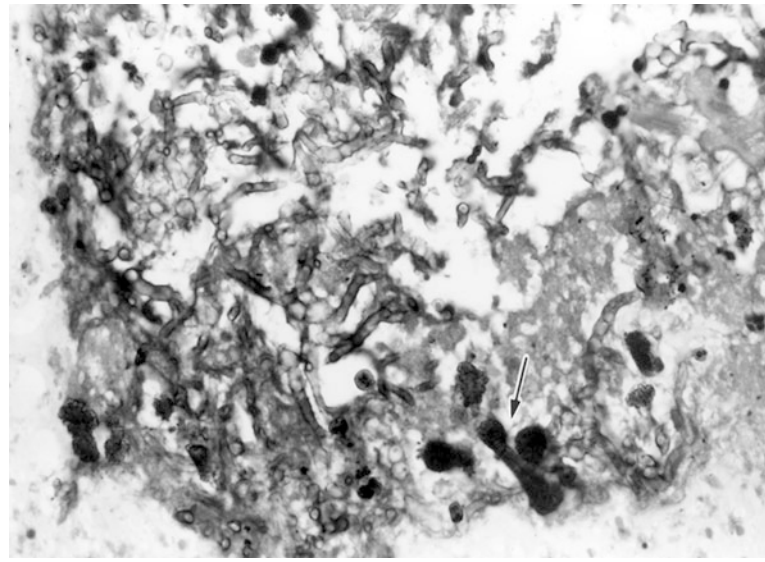

**Fig. 9.15** Hematoxylin- and eosin-stained fine-needle aspirate cell block shows a clump of branching septate hyphae, compatible with *Aspergillus* spp., and a nearly *dumbbell-shaped* asbestos body (*arrow*), one of many identified in the aspirated material

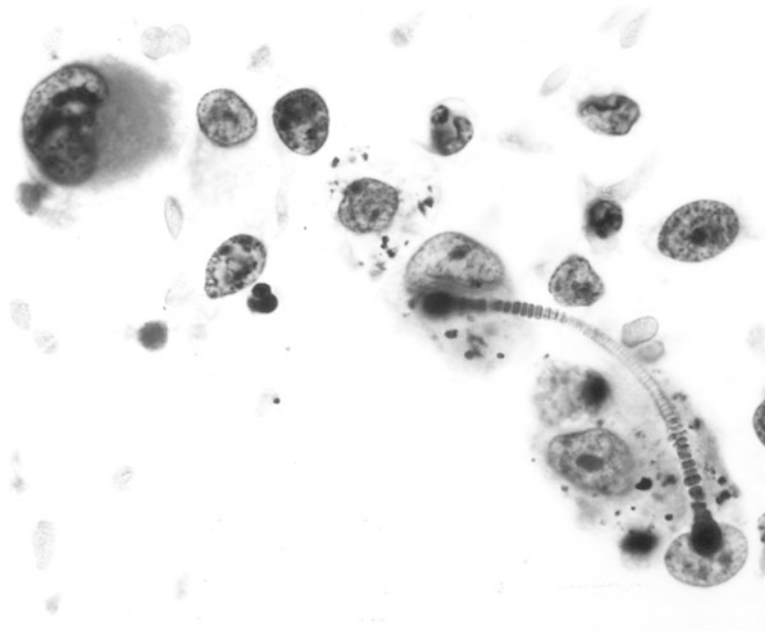

**Fig. 9.16** Papanicolaou-stained fine-needle aspirate smear demonstrates an asbestos body associated with several macrophages. Numerous asbestos bodies were identified in the specimen. A malignant cell is present at *upper left*. Original, ×1,000 (Reprinted from Ref. [112], with permission)

Leiman reviewed a series of 1,256 thoracic aspiration biopsies, which yielded asbestos bodies in 52 cases [113]. Significant occupational exposure was documented in all but eight patients. Malignant neoplasms were diagnosed in 30 of these cases and required additional diagnostic studies for confirmation in 20 % of the cases. The remaining 22 cases were benign lesions, typically abscesses or tuberculosis, and required additional confirmatory studies in 50 % of cases. The author concluded that the demonstration of asbestos bodies is highly associated with pulmonary pathology other than asbestosis, and the detection of the latter using aspiration

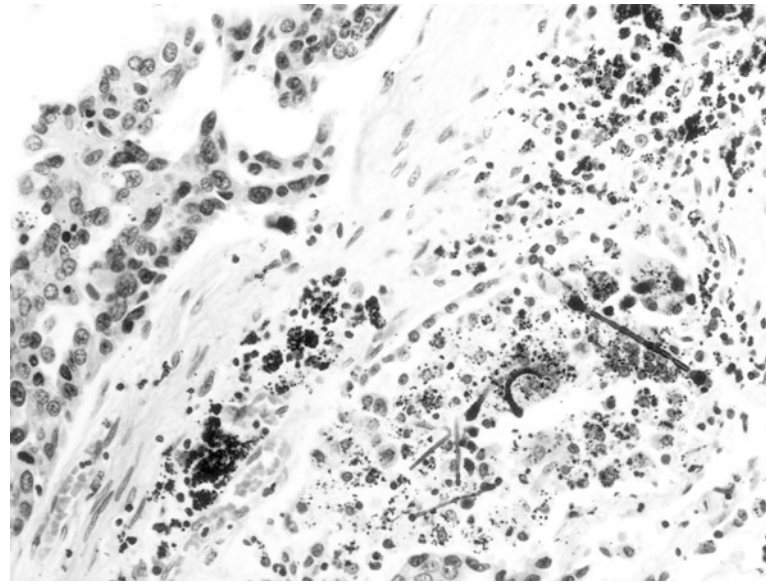

**Fig. 9.17** Sections of primary lung tumor at autopsy (same case as Fig. 9.15) demonstrate sheets of malignant cells with ill-defined lumen formation and adjacent desmoplastic stroma. Several asbestos bodies are visible adjacent to the tumor at *lower left*. Hematoxylin and eosin, ×400 (Reprinted from Ref. [112], with permission)

biopsy technique is diminished possibly due to parenchymal fibrosis related to asbestos.

The demonstration of asbestos bodies in aspiration biopsy specimens is best considered a marker of significant exposure for that patient and suggests that the radiographic lesion that prompted the aspiration biopsy may be asbestos related.

## 9.6 Summary and Conclusions

The evaluation of patients with respiratory disease suspected or alleged to complicate exposure to asbestos requires the synthesis of clinical, radiographic, and laboratory data, as well as data gleaned from the inspection of pathologic specimens. The examination of cytologic materials including body cavity fluids, bronchial lavages, and sputa (often obtained with a minimum of expense and attendant morbidity) may provide a wealth of information regarding the various disease states believed to be related to prior asbestos exposure. Moreover, these specimens may lend themselves to the application of special techniques discussed elsewhere in this book to identify and quantify asbestos fibers and thereby implicate them in the causation of disease.

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# Experimental Models of Asbestos-Related Diseases

10

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## 10.1 Introduction

Much of our understanding of the mechanisms by which asbestos injures the lung has been derived from experimental animal studies. Such studies have confirmed the fibrogenic and carcinogenic properties of asbestos fibers that have been surmised from human observations and have provided insights into the ways in which asbestos fibers interact with biological systems. Models commonly used to study asbestos-induced disease involve inhalation exposure to asbestos, intratracheal instillation, and in vitro studies of various cellular systems. Each of these techniques has particular advantages and disadvantages.

Inhalation studies, being more physiologic, more closely approximate the actual human exposure. While many facilities have methods for performing these studies, they are time-consuming and expensive. Conversely, experiments involving intratracheal instillation of asbestos fibers are simpler to perform, less time-consuming as the time to reach thresholds for fiber accumulation necessary to cause disease are accomplished with a single dose, and less expensive. However, there are disadvantages in that the normal defense mechanisms of the respiratory tract are bypassed and the distribution is more heterogeneous. Hence, the results are not completely comparable to inhalation exposures. In vitro studies of cellular systems permit the investigation of direct effects of asbestos and other particulates on cellular function and cell signaling under carefully controlled conditions. However, it is not always clear how the results apply to the more complex in vivo conditions or whether the particular mechanisms under investigation contribute significantly to the overall pathogenesis of asbestos-induced tissue damage. These limitations notwithstanding, each of these approaches has contributed substantially to our understanding of the mechanisms' underlying asbestos-related disease.

This chapter reviews the current understanding of the pathogenesis of asbestos-related diseases as derived from experimental models. To understand asbestos-related tissue injury, it is first necessary to understand the patterns of deposition of asbestos fibers within the lung parenchyma and the subsequent clearance of fibers from the lung through the mucociliary escalator, the macrophage defense system, and the pulmonary lymphatics. The pathogenesis and mechanisms involved in the development of pulmonary fibrosis and thoracic neoplasms in animal models will also be reviewed. Although asbestos-induced fibrogenesis and carcinogenesis share many common features and may involve similar molecular

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T.D. Oury et al. (eds.), *Pathology of Asbestos-Associated Diseases*,
DOI 10.1007/978-3-642-41193-9_10, 

mechanisms of tissue injury, these two processes will be reviewed separately for clarity.

## 10.2 Historical Background

Experimental models of asbestos-induced tissue injury were established in the 1930s and 1940s by the pioneering work of Gardner [1] and King et al. [2]. In 1951, Vorwald et al. [3] published the results of the classic inhalation studies performed at Saranac Lake, NY. These investigators showed that inhalation (or intratracheal instillation) of chrysotile, crocidolite, and amosite asbestos produced interstitial fibrosis similar to that observed in human asbestosis. Long asbestos fibers were found to be more injurious than short fibers, and the duration of exposure required for disease to develop varied inversely with the concentration of long fibers in the atmosphere [3]. The development of a dependable and reproducible fiber aerosolization system by Timbrell [4] paved the way for the inhalation studies of Wagner et al. [5] that were reported in 1974. These studies performed with SPF Wistar rats showed that both amphibole and chrysotile forms of asbestos produced asbestosis in a dose-dependent fashion and that the fibrosis continued to progress after removal from exposure. Furthermore, inhalation exposures to all forms of asbestos tested resulted in the production of thoracic neoplasms, including adenomas, carcinomas, and mesotheliomas. There was a positive correlation between the severity of asbestosis and the development of pulmonary neoplasms. These early studies provided the basis for subsequent investigations and more detailed analysis of pathogenetic mechanisms of asbestos injury at the cellular level [6].

## 10.3 Asbestos Fibers

### 10.3.1 Deposition of Asbestos Fibers

The mammalian respiratory system is equipped with a variety of defense mechanisms for protection against foreign matter, and these mechanisms in turn affect the size, shape, and number of particles that are deposited and that ultimately accumulate in the lower respiratory tract. These defense mechanisms include four major components: (a) the fine hairs, or vibrissae, in the nasal cavity that filter out most of the larger particles [>10-μm aerodynamic equivalent diameter (AED)] that are inhaled; (b) the mucociliary escalator of the tracheobronchial tree, which carries any particles that impact the surface of the airways upward toward the mouth; (c) the alveolar macrophages, which phagocytose particles that make their way past the first two levels of defenses and are deposited in the gas-exchange regions of the lung; and (d) the pulmonary lymphatics, through which many deposited particles are transported to regional lymph nodes or the pleura [7].

Particles that have the greatest probability of deposition and retention in the gas-exchange regions of the lung are in the size range of 1- to 5-μm AED. Particles less than 0.5 μm in maximum dimension are deposited by Brownian motion or diffusion. Deposition patterns may be influenced by such factors as tidal volume, respiratory rate, and pattern of breathing (nose versus mouth).

A unique feature of fibrous dusts is that fibers of considerable length can be deposited in the lower respiratory tract, even though most particles 5 μm or greater in size are excluded. This is because of the tendency for fibrous dusts to line up along the direction of laminar airflow, so that the diameter of a fiber rather than its length is the primary determinant of respirability [8, 9]. As a result, most fibers deposited in the lungs of humans or experimental animals are 1 μm or less in diameter, but may exceed 200 μm in length. In this respect, important differences exist between the amphibole fibers and the serpentine chrysotile fibers. The very long fibers of chrysotile tend to be curly and are thus more likely to interact with the upper respiratory tract, where they are subsequently removed by the mucociliary escalator [8, 9]. Very long amphibole fibers tend to be straight and have a greater likelihood of penetrating into the gas-exchange regions of the lung. Differences between the accumulations of amphibole versus chrysotile fibers within the lungs of experimental animals following long-term inhalational exposure were noted by Wagner et al. [5].

These observations stimulated the investigation of the pulmonary deposition and clearance of asbestos fibers, with particular attention to differences between chrysotile and the amphiboles.

The development of methods for producing radiolabeled asbestos fibers [10] has greatly facilitated the determination of total lung fiber burden after administration [11], as well as the patterns of deposition in the respiratory tract. Early studies using these techniques demonstrated a tendency for fibers to deposit and concentrate at bifurcation points in the conducting airways with a relatively uniform distribution throughout the alveolar regions [12, 13]. Studies using scanning electron microscopy have shown that this tendency for deposition at bifurcation points extends to the alveolar regions of the lungs [14–17]. In rats exposed to aerosolized chrysotile asbestos, fibers in the distal anatomic regions of the lung were localized primarily at alveolar duct bifurcations. The greatest concentration of fibers occurs at the more distant (e.g., second- and third-order) alveolar duct bifurcations [6, 15, 16]. Very few fibers are observed on the surfaces of adjacent alveoli. A similar pattern is observed for chrysotile and amphibole asbestos fibers [17].

These observations indicate that the geometry of the tracheobronchial tree is an important determining factor in the deposition of particulates in the lower respiratory tract [18]. Studies in which meticulous dissections of the tracheobronchial tree in asbestos-exposed rats were performed demonstrate that the quantity of asbestos deposited in the lung parenchyma is inversely related to airway path length and the number of airway bifurcations [19]. Variations in airway geometry among different species could result in different patterns of deposition, which in turn could account for some of the variation in species response to asbestos inhalation [20]. In this regard, it should be noted that marked differences in deposition pattern are obtained for dust administered by inhalation versus intratracheal instillation [21]. The distribution of dust resulting from instillation is much less homogeneous than that from inhalation, and penetration to the lung periphery is minimal. The resultant inflammatory responses are also quite different [22], so that one must use caution in extrapolating results based on intratracheal instillation in experimental animals to human inhalation exposures [6].

Opinions differ regarding the fractional deposition of chrysotile versus amphibole asbestos fibers in the lower respiratory tract. Morgan et al. [13], in a study in which rats were exposed to three concentrations (4, 11, and 32 μg/l) of two different samples of radiolabeled chrysotile asbestos for 30 min in nose-only chambers, found that 12 and 15 % of the respirable mass was deposited in the lower respiratory tract. Roggli and Brody [23], in a study in which rats were exposed to 15 μg/l of Jeffery mine chrysotile asbestos (a standardized preparation) for 1 h in nose-only chambers, found that 23 % of the respirable mass was deposited in the lower respiratory tract. In studies in which rats were exposed to UICC (Union Internationale Contre le Cancer) asbestos samples by inhalation for 6 weeks, Middleton et al. [24] found that the relative retention of chrysotile in the lungs decreases with increasing aerosolized concentrations. For the highest concentration employed in their study (7.8 μg/l), the fractional deposition of chrysotile in the lungs was 17 %. Short-term inhalation studies result in a similar fractional deposition for crocidolite as compared to chrysotile asbestos: 16 % of the respirable mass in the study by Morgan et al. [13] and 19 % in the study by Roggli et al. [25]. In contrast, Middleton et al. [24] determined that, for rats exposed to amphibole fibers for 6 weeks, the fractional deposition for amosite was 65 % and for crocidolite approached 100 %. These comparisons are summarized in Table 10.1. Although the reason for these discrepancies is unclear, it is apparent that, with durations of exposure of 6 weeks or longer, the relative retention of amphibole fibers in the lungs is considerably greater than that of chrysotile [5, 24]. This may reflect the much shorter half-life of chrysotile compared to amphiboles that may only become apparent with longer exposures.

### 10.3.2 Fiber Clearance

The clearance of asbestos fibers deposited in the lung is dependent upon several factors, including the anatomic site of deposition, particle

**Table 10.1** Fractional deposition of chrysotile versus amphibole asbestos fibers in lungs of rats following inhalational exposure

| Authors | Exposure dose (μg/l) | Exposure duration | Fractional deposition | |
|---|---|---|---|---|
| | | | Chrysotile | Amphibole |
| Morgan et al. [13] | 4, 11, and 32 | 30 min | 12–15 % | 16 % |
| Roggli and Brody [23] | 15 | 1 h | 23 % | – |
| Roggli et al. [25] | 3.5 | 1 h | – | 19 % |
| Middleton et al. [24] | 1, 5, and 10 | 6 weeks | 17–36 % | 65–100 % |

The studies by Morgan et al. [13], Roggli and Brody [23], and Roggli et al. [25] employed nose-only exposure chambers, whereas the studies by Middleton et al. [24] used open-chamber (i.e., whole animal) exposures

solubility, and the efficiency of the host's phagocytic system. In addition, cigarette smoking has been shown to interfere with particle clearance from the lower respiratory tract [26]. The fate of a fiber that has been deposited in the respiratory tract is dependent to some degree on the site of deposition. Fibers deposited on the surface of the large or small airways may become trapped in the mucous layer, where they will be transported upward by ciliary motion at a rate as high as several millimeters per minute [8]. Fibers deposited on the alveolar epithelium may be transported across the epithelium into the underlying interstitium via a mechanism that likely involves an actin-containing microfilament system [6, 27, 28]. Thus, within hours of a brief inhalational exposure, asbestos fibers are observed within the cytoplasm of type I epithelial cells. Within 24 h, fibers have been translocated into the interstitial compartment, including basal lamina, connective tissue, and cytoplasm of interstitial cells [6]. In addition, there is evidence that transepithelial transport occurs to some extent in the airways as well [29, 30]. Once within the interstitium, fibers may then penetrate the cytoplasm of endothelial cells [15, 16] and gain access to the vascular and lymphatic systems [8]. Fibers within lymphatic channels may then be carried to the visceral pleura [31] and hence gain access to the pleural space [32] or be transported to hilar or mediastinal lymph nodes [33, 34]. Within 24 h of a brief inhalational exposure to asbestos, there is an influx of alveolar macrophages that proceed to phagocytize any free asbestos fibers on the alveolar surfaces. These macrophages accumulate at the site of initial fiber deposition and are found on more than 90 % of alveolar duct bifurcations by 48 h postexposure [35]. Fibers that have been transported to the pulmonary interstitium may similarly be phagocytized by interstitial macrophages. Once ingested within the macrophage, fibers may remain for prolonged periods within alveoli or the interstitium. Alternatively, phagocytized fibers may be removed from the lung when macrophages enter onto the mucociliary escalator of the small airways or into the pulmonary lymphatics [8].

A number of studies have demonstrated that the average length of fibers retained within the lung increases with time postexposure and that this effect is observed for both chrysotile and amphibole asbestos [23, 25, 36–41]. The presumed mechanism of this effect is the more efficient clearance of short fibers, with preferential retention of longer fibers [14, 41, 42]. This phenomenon can be well demonstrated by measuring the half-times for clearance of fibers in various size categories after a single exposure. In these studies, it can be seen that the residence time within the lung for fibers 10 μm or more in length is particularly prolonged [37]. More direct evidence for the more efficient clearance of shorter fibers comes from the studies of Kauffer et al. [39], which showed a progressive decrease in mean length of fibers recovered by bronchoalveolar lavage following a brief inhalational exposure, with a concomitant increase in mean length of fibers remaining in the lungs.

With regard to fiber type, short-term inhalation studies have shown similar clearance rates for chrysotile versus amphibole asbestos fibers. Following a 1-h exposure period, the percentage of the original deposited mass remaining 1 month postexposure was 25 % for crocidolite

**Table 10.2** Accumulation of chrysotile versus crocidolite asbestos in rat lungs following inhalational exposure

| Fiber type | Fibers/g[a] | Asbestos/rat (μg)[b] |
|---|---|---|
| Crocidolite | $1.85 \times 10^8$ ($\pm 1.12 \times 10^8$) | 814 (±435) |
| Chrysotile | $2.50 \times 10^7$ ($\pm 8.4 \times 10^6$) | 71.6 (±28.9) |
| Sham[c] | $3.5 \times 10^4$ ($\pm 4.9 \times 10^4$) | 0.045(±0.025) |

Rats sacrificed following 3 months' exposure in inhalation chambers to 10.7 mg/m$^3$ chrysotile or 11.2 mg/m$^3$ crocidolite asbestos

[a]Fibers per gram of wet lung ±1 SE (4 animals in each group)

[b]Calculated mass of asbestos in both lungs ±1 SE

[c]Animals exposed to room air only

[25] and 19 % for chrysotile asbestos [23]. Middleton et al. [24] also reported that the rate of clearance is similar for chrysotile and amphibole types of asbestos and that the clearance could be expressed in terms of a three-compartment model with half-lives of 0.38, 8, and 118 days for each respective compartment. These observations are difficult to reconcile with the results of long-term inhalational studies, in which amphibole asbestos fibers accumulate within the lungs to a much greater extent than chrysotile fibers [5, 43]. In fact, the lung content of chrysotile appears to level off and remain constant after 2 or 3 months of exposure, whereas amphibole fibers continue to accumulate progressively with continued exposure [5]. Middleton et al. [24, 44] also noted substantially greater accumulation of amphiboles as compared to chrysotile following a 6-week exposure and attributed the difference to a greater fractional deposition of amphibole fibers (Table 10.1). Substantial differences in the pulmonary content of crocidolite versus chrysotile asbestos in rats exposed to similar doses of the two fiber types for a 3-month period have also been observed (Table 10.2). Observations using intratracheal instillation of asbestos have demonstrated more rapid clearance of chrysotile fibers as compared to amphiboles beginning almost immediately after exposure [45, 46]. More recent studies have also confirmed the much shorter half-life of chrysotile compared to amphiboles [47, 48].

Although the reasons for the preferential retention of amphibole fibers are not entirely clear, one very important factor is undoubtedly the tendency for chrysotile to divide longitudinally into individual fibrils [42]. Roggli and Brody [23] reported a progressive decrease in mean fiber diameter following a 1-h inhalational exposure to chrysotile asbestos, an observation that has been confirmed by a number of investigators [36, 39, 45]. In comparison, no significant alteration in mean fiber diameter is observed for amphibole fibers [25, 36, 45, 49]. The longitudinal splitting of chrysotile fiber bundles creates fibrils with a very fine diameter. Short fibrils created by this splitting process are readily cleared from the lung, whereas long, thin chrysotile fibrils are retained [14, 23, 49]. Kimizuka et al. [49] reported a further fragmentation of long, thin chrysotile fibers 2 years postexposure in hamsters, with a concomitant increase in the percentage of fibers less than 5 μm in length from 13 % 1 year postexposure to 56 % at 2 years. The decrease in mean fiber diameter of chrysotile has been associated with leaching of magnesium by some investigators [36, 49], but not others [45]. No significant change in elemental composition is observed for amphibole fibers with increasing time postexposure [36, 49]. Progressive leaching of magnesium from chrysotile fibers occurring in an acidic environment could result in fiber dissolution, and some investigators believe that this may be an important mechanism of chrysotile clearance from the lung, especially for very small fibrils [50, 51]. In this regard, in vitro studies with alveolar macrophages have shown a rate of magnesium leaching from chrysotile asbestos that is comparable to the leaching rate in an acid solution with a pH of 4 [52]. Although the in vivo significance of magnesium leaching from chrysotile asbestos fibers is controversial, it is of potential importance because the cytotoxicity and carcinogenicity of chrysotile asbestos is significantly reduced by in vitro depletion of magnesium [53].

Additional factors may significantly influence the clearance of asbestos fibers from the lower respiratory tract. Bolton et al. [54] have shown that, once a critical pulmonary burden of asbestos has been reached, there is an overload of the clearance mechanism. This phenomenon occurs at relatively high lung burdens and may be related to inhibition of clearance by alveolar

macrophages. Other studies have shown that administration of a toxic dust such as asbestos or quartz can interfere with the subsequent clearance of a nontoxic dust such as titanium dioxide [55, 56]. However, when looking at clearance of asbestos fibers (particularly chrysotile), titanium dioxide appeared to increase fiber retention, while quartz reduced it [57]. In addition, there is evidence that cigarette smoke interferes with the clearance of asbestos fibers from the lower respiratory tract, largely by increasing the retention of short fibers [58–60]. Exposure to low levels of ozone also enhances pulmonary retention of inhaled asbestos fibers by interfering with fiber clearance [61]. Fiber clearance may play an important role in the development and severity of asbestosis following inhalation of asbestos fibers. Experimental studies have shown that high alveolar dust retention precedes the development of asbestosis and that individual variability in alveolar dust clearance capacity may be a major determinant in the development of asbestos-induced pulmonary fibrosis [62].

## 10.4 Fibrogenesis

### 10.4.1 Role of Fiber Dimensions

The importance of fiber length in asbestos-induced fibrogenesis has been addressed in a number of studies. The classic studies reported by Vorwald et al. [3] suggested that fibers greater than 20 μm in length are the most fibrogenic, an opinion supported by the studies of Davis et al. [43]. Other investigators have also concluded that long-fiber asbestos results in considerably more lung injury than short-fiber asbestos [63–69] and that there is progression of injury after cessation of exposure only with the long-fiber inhalation [66]. It is difficult to determine a fiber length below which no significant fibrosis will occur regardless of intensity or duration of exposure, in part because of the problem of contamination of "short-fiber" samples with a small percentage of "long fibers" [68]. However, LeMaire et al. [70] studied rats injected intratracheally with 5 mg of a preparation of very short chrysotile fibers (100 % <8 μm) and found an alveolitis 60 days postexposure but no apparent fibrosis.

Platek et al. [71] exposed rats by inhalation of short chrysotile asbestos with a mean concentration of fibers in the chamber of 1.0 mg/m$^3$ and only 0.79 fibers/ml with length exceeding 5 μm. These investigators showed that a concentration of $23 \times 10^6$ chrysotile fibers >5 μm in length per gram of dry lung or $272 \times 10^6$ chrysotile fibers <5 μm in length per gram of dry lung or a combination of the two is insufficient to produce pulmonary fibrosis in the rat 18–24 months after initiation of exposure [71]. Adamson and Bowden found no appreciable fibrosis in the lungs of mice following intratracheal instillation of 0.1 mg of short crocidolite asbestos fibers (mean length 0.6 μm, with 98.8 % of fibers less than 2.5 μm in length) [63], whereas peribronchiolar fibrosis and significantly increased collagen levels were observed following instillation of 0.1 mg of long crocidolite asbestos fibers (mean length 24.4 μm, with 88 % of fibers greater than 2.5 μm in length) [64]. Contrary to the findings of most other investigators, [72] reported the production of interstitial fibrosis following intratracheal instillation of ultrashort-fiber chrysotile asbestos (fiber length between 0.05 and 0.2 μm). However, the one published light micrograph shows dense fibrosis at too high a magnification to determine whether the pattern is typical for that observed with asbestosis [6].

Recently, Tomatis et al. [73] examined short versus long amosite fibers to determine if differences in their surface properties lead to different biologic responses that may explain why longer fibers are more toxic. This study found that long amosite fibers lead to increased free radical and oxidizing potential than short fibers. This was explained in part by higher levels of reduced iron ($Fe^{2+}$) on the surface of long fibers compared to short fibers, which is an important factor in promoting the generation of free radicals. They further showed that long fibers also induce greater release of nitric oxide from lung epithelial cells that can react with superoxide to promote further oxidative stress. They suggest that these surface properties need to be considered in addition to

just aspect ratios when investigating pathogenic properties of fibers.

Another recent study also revisited the role of fiber length in promoting pathology [74]. The authors suggest that longer fibers are retained in the parietal pleura due to their inability to negotiate their way through stomata in the parietal pleura, which would have led to lymphatic clearance. This will promote pleural inflammation and mesothelioma due to the persistence of these fibers in the pleura.

In contrast to fiber length, relatively few studies have examined the role of fiber diameter in the pathogenesis of asbestos-induced tissue injury. The major importance of fiber diameter appears to be its role as a limiting factor for fiber deposition. For fibers with an aspect ratio between 10 and 100, the aerodynamic equivalent diameter is approximately three to four times the actual fiber diameter [8]. Hence, for fibers with an aspect ratio of 10 or more, 2 μm is about the maximum diameter of a fiber that may be deposited in the lower respiratory tract of the rat [8]. Other physical parameters of fibers are also potentially important. Some studies have indicated that fiber surface area is the most important determinant of the severity of pulmonary fibrosis [75]. In this regard, the progressive decrease in mean fiber diameter of chrysotile may be an important feature in its pathogenicity. This decrease in fiber diameter is believed to be due to longitudinal splitting of chrysotile fibers in vivo, which would result in both increased fiber number and increased surface area [76]. Each of these factors has been shown to correlate positively with the severity of fibrosis. Finally, another physical feature of importance is fiber charge, with highly charged fibers being more likely to be deposited in lung tissue [77]. This effect is probably greatest for long fibers, and electrostatically charged chrysotile asbestos produces more fibrosis than a similar level of asbestos that has been charge neutralized [77].

### 10.4.2 Cellular Modulation

Experimental animals have shown that asbestos produces a progressive, peribronchial, and interstitial pulmonary fibrosis (Fig. 10.1). Three specific lesions have been identified: [3, 5, 43] (a) peribronchiolar accumulation of acute inflammatory cells (neutrophils, macrophages, and giant cells) that contain engulfed asbestos fibers, and fibrous tissue in association with respiratory bronchioles and alveolar ducts; (b) extension of bronchiolar epithelium into adjacent alveolar ducts and alveoli producing a pattern referred to as bronchiolar metaplasia (or the older term pulmonary adenomatosis); and (c) diffuse stromal thickening of the alveolar septa with temporal heterogeneity associated with proliferation of type II pneumocytes and fibroblasts. Initially, the sites of particulate deposition are rich in reticulin fibers and are sites at which eventual collagen deposition occurs. Fibrotic lesions develop in the vicinity of respiratory bronchioles and, with continuing exposure, appear to extend to involve alveolar ducts and adjacent alveoli [3, 5]. All types of asbestos produce asbestosis in experimental animal models, including chrysotile [3, 5, 43], amosite [5, 43], crocidolite [5, 43, 78], anthophyllite [5, 43], and tremolite [79]. There is a dose–response relationship for each of the fiber types tested and the resulting fibrosis [5, 43]. Although there is a variation in species response to either intratracheal instillation or inhalation of asbestos [3, 6, 20], asbestosis has been produced in a wide range of experimental animals including baboons [50], sheep [80–83], mice [63, 64, 84], guinea pigs [3, 59, 65, 85], hamsters [44], and the white rat [3, 5, 46].

Electron microscopic studies following brief (1-h) inhalation exposures allow more detailed evaluation of the earliest events in asbestos-induced tissue injury [6]. Within 24 h of a brief exposure to aerosolized asbestos fibers, the lung responds with an influx of alveolar macrophages at the site of initial fiber deposition [35]. This accumulation of macrophages persists for at least 30 days and is associated with a significantly increased bifurcation tissue volume as assessed by morphometric studies [86]. In the interstitium adjacent to these alveolar duct bifurcations, asbestos fibers can readily be identified 1 month postexposure, both intracellularly and extracellularly, and are often associated with

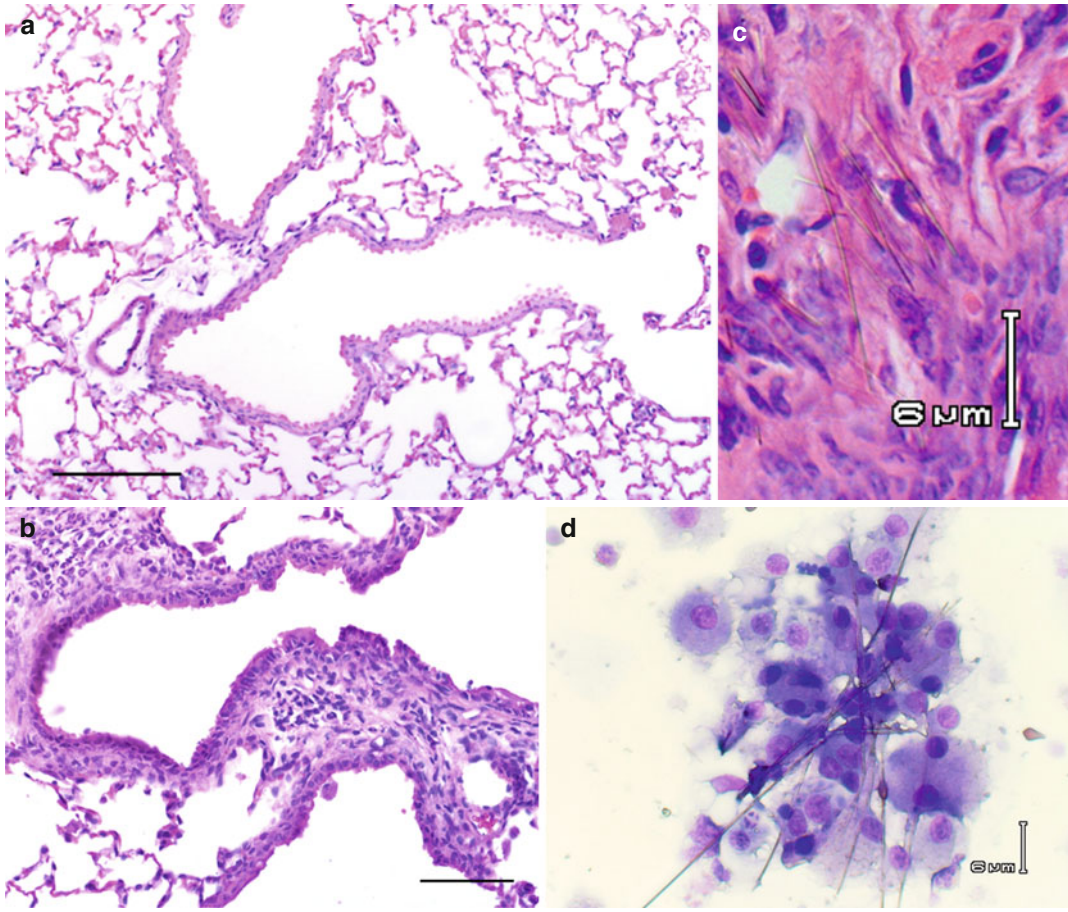


**Fig. 10.1** Hematoxylin and eosin stain of lungs from mice 14 days after intratracheal instillation of saline control (**a**) or 0.1 mg of crocidolite asbestos (**b**). Asbestos exposure led to marked peribronchial fibrosis with extension of fibrosis into alveolar septa. Higher magnification demonstrates accumulation of asbestos fibers in the interstitium (**c**). Bronchoalveolar lavage fluid from asbestos-treated mice also demonstrates asbestos fibers associated with macrophages (**d**)

microcalcifications [87]. These microcalcifications consist of calcium and phosphate and may be the consequence of fiber-induced membrane injury of interstitial cells [87].

Transmission electron microscopy correlated with autoradiography shows that epithelial proliferation is associated with bronchiolar Clara cells and alveolar type II cells, whereas interstitial proliferation is related to division of interstitial macrophages and fibroblasts [88, 89]. Furthermore, blood vessels adjacent to alveolar duct bifurcations show increased labeling of both endothelial and smooth muscle cell nuclei by H3-TdR 19–72 h following a brief inhalation exposure to chrysotile asbestos [90]. This mitogenic response may be the result of the release of diffusible growth factors derived from asbestos-stimulated alveolar macrophages. Ultrastructural examination of alveolar duct bifurcations of rats exposed to asbestos for 1 day has shown persistence of fibers at these sites as long as 1 year postexposure [19].

### 10.4.3 Alveolar Pneumocytes

The classic observations regarding experimental asbestos-induced lung injury [3, 5, 46] have been extended to the cellular level by means of ultrastructural morphometry of animals exposed

to asbestos fibers by chronic inhalation [6]. Examination of the lungs of animals exposed to chrysotile asbestos for 1 week, 3 months, or 1 year has demonstrated that the most significant changes occur in the epithelial and interstitial compartments [66, 91, 92]. Type I epithelial cells comprise 95 % of the alveolar surface area in the lung. They create the tight junctions in the lung and are critical for gas exchange. Type II cells are cuboidal pneumocytes that are progenitor cells for type I cells and function to produce proteins for the lung such as surfactant. Epithelial injury is thought to be one of the initial steps in the pathogenesis of pulmonary fibrosis [93]. Within the epithelial compartment, an increase in cell number and average cell volume can be largely attributed to alveolar type II pneumocyte hyperplasia. Similarly, the interstitial compartment shows an increase in cell number and average cell volume, most of which can be attributed to accumulation of interstitial macrophages [92] and myofibroblastic cells. Increases in smooth muscle cell numbers surrounding the arterioles and venules near alveolar duct bifurcations have also been noted [90]. In addition to morphometric analysis, several studies have looked at proliferation of specific subsets of cell populations by BrdU and $^{3}$H-thymidine incorporation. Animals exposed to chrysotile and crocidolite asbestos had significantly increased incorporation of BrdU in the nuclei of epithelial and interstitial cells located in the bronchiolar/alveolar regions of the lung [94–96] and visceral pleural mesothelial cells [94]. In models of both acute and chronic lung injury by asbestos, $^{3}$H-thymidine incorporation by mesothelial cells, subpleural fibroblasts, and interstitial macrophages was demonstrated [97, 98].

Asbestos fibers may be identified within pulmonary epithelial cells and interstitial macrophages via transmission electron microscopy. A decrease in the ratio of magnesium to silicon in some of these fibers, as determined by energy-dispersive x-ray analysis, is indicative of some leaching of magnesium [66]. Microcalcifications also are identified within some interstitial cells. The endothelial and capillary compartments of the lung are for the most part unaffected. Fibers are gradually cleared from epithelial cells and macrophages following cessation of exposure, and these compartments then resolve toward unexposed-control levels. However, significant clearance of fibers from the pulmonary interstitium does not occur even 1 year following cessation of exposure. This persistence of fibers in the interstitial compartment is associated with continuing fibrogenesis [91]. Long-term studies following intratracheal instillation of chrysotile asbestos in rats have shown, by means of biochemical analysis, significant increases in collagen and elastin content per unit lung weight [99].

Alveolar epithelial cells can take up asbestos. This process is thought to be mediated at least in part by endocytosis mediated by the $\alpha v\beta 5$ integrin receptor [100]. Asbestos contributes to epithelial apoptosis and may also promote epithelial to mesenchymal transition. Both processes are believed to play a central role in the pathogenesis of asbestosis [101].

### 10.4.4 The Role of Inflammatory Cells

In addition to the direct cytotoxicity of asbestos fibers, the inflammatory response to asbestos exposure is an extremely important mechanism of asbestos-induced tissue injury. Aerosolized chrysotile asbestos exposure produces a dose-related bronchiolitis and fibrosis associated with significantly elevated numbers of alveolar macrophages, neutrophils, and lymphocytes in bronchoalveolar lavage fluid [102]. Macrophages have been shown to be important mediators of asbestos-induced injury. Asbestos activates complement through the alternative pathway, resulting in the production of $C_{5a}$ from $C_5$ and the subsequent accumulation of macrophages at first alveolar duct bifurcations [103–105]. This chemoattraction of macrophages is reduced or abolished by depletion of circulating complement, as shown by decreased numbers of macrophages at alveolar duct bifurcations in asbestos-exposed, complement-depleted rats [104, 106]. Alterations in macrophage cytoplasmic and surface morphology are observed in animals exposed either briefly or chronically to aerosolized asbestos fibers

[35, 106–109]. These cells demonstrate diminished phagocytic capacity as assessed by carbonyl iron bead uptake [35, 103].

Alveolar macrophages can produce a wide variety of substances that are potential mediators of asbestos-induced tissue injury and repair (Table 10.3). It has been shown that phagocytosis of asbestos fibers by macrophages can result in the generation of reactive oxygen species [110–114], which can in turn produce alterations in membrane fluidity, lipid peroxidation, and breakage of DNA. Alveolar macrophages also secrete a number of hydrolytic enzymes, including aminopeptidase, acid phosphatase, esterase, lysozyme, cathepsin, RNase, lipase, phospholipase $A_1$ and $A_2$, elastase, hyaluronidase, β-glucuronidase, and catalase [111, 115–122], which can enhance tissue breakdown and destruction. In addition, alveolar macrophages may be stimulated to produce a broad spectrum of regulatory molecules that could in turn modulate the activity of other cells within the lung. These include the arachidonic acid metabolites, leukotriene $B_4$, prostaglandin $E_2$, and prostaglandin F2a [123, 124], as well as certain growth factors, such as platelet-derived growth factor, interleukin-1, fibroblast growth factor, and tumor necrosis factor [125–127]. Asbestos exposure in vitro [126] and in vivo [123] stimulates alveolar macrophages to release leukotriene $B_4$, a potent chemotaxin for neutrophils and eosinophils. Furthermore, both in vitro [126] and in vivo [128] asbestos exposure stimulates the release of tumor necrosis factor, which can augment neutrophil and eosinophil functional activity and stimulate fibroblast growth. In vivo studies have also shown increased replication of interstitial fibroblasts in asbestos-exposed animals, as determined by autoradiography [89, 129]. This latter effect is probably modulated by the release of fibroblast growth factor [130, 131], tumor necrosis factor [125, 126], interleukin-1 [124, 132–135], prostaglandin $F_{2a}$ [124], and/or fibronectin [128] by asbestos-activated macrophages.

Granulocytes (including neutrophils and eosinophils) have been shown to be present in increased numbers in bronchoalveolar lavage fluid obtained from experimental animals exposed to asbestos [102] and in patients with asbestosis [123]. Alveolar macrophages may play a key role in this influx of granulocytes [136] through the production and release of leukotriene $B_4$ [123, 126]. In vitro studies have shown that asbestos fibers have both a cytotoxic and an activating effect on neutrophils [137, 138]. This can result in amplification of asbestos-induced tissue injury by release of potent proteolytic enzymes and reactive oxygen species. In the presence of extracellular calcium, asbestos fibers stimulate the release of granule-associated enzymes by exocytosis [138]. Incubation of asbestos fibers with normal human neutrophils also results in generation of reactive oxygen species as measured by

**Table 10.3** Potential mediators of asbestos-induced tissue injury and repair produced by alveolar macrophages

| Mediator | References |
|---|---|
| *Reactive oxygen species* | |
| Superoxide anion | [110, 112, 113, 181, 189, 196] |
| Hydroxyl radical | [113, 114, 180] |
| Nitric oxide/peroxynitrite | [185–188] |
| *Hydrolytic enzymes* | |
| Matrix metalloproteinases | [225–228] |
| Aminopeptidase | [116] |
| Acid phosphatase | [115, 116, 122] |
| Esterase | [116] |
| Lysozyme | [115] |
| Cathepsin | [115, 122] |
| Ribonuclease | [115] |
| Lipase | [115] |
| Phospholipase $A_1$ and $A_2$ | [117] |
| Elastase | [120] |
| Hyaluronidase | [119] |
| Beta-glucuronidase | [115] |
| Catalase | [118, 121, 159] |
| *Arachidonic acid metabolites* | |
| Leukotriene $B_4$ | [123, 126] |
| Prostaglandin $E_2$ | [124] |
| Prostaglandin $F_2\alpha$ | [124] |
| *Growth factors* | |
| Platelet-derived growth factor | [127, 157, 179, 206, 218] |
| Interleukin-1 | [124–127, 132–135] |
| Fibroblast growth factor | [127, 130, 131] |
| Tumor necrosis factor | [125–127, 132, 133, 203–205, 207–209] |
| Transforming growth factor | [150, 208, 210–215] |
| NF-kappaB | [201, 219] |

chemilumenescence [137]. Furthermore, asbestos fibers and neutrophils interact to injure cultured human pulmonary epithelial cells in vitro through a mechanism that probably involves hydrogen peroxide production [139]. Fiber dimensions are once again an important factor, with long fibers producing greater neutrophil recruitment than short fibers [140].

A number of immune derangements have been described in individuals with asbestosis, as well as through in vitro studies of lymphocyte function that may contribute to asbestos pathogenesis [141–146]. However, the bulk of the immunologic abnormalities seen in vitro and in animal studies correlate poorly with clinical and radiographic parameters of asbestosis and may thus represent epiphenomena unrelated to the pathogenesis of asbestos-induced lung disease [142]. Moreover, low-dose cyclophosphamide treatment in a sheep model of experimental asbestosis accelerated, rather than suppressed, the fibrotic process [147]. These results suggest that some of the asbestos-induced immune responses may be adaptive rather than a direct contributor to fibrogenesis. While the dysregulation of the adaptive immune response may not contribute directly to fibrogenesis, the impaired cell-mediated immunity that develops in patients with asbestosis undoubtedly contributes to the increased susceptibility to neoplasia seen in these individuals.

### 10.4.5 Fibroblasts

The mesenchymal cell population is an active participant in remodeling of the lung in idiopathic pulmonary fibrosis, asbestosis, and asbestos animal models. Fibroblast expansion and excessive productivity of matrix components are features of pulmonary fibrosis [148, 149]. Myofibroblasts appear during the active phases of fibrosis, and the presence of these specialized cells has been documented in both human pulmonary fibrosis and animal models [148–151], often forming active clusters called fibroblastic foci [149]. Spindle-shaped myofibroblasts are characterized by the expression of α-smooth muscle actin, increased collagen production and cytokine gene expression, and increased contractile properties [152]. Myofibroblasts are potentially derived from three origins: (1) fibroblasts directly differentiating into myofibroblasts by gaining additional characteristics of smooth muscle cells, (2) epithelial-mesenchymal transition (epithelial cell transdifferentiation to myofibroblasts) [153, 154], and (3) from circulating fibrocytes or bone marrow progenitor cells [155].

It is possible that asbestos fibers may exert a direct effect on fibroblasts through the transport of fibers to the interstitium, where they may persist for prolonged periods [91]. In vitro studies in which a normal fibroblast cell line derived from rat lung was exposed to various concentrations of crocidolite asbestos showed enhanced synthesis of total cellular collagen per ng of DNA [129]. Collagen deposition by fibroblasts in vivo has been confirmed in rat and mouse models exposed to crocidolite asbestos as assessed by hydroxyproline content [96]. In addition to increased synthesis, collagen turnover may also be an important contributor to fibrosis [156]. Asbestos-induced production of cytokines and cell-modulating proteins are likely closely connected to the production of collagen by fibroblasts and are discussed in detail in later sections. It has been shown that exposure of rat lungs to chrysotile asbestos results in upregulation of PDGF-receptor mRNA and protein [157]. Furthermore, it has recently been suggested that asbestos exposure induces the production of antibodies that are capable of activating the PDGF-receptor pathway contributing to fibroblast to myofibroblast differentiation [158].

### 10.4.6 Cytotoxicity

There is abundant evidence both in vitro and in vivo that asbestos is directly cytotoxic to a variety of cells and tissues [159, 160]. The mechanisms of asbestos-induced cytotoxicity were first explored in red blood cells [161–163] and later in cell and tissue cultures [29, 30, 164, 165]. Photoelectron spectrometry analysis demonstrated that phospholipid membranes are adsorbed as a bilayer onto the surface of

chrysotile asbestos fibers [163]. Scanning electron microscopic examination of red blood cells treated with chrysotile asbestos showed distortion of the cells, and this effect was almost totally ablated by pretreatment of the cells with neuraminidase [161]. Similar observations have been reported for the binding of chrysotile fibers to alveolar macrophages in vitro [166]. These studies suggest that chrysotile binds to sialic acid residues of membrane surfaces. In contrast, neuraminidase treatment had no demonstrable effect on crocidolite binding. Other investigators using cell cultures of rat tracheal epithelium concluded that membrane damage was only a minor component of fiber-induced toxicity and that a sequence of fiber binding, phagocytosis, nuclear damage, disruption of mitosis, and inhibition of proliferation or cell death is an important alternative pathway of fiber toxicity [101, 167].

Although all forms of asbestos have been shown to be cytotoxic in vitro, results have varied as to which fiber type is the most cytotoxic. Early studies indicated that the order of cytotoxicity is chrysotile > crocidolite > amosite [165]. Other studies indicated that the order of cytotoxicity depends on the target cell type [164]. Most of these studies compared fiber toxicity on an equal-mass basis. However, when cytotoxicity in a cultured fibroblast cell line is compared on an equal-number basis (i.e., equal numbers of fibers per dish), it is found that crocidolite is more potent in causing cell death than chrysotile [168]. Of particular interest is the observation that erionite, a fibrous zeolite that is a potent cause of mesotheliomas in humans, is several orders of magnitude more potent on an equal-number basis in causing cell death than either crocidolite or chrysotile [168]. Erionite has also been shown to cause similar pathologic endpoints as asbestos including mesothelioma, pleural plaques, and pulmonary fibrosis [169]. Fiber size is also an important factor, with longer and thinner fibers having the greatest cytotoxic effect [164]. Fibers with lengths greater than 8–10 μm and diameters less than 0.25 μm result in greater induction of ornithine decarboxylase activity in tracheal epithelial cells [170] and greater generation of reactive oxygen species in alveolar macrophages [110] than is observed with shorter, blunter fibers.

## 10.4.7 Oxidative Stress

### 10.4.7.1 Reactive Oxygen Species

One mechanism by which asbestos can injure cells is through the generation of reactive oxygen species [171], which can produce alterations in membrane fluidity, lipid peroxidation [171, 172], breakage of DNA, and induction of programmed cell death [173]. Asbestos fibers have been shown to directly generate reactive oxygen species such as superoxide [174], but may also catalyze the production of hydroxyl radicals and superoxide anions from hydrogen peroxide in cell-free systems as well as in animal models [175], which may occur by a modified Haber-Weiss (Fenton-like) reaction [176–178]. These iron-catalyzed reactions may play key roles in the pathogenesis of asbestos-induced tissue damage. For example, in rat tracheal explants treated with asbestos fibers that were increasingly loaded with $Fe^{++}$/$Fe^{+++}$, increases in procollagen gene expression were evident after 7 days of treatment. Increased iron loading also resulted in increased expression of mitogenic and fibrogenic cytokines such as PDGF-α and TGF-β but did not affect the expression of other cytokines such as PDGF-β, TNF-α or TGF-α [179]. Phytic acid, an iron chelator, reduces asbestos-induced hydroxyl radical generation, DNA strand breaks, and injury to pulmonary epithelial cells [180]. When asbestos fibers were pretreated with phytic acid, fewer alveolar macrophages (AM) and polymorphonuclear leukocytes (PMN) accumulated in the BAL fluid of treated rats [180]. In addition, pretreatment of asbestos fibers with another iron chelator, deferoxamine, prevented AM-induced superoxide release [181] and asbestos-induced cell death of alveolar type I and II cells [173]. Notably, some flavonoid compounds such a quercetin and dihydroquercetin can also protect rat peritoneal macrophages against oxidative cellular injury through both their ability to scavenge superoxide and their ability to chelate iron [182]. ROS have recently been shown to be key activators of the Nalp3 inflammasome of the lung through NADPH oxidase [183].

A recent study has also demonstrated that asbestos fibers can directly activate latent TGF-β in vitro [174]. Addition of superoxide dismutase prevented this activation of TGF-β. As TGF-β

is known to be important in the pathogenesis of asbestosis (see below), this oxidative activation of TGF-β is another mechanism in which asbestos-induced reactive oxygen species contribute to the pathogenesis of asbestosis.

#### 10.4.7.2 Reactive Nitrogen Species

A second pathway for ROS generation independent of the metal-catalyzed pathways has also been described [184]. Various cell types including lung endothelial, alveolar, and airway epithelial cells, as well as macrophages, produce both nitric oxide (NO) [185] and superoxide anion ($O^{-2}$) [181] in response to asbestos. These two radicals can react with one another at diffusion-limited rates to produce the highly toxic peroxynitrite anion ($ONOO^{-}$), which can oxidize or nitrate specific amino acids in key lung proteins and inhibit their function [186]. The nitration of proteins is seen in inflammatory states, and studies have shown that elevated levels of nitrosylated proteins occur after inhalation of asbestos fibers in rat lungs. In inhalation and intratracheal instillation studies, rats exposed for 2 weeks to chrysotile or crocidolite asbestos showed significant increases in inducible nitric oxide synthase protein levels [110] and activity [185] as well as strong nitrotyrosine staining [187]. In addition, macrophages also show an increase in iNOS mRNA in response to asbestos [188]. Alveolar macrophages from these rats generated increased levels of nitrite/nitrate [185, 187, 188] which was inhibited by NG-monomethyl-L-arginine (NMMA), an inhibitor of nitric oxide synthase [188]. These findings suggest that asbestos inhalation can induce nitric oxide synthesis and peroxynitrite formation in vivo.

### 10.4.8 Enzymatic and Nonenzymatic Antioxidants

As discussed above, ROS play an integral part in the pathogenesis of asbestos-mediated disease. Studies exploring the effectiveness of both enzymatic and nonenzymatic antioxidant scavengers on the cytotoxic effects of asbestos can shed significant light on the mechanisms involved in the development of asbestosis [189]. Under pathologic conditions, significant depletion of glutathione (GSH) and alterations in other GSH redox system enzymes were observed in both the alveolar macrophages and lung tissue of chrysotile-exposed animals [190]. In addition, both short- and long-term exposure of Wistar rats to asbestos fibers resulted in decreased levels of antioxidants (ascorbic acid, retinol, α-tocopherol, glutathione peroxidase) and increases in markers of lung injury (lipid peroxidation, total protein, alkaline phosphatase) [191]. However, studies examining rats exposed to crocidolite asbestos demonstrated increased mRNA expression of several antioxidant enzymes including Mn- and Cu/Zn-superoxide dismutases, glutathione peroxidase, and catalase [192–194]. Protein levels and enzymatic activity of these antioxidants after similar exposures were also found to increase [192, 194, 195], suggesting a compensatory mechanism for tissues under oxidative stress.

The potential importance of ROS is well illustrated by the prevention of asbestos-induced cell death in rat lung fibroblasts and alveolar macrophages by catalase, Mn- and Cu/Zn-superoxide dismutase, glutathione peroxidase, and dimethylthiourea, all scavengers of reactive oxygen species [181, 189, 196]. In an in vivo model of crocidolite-induced pulmonary interstitial fibrosis, the absence of extracellular superoxide dismutase leads to enhanced fibrosis, related to several mechanisms including increased oxidative stress [78, 187], oxidative degradation of the extracellular matrix [197, 198], and enhanced inflammation [197, 199, 200]. Furthermore, it has been shown that intratracheal treatment with purified extracellular superoxide dismutase can prevent these pathways in the asbestos-induced mouse model of pulmonary fibrosis [197]. Also, continuous administration of polyethylene glycol-conjugated catalase has been shown to significantly reduce the inflammatory response and severity of fibrosis secondary to inhalation of aerosolized asbestos fibers [159].

### 10.4.9 Cytokines, Growth Factors, and Cellular Signaling

Much progress has been made with regard to the effect of soluble mediators produced and secreted

by macrophages on the proliferation of other lung cells. It is now known that several cytokines including TNF-α, TGF-β, PDGF, and IL-1 are involved in triggering both the initial inflammatory reaction and the later formation of fibrotic lesions. A summary of the importance of several of these mediators is provided below.

### 10.4.10 TNF-α

In intratracheal instillations studies in rats, alveolar and pleural macrophages isolated from animals exposed to asbestos show increased production of the cytokine tumor necrosis factor alpha (TNF-α) [132, 133]. TNF-α production by macrophages can be stimulated by a variety of asbestos fibers and can initiate a cascade of responses involving adhesion molecule expression and production of chemotactic cytokines which ultimately result in the infiltration of inflammatory cells into sites of tissue injury in the respiratory tract [201, 202]. Several studies have shown that TNF-α can stimulate chemokine expression in both immune and nonimmune cells. It appears that TNF-α production by alveolar macrophages is biphasic, because studies examining the effects of intratracheal injection of chrysotile asbestos in rats demonstrated a significant decrease in both TNF-α mRNA and protein levels at 1 and 3 weeks postexposure, but higher levels of TNF-α by 6 weeks postexposure [203]. Similar results were also obtained for TNF-α production in pleural macrophages [204]. Since TNF-α seems to increase over time postexposure, investigators have hypothesized that this cytokine is primarily important for the development of chronic inflammation and fibrosis [133, 205].

TNF-α may play a key role in modulating the expression of other inflammatory and fibroproliferative cytokines. Studies involving TNF-α receptor knockout mice have shown that these mice fail to develop fibroproliferative disease in response to asbestos exposure, even though the levels of TNF-α gene expression and protein production increase on exposure of the knockout animals to asbestos. In situ hybridization studies on lung tissue from the knockout mice demonstrate reduced TGF-α, TGF-β, and PDGF expression upon asbestos treatment [206]. It is thought that TNF-α may mediate its effects through induction and activation of other growth factors, which in turn control cell growth and matrix production [207, 208]. TNF-α may also regulate other cytokines and chemokines such as MCP-1 [209] and may act synergistically with IL-1β to promote pulmonary inflammation and fibrosis [205, 209].

### 10.4.11 TGF-β and PDGF

Studies have demonstrated that TGF-β is rapidly upregulated specifically at sites of asbestos fiber deposition (particularly bronchiolar-alveolar duct regions) in the lungs of rats exposed to asbestos and remain elevated for extended periods of time [210]. In situ hybridization studies have demonstrated mRNA for both proteins in fibrotic lungs. TGF-β is a profibrotic protein that is rapidly expressed in bronchiolar-alveolar epithelial cells, fibroblasts, and alveolar macrophages in exposed rats and mice [208, 211]. TGF-α is a potent mitogen for epithelial cells, while TGF-β, although inhibitory for fibroblast growth, stimulates extracellular matrix production [208, 212, 213]. Studies examining lungs of sheep treated intratracheally with chrysotile asbestos showed increased immunohistochemical staining for all three TGF-β isoforms in fibrotic lesions, associated with areas of extracellular matrix deposition and little staining of the interstitial cells. This study also demonstrated prominent IGF-1 staining in macrophages and proliferating epithelial cells, but not in the extracellular matrix. It is believed that TGF-β and IGF-1 have complementary roles in stimulating interstitial fibroblast proliferation and new collagen deposition in active fibrotic lesions [214]. TGF-β has multiple functions including inducing the expression of collagens, proteoglycans, and matrix components by fibroblast/myofibroblasts [150, 215]; is chemotactic to macrophages and fibroblasts; and can stimulate further cytokine production. Studies show that increased expression of this protein in alveolar epithelial cells leads to the development of fibrotic lesions [215]. In addition, reactive

oxygen species produced by asbestos fibers have been shown to activate profibrotic TGF-β in the lung [174]. TGF-β has also been shown to regulate inflammatory responses. It can drive T cells toward a $T_H$-helper 17 phenotype by increasing the cellular response to cytokines, such as IL-23, which promote the resolution of inflammation [216]. It also decreases superoxide production by macrophages [217], which have a key role in inflammatory responses.

Platelet-derived growth factor (PDGF) may also contribute to the development of asbestos-induced lung disease. Alveolar and pleural macrophages are known to secrete growth factors, including PDGF, that stimulate proliferation of fibroblasts [218]. PDGF-α and its matching receptor (PDGF-Rα) are upregulated in rat lung fibroblasts after exposure to chrysotile asbestos in vitro, which leads to fibroblast proliferation. There is also increased expression of PDGF-Rα mRNA and protein in asbestos-exposed rat lungs in vivo. Immunohistochemistry studies have shown that the receptor is located in the interstitial and subpleural regions of the lung. This suggests that a potent lung cell mitogen, PDGF-α, and its receptor are upregulated prior to the development of a fibroproliferative lesion and may play a key role in asbestos-induced lung fibrosis [157].

### 10.4.12 Other Signaling Pathways

Asbestos fibers can trigger alterations in gene expression in the lung by initiating signaling events upstream of gene transactivation. There have been at least two signaling cascades linked to activation of transcription factors that are stimulated after exposure of lung cells to asbestos fibers in vitro and in vivo. These include the NF-kB pathway and the MAPK signaling cascade, which ultimately leads to activation of the transcription factor AP-1. Both NF-kB and AP-1 bind to specific DNA sequences within the regulatory or promoter regions of genes that are critical to cell proliferation and inflammation [219]. The murine chemokine MIP-2 is expressed in response to inflammation induced by asbestos fibers in both epithelial cells and macrophages in the rodent lung. Notably, MIP-2 is regulated by NF-kB [201]. Crocidolite exposure causes a dose- and time-dependent induction of AP-1 activity both in vitro and in vivo. Initial activity was noted at 2 days postexposure and was increased tenfold over control by day 3. AP-1 upregulation appeared to be mediated through the activation of MAPK family members including Erk-1 and Erk-2 [220]. Activation of ERK has been associated with asbestos-induced apoptosis and proliferation in mesothelial and alveolar epithelial cells. ERK phosphorylation increases with the accumulation of inflammatory cells in the lung and in areas of fibrosis [221].

### 10.4.13 Matrix Deposition and Matrix Metalloproteinases

The extracellular matrix (ECM) is critical for maintaining a strong structure that can withstand mechanical stretch and recoil of the lung. Matrix deposition occurs primarily through fibroblasts and myofibroblasts but can involve endothelial and epithelial cells. The ECM is composed of various molecules including collagen, elastin, fibronectin, proteoglycans, hyaluronan, and laminin [222]. Pulmonary fibrosis induced by asbestos is characterized by often drastic changes in the extracellular matrix, which can be the result of excessive deposition of collagen, an impairment in ECM degradation and resolution or a combination of these two. Thus, ECM changes become very complex over the pathogenic course.

ECM degradation and turnover is regulated by the activity of matrix metalloproteinase enzymes (MMPs) and their tissue inhibitor counterparts (TIMPS). MMPs are matrix-degrading proteinases (currently a total of 22) that have been shown to be upregulated in models of pulmonary fibrosis [223, 224]. The substrates of MMPs are extracellular matrix components and soluble factors and include, but are not limited to, the following: (1) MMPs 1, 8, and 13 are collagenases targeting collagens I, II, III, VII, and X, gelatin, and pro-TNF-α; (2) MMPs 2 and 9 are gelatinases targeting type IV and V collagen, gelatin, elastin, fibronectin, pro-TGF-β, and

pro-TNF-α; (3) MMPs 3, 10, and 11 are stomelysins that target proteoglycans, laminin, fibronectin, gelatin, and pro-TNF-α; and (4) MMP 7 (matrilysin) targets proteoglycans, collagens, laminin, decorin, gelatin, and fibronectin [225]. The majority of MMPs are synthesized as proenzymes and activated by proteolysis of a cysteine-zinc pro-domain, called a "cysteine switch" [225, 226]. Reactive oxygen species are also capable of activating MMPs, increasing their transcription, and deactivating proteases [226–228]. Thus, oxidants may play a significant role in unregulated activity of MMPs in pulmonary fibrosis. Tissue inhibitors of metalloproteinases (TIMPs 1–4) are extracellular or membrane-bound enzymes that bind tightly to MMPs to inhibit their degradative activity [225].

### 10.4.14 Summary of Asbestos-Induced Fibrogenesis

Based on the foregoing discussion, a hypothetical scheme can be proposed for the mechanism of asbestos-induced fibrogenesis [229–231]. It is evident that asbestos induces ROS-dependent tissue destruction, cell signaling, and matrix remodeling that involve multiple key mechanisms to lead to asbestos-induced pulmonary fibrosis including direct production of ROS or indirectly by frustrated phagocytosis via macrophages. These processes in turn lead to activated cell signaling, cytokine/growth factor production, and cycled inflammation. According to the pathogenesis scheme, asbestos is deposited on the alveolar surfaces, especially on first alveolar duct bifurcations, where transport across the epithelium begins almost immediately. Asbestos releases ROS that leads to matrix degradation, cell signaling, and activation, which results in chemoattraction of macrophages and neutrophils to the site of asbestos deposition. These inflammatory cells proceed to phagocytize the asbestos fibers, stimulating the release of further reactive oxygen species and various hydrolytic enzymes. Also released are factors that amplify the inflammatory response through the attraction of additional inflammatory cells. In addition, activated macrophages release growth factors that stimulate the replication of interstitial macrophages and fibroblasts. Asbestos fibers translocated to the interstitium produce tissue injury by a combination of generating reactive oxygen species and direct interaction with cellular membranes of interstitial macrophages and fibroblasts as well as ROS-induced matrix degradation and TGF-β activation. As a result of some combination of soluble growth factor release from macrophages and direct tissue injury by translocated asbestos fibers, fibroblasts are stimulated to replicate and to synthesize collagen and other extracellular matrix components in increased amounts. Ongoing release of growth factors by activated macrophages, epithelial cell death, and persistence of asbestos fibers within the interstitium would result in the continuing fibrogenesis long after the cessation of exposure. Figure 10.2 illustrates several of the pathogenetic mechanisms involved in the development of asbestosis.

## 10.5 Carcinogenesis

### 10.5.1 In Vivo Inhalation Studies

Inhalation studies in experimental animals have shown that asbestos produces neoplasms of the lung and pleura [232]. These include pulmonary adenoma (Fig. 10.3), adenocarcinoma, squamous cell carcinoma [5, 43, 67, 68], and malignant mesotheliomas of the pleura and peritoneum [5, 43, 68]. These tumors have a prolonged latency period (300 days or more in the rat and 50 weeks or more in the mouse) [232, 233], and there is some evidence of a dose–response relationship, with a greater incidence of tumors in rats exposed for 12 months as compared to 6 months, but no further increase in incidence from 12 to 24 months of exposure [5]. All types of asbestos, including chrysotile [5, 43, 67, 68], amosite [5, 67, 69, 234], crocidolite [5], anthophyllite [5], and tremolite [79], produce pulmonary and pleural neoplasms in experimental animals. In the classic studies of Wagner et al. [5], chrysotile was as potent as crocidolite in the production of mesotheliomas by dust inhalation, with four

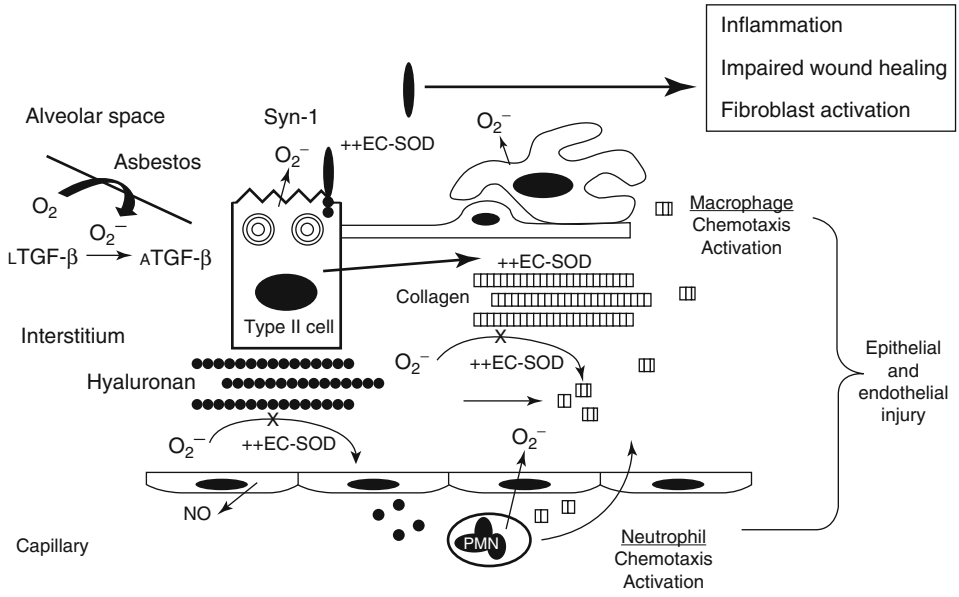


**Fig. 10.2** Hypothetical schema illustrating the pathogenesis of asbestos-induced pulmonary interstitial fibrosis. Fibers deposited on the surfaces of alveolar duct bifurcations stimulate the release of chemoattractants for alveolar macrophages and neutrophils. These cells phagocytose the fibers and become activated, releasing reactive oxygen species (ROS), arachidonic acid (AA) metabolites, and various growth factors. ROS can also be formed directly on the fibers. While antioxidants such as extracellular superoxide dismutase (*EC-SOD*) offer protection, once sufficient fibers are present, they can overwhelm natural defenses. ROS can then induce oxidative fragmentation of several components of the extracellular matrix including hyaluronan and collagen as well as shedding of syndecan-1 (*Syn-1*) from cell surfaces. These fragmented and shed components can then induce further pro-inflammatory and profibrotic responses including release and activation of TGF-β. TGF-β and other mediators then stimulate fibroblast replication and collagen synthesis, which eventuate in pulmonary interstitial fibrosis (i.e., asbestosis)

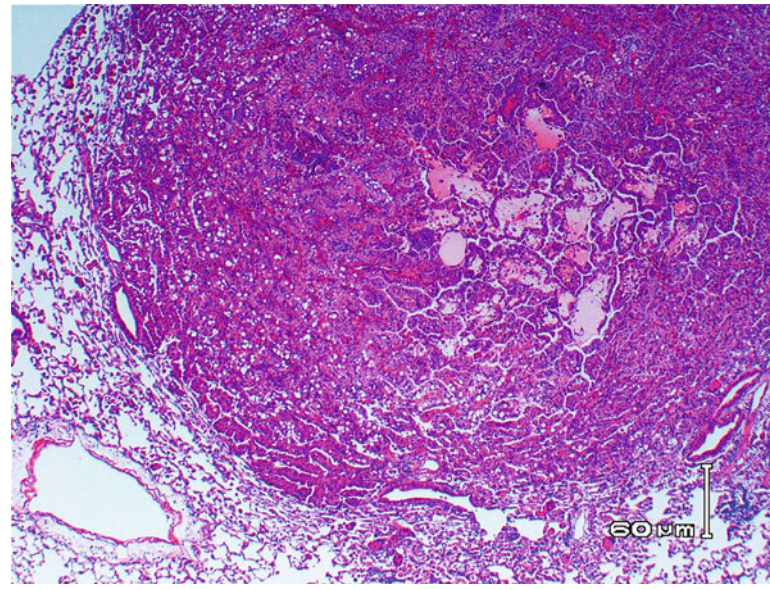


**Fig. 10.3** Pulmonary adenoma in a mouse lung

mesotheliomas developing in 137 animals at risk with chrysotile exposure and four in 141 animals at risk due to crocidolite. However, exposures were based on fiber mass, which means that the chrysotile-exposed animals received ten times as many fibers as the crocidolite exposed. Two animals developed mesotheliomas with only 1 day of exposure, one following exposure to crocidolite and the other, to amosite asbestos [5]. The mesotheliomas occurring in experimental animals exposed to asbestos are histologically, histochemically, and ultrastructurally similar to those occurring in humans (Fig. 10.4) [235, 236]. In addition, experimental pulmonary

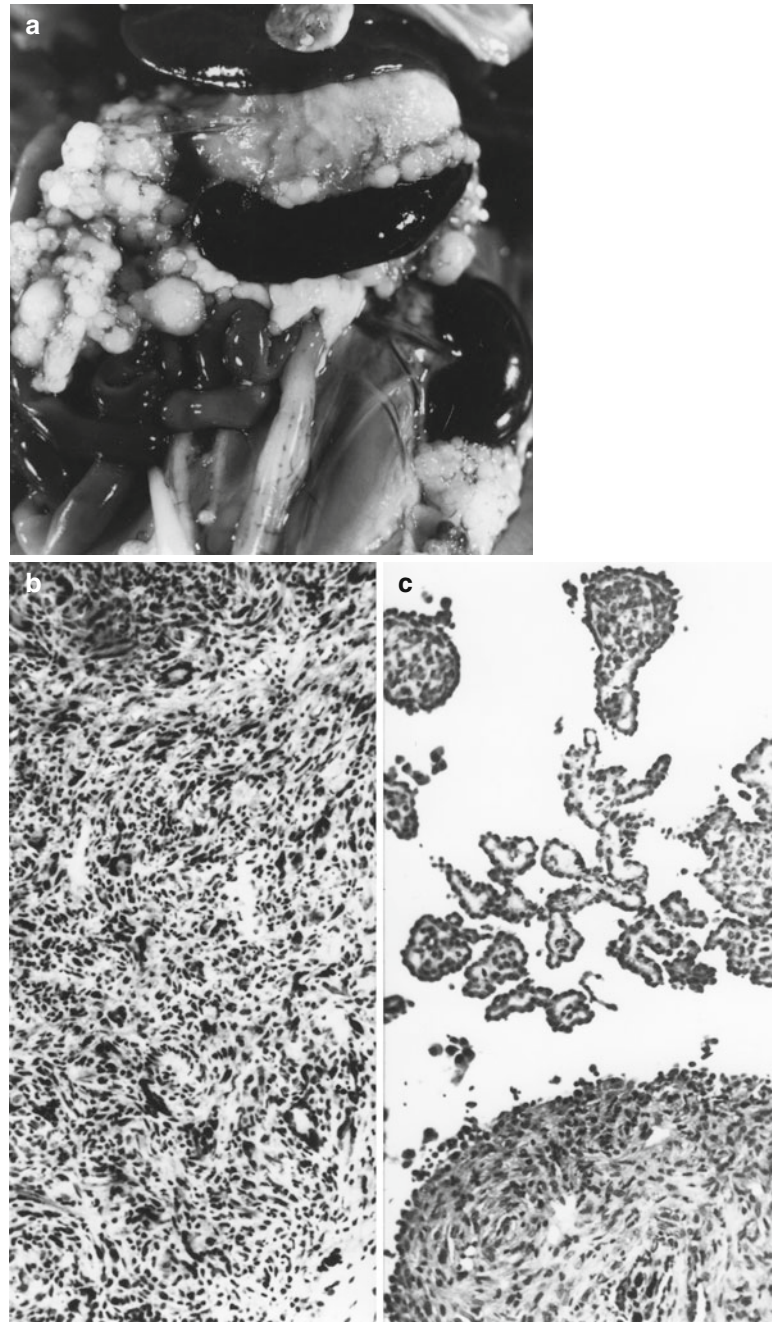


**Fig. 10.4** (**a**) Diffuse malignant mesothelioma developing in the abdomen of an asbestos-inoculated rat. These lesions exhibit a mixture of epithelial and fibrosarcomatous patterns (**b**, **c**) (Reprinted from Craighead [31], with permission)

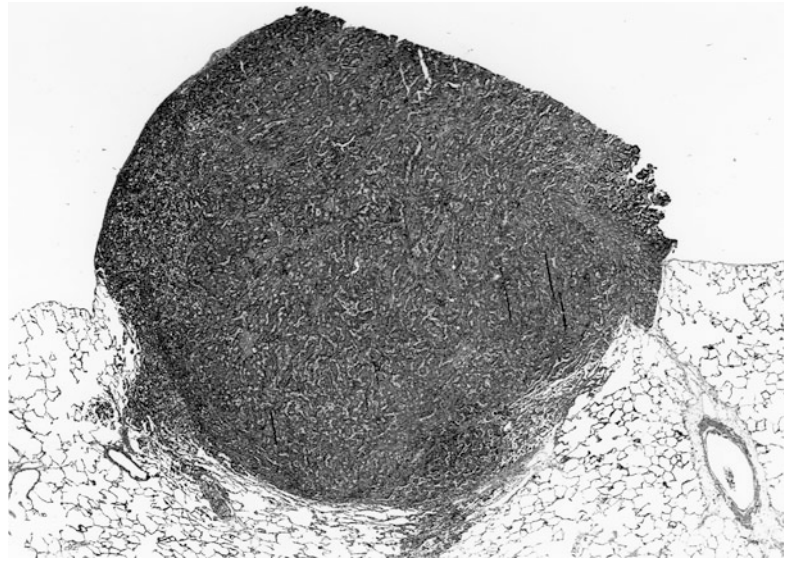

**Fig. 10.5** Pulmonary adenocarcinoma induced in a male Fischer 344 rat exposed to chrysotile asbestos. H&E, ×22 (Courtesy Dr. Gene McConnell, Raleigh, NC)

adenocarcinomas and squamous cell carcinomas are similar histologically to those occurring in humans [5, 69, 236]. However, one should always be cautious in extrapolating animal models to human risk assessments. Several studies have shown, for example, that humans suffer a tumor risk at approximately 100–1,000 times lower concentration of asbestos fibers than those needed to produce the same risk in a rat inhalation model [237, 238].

The studies reported by Wagner et al. [5] and by Davis et al. [43] both showed a close association between the severity of interstitial fibrosis (i.e., asbestosis) and the development of pulmonary neoplasms. This finding suggests that pulmonary parenchymal tumors in asbestos-exposed animals derive from a metaplastic and hyperplastic epithelial response in areas of interstitial fibrosis that in some instances progressed to neoplasia. Davis and Cowie [239] have addressed this question in greater detail. These authors note that when adenomas or very early carcinomas are found, they are frequently in the center of areas of advanced asbestosis with exuberant epithelial metaplasia/hyperplasia. In studies comparing the pathologic effects of various mineral fibers, there has also been a close association between the severity of pulmonary fibrosis and tumor development [102, 240, 241]. In an analysis of data from several different studies [43, 68], a strong correlation was observed between the percentage of lung occupied by fibrosis and the occurrence of pulmonary tumors ($p<0.001$) [239]. Tumors which developed in association with low-recorded levels of fibrosis (involving less than 4 % of the lung area) were either advanced tumors occupying a single lung lobe or early tumors originating from the center of areas of interstitial fibrosis (Fig. 10.5). While these studies support a role for fibrosis in the development of asbestos-associated tumors, they do not definitively answer the question as to whether fibrosis is an absolute prerequisite for the development of pulmonary tumors in experimental animals, which would require examination of a relatively large population of rats during the period of early tumor development [239]. Furthermore, the results may not be relevant to the great majority of lung cancers occurring in asbestos workers, in which cigarette smoke is an important cofactor [242, 243].

### 10.5.2 Role of Immune Function

The role of an impaired immune system in the development of asbestos-associated pulmonary tumors has developed growing interest as the field of tumor immunology expands. A normal immune system performs tumor surveillance and has the ability to both prevent and limit cancer

development. Notably, individuals with asbestosis have a number of immune derangements, including impaired cell-mediated immunity and hyperactive B-cell function, resulting in polyclonal hypergammaglobulinemia, elevated levels of secretory IgA, high frequency of autoantibodies and circulating immune complexes, and lymphoid neoplasms of B-cell lineage [141, 142].

One of the most important immune cells in tumor surveillance is the natural killer (NK) cell. This cell is derived from a lymphoid progenitor and is capable of eliminating mutated cells and controlling tumor growth [244]. One study found that NK cells were able to lyse and kill malignant mesothelioma cells in culture [245]. This function was impaired when researchers treated normal human NK cells with asbestos in vitro [246]. In a separate experiment, researchers isolated NK cells from the blood of patients with asbestosis. They found that the cells had decreased cytotoxic activity, despite the cells not having obvious interaction with the asbestos fibers [247–249]. In animal models, the instillation of chrysotile asbestos into the lungs of mice resulted in impaired function of their pulmonary NK cells resulting in decreased cytotoxic ability. Notably, a similar mechanism of impaired NK cells has also been found to contribute to pulmonary tumor formation in a mouse model of cigarette smoke injury [250]. This mechanism may in part explain why smokers exposed to asbestos have greater incidence of pulmonary carcinomas as compared to nonsmokers [251].

### 10.5.3 Role of Fiber Dimensions

Inhalation studies have indicated that in an analogous fashion to fibrogenic potential, long fibers have the greatest carcinogenic potential in experimental animal models [5]. Davis and Jones [68], using an amosite preparation with extremely few fibers greater than 5 μm in length, reported no tumors in rats following long-term inhalation, whereas a clear excess of lung carcinomas and pleural mesotheliomas developed in rats breathing an amosite cloud containing considerable numbers of fibers 5 μm or greater in length. Similar but less clear-cut results were obtained in a study of long and short preparations of chrysotile asbestos [68]. In this latter study, some longer fibers were still present in the "short-fiber" chrysotile preparation, although the "long-fiber" preparation (on an equal-mass basis) had five times as many fibers 5 μm or greater in length and 80 times as many fibers 30 μm or greater in length. Both long and short chrysotile preparations produced mesotheliomas in more than 90 % of rats following intraperitoneal injection of 25 mg. However, at a dose level of 2.5 mg, the short-fiber preparation produced only one-third as many mesotheliomas as the long-fiber preparation, which still produced mesotheliomas in more than 90 % of the animals injected. At a dose of 0.25 mg, the long-fiber preparation still produced tumors in 66 % of rats [68]. The dose of short-fiber chrysotile that resulted in no mesothelial tumors in 24 rats (injected intraperitoneally) was calculated to contain 57 million fibers greater than 8 μm in length [68]. Further complicating the carcinogenic potential of chrysotile are the relatively short half-lives of the longer, disease-causing fibers. Bernstein et al. conducted a 5-day inhalation study and found the half-life of chrysotile fibers >20 μm to be 16 days and the half-life of fibers 5–20 μm to be 29.4 days [252]. This was in contrast to amosite which had a half-life greater than 1,000 days for all lengths of fibers in rat lungs [253]. Studies using mineral fibers other than asbestos have also shown a strong association between fiber length and carcinogenicity [68, 241, 254–256]. Given all of these findings as well as other epidemiologic studies, the Agency for Toxic Substances and Disease Registry published an expert report in 2003 concluding that asbestos fibers less than 5 μm were "unlikely to cause cancers in humans" [257].

The classic studies of Stanton et al. [258, 259] showed that, in addition to fiber length, fiber diameter is also an important determinant of carcinogenic potential. The "Stanton hypothesis" has emphasized the dimension and durability of fibers with regard to carcinogenicity and states that, irrespective of chemical composition, the probability of developing mesotheliomas following implantation of mineral fibers into the pleural

cavity correlates best with the numbers of fibers 8 μm or greater in length and 0.25 μm or less in diameter [258]. Hesterberg and Barrett [260] reported that in vitro studies with cultured Syrian hamster embryo cells showed the transforming potency to be greatest for long, thin fibers. Furthermore, in organ cultures of rodent tracheobronchial epithelial cells, long fibers (≥8 μm) cause enhanced incorporation of tritiated thymidine, increased biosynthesis of polyamines, and increased amounts of squamous metaplasia and keratinization [159]. These effects are only observed with short fibers (≤2 μm) at several-fold higher concentrations. In a review of asbestos exposure indices, Lippman proposed, on the basis of the available data, that fibers 5 μm or greater in length and 0.1 μm or less in diameter are the most important in the production of mesotheliomas. In contrast, fibers 10 μm or greater in length and 0.15 μm or greater in diameter are the most important in the production of pulmonary carcinomas [75].

Although fiber dimensions may be the most well-characterized determinant of carcinogenic potential, several other fiber characteristics may contribute to the overall incidence of tumor formation. It has been suggested that surface properties of mineral fibers may be an additional contributing factor to carcinogenic potency [261, 262]. For example, fibrous erionite, which appears to have many times greater potential for mesotheliomas induction than asbestos, has an internal surface area (due to "pores" in the crystal lattice) of 200 $m^2/g$, as compared to a total surface area of 8–10 $m^2/g$ for crocidolite asbestos [263]. The mechanism by which this increased surface area enhances the carcinogenic potential of a fiber is unknown. Chemical composition of the fibers may also determine carcinogenic potential. Rats treated with amphibole asbestos fibers coated with either magnesium or cobalt had increased incidence of pleural mesotheliomas when compared to animals treated with uncoated fibers [264].

In addition to the abovementioned asbestos studies, there have been recent reports on the carcinogenic effects of various man-made mineral fibers, some commonly used as substitutes for asbestos. Some of these fibers, such as basic magnesium sulfate fiber [265] and kaolin refractory ceramic fibers (RCF) [266], produced significantly higher numbers of mesotheliomas and lung tumors in hamsters than chrysotile asbestos. On the other hand, other synthetic vitreous fibers such as fibrous glass or other insulation wools produced few if any tumors in these animals. These studies have shown that the fibers with the greatest biopersistence (i.e., least solubility) in vivo have the greatest carcinogenic potential.

### 10.5.4 Mechanisms of Asbestos-Induced Carcinogenicity

A great deal has been learned regarding the mechanisms by which fibers interact with cells and produce heritable alterations in cellular genetic material. Asbestos differs from most chemical carcinogens in that in in vitro studies it tests negative in bacterial mutation assays [267–269] and is not mutagenic in liver epithelial cells [270] or Syrian hamster embryo fibroblasts [271]. However, an in vivo study in which rats were treated with amosite by intratracheal instillation found that amosite did induce a mild mutagenesis, but only 16 weeks postexposure [272]. In this study, animals were treated intratracheally with amosite asbestos and then sacrificed at either 4 or 16 weeks. The researchers found an increased number of double-strand DNA breaks as well as an increase of approximately two-fold in the DNA mutation frequency compared to controls. The authors concluded that asbestos leads to slow and mild continual DNA damage. While the mutagenic potential is significantly less robust compared to other chemical carcinogens, asbestos fibers have the ability to persist in the lung tissue and cause DNA damage for longer periods. It is likely this cumulative effect that ultimately results in the development of a cancer.

A potential breakthrough in our understanding of asbestos-induced carcinogenicity is the development of methods for growing mesothelial cells in culture [273–276]. Cultured mesothelial cells have been shown to phagocytize chrysotile asbestos fibers in vitro [274], which results in a

slow leaching of magnesium from the chrysotile at a rate comparable to that which occurs in solution at pH 7 [52]. Chrysotile asbestos produces intense vacuolization of cultured mesothelial cells [277] and also induces morphologically transformed colonies [278]. Incubation of mesothelial cells with either chrysotile or crocidolite asbestos fibers prolonged the doubling time in culture, although this effect occurred at lower doses of chrysotile as compared to crocidolite. With either fiber type, asbestos fibers were often observed within dividing cells [277]. Wang et al. [279] used scanning electron microscopy to demonstrate the interaction between asbestos fibers and metaphase chromosomes of rat pleural mesothelial cells. Chromosomes were frequently entangled with, adherent to, or severed or pierced by long curvilinear fibers, and this effect was more pronounced for chrysotile than for crocidolite asbestos [279].

These observations are intriguing, considering that nonrandom chromosomal abnormalities, including translocations, rearrangements, and marker chromosomes, have been identified in both experimental (asbestos-induced) [280–282] and human malignant pleural mesotheliomas [283–285]. Furthermore, studies with nonneoplastic human pleural mesothelial cells in culture have shown aneuploidy with consistent specific chromosomal losses in mesothelial cells surviving two cytotoxic exposures with amosite fibers [280, 286]. These aneuploid cells exhibited altered growth control properties as well as a population doubling potential beyond the culture life span of control cells. Other studies using crocidolite, chrysotile, or amosite asbestos reported significant increases in numerical and/or structural chromosomal abnormalities in short-term cultured normal human mesothelial cells [287]. Also, crocidolite asbestos has been shown to induce sister chromatid exchanges in rat pleural mesothelial cells in vitro [288]. Specific DNA mutations, such as the formation of hydrophobic DNA adducts, have also been reported [289, 290].

Other in vitro approaches have also provided interesting information with respect to asbestos-induced carcinogenicity. Asbestos fibers have been shown to mediate the transfection of exogenous DNA into a variety of mammalian cells in vitro [291, 292]. Exposure of Chinese hamster ovary cells to crocidolite asbestos fibers in cell culture resulted in an increased frequency of multinucleated cells, and various mitotic abnormalities were observed in cells containing fibers 20 μm or greater in length [293]. Some studies have shown a strong correlation between fiber-induced cytotoxicity in a macrophage-like cell line and the probability of fiber-induced mesotheliomas [294], whereas others have reported no direct relationship between cytotoxicity and carcinogenic potency [295].

In vitro models have provided useful information regarding the early events of asbestos interaction with the mesothelium. Studies by Moalli et al. [296] using stereomicroscopy and scanning electron microscopy demonstrated the rapid clearance of short asbestos fibers through the opening of diaphragmatic stomata, whereas long fibers (60 % ≥2 μm in length) were trapped on the peritoneal surface, invoking an intense inflammatory reaction. This was associated with mesothelial cytotoxicity and regeneration at the periphery of asbestos fiber clusters. Maximal incorporation of tritiated thymidine by mesothelial cells occurred 7 days after exposure, and it was hypothesized that repeated episodes of injury and regeneration might promote the development of mesotheliomas [296]. Furthermore, asbestos fiber clusters on the peritoneal surface induce angiogenesis in the form of a capillary network radiating toward the center of the lesion, first notable 14 days after injection [297]. Recent studies detailing the early changes in the mesothelium following in vivo exposures to mineral fibers have been performed. Studies by Fraire et al. [298, 299] suggest that there may be a gradual progression from mesothelial hyperplasia/dysplasia to mesothelioma, with intermediate stages characterized by the presence of fibrous adhesions and gross pleural nodular lesions [298]. The capability of growing mesotheliomas as xenographs in athymic rodents [300–302] should enhance the opportunity for investigators to study the properties of malignant mesothelial cells.

Much of the foregoing discussion has focused on the carcinogenic effects of asbestos fibers on

mesothelial cells. However, asbestos is also carcinogenic for the respiratory epithelium and much knowledge has been gained by the study of the effects of asbestos on tracheal explants and organ cultures [29, 30]. Crocidolite asbestos causes necrosis and desquamation of surface epithelial cells, with subsequent basal cell hyperplasia and squamous metaplasia [29, 303]. Furthermore, asbestos-induced squamous metaplasia is inhibited by retinoids (retinyl methyl ether) [304] and vitamin C (ascorbic acid) [305]. These studies may have implications regarding the prevention and prophylaxis of respiratory tract malignancies in workers who have been heavily exposed to asbestos in the past.

### 10.5.5 The Effect of Asbestos on Tumor Suppressors and Oncogenes

Given the relatively low incidence of malignancy in people exposed to asbestos, it is unlikely that asbestos causes a "single hit" leading to the development of carcinomas and mesotheliomas. It is more likely that asbestos injury in a predisposed state results in malignancy or given the long period of latency results in alterations in the genetic environment ultimately culminating in malignancy. Most of the early experimental models utilized only asbestos rather than some combination of injury or genetic predisposition. This provided cleaner models to study but like the human disease resulted in low incidence of tumors with some studies reporting as low as 0 % and few going beyond 20 % of animals developing tumors. This led to the idea that the development of asbestos-induced cancers in rodents, like in humans, may require an adjuvant injury or genetic predisposition. Numerous studies have investigated the role of oncogenes and tumor suppressors on the role of asbestos-induced cancers.

One tumor suppressor that was found to be inactivated in human mesothelioma was *NF2* which is typically associated with the genetic syndrome of neurofibromatosis [306]. Investigators wanted to determine if loss of *NF2* was a result of asbestos injury or the cause of malignancy. Fleury-Feith et al. [307] utilized a mouse with heterozygous NF2 expression to study the effect of asbestos. They found that after intraperitoneal injection of crocidolite that 46.5 % of the NF2 +/− animals versus 16.7 % of the NF2 +/+ animals developed peritoneal tumors. This suggested that lack of *NF2* would predispose those exposed to asbestos. Indeed, a case report published in 2002 described a patient with NF2 and asbestos exposure who developed malignant mesothelioma [308]; however, additional controlled studies are needed to determine if NF2 alterations are directly contributing to mesothelioma pathogenesis. These findings however did not exclude the possibility of asbestos leading to the loss of the tumor suppressor gene. The researchers found that asbestos had led to the inactivation of the remaining wild-type NF2 allele in 86 % of malignant cells in the ascitic fluid of the treated mice [307]. Altomare et al. [309] utilized these animals in a second set of experiments and found that NF2 +/− mice had decreased survival (44 versus 56 weeks) and increased incidence of tumors at 52 weeks (85 % versus 33 %). Additionally, in their experiments, 50 % of the wild-type mice developed total inactivation of the *NF2* gene on both alleles. This inactivation led to the loss of function in other tumor suppressor genes as well as AKT activation, a well-known hallmark of malignant mesothelioma making it a biologically relevant experimental model.

A second tumor suppressor gene implicated in malignant mesothelioma is *Arf*. This tumor suppressor has been implicated in numerous other cancers, most notably melanoma. Utilizing a model similar to their previous study, Altomare et al. [310] exposed Arf (+/−) mice and wild-type controls to repeated intraperitoneal injections of crocidolite asbestos. They found that the mice heterozygous for *Arf* had reduced survival and loss of heterozygosity of their remaining copy of the Arf gene. However, unlike in the previous studies of *NF2*, they did not find that this led to the alteration of other tumor suppressor genes.

Asbestos has also been found to mediate its carcinogenicity via a p53-mediated pathway. p53 has been shown to mediate cell cycle arrest as well as DNA repair and is one of the most studied

tumor suppressors. Vaslet et al. [311] treated p53 (+/–) and wild-type mice with repeated intraperitoneal injection of crocidolite asbestos and found that heterozygosity for p53 led to decreased latency with 76 % of the heterozygous mice having mesothelioma at 44 weeks versus 32 % of the wild-type mice at 67 weeks. In addition, they found that asbestos injury led to loss of the p53 wild-type allele in 50 % of the heterozygous mice resulting in increased tumor size and invasion compared to those tumors that still expressed the p53 protein. These three studies demonstrate that while asbestos is capable of inducing malignancy in a predisposed genetic state, it is also capable of altering the genetic environment to make the host more susceptible to the development of malignancy.

More recently, germline mutations in the BAP1 gene have been linked to familial malignant mesothelioma [312]. Heterozygous individuals for BAP1 mutations have a very high risk of developing mesothelioma and uveal melanoma [312]. BAP1 is a tumor suppressor gene that codes for a deubiquitinating enzyme. The deubiquitinating function and the nuclear localization are important in modulating the tumor suppressor activity of this protein [313]. However, the exact target of BAP1 remains unknown. BAP1 appears to induce early exit out of G1 causing accumulation of DNA damage and cell death. Carbone et al. have proposed that BAP1 may help prevent environmental carcinogenesis by asbestos by influencing DNA damage/repair. Notably, BAP1 mutations have also been identified in 25 % of sporadic mesotheliomas [314] and are rare in other cancers other than uveal melanomas [313–316]. This suggests that BAP1 may play an important role not only in familial mesotheliomas but also in nonfamilial mesothelioma as well.

One of the challenges that have plagued researchers' ability to study mesothelioma has been the low incidence of malignancy in experimental asbestos models. This has made it difficult to further elucidate the pathogenesis and study therapeutic approaches. Robinson et al. [317] generated a transgenic mouse that expressed the oncogene SV40 large T antigen downstream of the mesothelin promoter. Utilizing this genetic model and two intraperitoneal injections of 3 mg of crocidolite asbestos separated by 1 month, they were able to increase the incidence of mesothelioma from 20–30 % up to 100 % as well as reduce the time to disease onset from 50–100 weeks down to 20–40 weeks [233]. The mice had no expression of the transgene at baseline or with intraperitoneal injection of the pro-inflammatory agent thioglycolate. Therefore, the malignant potential was not merely in response to inflammation but the asbestos. By combining the carcinogenic potential of asbestos with a known powerful oncogene, the researchers were able to create a more reliable animal model for the study of malignant mesothelioma.

### 10.5.6 The Effect of Smoke Exposure on Asbestos Injury

In the previous discussion, mechanisms by which asbestos fibers might interact directly with DNA and chromosomes, and thus as an initiator of carcinogenesis, were emphasized. However, there is considerable epidemiologic data indicating that, with respect to carcinoma of the lung, asbestos interacts in a multiplicative fashion with cigarette smoke to enhance greatly the rate of neoplastic transformation. In this sense, asbestos behaves as a classic promoter of carcinogenesis. Numerous studies have explored various mechanisms by which asbestos could interact with cigarette smoke components in the process of carcinogenesis [318].

One mechanism of interaction might be the adsorption of polycyclic aromatic hydrocarbons or other carcinogenic compounds within cigarette smoke onto the surface of the asbestos fiber, which then could act as a carrier particle, providing prolonged and intimate contact of the adsorbed carcinogens with respiratory epithelial cells. In vitro studies have demonstrated the adsorption of benzo[a]pyrene, nitrosonornicotine, and N-acetyl-2-aminofluorene onto the surface of all types of asbestos as well as other mineral fibers, with chrysotile binding significantly more

carcinogen than the other mineral fibers tested [319]. It has also been shown that intratracheal instillation of either chrysotile or amosite asbestos concomitantly with benzo[a]pyrene over a 6-week period significantly increased the number of tumors in hamsters as compared to any of these treatments alone [320]. Carcinogen binding is greatly enhanced by the prior adsorption of phospholipids (such as occur in surfactant) onto the asbestos fibers [321]. Studies using cell and organ cultures of tracheobronchial epithelium exposed to asbestos with and without adsorbed 3-methyl-cholanthrene (3-MC) show increased aryl hydrocarbon hydroxylase activity in cells treated with both asbestos and 3-MC as compared to 3-MC alone [322]. Furthermore, asbestos fibers and adsorbed carcinogens display a synergistic effect in the production of cell transformation in BALB/3 T3 cells in vitro [323] and in the formation of malignant tumors from treated tracheal explants that were subsequently implanted into syngeneic animals [324]. Intratracheal instillation studies using chrysotile administered concomitantly with subcutaneously injected N-nitrosoheptamethyleneimine have also demonstrated a synergistic effect between these two agents in the induction of pulmonary neoplasms in rats [325]. Although cigarette smoking is not thought to be a cofactor in the production of malignant mesotheliomas in humans, the administration of 3-MC along with chrysotile asbestos by intrapleural or intraperitoneal injection in rats greatly enhances the production of mesotheliomas over that observed by chrysotile injection alone [326].

Additional mechanisms whereby asbestos might interact with cigarette smoke or other environmental agents have also been explored. A novel hypothesis regarding the synergistic effect between asbestos fibers and polycyclic aromatic hydrocarbons suggests that the adsorption of lung surfactant phospholipids onto asbestos fibers [167, 321] provides the opportunity for lipophilic carcinogens to diffuse within an all-lipid environment, with the asbestos fiber behaving like a bronchial lining layer [327]. Others have reported results indicating that cigarette smoke and asbestos synergistically increase DNA damage and cell proliferation [328, 329] possibly by means of reactive oxygen species, such as hydroxyl radical formation [114, 328]. Furthermore, cigarette smoke potentiates the uptake of asbestos fibers by the tracheobronchial epithelium [59]. This latter effect is blocked by prior treatment with superoxide dismutase, catalase, and deferoxamine, which are inhibitors of reactive oxygen species [330]. Studies have also suggested that asbestos might interact with ionizing radiation in the process of oncogenic transformation [331]. In this regard, ionizing radiation has been reported to augment the production of mesotheliomas in rats injected with chrysotile asbestos as compared to animals treated with chrysotile alone [326].

### 10.5.7 The Role of Age on Malignancy Potential

The age of exposure to asbestos and its effect on the development of mesothelioma was recently investigated in a retrospective study in Australia. Reid et al. [332] discovered that people who had their first exposure to asbestos at less than 15 years of age were less likely to develop mesothelioma even when exposed to similar fiber burdens. While no exact cause for this phenomenon was identified, these results support the findings of Berry and Wagner [333] who demonstrated that older rats were more susceptible than younger rats to the carcinogenic potential of asbestos. This study gave intrapleural injection of asbestos and found both a higher incidence (39.6 % versus 18.9 %) and shortened time to onset of 4 months in the development of mesotheliomas when using older rats. This phenomenon was replicated in mice where older mice had more rapid development of their mesotheliomas [233]. While no exact cause for this has been identified, most have speculated that younger species have increased biological resilience and better repair mechanisms compared to older tissues. This poorly understood yet well-documented finding has numerous implications for those conducting asbestos research in vivo.

### 10.5.8 Summary of Carcinogenesis

It appears that asbestos is a complete carcinogen, possessing both initiating and promoting properties [267, 309–311, 334]. The carcinogenic potential of the various fiber types remains debated, but most agree that longer asbestos fibers have a greater carcinogenic potential and amphiboles more so than chrysotile. The carcinogenic mechanisms are multifactorial and include inactivation of tumor suppressor genes, activation of oncogenes, mutagenesis, and external environmental factors. In addition, suppression of cell-mediated immune function and age may contribute by decreased capacity for tumor surveillance and cellular repair, respectively.

In the tracheobronchial tree, asbestos acts primarily as a promoter agent. Important steps in this process include epithelial cell injury, with subsequent basal cell hyperplasia and squamous metaplasia, cocarcinogenic effect of asbestos as a carrier for polycyclic aromatic hydrocarbons, and stimulation of DNA synthesis [322]. Squamous metaplasia interferes with mucociliary clearance mechanisms and thus may encourage the transepithelial uptake of fibers otherwise cleared from the lung. This uptake in turn would bring fibers in contact with basal epithelium, where these cells would then be exposed to any carcinogens adsorbed to the surface of the asbestos fibers [335]. Asbestos exposure alone may also produce carcinomas in the lung periphery through the poorly understood mechanism of interstitial fibrosis with bronchiolar and alveolar cell hyperplasia proceeding to neoplastic transformation [239, 334].

In the pleural and peritoneal cavities, asbestos acts like a complete carcinogen, exhibiting both initiating and promoting activities [334]. A critical step appears to be the transport of durable fibers of appropriate dimensions to the pleura, through either the air spaces or the interstitial lymphatics (or both) [31]. Peritoneal transport mechanisms include direct penetration of the intestinal wall by swallowed fibers or diaphragmatic penetration. Once fibers have come into contact with mesothelial cells, these cells seem to be particularly susceptible to asbestos-mediated cellular injury, as compared, for example, to bronchial epithelial cells or fibroblasts [286].

Important steps in the neoplastic transformation of mesothelial cells probably include cellular injury with DNA damage and mutation. The mechanism by which this occurs is still not fully understood, but either direct interaction of asbestos fibers with mesothelial cells during cellular division or indirectly through the generation of reactive oxygen species by asbestos fibers is likely.

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# Analysis of Tissue Mineral Fiber Content

# 11

Victor L. Roggli and Anupama Sharma

## 11.1 Introduction

The development of techniques for assaying the mineral fiber content of tissues has provided the opportunity to correlate the occurrence of various fiber-related diseases with the cumulative fiber burdens in the target organ. Exposure to mineral fibers generally occurs through the inhalation of airborne fibers, and thus the respiratory tract is the site of most asbestos-related diseases. Consequently, most studies of tissue fiber burdens have concentrated on the analysis of lung parenchyma [1]. It is the purpose of this chapter to review the various techniques which have been developed for the analysis of tissue fiber burdens, noting the advantages and limitations of each. The morphologic, crystallographic, and chemical features of the various types of asbestos are reviewed in Chap. 1 and the structure and nature of asbestos bodies in Chap. 3. In addition, the relationship between tissue asbestos burden and the various asbestos-associated diseases (see Chaps. 4, 5, 6, and 7) and the various categories of occupational and environmental exposures (see Chap. 2) will also be explored in the present chapter. Finally, the overall contribution of the various types of asbestos and non-asbestos mineral fibers to the total mineral fiber burden will be discussed in relationship to the biological activity and pathogenicity of the various fiber types.

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## 11.2 Historical Background

During the past several decades, there has been considerable interest in the correlation of dusts in the workplace environment with lung diseases resulting from the inhalation of these dusts (i.e., the pneumoconioses). Analysis of lung dust burdens required considerable cooperation between the basic physical sciences and the biological and medical sciences [2]. The distinctive behavior of fibrous materials as compared to other particulates has required many pointed studies as to inhalability, deposition, and subsequent disposal or accumulation of airborne fibers (see Chap. 10). The techniques employed were generally bulk analytical techniques such as x-ray diffraction, chemical analysis, or polarizing microscopy, which were adequate in most circumstances because of the well-defined source of the dust in the workplace and the relatively large amounts of dust recoverable from the lungs of patients dying with pneumoconiosis. However, analysis of dust content from individuals exposed to asbestos posed a number of difficulties for the traditional bulk analytical approaches. First, the

T.D. Oury et al. (eds.), *Pathology of Asbestos-Associated Diseases*,
DOI 10.1007/978-3-642-41193-9_11, © Springer-Verlag Berlin Heidelberg 2014

quantities of dust present within the lung samples were often relatively small. Second, the size of the particles posed some difficulty, since most of the asbestos fibers were less than a micron in diameter. Third, other dusts were often present in similar or even greater amounts than the asbestos component. Fourth, alteration of the chemical or crystalline properties of some types of fibers during prolonged residence in tissues complicated the precise identification of such agents. Furthermore, many of the techniques used for microfiber extraction from tissues tended to alter or destroy some of the mineral phases that were present [2].

Clearly, the development of unique approaches for the identification of asbestos fibers in tissues was necessary before progress in this area could become possible. In the 1970s, the use of analytical electron microscopy for the identification and characterization of individual asbestos fibers isolated from human tissues was pioneered in large part by the innovative studies of investigators such as Arthur Langer in the USA and Fred Pooley in Great Britain [3–8]. The usefulness of these techniques has since been confirmed by other investigators [9–14], and from the 1980s to the present time, these techniques have been employed to correlate the tissue asbestos burden with various asbestos-related diseases [11, 15–21].

## 11.3 Methods for Analysis of Tissue Mineral Fiber Content

### 11.3.1 Tissue Selection

As noted in the introduction, most studies of tissue mineral fiber content have examined lung parenchyma. There is no inherent reason why the techniques developed for this purpose cannot be applied to other tissues. However, there is little published information on the expected values of mineral fiber content of tissues other than the lung. Therefore, any investigator wishing to study such tissues must establish normal ranges for his or her laboratory and the analytical technique employed. In this regard, it should be noted that the expected levels of fibers in extrapulmonary tissues would be at or below the limits of detection for current techniques, and background contamination can be a considerable problem. In this section, comments regarding selection of tissue for mineral fiber analysis will be confined to lung parenchyma.

In most circumstances, formalin-fixed lung tissue is utilized, although fresh specimens work just as well. In some instances, paraffin-embedded tissue is all that is available. Such samples can be deparaffinized in xylene and rehydrated to 95 % ethanol. The dehydration process removes some components of tissue, mainly lipids, so that a correction factor must be applied to equate the values obtained from paraffin blocks to those obtained from formalin-fixed tissue. In the author's laboratory, the correction factor has been determined to be approximately 0.7 (i.e., the asbestos fiber concentration determined from a paraffin block should be multiplied by 0.7) [17].

In selecting tissue for digestion, areas of consolidation, congestion, or tumor should be avoided as much as possible. Such pathologic alterations would affect the denominator in calculations of the tissue concentration of fibers or asbestos bodies. Since there is some site-to-site variation of mineral fiber content within the lung, the more tissue that is available for analysis the better. Ideal specimens include autopsy, pneumonectomy, or lobectomy specimens, with analysis of multiple sites. In the authors' laboratory, two or three samples are typically analyzed for a lobectomy or pneumonectomy specimen, whereas four sites (upper and lower lobes of each lung) are sampled when both lungs are available at autopsy. Samples usually include lung parenchyma abutting against the visceral pleura, with each sample typically weighing 0.25–0.35 g (wet weight). However, analyses may be performed on as little as 0.1 g or less of wet tissue. Although some studies have reported analyses of transbronchial biopsy specimens [22–24], the small size of such samples (usually 2–5 mg of tissue at best) makes them unlikely to be representative [25, 26].

### 11.3.2 Digestion Technique

Techniques for mineral fiber analysis generally involve three basic steps. First, there is dissolution and removal of the organic matrix material of the lung in which the fibers are embedded. Second, the mineral fibers are recovered and concentrated. Third, the fiber content is analyzed by some form of microscopy [1]. Dissolution steps involve either wet chemical digestion or ashing. Wet chemical digestion can be accomplished with sodium or potassium hydroxide, hydrogen peroxide, 5.25 % sodium hypochlorite solution (commercial bleach), formamide, or proteolytic enzymes [12]. Most investigators prefer an alkali wet chemical digestion using either sodium hypochlorite or sodium or potassium hydroxide. Tissue ashing is an alternative approach. Ashing in a muffle furnace at 400–500 °C is unsuitable, because the drying and shrinkage of the tissue causes fragmentation of the fibers, artifactually increasing fiber numbers and decreasing mean fiber lengths. This problem is largely avoided by ashing the sample in a low-temperature plasma asher [27].

Once the digestion of the tissue is complete, the inorganic residue may then be collected on an acetate or polycarbonate filter or an aliquot can be transferred into a Fuchs-Rosenthal counting chamber for direct counting of fibers by phase-contrast light microscopy [15]. However, a permanent sample cannot be prepared with this latter technique, so most investigators prefer filtration, with a pore size of 0.2–0.45 μm. Use of a pore size which is too large in relation to the size of the fibers to be analyzed can result in significant loss of fibers and underestimation of the mineral fiber content of the sample [28]. Details of the digestion procedure employed by the authors are provided in the Appendix.

### 11.3.3 Fiber Identification and Quantification

A number of analytical techniques have been used for the identification of asbestos fibers in bulk samples, including x-ray diffractometry, infrared spectroscopy, differential thermal analysis, and polarization microscopy with dispersion staining [2, 6, 11]. For a variety of reasons as noted above in the section on "Historical Background," these techniques have severe limitations in regard to the identification of fibers from human lung tissue samples, and in practice bulk analytical techniques have been ineffective for this purpose [2, 6, 11]. As a result, investigators have turned to the use of various forms of microscopy for the analysis of pulmonary mineral fiber content. These include conventional bright field light microscopy, phase-contrast light microscopy, scanning electron microscopy, and transmission electron microscopy.

Conventional bright field light microscopy is a simple, inexpensive technique that requires no special instrumentation. This technique, detailed in Chap. 3, is ideal for the quantification of asbestos bodies [1–14, 17]. A few uncoated asbestos fibers can also be observed, but the vast majority of fibers are beyond the resolution of this technique. Furthermore, conventional light microscopy cannot distinguish among the various fiber types. Asbestos bodies can be counted at a magnification of 200–400× and the results reported as numbers/gram of wet lung tissue [12, 17]. Alternatively, a piece of lung tissue adjacent to the one actually analyzed can be dried to constant weight to obtain a wet-to-dry weight ratio and the results reported as asbestos bodies/gram of dry weight [11, 14].

Phase-contrast light microscopy (PCLM) has also been used by investigators to quantify the tissue mineral fiber burden [15, 18, 19]. This technique can resolve fibers with a diameter of 0.2 μm or greater, and it reveals that uncoated fibers greatly outnumber the coated ones (i.e., asbestos bodies) [29]. However, a substantial proportion of asbestos fibers have diameters less than 0.2 μm, and thus are not detectable by PCLM. As is the case for conventional light microscopy, one cannot distinguish among the various types of asbestos fibers or differentiate asbestos from non-asbestos fibers by PCLM. Investigators using this technique have generally reported results as total fibers/gram of dry lung tissue or separately as asbestos bodies and

uncoated fibers/gram of dry lung tissue [15, 30]. Some investigators have also reported results as fibers/cm$^3$ of lung tissue [31]. As a rule of thumb, 1 fiber/g of wet lung ≅ 1 fiber/cm$^3$ ≅ 10 fibers/g of dry lung [25].

Scanning electron microscopy (SEM) has been used by some investigators for the quantification of tissue mineral fiber content [17, 21, 26, 32]. This technique offers several advantages over PCLM and conventional bright field light microscopy. At low magnifications (1,000×), asbestos bodies and uncoated fibers can be counted (Fig. 11.1) yielding quantitative results similar to those obtained with PCLM [17]. At higher magnifications (10,000–20,000×), the superior resolution of SEM permits the detection of fibers not visible by PCLM, with fibers as small as 0.3 μm long and 0.05 μm in diameter detected by this technique [33]. Furthermore, SEM can be coupled with energy dispersive x-ray analysis (EDXA) to determine the chemical composition of individual fibers (Fig. 11.2). This information can in turn be used to classify a fiber as asbestos or non-asbestos and to determine the specific asbestos fiber type [17, 33–35]. Sample preparation for SEM is relatively simple, requiring only that the filter be mounted on a suitable substrate (such as a carbon disc) with carbon paste and then coated with a suitable conducting material (such as carbon, platinum, or gold). Also, SEM analysis of mineral fibers has the potential for automation using commercially available automated image x-ray analyzers [36] and software programs which discriminate between fibers and other particles [37, 38]. Disadvantages of SEM include the high cost of the instrumentation and the considerable time required for analysis (an hour or more per sample).

Transmission electron microscopy (TEM) is the analytical technique that has been preferred by many investigators for the determination of mineral fiber content in tissue digest preparations [7, 8, 11, 16, 18, 23, 30]. This technique provides the highest resolution for the identification of the smallest fibrils and can be coupled with EDXA for determination of the chemical composition of individual fibers. TEM has the further advantage that selected area electron diffraction (SAED) can be readily performed, providing information regarding the crystalline structure of an individual particle. The diffraction pattern of a fiber (Fig. 11.3) can provide information useful for identification purposes, especially when the chemical compositions of two fibers are similar [39, 40]. For example, SAED can readily distinguish chrysotile from anthophyllite asbestos or anthophyllite from talc [40]. Methods for preparation of tissue samples for TEM analysis have been described [40, 41]; however, these techniques are more complex than preparative steps

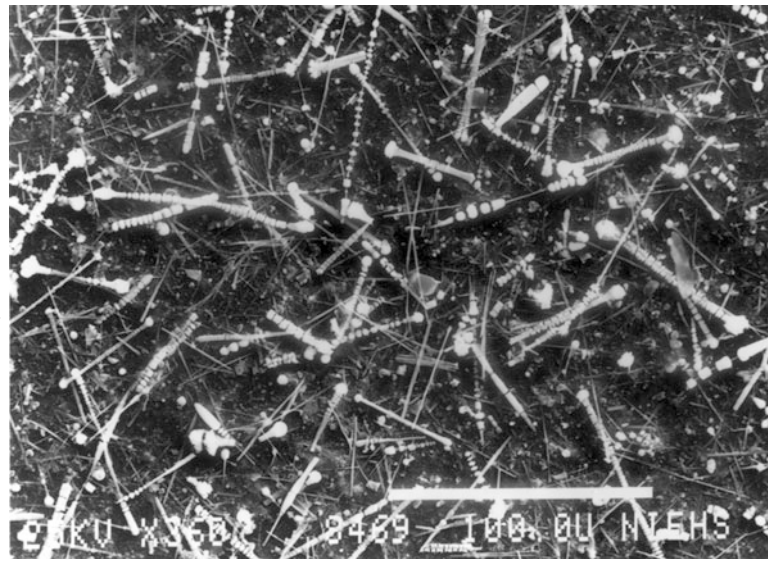

**Fig. 11.1** Scanning electron micrograph of Nuclepore filter preparation of lung tissue from an asbestos insulator with malignant pleural mesothelioma and asbestosis. Numerous asbestos bodies and uncoated asbestos fibers are visible. This patient's lung tissue contained nearly three million asbestos bodies and more than nine million uncoated fibers 5 μm or greater in length/gram of wet lung. Magnified ×360

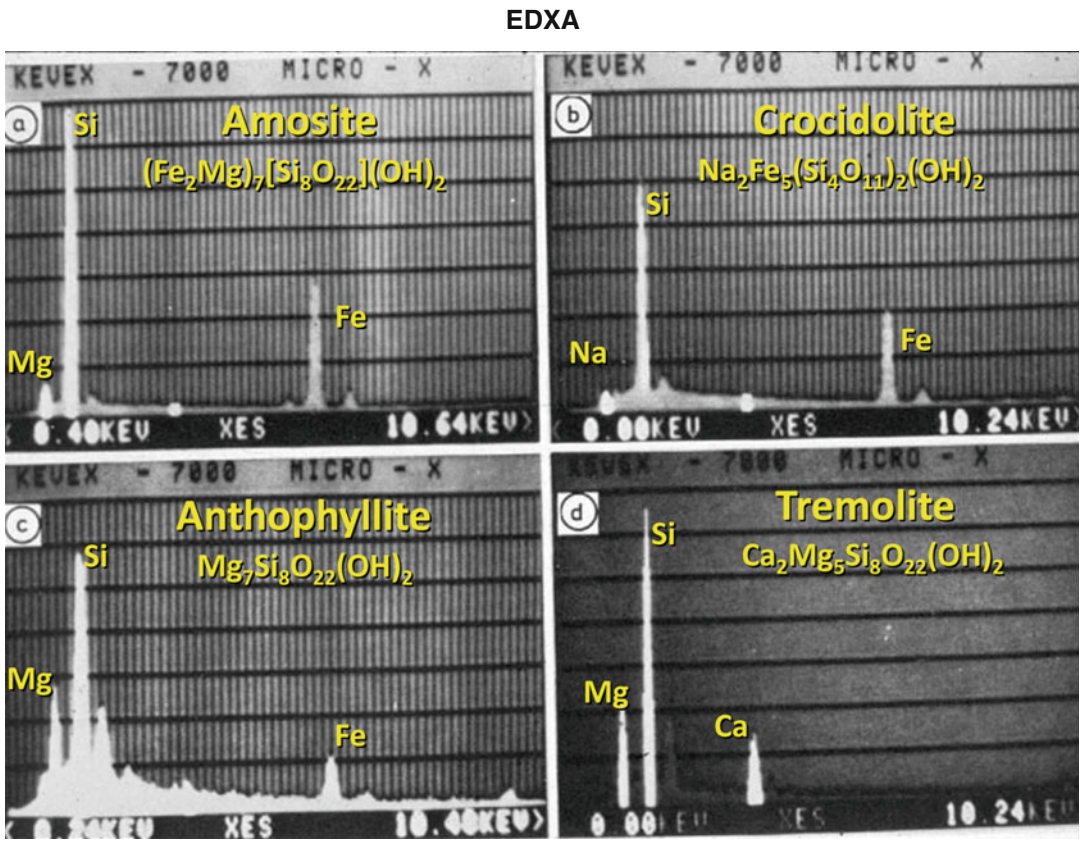


**Fig. 11.2** Energy dispersive x-ray spectra of four different amphibole asbestos fibers. (**a**, *upper left*) Amosite has peaks for Si, Fe, Mg, and sometimes Mn. (**b**, *upper right*) Crocidolite has peaks for Si, Fe, Na, and Mg. (**c**, *lower left*) Anthophyllite has peaks for Si, Mg, and Fe. (**d**, *lower right*) Tremolite has peaks for Si, Mg, and Ca. Peak in each spectrum immediately to right of Si is due to Au used to coat specimen (Reprinted from Ref. [17], with permission)

for light microscopy or SEM [32, 42]. Therefore, there is increased opportunity for loss of fibers or contamination of the sample. Also, only a small proportion of a filter can be mounted on a TEM grid, so that one must be concerned with whether the portion of the filter sampled is truly representative [25]. As is the case for SEM, analysis of mineral fiber content of tissue by TEM is both time-consuming and expensive. Results are generally reported in terms of fibers/gram of wet or dry lung tissue. The magnifications used are generally too high to accurately assess the tissue asbestos body content by TEM.

Two other techniques deserve brief mention as potentially useful for tissue mineral fiber analysis. The confocal scanning optical microscope uses a focused light beam to scan across the sample and the image is detected and processed electronically [43, 44]. This light microscopic technique has a resolution of 0.1 μm or better, which is superior to that of PCLM and thus would permit detection of considerably more fibers. The image is focused in a discrete plane with a thickness of less than 1 μm. Because asbestos fibers may be present at different depths in a filter preparation, quantitative examination of a filter with this imaging technique could be time-consuming. Another technique with potential value is scanning transmission electron microscopy (STEM). This technique has the high resolution and ease of performance of electron diffraction which are characteristic of TEM [45, 46]. Furthermore, the

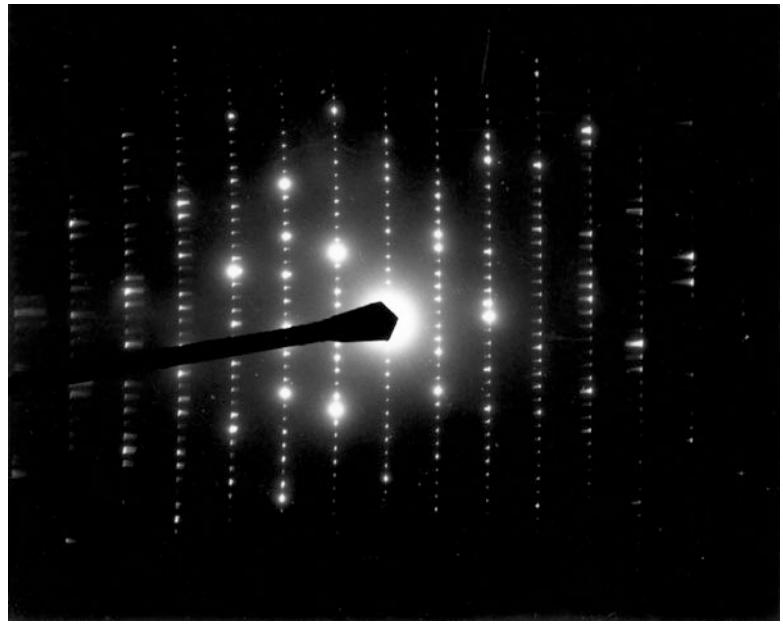

**Fig. 11.3** Selected area electron diffraction pattern obtained from an asbestos body core of an insulation worker. The pattern shows discrete dots along each layer line, with a calculated 5.3A interlayer-line spacing typical for amphibole asbestos (Reprinted from Roggli et al. [205])

scanning mode of operation produces an image which is amenable to automated analysis. Hence STEM has many characteristics which would be ideal for a standardized and automated approach to mineral fiber analysis.

### 11.3.4 Variability of Results

The wide variety of preparative techniques and analytical methodologies that have been employed by various investigators make it difficult to extrapolate results from one laboratory to another. The actual analytical result obtained on any one sample can be profoundly influenced by the steps employed in the analytical procedure (Table 11.1) [1]. Interlaboratory comparison trials demonstrate that striking differences can occur among laboratories even when the same sample is analyzed [47]. Some asbestos bodies and fibers may be lost during the preparation process [48, 49], and some of the smallest fibers are difficult to recognize and count in a reproducible fashion [50]. On the other hand, use of a sonication step or ashing of the specimen can enhance the fragmentation of chrysotile fibers, artifactually increasing fiber numbers [24, 48]. Nonetheless, there is evidence for internal consistency within individual laboratories, with similar ranking of samples among different laboratories from the lowest to the highest tissue fiber concentration. Still, one must use caution in comparing results

**Table 11.1** Factors affecting fiber burden data

| |
|---|
| I. Digestion procedure |
| (A) Wet chemical digestion (alkali, enzymes) |
| (B) Low-temperature plasma ashing |
| (C) Number of sites sampled |
| II. Recovery procedure |
| (A) Use of centrifugation step |
| (B) Use of a sonication step |
| (C) Filtration step (type of filter, pore size) |
| III. Analytical procedure |
| (A) Microscopic technique (LM, PCLM, SEM, TEM) |
| (B) Magnification used |
| (C) Sizes of fibers counted and other "counting rules" |
| (D) Numbers of fibers or fields actually counted |
| IV. Reporting of results |
| (A) Asbestos bodies or fibers (or both) |
| (B) Sizes of fibers counted |
| (C) Concentration of fibers (per gram wet or dry lung or per $cm^3$) |

Reprinted from Ref. [1]
*LM* light microscopy, *PCLM* phase-contrast light microscopy, *SEM* scanning electron microscopy, *TEM* transmission electron microscopy

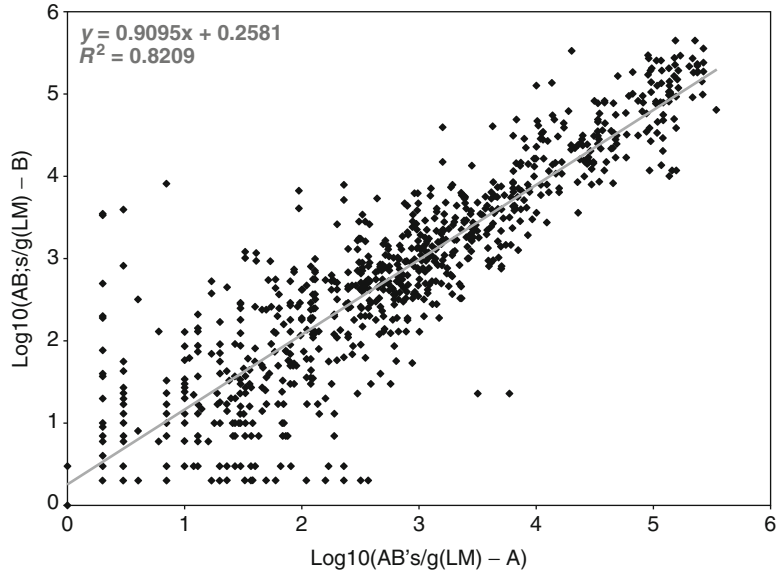


**Fig. 11.4** Correlation of asbestos body counts by light microscopy in 463 cases where multiple sites were sampled. *Graph* shows all pairwise comparisons, with the linear regression equation given by $\log y = 0.91 \log x + 0.26$ (correlation coefficient $r = 0.91$, $p = 0.0000$)

between laboratories, bearing in mind any differences in the analytical procedures employed [1].

In addition to interlaboratory variation, intralaboratory variation can occur, which may be either due to changes in a laboratory's procedures over time [51] or to variation in fiber content from one site to another within the lung [29, 52]. Morgan and Holmes [29, 52] have reported a five- to tenfold site-to-site variation based on analyses of multiple samples from a single lung using phase-contrast light microscopy. In the author's experience using light microscopy for asbestos body quantification (Fig. 11.4) or SEM for asbestos body and uncoated fiber quantification (Fig. 11.5), paired samples have asbestos body and fiber concentration values ranging from identical to within a factor of two or three. Rarely, two samples from the same patient may differ by as much as a factor of 10. The coefficient of variation for counting the same sample on multiple occasions is on the order of 10 % [53]. When interpreting fiber burden data, one must keep in mind that the analysis is occurring at a single point in time, usually when advanced disease is present. The fiber burden at that time may or may not relate to the tissue fiber content at the time when disease was actively evolving [1]. Nonetheless, there is a growing consensus that the fiber burdens that persist in the lung are the primary determinant of subsequent disease [21, 54, 55].

## 11.4 Asbestos Content of Lung Tissue in Asbestos-Associated Diseases

### 11.4.1 Asbestosis

Relatively few studies have been published in which the asbestos content of lung tissue was examined in a series of patients with asbestosis [15, 16, 18, 30, 56]. The data from these studies are summarized in Table 11.2. Except for the unusually high median count for asbestos bodies in the study by Ashcroft and Heppleston [30] and the high mean count for uncoated fibers by electron microscopy in the study by Wagner et al. [18], the values are roughly similar among the reported series. This is rather remarkable when one considers the wide range of values obtained when different laboratories examine the same sample [47] and the different techniques employed in the various studies referred to in Table 11.2. For example, Whitwell et al. [15] used PCLM and counted all fibers greater than or equal to 6 μm in length, counting asbestos bodies and uncoated fibers

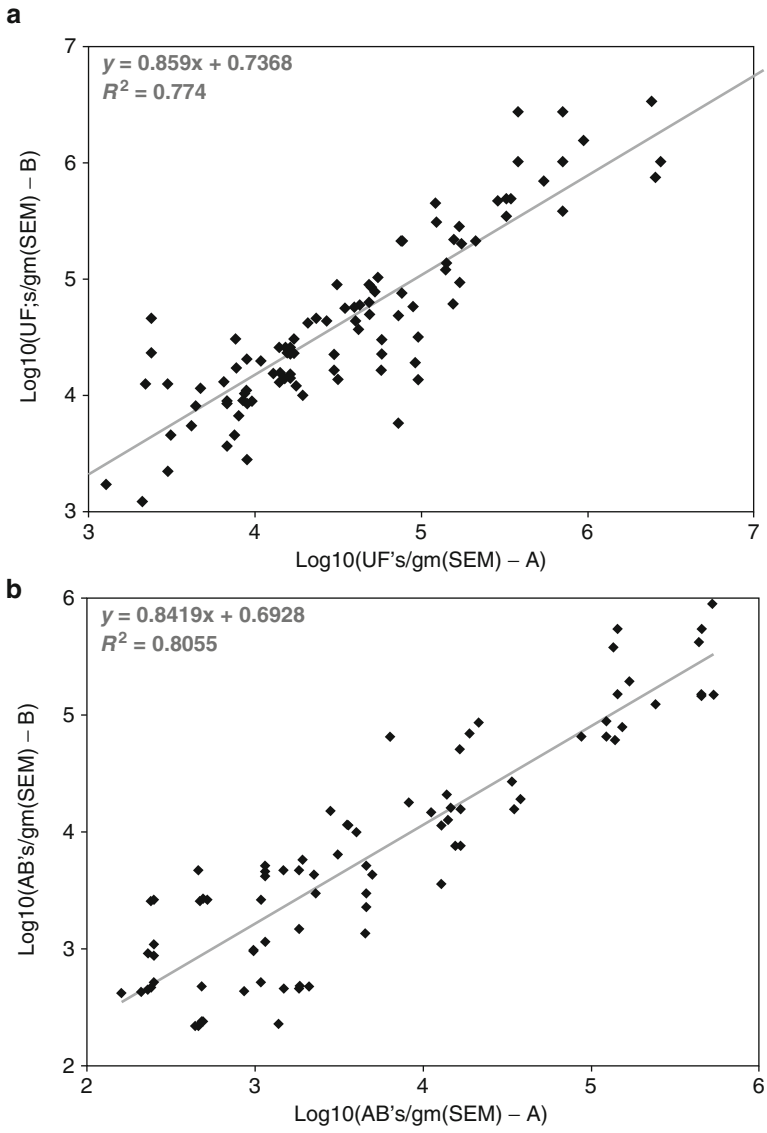


**Fig. 11.5** (**a**) Correlation of uncoated fiber concentrations for fibers 5 **µ**m or greater in length by SEM in 68 cases where multiple sites were sampled. *Graph* shows all pairwise comparisons, with the linear regression equation given by log $y = 0.86 \log x + 0.74$ ($r = 0.88$, $p = 0.0000$). (**b**) Correlation of asbestos body counts by scanning electron microscopy (SEM) in 52 cases where multiple sites were sampled. *Graph* shows all pairwise comparisons, with the linear regression equation given by log $y = 0.84 \log x + 0.69$ (correlation coefficient $r = 0.89$, $p = 0.0000$)

together. Ashcroft and Heppleston [30] used PCLM at a magnification of 400× and counted all visible fibers, reporting coated and uncoated fibers separately. Warnock et al. [16] used TEM and counted all fibers exceeding 0.25 µm in length and with an aspect ratio (length to width) of 3 or greater. The study by Warnock et al. [16] also counted asbestos bodies by conventional light microscopy. Wagner et al. [18] used the PCLM method of Ashcroft and Heppleston [30] as well as TEM. Churg and Vedal [56] used TEM and counted all fibers exceeding 0.5 µm in length. The median uncoated fiber count exceeds one million fibers/gram of dried lung tissue in all four studies.

The asbestos content of the lung in 165 patients with histologically confirmed asbestosis (using recently published criteria [57]) from the author's laboratory is summarized in Table 11.3. Our laboratory employs SEM at a magnification of 1000×, counting all fibers with a length greater than or equal to 5 µm. Asbestos bodies are counted also by light microscopy. The median asbestos body count for patients with asbestosis is 25,700 asbestos bodies/gram of wet lung tissue. The median asbestos fiber count

**Table 11.2** Asbestos content of lung tissue in reported series of patients with asbestosis

| Source | No. of cases | Method[a] | Asbestos bodies/g | Uncoated fibers/g |
|---|---|---|---|---|
| Whitwell et al. [15] | 23 | PCLM | – | 8 (1.0–70) |
| Ashcroft and Heppleston [30] | 22 | PCLM | 12.2 (0.49–192) | 32 (1.3–493) |
| Warnock et al. [16] | 22 | TEM[b] | 0.123 (0.001–7.38) | 5.68 (1.6–121) |
| Wagner et al. [18] | 100 | PCLM | – | 1.5 (0.001–31.6) |
| | 170 | TEM | – | 372 (<1.0–10,000) |
| Churg and Vedal [56] | 23 | TEM | – | 10 (±6.6) |

Values reported as the median counts for millions ($10^6$) of asbestos bodies or uncoated fibers/gram of dried lung tissue, with ranges indicated in parentheses, except for the study of Wagner et al. [18], where only the mean value could be determined from the data presented, and the study by Churg and Vedal [56], where results are reported as geometric mean and standard deviation

[a]*PCLM* phase-contrast light microscopy, *TEM* transmission electron microscopy

[b]In this study, asbestos bodies were counted by conventional light microscopy

**Table 11.3** Asbestos content of lung tissue in 165 cases of asbestosis

| | *N* | AB/g (LM) | *N* | AF/g (SEM) |
|---|---|---|---|---|
| Asbestosis[a] | 48 | 20,800 (910–1,400,000) | 47 | 278,000 (26,500–7,530,000) |
| Asbestosis plus lung cancer | 77 | 34,700 (840–343,000) | 75 | 300,000 (14,700–8,540,000) |
| Asbestosis plus peritoneal mesothelioma | 7 | 159,000 (23,000–207,000) | 7 | 505,000 (247,000–1,010,000) |
| Asbestosis plus pleural mesothelioma | 29 | 17,700 (2,160–1,600,000) | 29 | 131,000 (31,500–11,900,000) |

Asbestos bodies/gram of wet lung tissue as determined by light microscopy (LM) and total asbestos fibers 5 μm or greater in length/gram of wet lung tissue as determined by scanning electron microscopy (SEM). Values reported as median with range in parentheses

[a]Cases of asbestosis with neither lung cancer nor mesothelioma

in 159 asbestosis cases examined by SEM is 260,000 fibers/g of wet lung. The results can be approximately converted to bodies or fibers/gram of dry lung tissue by multiplying by a factor of 10 [11]. For comparison, the median asbestos body count from individuals with normal lungs and no asbestos-related disease is 2.8 AB/g. The median count by SEM for asbestos fibers 5 μm or greater in length for our control cases is fewer than 600 fibers/g. In 95 % of the cases of asbestosis, the asbestos body content is 1,840 AB/g or greater. At this tissue asbestos body concentration, several asbestos bodies should be observed on most 2×2 cm histologic sections stained for iron and examined systematically (Chap. 3) [58]. Thus the finding of two asbestos bodies/$cm^2$ in iron-stained histologic sections is a reasonable histopathologic criterion for the diagnosis of asbestosis (Chap. 4) [57].

Roggli and Vollmer examined time trends in a series of patients with asbestosis [21]. These authors noted that there was an increase in age of asbestosis patients over time, and this was associated with a fall in average asbestos fiber concentration. These observations are consistent with an inverse relationship between dose and latency for asbestosis and with decreased exposures as a consequence of regulation of workplace fiber levels. There was also a trend toward decreasing grade of fibrosis over time and fewer cases with grade 4 disease (honeycomb changes), although this did not reach statistical significance.

A few studies have investigated the relationship between tissue asbestos burden and the fibrotic response in human lungs (Table 11.4). Whitwell et al. [15] found a progressive increase in median total coated and uncoated fiber count from patients with mild (1+) to severe (3+) fibrosis. Ashcroft and Heppleston [30] also reported a progression in the severity of fibrosis with increasing uncoated fiber count from no fibrosis to moderate (2+) fibrosis, but no further increase in fiber count from moderate to severe disease. These authors concluded that additional factors other than tissue fiber burden

**Table 11.4** Severity of asbestosis versus tissue asbestos content as total fibers/gram of dried lung

| | Asbestosis grade | | | | |
|---|---|---|---|---|---|
| Study | 0 | 1/2+ | 1+ | 2+ | 3+ |
| Whitwell et al. [15] | | | $8\times10^6$ | $14\times10^6$ | $37\times10^6$ |
| Ashcroft and Heppleston [28] | $2.4\times10^6$ | | $20\times10^6$ | $200\times10^6$ | $144\times10^6$ |
| Warnock et al. [16] | $4.8\times10^6$ | $5.7\times10^6$ | $48\times10^6$ | $11\times10^6$ | $3.6\times10^6$ |
| Wagner et al. [18] | $0.005\times10^6$ | $0.009\times10^6$ | $0.015\times10^6$ | $0.12\times10^6$ | $1.2\times10^6$ |
| | $1.3\times10^6$ | $31.6\times10^6$ | $44\times10^6$ | $68\times10^6$ | $464\times10^6$ |

Reprinted from Ref. [54], with permission

Values indicated represent the median counts derived from the data presented in the reference that is cited. Asbestosis grade is as defined in each original source. The first two studies employed phase-contrast light microscopy, whereas the study by Warnock et al. [16] used transmission electron microscopy and the study of Wagner et al. [18] used both (phase-contrast results from Fig. 11.2 of the latter study listed first and EM results from Fig. 11.1 listed below). Wagner et al. [18] grading scheme of 0–4 has been modified to 0–3 simply for purposes of tabulation

must be involved in progression from moderate to severe fibrosis [30]. Warnock et al. [16] graded the severity of fibrosis on a scale of 0–3+ based on visual inspection of the cut surface of inflation-fixed specimens, with 1/2+ defined as microscopic fibrosis only. These authors found no apparent correlation between the severity of fibrosis and total fiber content for all fibers 0.25 μm or greater in length as assessed by TEM [16]. Wagner et al. [18] graded the severity of fibrosis microscopically on a scale of 0–4. Their data are summarized in Table 11.4 and, for the sake of convenience, have been tabulated as 0–3 with their grade 1 fibrosis listed under 1/2+. These authors found a progressive increase in optically visible and electron microscopically enumerated fibers with increasing severity of asbestosis [18].

The relationship between the tissue content of commercial amphibole fibers 5 μm or greater in length as assessed by SEM in the authors' laboratory and the histologic asbestosis score as determined by the method proposed by the Asbestosis Committee of the College of American Pathologists [57] is shown in Fig. 11.6. These data are based on 119 cases of asbestosis for which tissue was available for analysis of asbestos content, as revised using the diagnostic criteria for asbestosis recommended by the Asbestosis Committee [57, 59]. There is a statistically significant relationship between fiber content and histologic score, although there is a wide range of scatter of the data points. It is likely that the degree of correlation would improve with more extensive histologic and mineralogical sampling of the lungs and expression of the data as total lung burden rather than fiber concentration [17]. This is due to the fact that accumulation of collagen and other cellular components as a result of the scarring process increases the weight of the lungs and hence dilutes the concentration of fibers in the parenchyma, a point often overlooked in dust analysis studies [60]. The severity of fibrosis correlates best with the concentration of uncoated commercial amphibole fibers (amosite plus crocidolite) as determined by SEM ($p=0.011$) [59]. There was also a statistically significant association between histologic score and total (coated plus uncoated) commercial amphibole fibers and total uncoated (asbestos and non-asbestos) fibers as determined by SEM as well as between histologic score and asbestos body content as measured by light microscopy ($p<0.05$ in all instances). In a prior smaller study, there was no significant association between histologic score and patient age, duration of occupational exposure, or pack-years of smoking [61].

Although the best correlation with severity of fibrosis was with uncoated commercial amphibole fibers, we also observed six cases in which noncommercial amphiboles were present in the highest concentrations. These cases are summarized in Table 11.5, and five have been previously reported [59, 62]. The source of the noncommercial amphiboles varied among these six cases. In two cases (Cases 1 and 4), the source was tremolite contamination of chrysotile-containing products. In two cases (Cases 2 and 6), the source was environmental contamination by tremolite and

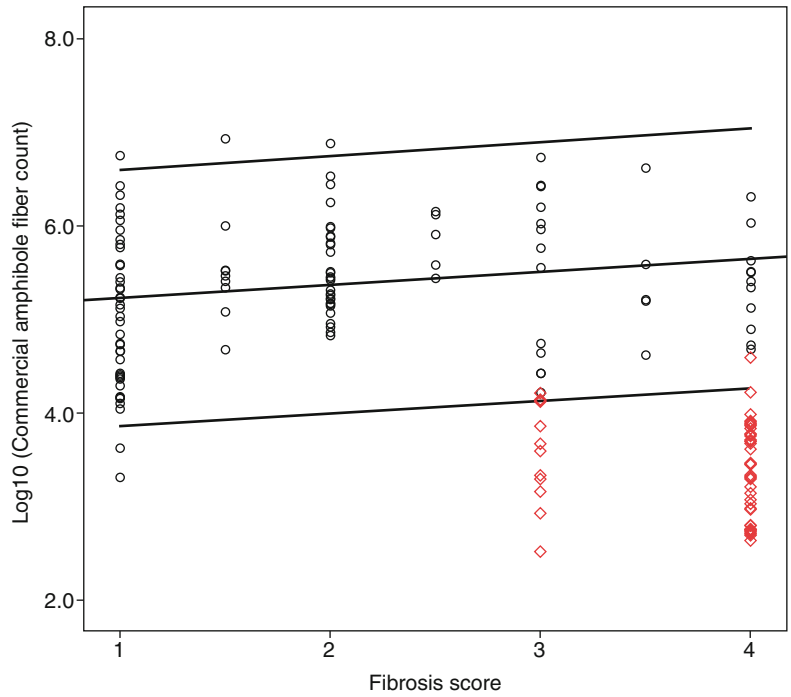


**Fig. 11.6** Linear regression analysis of the relationship between the severity of fibrosis and the pulmonary concentration of commercial amphibole fibers 5 μm or greater in length as determined by SEM in 118 cases of asbestosis and 86 cases of diffuse pulmonary fibrosis of unknown cause. The regression equation is given by $\log y = 0.142\,x + 5.08$ ($r = 0.199$, $p = 0.03$) and is indicated by the heavy *black line*. *Thin lines* mark the 95 % confidence limits. Asbestosis (*white circle*), diffuse pulmonary fibrosis of unknown cause (*red diamond*)

actinolite in individuals living in the Southern Anatolian region of Turkey [63]. In one case (Case 5), the source was tremolite and actinolite contamination of vermiculite. In the final case (Case 3), the source was anthophyllite used in the manufacture of cement pipe. The median noncommercial amphibole fiber concentration in these six cases (491,000 fibers/g of wet lung) is similar to the median asbestos fiber count of other cases of asbestosis that we have examined (Table 11.3).

Figure 11.6 also shows the commercial amphibole fiber content of the lung in 86 cases with diffuse pulmonary fibrosis and some history of asbestos exposure, but lacking histologic criteria for the diagnosis of asbestosis (Chap. 4). There is minimal overlap between the fiber counts in these cases and the 95 % confidence interval for bona fide cases of asbestosis. One case classified as idiopathic was a shipyard sheet metal worker for 24 years with grade 4 fibrosis who also had lung cancer and plaques; the other case had grade 3 fibrosis but no other information was available. These two may very well represent cases of occult asbestosis. It should be noted that the intercept of the regression line in Fig. 11.6 is above 100,000 fibers/g of wet lung (or one million fibers/gram of dried lung), which coincides with the lower limit of the range of values shown in Table 11.2. The median commercial amphibole fiber burden for 20 cases with normal lungs and no history of asbestos exposure (<600 fibers/g of wet lung) is more than two orders of magnitude less than the intercept at zero fibrosis for asbestosis cases. This observation indicates that there is likely a threshold for asbestos exposure and the development of any degree of lung fibrosis.

Thus, there generally appears to be a correlation between the severity of fibrosis in patients with asbestosis and the tissue mineral fiber burden, although there is a wide scatter in the data [30, 61]. In this regard, studies by Timbrell et al. [64] (reviewed by Lippmann [65]) have shown that among individuals exposed to the various types of amphibole asbestos, the severity of pulmonary fibrosis correlates better with the relative fiber surface area per unit weight of tissue than with the relative fiber number or mass, as determined by magnetic alignment and light scattering. On the other hand, Churg et al. [66] in a study of asbestosis among chrysotile miners and millers found no correlation between fibrosis and

**Table 11.5** Fiber burden[a] of six asbestosis patients whose noncommercial amphibole fiber count was higher than the commercial amphibole fiber count

| Case no. | Age, sex | Diagnosis (in addition to asbestosis) | Asbestos exposure, duration | Coated commercial amphiboles | Uncoated commercial amphiboles | Noncommercial amphiboles | Chrysotile | NAMF |
|---|---|---|---|---|---|---|---|---|
| 1[b] | 74, M | Unilateral diffuse pleural fibrosis | Manufactured asbestos blankets and gaskets, 7 years | 2,100 | <29,000 | 471,000 | 68,000 | 21,300 |
| 2 | ND[c], M | Necrotizing granulomatous inflammation | Lived in Turkey, ND[c] | <10,700 | <37,200 | 499,000 | <22,000 | 43,500 |
| 3 | 75, M | Centrilobular emphysema | Anthophyllite cement pipe plant, 2 years; automobile industry, 25 years | <3,500 | <37,200 | 779,000 | <37,200 | <37,200 |
| 4 | 66, M | Malignant pleural mesothelioma | Plasterer, dry wall, 11 years | <2,400 | <24,200 | 483,000 | <24,200 | 24,200 |
| 5[b] | 44, M | Lung adenocarcinoma | Lived near vermiculite processing plant, 20 years | <3,000 | <8,200 | 150,000 | <8,200 | <8,200 |
| 6 | 45, F | None | Lived in Turkey, ND | <790 | <86,000 | 1,640,000 | <86,000 | 86,000 |

[a]Fibers 5 μm or greater in length/gram of wet lung tissue
[b]Previously published [62]
[c]*ND* not documented

fiber size, surface area, or mass for chrysotile and an inverse correlation with fiber length, aspect ratio, and surface area for contaminating tremolite asbestos. These authors did show a direct correlation between fiber concentration and severity of fibrosis for both chrysotile and tremolite fibers [66]. Further studies of the mineralogic correlates of fiber-induced pulmonary fibrosis would be required to resolve these discrepancies [1].

In summary, analyses of tissue mineral fiber burdens in patients with asbestosis indicate a heavy lung asbestos burden in the vast majority of cases. This observation is consistent with epidemiologic evidence that asbestosis occurs primarily in individuals with direct and prolonged occupational exposure to asbestos [1]. Since no uniform method for the analysis of tissue mineral fiber content has been established, it is not presently possible to recommend a specific tissue asbestos fiber content to be used as a criterion for the pathologic diagnosis of asbestosis. Whereas the fibrogenicity of asbestos fibers 5 μm or greater in length is well established [67–71], the fibrogenicity of fibers less than 5 μm in length remains unproven [72] (see Chap. 10). Therefore, no tissue level of fibers in the latter size range should be proposed as a criterion for the diagnosis of asbestosis [61].

### 11.4.2 Malignant Mesothelioma

Several studies have examined the asbestos content of lung tissue in patients with mesothelioma [15, 56, 73–79]. The data from these studies are summarized in Table 11.6. Whitwell et al. [15] studied 100 patients with malignant mesothelioma by means of PCLM. The median count was 750,000 combined fibers and bodies/gram of dried lung, with a range of 0–70 million fibers/g. In only seven cases was the combined count less than 20,000/g, and in six of these, there was no

identifiable occupational exposure to asbestos. In contrast, the count was less than 20,000/g in 71 % of the normal control series in the same study [15]. Gylseth et al. [73] examined 15 cases of malignant mesothelioma counting fibers by means of SEM at a magnification of 4500× and compared the results with those of 14 cases of parietal pleural plaques and 12 control cases without cancer or chronic respiratory disease. The median fiber count in the patients with mesothelioma was 11 million/g of dried lung as compared to 2.2 million/g in the pleural plaque cases and 0.6 million/g among the control cases. Mowe et al. [74] used SEM at a magnification of 4,500× to analyze the asbestos fiber content of lung tissue from 14 cases of mesothelioma and 28 controls matched for age, sex, year of death, and county of residence. These investigators reported a median fiber count of 2.4 million fibers/g of dried lung among the mesothelioma patients as compared to 0.25 million fibers/g among the controls. Dodson et al. [75] used TEM to study the asbestos content of 55 patients with mesothelioma from the Northwestern USA, many of whom had been exposed to asbestos in shipyard-related activity. The authors reported a median value of 698,000 fibers/g. Churg and Vedal [56] examined the mineral fiber content of lung tissue by TEM from 83 patients with malignant mesothelioma and heavy mixed amosite and chrysotile exposure, also from the Pacific Northwest. The geometric mean asbestos fiber count was 920,000 fibers/g of dried lung. Churg et al. [76] also studied 15 cases of mesothelioma in chrysotile miners and millers. The geometric mean asbestos fiber count in this group was 214 million fibers/g of dried lung. These authors concluded that patients with mesothelioma due to exposure to chrysotile from mining and milling have large pulmonary fiber burdens relative to patients with mesothelioma secondary to exposure to amosite or crocidolite asbestos.

Gaudichet et al. [77] examined lung tissue in 20 patients with mesothelioma and compared the results with those from 40 lung cancer cases and 20 patients who died from nonmalignant, non-asbestos-related processes. The mean total fiber burden for the patients with mesothelioma was 18 million fibers/g of dried lung, as compared to 16 million fibers/g for the lung cancer cases and 11.2 million fibers/g for the nonmalignant control group. The main difference between the mesothelioma cases and the other comparison groups was in regard to the greater numbers of commercial amphibole fibers (amosite and crocidolite) in the former as compared to the latter. Warnock [78] studied the mineral fiber content of lung tissue in 27 shipyard and construction workers with mesothelioma. The median total fiber count by TEM was 4.9 million fibers/g of dried lung. In contrast, the median count in 19 unexposed controls was 0.85 million fibers/g. Dodson et al. [79] reported on an additional 54 cases from various regions of the USA and found a median count of 77,500 asbestos fibers/g of wet lung tissue. Wet-to-dry weight ratios were available in 43 cases, and these cases are included in Table 11.6. The median fiber count for these was 382,000 fibers/g of dry lung.

Although valuable information can also be obtained from an analysis of the tissue asbestos body content in mesothelioma cases, only a few studies have reported such data in a series of patients with malignant mesothelioma. Dodson et al. [75, 79] reported a median asbestos body count of 9,560 asbestos bodies/g of dried lung in 55 patients with mesothelioma primarily from the Pacific Northwest and 1,500 asbestos bodies/g of dried lung in 54 mesothelioma patients from various regions of the USA. Gaudichet et al. [77] reported a median asbestos body concentration of 3,200 asbestos bodies/g of dried lung tissue in 20 patients with mesothelioma. The concentration exceeded 1,000 asbestos bodies/g in 70 % of mesothelioma patients but in only 10 % of 80 age- and sex-matched control cases. Warnock [78] found a median asbestos body count of 18,500 bodies/g of dried lung in 27 patients with mesothelioma, as compared to 300 bodies/g in 19 nonexposed controls. Kishimoto et al. [80] reported a median asbestos body concentration of 1,360 asbestos bodies/g of wet lung (approximately 13,600 bodies/g of dried lung) in eight Japanese workers with mesothelioma.

The asbestos content of the lung in 524 patients with malignant mesothelioma from the author's

**Table 11.6** Asbestos content of lung tissue in reported series of patients with mesothelioma

| Source | No. of cases | Method[a] | Asbestos bodies/ gram dried lung | Uncoated fibers/ gram dried lung |
|---|---|---|---|---|
| Whitwell et al. [15] | 100 | PCLM | – | 0.75 (0–70) |
| Gylseth et al. [73] | 15 | SEM | – | 11 (2–490) |
| Mowe et al. [74] | 14 | SEM | – | 2.4 (0.4–37) |
| Dodson et al. [75] | 55 | TEM[b] | 9.56 (0.06–1,250) | 0.70 (0.022–69.0) |
| Churg and Vedal [56] | 83 | TEM | – | 0.92 |
| Churg et al. [76] | 15 | TEM | – | 214 (SD ± 9) |
| Gaudichet et al. [77] | 20 | TEM[b] | 3.2 (0.04–450) | 18 |
| Warnock [78] | 27 | TEM[b] | 18.5 (1.9–3,800) | 4.9 (0.57–137) |
| Dodson et al. [79] | 43 | TEM[b] | 1.5 (0–1,060) | 0.38 (0.017–539) |

Values reported are the median counts for thousands ($10^3$) of asbestos bodies or millions ($10^6$) of uncoated fibers/gram of dried lung tissue, with ranges indicated in parentheses. The studies by Churg and Vedal [56], Churg et al. [76], and Gaudichet et al. [77] provided the mean value for total fibers/gram of dried lung. Geometric standard deviation is given in parentheses for the study by Churg et al. [76]

[a]*PCLM* phase-contrast light microscopy, *SEM* scanning electron microscopy, *TEM* transmission electron microscopy

[b]In these four studies, asbestos bodies were counted by conventional light microscopy

**Table 11.7** Asbestos content of lung tissue in 524 cases of mesothelioma

| | *N* | AB/g (LM) | *N* | AF/g (SEM) |
|---|---|---|---|---|
| *Pleural mesothelioma* | | | | |
| Asbestosis | 29 | 17,700 (2,160–1,600,000) | 30 | 131,000 (31,500–11,900,000) |
| PPP | 170 | 960 (2.2–74,500) | 164 | 13,800 (370–1,710,000) |
| Other | 284 | 100 (0.8–174,000) | 278 | 5,200 (340–1,420,000) |
| *Peritoneal mesothelioma* | | | | |
| Asbestosis | 7 | 159,000 (23,000–207,000) | 7 | 505,000 (247,000–1,010,000) |
| PPP | 11 | 490 (14.4–140,000) | 11 | 30,600 (263–1,160,000) |
| Other | 17 | 4 (1.0–684,000) | 17 | 1,090 (<490–1,960,000) |

Asbestos bodies/gram of wet lung tissue as determined by light microscopy (LM) and total asbestos fibers 5 μm or greater in length/gram of wet lung tissue as determined by scanning electron microscopy (SEM). Values reported as median with range in parentheses

*PPP* parietal pleural plaques, *other* cases with neither asbestosis nor plaques (or uninformative cases with regard to plaques or asbestosis)

laboratory is summarized in Table 11.7. The median asbestos body count for mesothelioma cases that also had asbestosis is much higher than cases that had parietal pleural plaques without asbestosis, which in turn is higher than cases that had neither plaques nor asbestosis. In addition, the median asbestos body count for asbestosis cases is much higher for patients with peritoneal as compared to pleural mesotheliomas, but less for patients with pleural plaques alone or for patients with neither plaques nor asbestosis (or uninformative cases). A similar trend is observed for total asbestos fibers as measured by SEM, with the exception of a higher asbestos fiber count for peritoneal mesothelioma patients with pleural plaques compared to pleural mesothelioma patients with plaques (Table 11.7). These findings are consistent with the observation that, on average, greater exposure to asbestos is necessary for the development of peritoneal mesothelioma than is needed to develop pleural mesothelioma [81]. The asbestos body counts and fiber levels in the author's laboratory are reported per gram of wet lung and can be compared with those of other investigators by multiplying by a factor of 10 in order to convert to counts per gram of dried lung.

The asbestos body content was within our normal range of 0–20 AB/g in 124 cases or 24 % of the total. In 41 of these 124 cases, the fiber content was found to be elevated by SEM [82]. Hence,

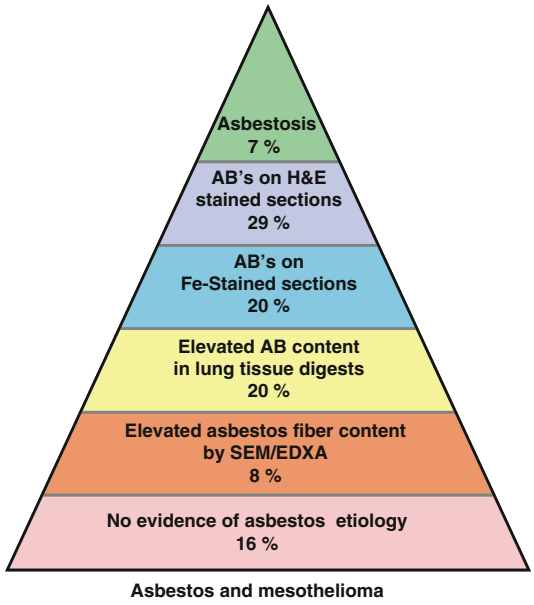


**Fig. 11.7** Pyramid showing the relationship between asbestos exposure and mesothelioma. At the upper range of exposures, 7 % of patients have histologically confirmed asbestosis using recently reported criteria [57]. An additional 49 % have asbestos bodies on hematoxylin and eosin (H&E) or iron-stained sections [58]. At the next level, 20 % of patients will have an elevated pulmonary asbestos body content even though asbestos bodies are not observed in histologic sections. A further 8 % will have an elevated lung fiber burden as determined by scanning electron microscopy even though asbestos body counts are within the background range of 0–20 AB/g. Finally, in 16 % of cases, there is no pathologic evidence for an asbestos etiology. Based on lung fiber analyses of 524 patients with mesothelioma (see Table 11.7)

the asbestos content was indistinguishable from that of a background population in 16 % of cases (Fig. 11.7). In 13 cases (12 pleural, one peritoneal), neither asbestos bodies by light microscopy nor asbestos fibers by SEM were identified. The median asbestos body count for 67 women with mesothelioma was 15 AB/g (range, 1.6–14,100 AB/g). The median total asbestos fiber count by SEM for 67 women was 4,160 fibers/g (range, 450–283,000 fibers/g). Thirty-eight cases (57 %) had asbestos body counts within the background range, and 11 of these had an elevated fiber count by SEM. Hence, about 40 % of mesotheliomas in women had an asbestos content indistinguishable from background. Most of the cases with a normal-range asbestos body count and elevated fiber content by SEM had predominantly noncommercial amphiboles (mostly tremolite). These fibers typically were in the size range between 5 and 20 μm. Asbestos bodies usually form on fibers that are greater than 20 μm in length (see Chap. 3). In five cases of mesothelioma in women (including one peritoneal), neither asbestos bodies by light microscopy nor asbestos fibers by SEM were identified.

The predominant fiber type identified in patients with mesothelioma is commercial amphibole (amosite or crocidolite) [56, 75, 77–79, 83–86]. In a study of 94 cases from the USA, Roggli et al. found that 58 % of more than 1,500 fibers analyzed were amosite, whereas only 3 % were crocidolite [83]. A subsequent study showed falling levels of amosite, but an increasing percentage of cases in which crocidolite was identified [21]. In a separate study, the concentration of commercial amphibole fibers showed a significant correlation with the duration of asbestos exposure [87]. Patients with direct exposures to asbestos had on average higher lung fiber burdens than patients with indirect (i.e., bystander) exposures, and shipyard exposures had on average higher burdens than non-shipyard exposures. When cases were grouped by exposure category, more than 94 % of 1,445 cases fit into one or more of 12 different industrial, six different occupational, or one nonoccupational categories (Table 11.8) [88]. The one nonoccupational category, that of a household contact of an asbestos worker, accounted for 6 % of all cases and more than half of mesotheliomas among women [89].

In view of the experimental observations that fibers 8.0 μm or greater in length and 0.25 μm or less in diameter are the most efficient at producing mesotheliomas [90], it is of interest to examine fiber dimension data in studies of human cases of malignant mesothelioma. In a study of amphibole asbestos-induced mesotheliomas, Churg and Wiggs [91] reported that 39 % of amosite fibers and 23 % of crocidolite fibers exceeded 5 μm in length. In contrast, a study of chrysotile-related mesotheliomas showed that only 11 % of chrysotile fibers and 13 % of tremolite fibers were 5 μm or greater in length [92]. The vast majority of fibers in both studies were less than 0.25 μm in

**Table 11.8** Exposure categories in 94 % of 1,445 cases with malignant mesothelioma

| Industry | Occupation | Nonoccupational exposure |
|---|---|---|
| Shipbuilding | Pipefitter/welder | Household contact |
| US Navy/merchant marine | Boiler worker | |
| Construction | Maintenance | |
| Insulation | Machinist | |
| Oil and chemical | Electrician | |
| Power plant | Sheet metal worker | |
| Railroad | | |
| Automotive | | |
| Steel/metal | | |
| Asbestos manufacture | | |
| Paper mill | | |
| Ceramics/glass | | |

Modified from Ref. [88]

diameter [91, 92]. The biopersistence of relatively long amphibole fibers in lung tissues is the likely reason for the greater potency of amosite and crocidolite fibers in the production of mesothelioma as compared to chrysotile. The latter tends to fragment into shorter fibers and has a much shorter half-life within the lung [56, 76].

It should be noted that most of the studies of fiber burdens in mesothelioma patients have examined lung parenchyma. It is reasonable to assume that fibers actually reaching the pleura are the ones responsible for pleural disease, and the dimensions and types of fibers accumulating in the pleura are of interest in this regard. Sebastien et al. [31] reported that in individuals exposed to mixtures of fibers, short chrysotile fibers (<5 μm) tended to accumulate in the pleura, whereas longer amphibole fibers accumulated in the lung parenchyma. Suzuki and Yuen [93] and Suzuki et al. [94] also reported primarily short chrysotile fibers in the pleura and in mesothelial tissues. Churg et al. [92], on the other hand, found no difference in the length, diameter, or type of fibers isolated from peripheral versus central lung parenchyma in Canadian chrysotile workers. Dodson et al. [95] found long commercial amphibole fibers in samples of pleural plaque from asbestos workers, and Gibbs et al. [96] also identified similar fibers in pleural samples of patients with diffuse visceral pleural thickening. Boutin et al. [97] found a preferential concentration of long commercial amphibole fibers in black spots on the parietal pleura. Dodson et al. [98] recovered long commercial amphibole fibers from samples of peritoneum and mesentery. Clearly, fibers of the type and size known to be associated with the greatest risk of mesothelioma do in fact migrate to pleural and peritoneal tissues. The identification of short chrysotile fibers in these tissues is of questionable relevance, since there is no convincing data that these fibers are pathogenic (see Chap. 10). Analysis of pleural tissues should not be substituted for lung tissue analyses for purposes of determination of causation, since the goal of such analyses is to determine if an individual has a cumulative fiber content different from that of a reference population. This can best be determined by analysis of lung tissue samples. Analysis of tumor tissue is uninformative since values expected for metastatic tumor to the pleura or peritoneum in patients with malignancies not known to be asbestos-related have not been reported.

In summary, patients with mesothelioma who do not also have asbestosis have on average smaller pulmonary asbestos burdens than do patients with asbestosis. This observation is consistent with epidemiologic evidence that mesothelioma can occur in individuals with brief, low-level, or indirect exposures to asbestos [1]. In over half of the patients with mesothelioma in the authors' series, asbestos bodies can be detected in histologic sections with careful scrutiny, and in more than 75 %, tissue digestion studies show an elevated tissue asbestos body content (Fig. 11.7). The distribution of asbestos

**Table 11.9** Asbestos content of lung tissue in reported series of patients with benign asbestos-related pleural disease

| Source | No. of cases | Method[a] | Asbestos bodies/ gram dried lung | Uncoated fibers/ gram dried lung |
|---|---|---|---|---|
| Gylseth et al. [73] | 14 | SEM | – | 2.2 (0.1–13) |
| Warnock et al. [99] | 20 | TEM[b] | 7.8[c] (0.3–9,600) | 0.54[c] (0.018–71) |
| Churg [100] | 29 | TEM | 17.3[c] (0–194) | 1.14[c] (ND) |
| Stephens et al. [101] | 7[d] | PCLM | – | 0.131 (0.029–0.378) |
| | | TEM | – | 28.9 (9.2–83.5) |
| Voisin et al. [102] | 6[d] | LM | 3[c] (0.1–40) | – |

[a]*PCLM* phase-contrast light microscopy, *SEM* scanning electron microscopy, *TEM* transmission electron microscopy, *LM* light microscopy

[b]Values reported are the median counts for thousands ($10^3$) of asbestos bodies or millions ($l0^6$) of uncoated fibers/gram of dried lung tissue, with ranges indicated in parentheses, except for the study of Churg [100], where only the mean value for total fibers/gram was given and a range could not be determined (ND)

[c]In these three studies, asbestos bodies were counted by conventional light microscopy

[d]Cases in series of Stephens et al. [101] are diffuse pleural fibrosis and those of Voisin et al. [102] are rounded atelectasis. All others are parietal pleural plaques

body counts in patients with mesothelioma has been reported to be bimodal, suggesting that there are two distinct populations [19, 26, 82]. One group has elevated tissue asbestos content and is asbestos-related, while the other has a tissue asbestos content indistinguishable from a reference population and may be considered to be "spontaneous" or idiopathic [15, 26]. Analysis of tissue asbestos content in an individual case can thus provide useful information with regard to an etiologic role for asbestos in the production of a mesothelioma.

### 11.4.3 Benign Asbestos-Related Pleural Diseases

Several studies have examined the asbestos content of lung tissue in a series of patients with benign asbestos-related pleural disease [73, 99–102]. Most of these have dealt with parietal pleural plaques, and the studies are summarized in Table 11.9. Gylseth et al. [73] studied 14 cases of parietal pleural plaques by means of SEM and found a median of 2.2 million fibers/g of dried lung as compared to 0.6 million fibers/g in 12 control cases. Warnock et al. [99] reported a median of 0.54 million fibers/g of dried lung in 20 cases of parietal pleural plaques studied by TEM, whereas Churg [100] found 1.14 million fibers/g in 29 cases of pleural plaques. Both studies showed a significant increase in the concentrations of commercial amphiboles (amosite or crocidolite) in the lungs of patients with plaques as compared to a reference population, but no significant differences for chrysotile or noncommercial amphiboles. Whitwell et al. [15] included 21 patients with pleural plaques in their normal control series of 100 cases and found that 55 % of the cases with more than 20,000 fibers/g as determined by PCLM but only 5.5 % of cases with fewer than 20,000 fibers/g had plaques. All of these observations support a role for asbestos fibers in the production of pleural plaques [1].

The authors have had the opportunity to examine the asbestos content of the lung in 356 patients with parietal pleural plaques, but with no evidence of parenchymal asbestosis (Table 11.10). The median asbestos body concentration by light microscopy in 348 patients was 600 AB/g (range, 1.5–140,000). This is similar to the median value of 780 bodies/gram in the study by Warnock et al. [99] and the value of 1,730/g of wet lung in the series reported by Churg [100]. The median total asbestos fiber count by SEM for fibers 5 μm or greater in length in 335 patients from the author's series was 13,300 fibers/g of wet lung, which is less than 10 % of the median level in patients with asbestosis [61] (Table 11.3). Among the 348 patients with plaques alone, 40 (11.5 %) had asbestos body counts within our normal range of 0–20 AB/g, as compared to 0 %

**Table 11.10** Asbestos content of lung tissue in 366 cases of benign asbestos-related pleural disease

| | *N* | AB/g (LM) | *N* | AF/g (SEM) |
|---|---|---|---|---|
| PPP + mesothelioma | 181 | 940 (2.2–140,000) | 175 | 14,600 (263–1,710,000) |
| PPP + lung cancer | 107 | 52 (1.5–23,000) | 108 | 14,900 (<440–1,430,000) |
| PPP (other) | 64 | 255 (3.0–95,300) | 59 | 8,340 (280–1,320,000) |
| Rounded atelectasis | 10 | 680 (5.5–1,980) | 7 | 49,200 (7,290–146,000) |

Asbestos bodies/gram of wet lung tissue as determined by light microscopy (LM) and total asbestos fibers 5 μm or greater in length/gram of wet lung tissue as determined by scanning electron microscopy (SEM). Values reported as median with range in parentheses

*PPP* parietal pleural plaques, *other* cases with neither mesothelioma nor lung cancer. Cases with asbestosis excluded

of patients with asbestosis (Table 11.3). Among these 40, an additional 18 had elevated fiber levels by SEM. Thus, in our series, 94 % of patients with pleural plaques (326/348) had an elevated tissue asbestos content. One additional case with no detectable asbestos had an elevated concentration of refractory ceramic fibers. Andrion et al. [103] reported a highly significant association between pleural plaques and the finding of asbestos bodies in 30 μm thick histologic sections by light microscopy in a study of 191 cases of pleural plaques from a series of 996 consecutive autopsies in Torino, Italy. The median asbestos body count tends to be higher in patients with bilateral plaques when compared to those with unilateral plaques [17]. In addition, the asbestos body count in histologic sections seems to correlate positively with the severity and extent of plaque formation [103].

Benign asbestos-related pleural diseases that occur less frequently than pleural plaques include diffuse pleural fibrosis, rounded atelectasis, and benign asbestos effusions (see Chap. 6). Stephens et al. [101] examined the pulmonary mineral fiber content in seven patients with diffuse pleural fibrosis (Table 11.9). The median uncoated fiber count in these seven cases by PCLM was 0.131 million fibers/g of dried lung and by TEM was 28.9 million fibers/g. These patients on the average have a greater fiber burden than patients with pleural plaques alone, but less than patients with asbestosis (Tables 11.2 and 11.9). The asbestos body content of lung parenchyma was examined in six patients with rounded atelectasis by Voisin et al. [102]. These authors found a median value of 3,000 AB/g of dry lung, with a range of 100–40,000 AB/g (Table 11.9). Ten cases of rounded atelectasis have been studied in the authors' laboratory (Table 11.10). All were men, and their age ranged from 42 to 80 years. Six patients also had parietal pleural plaques and one had bilateral areas of rounded atelectasis. The median asbestos body count by light microscopy was 680 AB/g of wet lung tissue (range, 5.5–1,980 AB/g). The median total asbestos fiber concentration (fibers 5 μm or greater in length) as assessed by SEM in 7 cases was 49,200 fibers/g of wet lung tissue (range, 7,290–146,000 fibers/g). These levels are similar to the values we have observed for patients with pleural plaques. We are not aware of any reports in the literature of pulmonary mineral fiber content in a series of patients with benign asbestos effusion.

In summary, patients with parietal pleural plaques who do not also have asbestosis have considerably smaller pulmonary asbestos burdens than patients with asbestosis and levels that are somewhat lower than but of about the same order of magnitude as patients with malignant mesothelioma. This observation is consistent with epidemiologic evidence that pleural plaques can occur in individuals with brief, low-level, or indirect exposures to asbestos [1]. Limited information is available regarding the pulmonary mineral fiber content of patients with other benign asbestos-related pleural diseases. Published findings in this regard seem to indicate that patients with rounded atelectasis have tissue asbestos levels similar to those of patients with plaques, whereas patients with diffuse pleural fibrosis have levels intermediate between those of patients with plaques and patients with asbestosis.

**Table 11.11** Asbestos content of lung tissue in reported series of patients with carcinoma of the lung

| Source | No. of cases | Selection criteria | Method[a] | Asbestos bodies/ gram dried lung | Uncoated fibers/ gram dried lung |
|---|---|---|---|---|---|
| Whitwell et al. [15] | 100 | General population | PCLM | – | 0.009 (0–0.115) |
| Gaudichet et al. [77] | 40 | General population | TEM[b] | 0.16 (0–290) | 16 |
| Warnock et al. [16] | 9 | Asbestos workers | TEM[b] | 35.6 (0.41-840) | 5.83 (3.10–73.3) |
| Warnock and Isenberg [104] | 75 | Asbestos workers | TEM[b] | 3.75 (0–1,000) | 2.18 (0.077–97) |
| Anttila et al. [105] | 22 | Construction workers | TEM | – | 2.1 (0.3–49.9) |
| Dodson et al. [106] | 20 | Asbestos exposed | TEM[b] | 1.44 (0–21,900) | 0.388 (0–20.1) |

Values reported are the median counts for thousands ($10^3$) of asbestos bodies or millions ($l0^6$) of uncoated fibers/gram of dried lung tissue, with ranges indicated in parentheses, except for the study of Gaudichet et al. [77], where only the mean value for total fibers/gram of dried lung could be obtained from the data presented

[a]*PCLM* phase-contrast light microscopy, *TEM* transmission electron microscopy

[b]In these four studies, asbestos bodies were counted by conventional light microscopy

### 11.4.4 Carcinoma of the Lung

The association between asbestos exposure and an increased risk for lung cancer has been well established in epidemiologic studies, and cigarette smoking and asbestos appear to act in a synergistic fashion to increase this risk [1]. The data supporting these observations and the pathologic features of lung cancers occurring among asbestos-exposed individuals are described in Chap. 7. Although the association between asbestos exposure and lung cancer among individuals with asbestosis is universally accepted, the causative role for asbestos among asbestos workers with lung cancer but without asbestosis is controversial. It is therefore of interest to review what has been learned from fiber burden analysis in this regard.

Studies which have examined the asbestos content of lung tissue in a series of patients with lung cancer are summarized in Table 11.11 [15, 16, 77, 104–106]. The values reported are influenced not only by the investigative and analytical techniques employed, but also by the way the cases were selected. Whitwell et al. [15] examined 100 consecutive cases of lung cancer by PCLM and found a similar distribution of fiber content between cancer cases and controls. Gaudichet et al. [77] included 20 patients with squamous carcinoma and 20 with adenocarcinoma of the lung and found similar asbestos body counts by light microscopy and fiber counts by TEM in these two groups as compared to 20 patients with pulmonary metastases and 20 with cardiovascular disease. The series of Warnock et al. [16] included 7 of 9 cases with histologically confirmed asbestosis, and the series of Warnock and Isenberg [104] included 12 of 62 cases with asbestosis. The authors of the latter study concluded that an asbestos body concentration of 1,000 or more per gram of dried lung tissue or a combined amosite and crocidolite fiber concentration of 100,000 or more per gram of dried lung should be used as an indication that a lung cancer may be asbestos related [104]. Anttila et al. [105] studied 22 cases of lung cancer among construction workers and concluded that fiber number and size correlated with location of tumors in the lower lobes. Dodson et al. [106] studied 20 individuals with lung cancer and a history of asbestos exposure and concluded that a mixture of asbestos fiber types is found in most cases.

We have had the opportunity to study the asbestos content of lung tissue in 408 cases of lung cancer, and the results of our analyses are summarized in Table 11.12. Seventy-eight patients also had asbestosis, 113 had parietal pleural plaques without asbestosis, and 217 had neither plaques nor asbestosis or were uninformative cases. All had some alleged degree of asbestos exposure. Smoking histories were available in 280 cases. All but 23 were cigarette smokers or ex-smokers. Three hunderd and ninty-seven of the cases occurred in men. The 11 women included none with asbestosis (using the newly defined criteria) [57], 2 with pleural plaques,

**Table 11.12** Asbestos content of lung tissue in 408 cases of lung cancer

| | *N* | AB/g (LM) | *N* | AF/g (SEM) |
|---|---|---|---|---|
| Lung cancer plus asbestosis | 77 | 34,700 (840–343,000) | 75 | 300,000 (14,700–8,540,000) |
| Lung cancer plus PPP | 107 | 52 (1.5–23,000) | 108 | 14,900 (<440–1,430,000) |
| Lung cancer (other)[a] | 214 | 224 (1.0–62,900) | 185 | 4,420 (330–285,000) |

Asbestos bodies/gram of wet lung tissue as determined by light microscopy (LM) and total asbestos fibers 5 μm or greater in length/gram of wet lung tissue as determined by scanning electron microscopy (SEM). Values reported as median with range in parentheses

[a]Cases of lung cancer with neither asbestosis nor PPP or uninformative cases with respect to asbestosis or plaques. *PPP* parietal pleural plaques

and 9 with "other" lung cancer cases. The data from Table 11.12 show that patients with asbestosis had a median asbestos body count that was about 700 times that of pleural plaque cases and a total asbestos fiber count that was about 20 times greater. Patients with pleural plaques alone had an asbestos body count that was about one-fourth that of lung cancer patients with neither plaques nor asbestosis and a total asbestos fiber content about three times as great. Although all cases had some history of asbestos exposure, 92 of the 321 patients (29 %) with neither plaques nor asbestosis had asbestos body counts within our normal range of 0–20 AB/g. In five cases (three adenocarcinomas and two large cell carcinomas), neither asbestos bodies by light microscopy nor asbestos fibers by SEM were identified.

Epidemiologic studies have generally failed to present convincing evidence that patients with pleural plaques alone have a significantly increased risk for developing lung cancer (see Chap. 6). Our 217 lung cancer patients with neither plaques nor asbestosis are a very heterogeneous group with regard to type, duration, and intensity of exposure to asbestos. Nonetheless, comparison of their pulmonary fiber burdens with that of the 113 patients with plaques alone would suggest that as a *group*, they would be unlikely to have a significantly increased risk for lung cancer as a result of exposure to asbestos. Others have argued that in an *individual* case, it is the fiber burden rather than the fibrogenic response that is likely the important determinant of carcinogenic risk. Therefore, according to this line of argument, patients with a fiber burden within the range of values observed for patients with asbestosis would have a similar lung cancer risk as patients with asbestosis [104] (see also Chap. 7).

Karjalainen et al. [107] in 1994 published the results of a study of the pulmonary asbestos concentration in 113 surgically resected specimens and compared them with 297 autopsy cases serving as referents. These authors were able to demonstrate that a fiber burden exceeding one million amphibole fibers/gram of dry lung as measured by SEM was associated with an overall lung cancer odds ratio of 1.7. When the value exceeded five million fibers/gram, the odds ratio increased to 5.3. The elevated risk persisted after controlling for smoking and asbestosis. The odds ratio was greatest for adenocarcinoma and lower lobe cancers. Roggli and Sanders [108] studied 234 cases of lung cancer by SEM, dividing them into patients with asbestosis, with plaques alone, or with neither asbestosis nor plaques. The fiber burden exceeded 50,000 amphibole fibers 5 μm or greater in length per gram of wet lung tissue in 82 % of cases with asbestosis, which is roughly equivalent to one million amphibole fibers/gram of dry lung as determined by Karjalainen et al. [107]. However, only 10 % of patients with plaques alone and 5 % of patients with neither plaques nor asbestosis had fiber burdens exceeding this level. The odds were approximately 100 to 1 against finding 50,000 or more amphibole fibers/gram of wet lung when asbestos bodies were not detected in H&E or iron-stained histologic sections. Fiber burden studies are most useful in lung cancer patients that do not meet histologic criteria for a diagnosis of asbestosis but for whom asbestos bodies are identified in histologic sections of the lung.

**Table 11.13** Asbestos content of lung tissue in reference or control populations

| Source | No. of cases | Method | Asbestos bodies/ gram dried lung | Uncoated fibers/ gram dried lung |
|---|---|---|---|---|
| Whitwell et al. [15] | 100 | PCLM | | 0.007 (0–0.521) |
| Mowe et al. [74] | 28 | SEM | | 0.25 (0–4.8) |
| Gaudichet et al. [77] | 20 | TEM[a] | 0.18 (0–3.2) | 11.2 |
| Churg and Warnock [110] | 20 | TEM[a] | 0.28[b] (0.02–0.84) | 1.29[b] (0.260–7.55) |
| Case et al. [111] | 23 | TEM | | 0.62 |
| Srebro et al. [82] | 20 | SEM[a] | 0.029[b] (0–0.22) | 0.030[b] (0.004–0.127) |

Values reported are the median counts for thousands ($10^3$) of asbestos bodies or millions ($10^6$) of uncoated fibers/gram of dried lung tissue, with ranges indicated in parentheses, except for the study of Gaudichet et al. [77], where only the mean value for total fibers/gram of dried lung could be obtained from the data presented

*PCLM* phase-contrast light microscopy, *SEM* scanning electron microscopy, *TEM* transmission electron microscopy

[a]In these three studies, asbestos bodies were counted by conventional light microscopy

[b]Values multiplied by a factor of 10 (approximate ratio of wet-to-dry lung weight) for purposes of comparison

In summary, tissue asbestos analysis has shown that in populations with no appreciable occupational exposure to asbestos and with substantial exposure to cigarette smoke, there is no evidence for a contributing role for asbestos in any lung cancers which occur [15, 77]. This observation is not surprising when one considers that 90 % or more of lung cancers occurring annually in the USA are attributable to cigarette smoking, whereas as few as 2 % of cases may be related to asbestos exposure [109]. In populations with some occupational exposure to asbestos, the presence of either histologically or clinically confirmed asbestosis or a tissue asbestos burden equivalent to that seen in asbestotic subjects is the most useful marker for an asbestos-related lung cancer. It should be noted that fiber dimensions are probably important with regard to the carcinogenic potential of asbestos. Lippman [65] concluded in his review of the human and animal data that it is primarily fibers greater than 10 μm in length and greater than 0.15 μm in diameter that are responsible for the development of lung cancer [1].

### 11.4.5 Normal Lungs (Nonexposed Individuals)

Determination of background levels of fibers to be expected in the general population is an extraordinarily difficult task, since it is no simple matter to define what is normal or to exclude unknown exposures. Several investigators have established ranges of fiber burdens identified in control or reference populations [15, 74, 77, 82, 110, 111], and these are summarized in Table 11.13. Methodological differences and patient selection criteria largely account for the variations in reported values. Our own control cases were selected on the basis of having macroscopically normal lungs at autopsy, no evidence of asbestos-related disease, and an asbestos body count within our previously determined normal range [17, 82]. Two cases with grossly normal lungs but with asbestos body counts of 620 and 300/g of wet lung were excluded. Although there is substantial difference in fiber counts between laboratories, there is a remarkable similarity for asbestos body counts. Three separate laboratories have identified 0–20 AB/g as the background or reference value. These include the laboratories of Ron Dodson in Tyler, TX; Sam Hammar in Bremerton, WA; and Victor Roggli in Houston, TX, and Durham, NC [112]. In any analysis of fiber burden data in a population or an individual case with a given disease, it is of paramount importance to compare the findings with those of an appropriate reference or control population for which the same analytical technique was employed [1].

## 11.5 Asbestos Content of Lung Tissue by Exposure Category

There have been relatively few studies that attempted to correlate tissue asbestos burdens with occupational exposures. Whitwell et al. [15] reported that the number of asbestos fibers found

in the lungs correlated closely with patient occupations but not with their home environment. Patients living near likely sources of atmospheric asbestos pollution had asbestos fiber counts that were similar to the remainder of the patients. Sebastien et al. [31] described the tissue asbestos content in six asbestos workers with heavy exposure, six subjects who handled small amounts of asbestos during their professional life, and six randomly selected cases with no known asbestos exposure history. The mean fiber count by PCLM (400× magnification) for these three groups was two million, two thousand, and two hundred fibers/cm$^3$ of lung parenchyma, respectively. However, the difference between the first two groups was much less striking in terms of fiber counts by TEM: ten million for the six heavily exposed as compared to one million fibers/cm$^3$ for the casually exposed subjects.

Churg and Warnock [113] reported pulmonary asbestos body counts in 252 urban patients over 40 years of age and found that 32 % of blue-collar men but less than 12 % of white-collar men and blue- or white-collar women had more than 100 asbestos bodies/gram of wet lung tissue. In addition, 45 % of steelworkers and 65 % of construction workers had more than 100 asbestos bodies/gram. Tuomi et al. [114] correlated occupational exposure to asbestos and lung fiber burdens in 23 Finnish mesothelioma cases. Two patients had definite and seven probable occupational exposure to asbestos. Six patients had possible and eight unlikely or unknown exposures. All nine patients with definite or probable asbestos exposure had one million or more fibers/gram of dry lung as determined by SEM, whereas only three of eight with unlikely or unknown exposure had more than a million fibers/gram.

The authors have had the opportunity to examine the pulmonary asbestos content by LM and SEM in more than 900 patients with diseases known to be associated with asbestos exposure whose occupational category was also known. The more common occupational categories for these patients are summarized by disease classification in Table 11.14. The results of tissue asbestos analysis for these more common occupational categories are summarized in Table 11.15 and compared with 20 patients with normal lungs at autopsy. The various categories are discussed in more detail in the following sections.

### 11.5.1 Insulators

The highest levels of pulmonary asbestos content were found in patients who were categorized as asbestos insulators. These included individuals whose job descriptions involved work as an insulator, pipe coverer, lagger, asbestos sawyer, and asbestos sprayer (Table 11.14). The median asbestos body content among 89 insulators was 26,600 AB/g, with a range of 3–1,600,000 AB/g, as determined by LM. The median total asbestos fiber content was 300,000 fibers 5 μm or greater in length/gram of wet lung, with a range of 930–11,900,000 fibers/g, as determined by SEM. Forty-eight of 92 insulators had histologically confirmed asbestosis (Table 11.14). In spite of early statements by Selikoff et al. [115] that insulators had relatively light and intermittent exposures to asbestos, this group of workers has higher asbestos body and total asbestos fiber content of the lung than other categories of asbestos-exposed individuals, even when the duration of exposure is similar [26]. These observations correlate well with the reported high prevalence of asbestos-associated diseases among asbestos insulators [116]. Furthermore, despite reports that insulators were exposed almost exclusively to chrysotile [117], the most abundant fiber type found in these workers lungs is amosite [56].

### 11.5.2 Shipyard Workers (Other than Insulators)

This category includes individuals whose job descriptions listed their primary occupation as a joiner, welder, rigger, sandblaster, fitter, shipwright, electrician, draftsman, handyman, engineer, painter, and estimator. Shipyard workers whose primary occupation was as an insulator are included in the previous category of asbestos insulators. There were 161 individuals in this group of shipyard workers (Table 11.15), and most of these

**Table 11.14** Exposure category for 664 patients with asbestos-associated diseases

| Exposure category | Asbestosis | Mesothelioma | PPP | Lung cancer |
|---|---|---|---|---|
| Asbestos insulator | 48 | 34 | 60 | 43 |
| Shipyard worker | 33 | 62 | 88 | 68 |
| Asbestos manufacturing | 8 | 8 | 11 | 11 |
| Power plant worker | 3 | 13 | 9 | 7 |
| Molten metal worker | 0 | 17 | 12 | 7 |
| US Navy/merchant marine | 0 | 40 | 23 | 16 |
| Construction worker | 8 | 60 | 37 | 25 |
| Oil/chemical refinery | 4 | 17 | 19 | 17 |
| Railroad worker | 3 | 12 | 13 | 13 |
| Automotive industry | 0 | 17 | 5 | 5 |
| Household contact | 2 | 44 | 12 | 5 |
| Bldg. occupant | 0 | 12 | 1 | 2 |

Asbestos insulator: insulator, pipe coverer, lagger, asbestos sawyer, asbestos sprayer
Shipyard worker: joiner, welder, rigger, sandblaster, fitter, shipwright, electrician, draftsman, handyman, engineer, and estimator (excluding asbestos insulator)
Molten metal worker: steel mill, iron foundry, aluminum plant worker, miscellaneous
Construction worker: brick mason, carpenter, construction worker, drywall finisher, electrician, laborer, machinist, painter, plasterer, project engineer, tile setter, and roofer
Bldg. occupant: worked in building containing asbestos materials as only known exposure

**Table 11.15** Asbestos content of lung tissue by exposure category

| | *N* | AB/g (LM) | AF/g (SEM) |
|---|---|---|---|
| Asbestos insulator | 89 | 26,600 (3–1,600,000) | 300,000 (930–11,900,000) |
| Shipyard worker (other than insulator) | 161 | 1,540 (2–1,400,000) | 27,800 (260–7,530,000) |
| Asbestos manufacturing | 29 | 760 (1–96,500) | 22,200 (280–2,300,000) |
| Power plant worker | 24 | 810 (2–58,800) | 20,500 (<490–221,000) |
| Molten metal worker | 25 | 180 (3–8,490) | 7,360 (<640–122,000) |
| US Navy/merchant marine | 64 | 310 (1.5–8,020) | 6,920 (830–219,000) |
| Construction worker | 96 | 160 (1.5–83,500) | 7,380 (195–2,610,000) |
| Oil/chemical refinery | 40 | 108 (1.5–3,620) | 6,310 (<490–283,000) |
| Railroad worker | 33 | 68 (1.7–14,200) | 7,670 (<480–434,000) |
| Automotive industry | 33 | 12.5 (1.5–7,740) | 1,040 (91–43,300) |
| Household contact | 56 | 43 (2.0–14,100) | 4,480 (450–283,000) |
| Bldg. occupant | 14 | 8.5 (<0.2–75) | 3,220 (570–6,100) |
| Reference population | 20 | 2.9 (0–22) | <600 (<170–2,540) |

Data are presented as median values, with range indicated in parentheses underneath, of asbestos bodies/gram of wet lung as determined by light microscopy or total asbestos fibers 5 μm or greater in length/gram of wet lung as determined by SEM. Exposure categories as defined in Table 11.14, with *N* representing the number of cases in each category

individuals did not work directly with asbestos products but were rather exposed as bystanders. The median asbestos body content among these shipyard workers was 1,540 AB/g, with a range of 2–1,400,000 AB/g, as determined by LM. The median total asbestos fiber content was 27,800 fibers 5 μm or greater in length/gram of wet lung, with a range of 260–7,530,000 fibers/g, as determined by SEM. Thirty-three of 161 shipyard workers had histologically confirmed asbestosis (Table 11.14). The relatively high pulmonary asbestos burden among individuals with a bystander type of exposure can be related to the fact that these individuals worked side by side with others who directly handled asbestos within the tight confines of the holds of ships. Shipyard workers represented the largest single exposure category in a study of 1,445 mesothelioma cases

by Roggli et al. [88], accounting for 289 (20 %) of the cases studied. The wide range of values observed in this and other categories of occupational asbestos exposure may be explained by variation in duration and intensity of exposure, cofactors such as cigarette smoking, and individual variability in clearance efficiency.

### 11.5.3 Asbestos Manufacturing Plant Workers

Exposure to asbestos in plants that manufactured asbestos products in the past was quite heavy, resulting in many cases of asbestos-related diseases [53, 118–120]. The authors have studied lung tissue from 29 workers at asbestos manufacturing plants with various diseases that have been associated with exposure to asbestos (Table 11.14). The median asbestos body content among these workers was 760 AB/g, with a range of 1–96,500 AB/g, as determined by LM. The median total asbestos fiber content was 22,200 fibers 5 μm or greater in length/gram of wet lung, with a range of 280–2,300,000 fibers/g, as determined by SEM. Eight of 29 cases had histologically confirmed asbestosis. One reason for the somewhat lower asbestos content for asbestos plant workers as compared to insulators is the shorter duration of exposure for the former (7 years for 20 cases with information; range, 4 months to 42 years) as compared to the latter (27 years for 78 cases; range, 1–49 years).

### 11.5.4 Power Plant Workers

Cross-sectional studies have demonstrated the presence of asbestos-related diseases among workers in power plants [121]. Exposures to these individuals derive from insulation used on turbines, generators, boilers, and pipes carrying steam. The authors have studied 24 power plant workers with various diseases associated with asbestos exposure (Table 11.14). The results of tissue asbestos analysis in these cases are summarized in Table 11.15. The median asbestos body content in this category was 810 AB/g, with a range of 2–58,800 AB/g. The median total asbestos fiber content was 20,500 fibers 5 μm or greater in length/gram of wet lung, with a range of <490–221,000 fibers/g. The asbestos body count and total asbestos fiber content in power plant workers are intermediate between that of shipyard workers and US Navy/merchant marine or oil and chemical refinery workers. Asbestosis was present in 3 of 24 cases. Amosite was the main fiber type identified. Exposure to asbestos in power plants was a common cause of mesothelioma in both the study of Roggli et al. [88] and the Australian Mesothelioma Surveillance study [122].

### 11.5.5 Molten Metal Workers

Industries such as steel manufacture, iron foundries, and aluminum smelting and manufacture involve intense heat, so it is not surprising that such industries might afford the opportunity for exposure to asbestos that had been used for insulating purposes. The authors have studied 25 workers from the molten metal industry (13 steel workers, 6 iron workers, 3 aluminum workers, and 3 miscellaneous) with various diseases associated with asbestos exposure (Table 11.14). The results of tissue asbestos analysis in these cases are summarized in Table 11.15. The median asbestos body content in this category was 180 AB/g, with a range of 3–8,490 AB/g. The median total asbestos fiber content was 7,360 fibers 5 μm or greater in length/gram of wet lung, with a range of <640–122,000 fibers/g. Asbestosis was not present in any of these cases. Amosite was the main fiber type identified.

### 11.5.6 US Navy/Merchant Marine

Due to the large amount of asbestos aboard ships, servicemen in the US Navy and seamen in the merchant marine had the opportunity for significant asbestos exposure. This is especially true for those who worked around boilers in the engine room. The authors have studied 64 such patients with various diseases associated with asbestos exposure (Table 11.14). The results of tissue asbestos analysis in these cases are displayed in Table 11.15. The median asbestos body content in this category was 310 AB/g, with a range of

1.5–8,020 AB/g. The median total asbestos fiber content was 6,920 fibers 5 μm or greater in length/gram of wet lung, with a range of 830–219,000 fibers/g. Asbestosis was not present in any of these cases. In the study of 1,445 mesothelioma cases by Roggli et al. [88], US Navy/merchant marine seamen were second only to shipyard workers as the major source of mesothelioma cases in the USA. In comparison, the median asbestos body count in shipyard workers is about five times as high and the total asbestos fiber content about four times as high (vide supra). As in the case of shipyard workers, amosite was the predominant fiber type identified.

### 11.5.7 Construction Workers

This group encompasses a variety of occupations in the construction industry not covered in any of the prior categories. Job titles include brick mason, carpenter, construction worker, drywall finisher, electrician, laborer, machinist, painter, plasterer, project engineer, tile setter, and roofer. The authors have studied 96 construction workers with various diseases associated with asbestos exposure (Table 11.14). The results of tissue asbestos analysis in these cases are summarized in Table 11.15. The median asbestos body content in this category was 160 AB/g, with a range of 1.5–83,500 AB/g. The median total asbestos fiber content was 7,380 fibers 5 μm or greater in length/gram of wet lung, with a range of 195–2,610,000 fibers/g. Asbestosis was present in 8 of 96 cases. Amosite was the main fiber type identified. Sixty of our construction workers had mesothelioma. In the Australian Mesothelioma Surveillance Program, construction trades were the most common cause of mesothelioma [122].

### 11.5.8 Oil and Chemical Refinery Workers

Studies have shown that oil and chemical refinery workers are at risk for asbestos-related diseases [123, 124]. This is due primarily to the presence of boilers and miles of pipeline in these facilities and the consequent requirement for pipe and boiler insulation. The authors have studied 40 refinery workers with various diseases associated with asbestos exposure (Table 11.14). The results of tissue asbestos analysis in these cases are summarized in Table 11.15. The median asbestos body content in this category was 104 AB/g, with a range of 1.5–3,620 AB/g. The median total asbestos fiber content was 6,310 fibers 5 μm or greater in length/gram of wet lung, with a range of <490–283,000 fibers/g. Asbestosis was confirmed histologically in 4 of 40 cases. Amosite was the predominant fiber type identified.

### 11.5.9 Railroad Workers

Railroad workers during the steam engine era often had the opportunity for occupational exposure to asbestos, especially workers in the machine shops or those involved with ripping out old insulation from the steam boilers and replacing it with new insulation. Such exposures virtually disappeared with the replacement of steam locomotives with diesel engines. The authors have had the opportunity to examine the tissue asbestos content of the lungs in 33 individuals whose primary exposure to asbestos was as a railroad worker (Table 11.15). The median asbestos body content among these workers was 68 AB/g, with a range of 1.7–14,200 AB/g. The median total asbestos fiber content was 7,670 fibers 5 μm or greater in length/gram of wet lung, with a range of <480–434,000 fibers/g. Only three of the 33 workers had histologically confirmed asbestosis (Table 11.14). Although some investigators have claimed that chrysotile was the primary type of fiber to which the railroad workers were exposed [125], railroad workers were also exposed to amosite asbestos [126]. Amosite fibers have been identified by the authors in many of these workers' lungs by means of EDXA [88, 108].

### 11.5.10 Automotive Industry

Large numbers of workers are involved with the repair and replacement of brake linings and clutch facings in the course of their daily work. Since these friction products contain asbestos,

there has been some concern that these workers are at risk for the development of asbestos-associated diseases. We have had the opportunity to examine the tissue asbestos content in 33 individuals whose only known exposure to asbestos was working in the automotive industry, including 30 cases in the service industry, two in manufacturing, and one "shade tree" mechanic. The results of tissue asbestos analysis of these individuals are summarized in Table 11.15. The median asbestos body count among these workers was 12.5 AB/g, with a range of 1.5–7,740 AB/g. The median total asbestos fiber content was 1,040 fibers 5 μm or greater in length/gram of wet lung, with a range of 91–43,300 fibers/g. The patient with the highest asbestos body count was a brake line grinder in a manufacturing plant for many years, who died at age 85 with advanced pulmonary fibrosis. At autopsy, the total asbestos fiber content was only 13,800 fibers/g, and most of these were amosite. None of the 33 patients had histologically confirmed asbestosis, although ten patients (including the 85-year-old man noted above) had interstitial lung disease, including eight idiopathic pulmonary fibrosis, one rheumatoid lung, and one desquamative interstitial pneumonia [59]. A few cases of mesothelioma among auto mechanics have been described in the literature [127–130]. Seventeen cases of pleural mesothelioma are also included among the 33 automotive workers we have studied [88, 131, 132]. Fiber analyses in these cases have shown either asbestos content within the range of a reference population or elevated commercial amphibole fibers (with or without elevated chrysotile or noncommercial amphiboles). Similar observations have been reported by others [79, 133–135].

Our fiber burden studies are in accord with epidemiologic studies, which have failed to demonstrate an increased risk for mesothelioma or lung cancer as a result of exposure to asbestos as an automotive mechanic [136, 137]. Furthermore, there is no evidence of an interaction between brake work and other occupational exposures [138]. The lack of significant risk of asbestos-related diseases among brake repair workers and their low pulmonary asbestos content are apparently related to the nature of brake dust. It contains a low level of asbestos (about 1 %), most of which is short chrysotile fibers (less than 1.0 μm in length). The crystalline structure of much of the chrysotile in the dust has been altered due to the heat generated during the braking process [139, 140]. Experimental animal studies have confirmed the lack of fibrogenic and carcinogenic potential of short asbestos fibers (see Chap. 10).

### 11.5.11 Household Exposures

An increased risk of developing an asbestos-associated disease has been reported among household contacts of asbestos workers [141, 142], apparently secondary to asbestos fibers brought home on the worker's clothing. Whitwell et al. [15] reported a case of mesothelioma in the son of a worker from a gas mask factory where the workers took crocidolite home to pack into canisters. The worker's son was found to have between 50,000 and 100,000 fibers/g of dry lung tissue as determined by PCLM. Huncharek et al. [143] reported another case of mesothelioma in the 76-year-old wife of a shipyard machinist who dismantled boilers and other shipyard machinery for 34 years. She was found to have 6.5 million fibers/g of dry lung as determined by TEM. Gibbs et al. [144] reported ten cases of malignant pleural mesothelioma among household contacts of asbestos workers. The total fiber count in these individuals ranged from 5.3 to 320 million per gram of dry lung tissue. Amosite and/or crocidolite were found at elevated levels in eight of the ten cases, whereas two cases had fiber counts within the range of a reference population. The occupations of the asbestos workers included shipyard working, lagging, building, and ordnance, and the household contacts were presumably exposed through fibers brought home on the workers' clothing. In general, the fiber burdens in the lungs of the household contacts were similar to other groups of workers with light or moderate direct industrial exposure to asbestos.

The authors have had the opportunity to examine the pulmonary asbestos content in 56 individuals whose only known exposure was household contacts of asbestos workers, includ-

ing 44 with mesothelioma and 5 with lung cancer (Table 11.14). Fifty of these cases were women [89], including 29 wives, 16 daughters, and one mother of an asbestos worker. The other six cases were sons of asbestos workers. The occupation of the worker was known in 48 cases and included 13 insulators, 8 shipyard workers, five oil or chemical plant workers, five auto mechanics, four power plant workers, three pipefitters, three construction workers, and one each tool grinder/glass plant worker, railroad worker, engine company worker, paper mill worker, mechanical engineer, machinist, and field technician. The median asbestos body count for these cases was 43 AB/g, with a range of 2.0–14,100 AB/g. The median total asbestos fiber content was 4,480 fibers 5 μ or greater in length/gram of wet lung, with a range of 450–283,000 fibers/g (Table 11.15). It should be noted that the tissue asbestos content in some household contacts is consistent with a low or moderate occupational exposure to asbestos (Table 11.15). Amosite was the major fiber type identified. Similar findings were reported by Dodson et al. in a series of 15 women with mesothelioma. [145] Comparing our 13 household contacts of insulators with our series of 89 insulators, we find that the former have about 4 % of the asbestos body and total asbestos fiber content compared to the latter. Asbestosis was confirmed histologically in 2 of 55 cases. In 16 cases (29 %), including all five household contacts of auto mechanics, the tissue asbestos content was within the range of our reference population.

### 11.5.12 Building Occupants

There has been considerable scientific and public debate concerning possible risks of asbestos-induced disease derived from living and working (or attending school) in buildings containing asbestos. Certainly the measured fiber levels in buildings are extremely low [146], and no adverse health effects have been observed in at least one study of workers in buildings with and without asbestos insulation [147]. There are two case reports in the literature of patients with pleural mesothelioma whose only known asbestos exposure was that of an occupant of a building with asbestos-containing materials. One is that of a 54-year-old woman with pleural mesothelioma whose only known exposure to asbestos was as an office worker in a building with ceiling material composed of 70 % amosite asbestos [148]. Analysis of lung tissue demonstrated 31 million fibers/g of dry lung by TEM, the vast majority of which were found to be amosite asbestos by EDXA. The other is that of a teacher's aide with pleural mesothelioma and parietal pleural plaques [149]. Analysis of lung tissue demonstrated elevated concentrations of tremolite asbestos and a particle content consistent with exposure to dust from acoustical ceiling tiles.

The authors have examined the pulmonary asbestos content in 14 patients whose only known exposure was in buildings containing asbestos. The median age was 54.5 years with a range of 28–70. Nine had been exposed within school buildings. Twelve patients had mesothelioma (11 pleural, one peritoneal), one was a nonsmoking man with pulmonary adenocarcinoma who had worked for 20 years in a building containing asbestos, and one was a 69-year-old superintendent of schools and cigarette smoker with pulmonary adenocarcinoma (Table 11.14). The median asbestos body count in these cases was 8.5 AB/g, with a range of less than 0.2–75 AB/g. The median total asbestos fiber content was 3,220 fibers 5 μ or greater in length/gram of wet lung, with a range of 570–6,100 fibers/g (Table 11.15). The asbestos body and asbestos fiber counts are slightly greater than those of 20 individuals with no known occupational exposure to asbestos. The tissue asbestos content was elevated in seven cases. Five were pleural mesotheliomas, and all five were exposed in schools. Three cases had elevated lung content of noncommercial amphibole fibers (e.g., tremolite), one had increased commercial amphiboles (crocidolite), and one had both commercial and noncommercial fiber counts elevated (amosite and tremolite). One additional case occurred in a teacher who had received radiation and chemotherapy as a child for Wilms' tumor [150]. This patient had a lung asbestos body count within the

range of our reference population. The other two cases were lung cancers, and both of these had elevated levels of commercial amphiboles.

The available information indicates that the asbestos content of the lungs in patients with building exposures is often within the background range (Table 11.15). In a series of 1,445 mesotheliomas, only three cases were identified that may be related to exposure as a building occupant [88]. No cases of asbestosis or asbestos-related lung cancer were identified among the patients we studied. It would appear that rare cases of mesothelioma may result from exposures that took place in some schools in decades past. Alternatively, as is the case for auto mechanics, these individuals may have had other exposures which had been forgotten or for which the individual was unaware. The exposure levels are too low to result in an increased risk of lung cancer (see Chap. 7 and the Sect. 11.4.4).

## 11.6 Identification of Fiber Types

As noted in the previous section on fiber quantification, analytical electron microscopy can be used to also identify the types of mineral fibers present in a tissue sample. A number of studies have reported the results of EDXA of mineral fibers from human lung samples [3–5, 16–19, 21, 26, 33, 48, 53, 55, 61, 78, 83, 84, 88, 91, 99, 100, 104, 108, 110, 111, 151–155]. These studies have confirmed the observations from animal experimentation, i.e., that amphibole fibers accumulate within the lung parenchyma to a much greater degree than chrysotile fibers, which over long periods of time are more readily cleared from the lungs. The observations regarding types of mineral fibers present in human lung tissue samples from our laboratory as well as results reported in the literature are summarized in the following sections.

### 11.6.1 Asbestos Fibers

McDonald et al. [156] examined the mineral fiber content of lung tissue in 99 mesothelioma cases and an equal number of age- and sex-matched controls. These investigators noted an excess of amphibole fibers (amosite and crocidolite) in cases as compared to controls, but equal quantities of chrysotile fibers in cases and controls. In a study of 78 additional cases of mesothelioma and matched referents in Canada, McDonald et al. [84] reported that relative risk was related to the concentration of long (≥8 μm) amphibole (amosite, crocidolite, or tremolite) fibers with no additional information provided by shorter fibers. The distribution of chrysotile, anthophyllite, and talc fibers and all other inorganic fibers in the two groups were quite similar. The relationship between commercial amphibole fibers and mesothelioma has been confirmed by other investigators, with amosite as the major fiber type identified in cases from North America [56, 75, 79, 83, 88]. Furthermore, studies have indicated that anthophyllite is also associated with the development of mesothelioma [157, 158].

Similar observations have been reported with regard to asbestos fiber types in the lungs of individuals with asbestosis. Warnock et al. [16] found large numbers of commercial amphiboles, noncommercial amphiboles, and chrysotile fibers in patients with asbestosis. Churg [159] reported the presence of both chrysotile and tremolite fibers in the lungs of chrysotile miners and millers with asbestosis, although tremolite fibers were more abundant in the lungs of these miners when compared to the mine dust. Wagner et al. [18], in a study of naval dockyard workers, found significantly elevated levels of commercial amphiboles in the lungs of workers with asbestosis, whereas chrysotile fibers did not show the same degree of elevation. An elevated pulmonary content of commercial amphibole fibers but not of chrysotile has also been reported for individuals with parietal pleural plaques [99, 100].

The situation with respect to chrysotile and mesothelioma is somewhat more complex. There is a consensus among investigators that a fiber gradient exists with respect to fiber type and mesothelioma, with crocidolite more potent than amosite and amosite more potent than chrysotile on a fiber per fiber basis. Some investigators have suggested that the ratio of crocidolite versus chrysotile potency is as low as twofold

[160], but more recent studies have indicated that it is far higher. Hodgson and Darnton suggested that the ratio is as high as 500 to 1 for crocidolite versus chrysotile and 100 to 1 for amosite versus chrysotile [161], and similarly high ratios between commercial amphiboles and chrysotile were reported by Berman and Crump [162]. In this regard, it is of interest that McDonald et al. have reported that chrysotile miners and millers have a 300-fold increased risk of mesothelioma (assuming a background rate of one case per million person-years) [163]. These workers have an estimated cumulative exposure of 1,000–1,500 fiber/cc-years (approximately 50 fibers/cc for 20–30 years). Reid et al. reported 710 cases of mesothelioma per million person-years in a cohort exposed to Wittenoom crocidolite at estimated median cumulative exposures of 5.5 fiber/cc-years [164, 165]. These observations add further support to the existence of a substantial fiber gradient between commercial amphibole fibers (such as crocidolite) and chrysotile with respect to the disease mesothelioma.

Investigators have debated as to whether chrysotile or its noncommercial amphibole contaminants are responsible for mesothelioma development. Despite the epidemiologic evidence to the contrary [166], some have gone so far as to claim that chrysotile is the primary cause of pleural mesothelioma [167]. Begin et al. [168] noted that the rates of mesothelioma were similar among miners in Thetford as compared to the township of Asbestos, although tremolite contamination was much less in chrysotile from the latter mines. However, subsequent studies showed that some of the mesothelioma cases from miners in Asbestos contained commercial amphibole fibers in their lung tissue samples [169]. In contrast, Thetford miners and millers with mesothelioma contained only chrysotile and tremolite in their lung samples. Tremolite levels usually exceeded chrysotile, even though tremolite accounted for only a small fraction of the mine dust. In addition, the five central mines around Thetford with the highest levels of tremolite contamination had the highest mesothelioma rates [163]. Although Yano et al. [170] reported that workers exposed to Chinese chrysotile that was tremolite-free had an elevated mesothelioma risk, subsequent studies showed that Chinese chrysotile is in fact contaminated with tremolite [171]. Furthermore, the ratio of tremolite to chrysotile among the Chinese workers was similar to that of Canadian chrysotile miners and millers. In addition, there have been no reported cases of mesothelioma from South African chrysotile mines, which are tremolite-free [172]. Taken together, these data strongly suggest that tremolite contamination is the major factor in the mesotheliogenic properties of chrysotile dust [173].

Indeed, in a study of 71 asbestos cohorts exposed to free asbestos fibers, Yarborough concluded that the evidence does not support the hypothesis that chrysotile uncontaminated by amphiboles causes mesothelioma in humans [174]. Berman and Crump examined fiber potency based on a number of different fiber parameters based on physical dimensions (metrics) and concluded that the hypothesis that pure chrysotile is nonpotent for mesothelioma was not rejected by any metric [175]. Although mesotheliomas associated with chrysotile exposures do occur [176], these studies are confounded by contamination with amphiboles [177]. Several studies have suggested that there is a no-effect level of exposure for lung cancer and mesothelioma causation by chrysotile, but there is no general agreement as to what that level may be [177].

Some investigators have claimed that tremolite is removed from chrysotile during processing, implying that exposure to chrysotile-containing products is unlikely to cause mesothelioma [178]. Churg studied the tremolite to chrysotile ratio in chrysotile miners and millers with mesothelioma as well as in mesothelioma patients with heavy mixed exposure to amosite and chrysotile as insulators or shipyard workers [56, 92]. The ratio of tremolite to chrysotile was indistinguishable in these two groups, and there was a strong correlation between tremolite and chrysotile concentrations. These findings suggest that there is little if any removal of tremolite from chrysotile during processing. Roggli et al. [179] performed lung fiber burden analyses in more than 300 mesothelioma patients exposed to asbestos as users of end products. Tremolite was detected

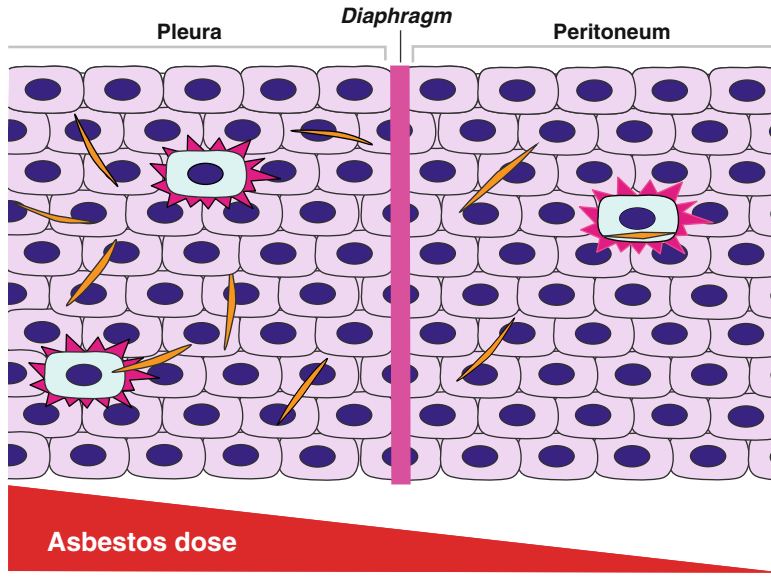


**Fig. 11.8** Artist's diagram of mesothelium shows the pleura and peritoneum as continuous sheets of mesothelial cells separated by the diaphragm. If asbestos were sprinkled evenly over the plain of mesothelial cells, the occurrence of transformation of a mesothelial cell to become mesothelioma (assuming a sufficient dose) would be equally as likely on the pleural or peritoneal side. However, the dose is uneven, since the pleura is more proximate to the original site of deposition (the lungs). Based on this model, the lack of chrysotile-induced peritoneal mesotheliomas would be due to an insufficient dose of tremolite reaching the peritoneal cavity (also see discussion in text)

in more than 50 % of the cases and was elevated above background levels in about 25 %. Cases with elevated tremolite levels represented a wide range of occupational exposures. In about 3 % of cases, tremolite was the only fiber type found in excess concentrations. Tremolite correlated with both talc and chrysotile levels within the lung. The weight of the evidence does not support the claim that chrysotile from end products is free of contamination by tremolite.

There is no convincing evidence for a causative relationship between peritoneal mesothelioma and exposure to chrysotile dust [161, 166, 180]. This apparently is a dosage phenomenon, since asbestos must be deposited in the lungs where it is cleared to the pleura and/or the gastrointestinal tract before it makes its way to the peritoneum (Fig. 11.8). Only about 3 % of the pleural mesotheliomas we have studied are related to chrysotile exposure (with its contaminating tremolite) alone, as compared to about 80 % related to commercial amphiboles (with or without tremolite) [179]. The likely explanation for a lack of peritoneal mesotheliomas in chrysotile cohorts is the inability for sufficient levels of tremolite from chrysotile dust to reach the peritoneal cavity (since tremolite accounts for less than 1 % of chrysotile dust from Canadian mines).

The results of analysis of more than 17,700 fibers from more than 900 patients in our laboratory are summarized in Table 11.16. The data reported are for fibers 5 μm or greater in length/gram of wet lung tissue. Values below the detection limits for any fiber type were recorded as half the detection limit for that case. Analysis of the types of fibers identified according to disease category indicates that as the pulmonary fiber burden increases, the proportion of commercial amphiboles (amosite or crocidolite) also increases. These fiber types are generally below detection limits from individuals with background exposure (i.e., controls), but as much as 100 % of the fiber burden among individuals with asbestosis. The highest levels occurred among patients with asbestosis and the lowest levels among patients with neither plaques nor asbestosis. Patients

**Table 11.16** Energy dispersive x-ray analysis of 17,731 fibers from 919 cases

| | *N* | AC | TAA | Chrys | NAMF |
|---|---|---|---|---|---|
| Asbestosis[a] | 47 | 163,000 (2,060–7,530,000) | 7,920 (740–1,650,000) | 5,630 (740–1,220,000) | 14,900 (2,100–188,000) |
| *Pleural mesothelioma* | | | | | |
| Asbestosis | 30 | 105,000 (8,740–11,900,000) | 6,720 (800–483,000) | 2,830 (800–142,000) | 16,000 (980–541,000) |
| PPP | 164 | 10,800 (120–1,710,000) | 2,070 (220–79,800) | 650 (60–124,000) | 6,700 (240–454,000) |
| Other[b] | 277 | 1,790 (120–1,420,000) | 1,540 (160–454,000) | 490 (120–37,200) | 6,370 (180–146,000) |
| *Peritoneal mesothelioma* | | | | | |
| Asbestosis | 7 | 505,000 (247,000–1,010,000) | 7,380 (4,640–16,900) | 9,850 (4,640–16,900) | 13,200 (5,280–59,200) |
| PPP | 11 | 27,300 (240–1,170,000) | 1,990 (26–20,100) | 710 (13–30,800) | 3,920 (13–39,400) |
| Other[b] | 17 | 330 (240–1,960,000) | 490 (240–49,000) | 290 (240–49,000) | 4,900 (500–49,000) |
| *Lung cancer* | | | | | |
| Asbestosis | 75 | 275,000 (3,710–8,540,000) | 9,580 (490–148,000) | 7,220 (240–133,000) | 14,700 (490–247,000) |
| PPP | 108 | 13,500 (150–1,430,000) | 1,440 (170–49,200) | 700 (150–33,800) | 4,940 (380–1,690,000) |
| Other[b] | 183 | 2,000 (190–113,000) | 1,120 (140–144,000) | 440 (80–22,800) | 5,720 (100–174,000) |
| *Reference population* | 20 | 290 (50–1,270) | 390 (85–2,540) | 300 (50–1,270) | 2,910 (210–10,200) |

Values reported are the median of fibers 5 μm or greater in length/gram of wet lung tissue, with range in parentheses, as determined by SEM

*AC* amosite and crocidolite, *chrys* chrysotile, *NAMF* non-asbestos mineral fibers, *PPP* parietal pleural plaques without asbestosis, *TAA* tremolite, anthophyllite, and actinolite

[a]Asbestosis cases without mesothelioma or lung cancer

[b]Other represents cases with neither asbestosis nor plaques (or uninformative cases)

with parietal pleural plaques tended to have intermediate values of commercial amphibole fibers. Patients with peritoneal mesothelioma had on average higher values than patients with pleural mesothelioma with the exception of the category that had neither plaques nor asbestosis. Noncommercial amphiboles were present at higher levels than chrysotile for most disease categories, and both were present at much lower levels than commercial amphiboles. For cases with neither plaques nor asbestosis, this distinction was less pronounced or absent. In general, the percentage of noncommercial amphiboles and chrysotile fibers correlated inversely with the total pulmonary fiber burden. These observations are consistent with the findings of others that asbestos-related disease correlates primarily with levels of commercial amphiboles [56, 84].

An important area of investigation and regulatory concern is the distinction between true mineral fibers and cleavage fragments of non-asbestiform minerals. Based upon the National Institute of Occupational Safety and Health definition of a fiber, i.e., a particle greater than or equal to 5 μm in length with roughly parallel sides and length-to-diameter ratio of at least 3:1, there may be considerable overlap between the dimensions of true fibrous minerals and cleavage fragments. A recent study indicated that very few cleavage fragments satisfy criteria of length greater than 10 μm *and* diameter less than 1 μm [181]. The great majority of fibers that we have counted (especially com-

**Table 11.17** Energy dispersive x-ray analysis of 7,834 non-asbestos mineral fibers from 1,038 cases

| Fiber type | *N* (%) | Fibers/g |
|---|---|---|
| Talc | 797 (77) | 3,000 (91–1,690,000) |
| Silica | 487 (47) | 1,460 (210–180,000) |
| Rutile | 420 (40) | 1,480 (80–98,000) |
| Aluminum silicate | 303 (29) | 1,270 (91–124,000) |
| Fibrous glass | 101 (9.7) | 980 (170–62,700) |
| Others[a] | 664 (64) | 1,860 (80–541,000) |

Values reported are the median of fibers 5 μm or greater in length/gram of wet lung tissue, with range in parentheses, as determined by SEM. *N* = number of cases with given fiber type detected, with percentage of total cases in which non-asbestos mineral fibers were detected, indicated in parentheses

[a]Other fiber types detected include various silicates, metal oxides (Fe, Al, Cr, CuZn, Sn), endogenous calcium fibers, barium sulfate, and rare earth metals

mercial amphiboles) meet these criteria, so that the issue of cleavage fragments versus true mineral fibers is of little concern for our data set.

### 11.6.2 Non-asbestos Mineral Fibers

If one defines a fiber as an inorganic particle with an aspect (length to diameter) ratio of three or more and with roughly parallel sides, then studies have shown that a number of non-asbestos mineral fibers (NAMF) can be recovered from human lung tissue samples [182]. Among members of the general population, NAMF actually outnumber asbestos fibers by a ratio of about three or four to one. In a study employing transmission electron microscopy with EDXA and electron diffraction, Churg reported that calcium phosphate (apatite), talc, silica, rutile, kaolinite, micas, feldspar, and other silicates (in decreasing order of frequency) accounted for most NAMF recovered from the human lung [182]. These minerals also account for the majority of nonfibrous particulates which can be recovered from human lung samples [183].

The authors have analyzed more than 7,800 NAMF from more than 1,000 patients (Table 11.17). Such fibers were detected in 1,038 out of 1,182 cases examined (87.8 %) and were the only fiber type identified in 74 cases (6.3 %). NAMF account for about 80 % of the fiber burden from individuals with background exposure, but for between 5 and 10 % of the fiber burden among individuals with asbestosis. In general, the percentage of NAMF correlated inversely with the total pulmonary fiber burden. The most commonly encountered fibers in decreasing order were talc, silica, rutile (titanium dioxide), aluminum silicate, fibrous glass, potassium aluminum silicates, and iron oxides [184]. The remainder consisted primarily of silicates, with various combinations of Si with Na, Mg, Al, K, Ca, and Fe. Metal oxides other than titanium or iron were uncommon and included aluminum, iron-chromium, iron-aluminum, copper-zinc, and tin fibers. Endogenous calcium fibers (mostly calcium phosphate or apatite) represented less than 1 % of the total. Fibrous erionite, a hydrated aluminum silicate belonging to the zeolite family of minerals and known to be associated with malignant mesothelioma and pleural calcification (see Chaps. 3, 5, and 6), has also been identified in human lung tissue samples as has recently been reported in a mesothelioma case from North America [185].

In addition to erionite, a number of other non-asbestos mineral fibers have received attention as contributors to asbestos-related disease, particularly mesothelioma. Chrysotile from the mines of Balangero, Italy, is contaminated with a mineral called balangeroite, which has dimensional characteristics similar to amphibole asbestos fibers with demonstrated biopersistence and in vitro cytotoxicity [186]. These fibers may be related to the increased risk of mesothelioma among the Balangero miners and millers [187]. A similar situation has occurred with respect to the vermiculite miners in Libby, Montana, where the vermiculite mineral is contaminated with amphibole fibers including tremolite, winchite and richterite [188, 189]. The latter two are not regulated as asbestos, although studies have shown that size-fractionated Libby amphibole fibers have dimensional characteristics similar to those of commercial amphibole asbestos fibers [190]. There has been concern regarding potential neighborhood exposure to these fibers at vermiculite exfoliation plants located in various sites around the USA [191], and we have reported an example of a case of lung cancer and asbestosis

related to such an exposure [62]. Similar considerations apply to fluoro-edenite, a mineral fiber found in parts of Sicily which has been implicated in some cases of mesothelioma [192–194].

Man-made mineral fibers (MMMF) represent another class of non-asbestos mineral fibers that occasionally are identified in human lung samples. Fibers with a morphology and composition consistent with fibrous glass represented 2.4 % of the NAMF that we have analyzed. These fibers are soluble in lung tissue and thus lack the biopersistence of amphibole asbestos fibers. However, some less common MMMF, namely, refractory ceramic fibers (RCF), are considerably more biopersistent and thus are of some concern [195, 196]. We have detected RCF in lung tissue from 24 cases, including 7 RCF manufacturing plant workers. Eight patients had lung cancer, and eight had pleural plaques. Among the 17 cases who were not RCF workers were eight patients with mesothelioma (including one peritoneal case) [88]. In most of these cases, RCF were incidental and asbestos fibers predominated. However, in two of these cases, RCF were the most frequent fibers identified. In both cases, however, elevated levels of commercial amphibole fibers were also detected. The occupations of the non-RCF workers included brick mason (3 cases), boiler worker (2 cases), pipefitter (two cases), paper mill worker (two cases), machinist (two cases), and insulator, shipyard worker, and railroad worker (one case each). There is currently insufficient information to implicate MMMF (including RCF) as a cause of mesothelioma in humans [197, 198].

In addition to man-made vitreous fibers, there has been interest in the possible human toxicity from exposure to man-made carbon fibers, including carbon composites and more recently carbon nanotubes [199, 200]. Both single-walled and multiwalled carbon nanotubes exist, and they may occur as tangles, ropes, and wires of intertwined tubes [201]. However, the multiwalled carbon nanotubes may also form relatively long thin fibers, and these have the potential for being retained in mesothelial tissues, where they may induce pro-inflammatory effects which could then lead to neoplasia. These observations are consistent with the studies of Boutin and colleagues [97] and have implications for the regulation of workplace exposures to these materials.

Although the biologic significance of non-asbestos mineral fibers is largely unknown, there is no evidence to date (with the exception of erionite and some amphiboles noted above) that they are of any significance in the causation of mesothelioma. A few studies have demonstrated a statistically significant increase in the pulmonary content of fibrous and nonfibrous particulates among patients with lung cancer as compared to noncancer controls matched for age, smoking history, and general occupational category [202, 203]. Although it has been suggested that these mineral fibers and particles may play a pathogenetic role in the development of lung cancer, these observations may merely imply that smokers who develop lung cancer have less efficient clearance mechanisms for fibers, particles, tars, and associated carcinogens that may find their way into the respiratory tract [1].

Non-asbestos mineral fibers may also be important as laboratory contaminants during the analysis of lung tissue samples for their asbestos content. Attanoos et al. reported a case in which fibers of sepiolite, a hydrated magnesium silicate used as an absorbant in pet waste (kitty litter), were found in large numbers in lung tissue from a patient with mesothelioma [204]. The lung tissue submitted for analysis had been immersed in absorbent granules containing sepiolite.

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# 12 Medicolegal Aspects of Asbestos-Related Diseases: A Plaintiff's Attorney's Perspective

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## 12.1 Background: History of Exposure and Disease

The widespread production and use of asbestos, combined with the ambient nature of asbestos fibers and the debilitating effects of asbestos-related diseases, have caused unprecedented human suffering. This is not simply an American tragedy and it is not going away. The use of asbestos and asbestos-containing products continues throughout the world, including in the USA, and while the deadly consequences may be latent, they are no less inevitable.

The association between exposure to asbestos fibers and disease is well documented. Human beings have been using asbestos, which is one of the most toxic substances on Earth, since prehistoric times, and for at least 2,000 years, humans have recognized that exposure to this mineral can have a toxic effect. Nonetheless, for decades, the working conditions in asbestos mills, mines, and manufacturing plants subjected workers to hazardous conditions on a daily basis.

In 1906, a London physician, Dr. H. Montague Murray, performed a postmortem examination on a 33-year-old man who worked 14 years in an asbestos textile factory [1]. The patient was suffering from pulmonary fibrosis. At autopsy, Dr. Murray found asbestos fibers in the lung tissue and linked the man's occupational exposure to asbestos to the disease that killed him. Increasingly throughout the early 1900s, asbestos-related disease became associated with occupations such as asbestos mining, milling, and manufacturing in which the workers were heavily exposed to asbestos fibers. It was during this time that reports of asbestosis began to appear in the published literature. The same year Dr. Murray linked his patient's disease to occupational exposure, 50 deaths were reported among French asbestos textile workers.

Nearly two decades after Dr. Murray's diagnosis, the first death due to asbestosis appeared in the medical literature in 1924 [2]. Dr. W. E. Cooke, an English physician, performed a postmortem autopsy on a 33-year-old woman who had worked in an asbestos textile plant. The autopsy showed extensive pulmonary fibrosis and dense strands of abnormal fibrous tissue connecting the lungs and the pleural membranes surrounding them.

In 1930, Merewether and Price, medical inspectors with the Factory Department, reported on their research of the British asbestos textile industry. This landmark study reported that of 363 asbestos textile workers examined, more than 25 % showed evidence of pulmonary fibrosis [3]. The data made clear that the incidence of fibrosis increased with the number of years of exposure. As a result, legislation was passed in England that required improved methods of ventilation and dust suppression in asbestos textile factories, instituted periodical medical examinations for workers engaged in dusty processes in

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T.D. Oury et al. (eds.), *Pathology of Asbestos-Associated Diseases*,
DOI 10.1007/978-3-642-41193-9_12, 

the asbestos textile industry, and made asbestosis a compensable disease.

In the USA, asbestos workers suffering from asbestosis began filing claims against Johns-Manville and Raybestos-Manhattan, the nation's largest asbestos manufacturers, in the early 1930s. However, asbestosis would not become a compensable disease in most states until 10 or 15 years after the English regulations.

As diagnostic technology and knowledge increased, so did the medical community's understanding of the asbestos tragedy's disastrous scope. By the mid-1930s, reports of asbestos-related lung cancers surfaced in the peer-reviewed literature [4]. By the 1940s and 1950s, asbestos exposure was associated with mesothelioma. In 1955, pathologists began examining cancer patients from a South African mining town, and the finding which associated the diseases with asbestos exposure were published in 1960. The more studies that were conducted, the more scientists demonstrated that shorter durations of exposure could still cause mesothelioma and longer periods of time could lapse from first exposure to the development of cancer. By 1964, the association between asbestos exposure and mesothelioma was generally accepted in the medical community. The New York Academy of Sciences conference that year brought together a compendium of scientists who attested to the fatal consequences of asbestos exposure as evidenced by the significant public health disaster unleashed by asbestos-using industries throughout the country.

The proliferation of products containing asbestos as a key ingredient caused the spread this disease throughout society. Scientists understood that the lethal effects of asbestos exposure would not be contained within asbestos-manufacturing industries. In addition to mining, milling, and manufacturing raw asbestos, the application and use of asbestos-containing products also subjected workers to breathing asbestos dust. The number of persons exposed from various trades and occupations using asbestos-containing products or working next to trades using asbestos-containing products was enormous. Inadequate controls and warnings subjected hundreds of thousands of workers to breathing asbestos fibers.

Our society and those throughout the world are now experiencing the long-term health problems caused by the expansion of the exposed population. Asbestos fibers are toxic to the extent that dangerous, even lethal, exposure is not confined to the plants and worksites. Dust-laden clothing brought home by workers serve as guided transportation vehicles for asbestos fibers which then expose entire households to these deadly asbestos-related diseases. The most innocent and unsuspecting of victims, the wives and children of asbestos-exposed workers, have also developed and died of asbestos-related diseases at alarming rates. Dust emitted from the mines and plants have exposed entire neighborhoods to environmental pollution.

According to the Center for Disease Control and Prevention, 10,000 people in the USA die each year from asbestos-related diseases. The 2,704 deaths from mesothelioma reported in 2005 represented an 8.9 % increase from 1999 [5]. In 2007 alone, roughly 1,730 metric tons of asbestos was imported into the USA for use in a variety of construction and transportation products [5]. At its peak, asbestos was incorporated into more than 3,000 products and applications. In Canada, the number of mesothelioma deaths rose 17 % between 2000 and 2003 [6]. These numbers are expected to continue rising, despite the elimination of asbestos from the manufacturing of many products, due to both the latency periods discussed earlier and the ongoing importation of asbestos into this country. Asbestos disease will continue to be a worldwide problem. Although the use of asbestos has declined over recent years, an estimated 125 million people continue to be occupationally exposed to asbestos each year [7]. Russia, China, Kazakhstan, Brazil, and Canada continue to mine asbestos for use in various products. India, Pakistan, Mexico, and Thailand are among the developing countries still importing asbestos. It is estimated that 250,000 cases of mesothelioma will occur in Europe and the USA in the next 35 years [8].

Until around 1970, the industry essentially regulated themselves as to permissible exposure levels. Although there were state regulations prior to this, state regulators did little to enforce health and safety laws enacted to protect workers.

Corporations chose to ignore regulations or plead ignorant to such regulation. Occupational and environmental asbestos exposures today are regulated by the Occupational Safety and Health Administration (OSHA) under provisions of the Clean Air Act (CAA) and the Toxic Substances Control Act (TSCA). Consumer asbestos exposures are regulated by the Consumer Product Safety Commission. In March 1971, the EPA, under the Clean Air Act, listed asbestos as a hazardous air pollutant.[1] In April 1973, spray application of products containing more than 1 % asbestos was banned except on equipment and machinery.[2] In 1975, demolition standards were revised and made more stringent concerning controls, use of asbestos in friable insulation, and waste disposal. That same year, friable insulation products containing more than one percent asbestos were banned in the USA.[3] In addition, the EPA under the Toxic Substances Control Act has issued numerous regulations under its authority to regulate toxic substances if it finds that the manufacture, processing, distribution in commerce, use, or disposal of the chemical substance, or any combination of such activities, presents or will present an unreasonable risk of injury to human health or the environment.[4] In May 1971, OSHA issued initial regulations adopting the ACGIH threshold limit value of 12 f/cc.[5] OSHA has continually reduced by regulation permissible exposures to asbestos in the workplace.[6] In 1986, OSHA's overview of asbestos-related diseases described the magnitude of the problem:

> OSHA is aware of no instance in which exposure to a toxic substance has more clearly demonstrated detrimental health effects on humans than has asbestos exposure. The diseases caused by asbestos exposure are life-threatening or disabling. Among these diseases are lung cancer, cancer of the mesothelial lining of the pleura and peritoneum, asbestosis, and gastrointestinal cancer.[7]

That same year, the World Health Organization's International Agency for Research on Cancer (IARC), as well as OSHA, concluded that all fiber types were carcinogenic [9].

Due to the constraints that require regulators to set standards within the confines of what is "feasible," regulations maintain a "permissible" level of exposure to asbestos. "Permissible" is not synonymous with safe, and it does not guarantee that persons exposed at the permissible levels will not develop disease. To the contrary, although the risk may decrease, disease will still occur at those levels as set out and noted in the regulations. There is ample evidence in the medical and scientific community that there is no safe level of exposure to any type of asbestos fiber and that regulation and standards for carcinogenic substances such as asbestos should be zero.

## 12.2 Evolution of Legal Claims

### 12.2.1 The Catalyst

The latent onset of disease from exposure has led to a catastrophic epidemic and a continuing onslaught of disease as a result of persons exposed

[1] 36 Fed. Reg. 3031 (March 1971).

[2] 38 Fed. Reg. 8820 (April 1973).

[3] 40 Fed. Reg. 48299 (1975).

[4] In October 1979, the EPA issued its Advanced Notice of Proposed Rule Making outlining EPA's intent to use section 6 of the TSCA to reduce the human health risk posed by asbestos. 44 Fed. Reg. 60061. In July 1982, EPA issued its reporting rule promulgated to collect information on industrial and commercial uses of asbestos. 47 Fed. Reg. 33207. In January 1986, EPA issued its Proposed Final Rule stating EPA's finding that asbestos exposure poses an unreasonable risk to human health. 51 Fed. Reg. 3738. In July 1989, EPA issued a Final Rule entitled Asbestos, Manufacture, Importation, Processing and Distribution in Commerce Prohibitions. 54 Fed. Reg. 29460. The EPA July 1989 TSCA regulations contain specific effective dates for various bans including the manufacture, import and processing ban, distribution in commerce ban, and the ban of different asbestos products banned in various stages.

[5] 36 Fed. Reg. 10466 (May 1971).

[6] December 1971, OSHA issued an emergency temporary standard of 5 f/cc as an 8-hour time-weighted average (TWA) and a peak exposure level of 10 f/cc, 36 Fed. Reg. 23207. In June 1972, OSHA issued its final TWA standard of 5 f/cc and a ceiling limit of 10 f/cc. The TWA automatically reduced to 2 f/cc effective July 1976, 44 Fed. Reg. 11504. In June 1986, OSHA issued its reduced final standard of 0.2 f/cc as a TWA. A short-term exposure limit (STEL) of 1.0 f/cc was promulgated in September of 1988, 51 Fed. Reg. 22612. In July 1990, OSHA proposed a reduction of the TWA to 0.1 f/cc, 55 Fed. Reg. 29712.

[7] 51 Fed. Reg. at 22615 (June 20, 1986).

decades ago. The US Supreme Court recognized the nightmare that asbestos exposure has inflicted on the American public and the American judicial system. In *Ortiz v. Fibreboard Corporation*, it commented that occupational asbestos exposure and its detrimental effects have created an "elephantine mass of asbestos cases."[8]

Although asbestos claims were filed before the 1960s and early internal corporate documents reveal companies implemented strategies to avoid liability, legal troubles mounted for the asbestos industry when Claude J. Tomplait, a 40-year-old Texas insulator sought legal representation from Ward Stephenson, an attorney in Orange, Texas, in 1969. For 20 years, Tomplait labored as an asbestos worker insulating steam pipes, boilers, turbines, and other high-temperature equipment in the shipyards, power plants, oil refineries, and petrochemical factories. Recently diagnosed with pulmonary fibrosis as a result of inhaling asbestos fibers, Tomplait asked Stephenson if he would file a worker's compensation claim on his behalf. Stephenson not only filed a worker's compensation claim but, for the first time, brought suit against the asbestos manufacturers under the newly recognized doctrine of strict liability.

Although unsuccessful in his suit against the manufacturers, this case served as a catalyst for future litigation. In 1969, Stephenson brought suit on behalf of a coworker of Tomplait, Clarence Borel, against 11 asbestos manufacturers.[9] *Borel v. Fibreboard Corporation* was filed in the District Court for the Eastern District of Texas and significantly changed the way the litigation would progress into the future. *Borel* was the first case in the country to recognize a manufacturer's duty to warn of asbestos product dangers. It became the seminal case standing for the manufacturer's responsibility to know and warn of the dangers of its products that may cause occupational disease.

When Judge John Minor Wisdom issued the opinion of the US Court of Appeals for the Fifth Circuit in *Borel* on September 10, 1973, little did anyone expect that this decision, extending the doctrine of strict product liability to asbestos-related disease caused by the use of insulation materials, would engender a wave of personal injury litigation never before seen in American jurisprudence. Asbestos exposure was ubiquitous throughout industrial facilities. Less than 10 years later, over 16,000 asbestos-related personal injury cases had been filed in the USA, and in the words of a subsequent opinion by the Fifth Circuit, asbestos litigation "had become the largest area of product liability litigation, far surpassing the number of cases generated by the controversies over Agent Orange, the drug DES, the Dalkon Shield intrauterine device, or even automobile defects."[10]

Today, there is no shortage of plaintiffs seeking compensation for asbestos-related disease. Filings of claims by persons exposed to asbestos fibers from a variety of sources continue to fill the Courts and impede their ability to achieve a speedy resolution. Many still proceed by traditional jurisprudence of tort litigation—a single plaintiff suing for injury against a number of asbestos premise, product manufacturer, or supplier defendants. A traditional setting requires the plaintiff to prove his injuries, that the injuries are a result of exposure to respirable asbestos fibers, that the exposure attributed to each defendant contributed to the disease, and that the asbestos defendant is legally liable for the asbestos-related injuries sustained by the plaintiff.

As the numbers of persons injured from exposure to asbestos grew, so did the flood of litigation in courts throughout the nation. It became obvious that to try each case one at a time would create a huge backlog of cases well past many of the lifetimes of the plaintiffs

[8] *Ortiz v. Fibreboard Corp.*, 527 U.S. 815, 821, 119 S.Ct. 2295 (1999) (stating that asbestos litigation "defies customary judicial administration" and "calls for national legislation." It is important to note, however, that all proposed national legislative solutions to date have been sponsored by manufacturing defendants and have been woefully inadequate in their terms in compensation to diseased individuals.)

[9] *Borel v. Fiberboard Corp.*, 493 F.2d 1075 (5th Cir. 1973), cert. denied, 419 U.S. 869 (1974).

[10] *Jackson v. Johns-Manville Sales Corp.*, *750* F.2d 1314, 1335-36 (5th Cir. 1985).

involved as well as the lawyers and judges.[11] In order to deal with the staggering numbers of cases, courts made efforts to streamline the cases by consolidating trials, conducting summary trials, and standardizing discovery and pleading forms. Still huge numbers of cases remained in the court system.

In an effort to deal with the growing number of claims, a national settlement class action was filed in 1993 in an attempt to resolve claims of present and future victims of asbestos disease.[12] Nevertheless, in 1997, the US Supreme Court overturned the $1.3 billion dollar class action settlement that was forged between plaintiffs and the Center for Claims Resolution, a consortium of twenty former asbestos manufacturers.[13] Some view this decision as contributing to the resurgence of asbestos litigation, and in striking down this historical class action settlement, plaintiffs and asbestos defendants were again forced to resolve these cases one at a time in what appears to be endless litigation.

### 12.2.2 State Courts

As in *Borel*, typically, a plaintiff files their action in state court. Ideally, a plaintiff would file their case in the home state and county in which they live and where a jury of their peers is selected to hear their case. The defendants, for the most part, do not have ties to the community as substantial as the plaintiff's ties. The plaintiff's lawyer will inevitably look to a jurisdiction that will be most favorable to their client's claims. There may be several jurisdictions to choose from because a typical asbestos plaintiff may have worked and been exposed in many different states at many different jobsites. Today, many jurisdictions in which asbestos litigation once flourished have been transformed into court systems in which asbestos claims are increasingly difficult to prove. Many states have enacted tort reform legislation limiting the plaintiff's access to the courts. Jurisdictions such as Ohio, Texas, and Florida which have large manufacturing bases have nonetheless enacted statutes which severely restrict potential plaintiffs' ability to recover for their asbestos-related injuries.[14] Throughout the country, tort reform front groups, organized to lobby legislatures and ensure that more defendant-friendly judges are elected or appointed to the bench, have flourished.

As a result, causation and threshold exposure requirements may differ from jurisdiction to jurisdiction. Although a defendant's conduct and a plaintiff's exposures may be identical in two separate jurisdictions, the laws of each state vary in how they may treat similarly situated parties so dramatically as to render some injured parties without a judicial remedy. With the number of asbestos-related malignancies rising dramatically, several jurisdictions created exigent dockets that allow for living claimants to have their cases set for trial on an expedited basis. As a result, many nonmalignant claims move slowly through the process, if at all.

By way of example, a person suffering from an asbestos-related disease as the result of fibers brought home on a family member's clothing will shy away from bringing an action in a jurisdiction that has previously ruled it was not foreseeable that family members could be exposed to such deadly fibers in this way. In addition, many states have enacted statutes of repose which create an unrealistic time limitation on the number of years a plaintiff has to purse a claim against certain defendants. These time limitations are unrealistic in that the latency period and first possible diagnosis of asbestos-related disease often occurs after the repose cutoff. Regardless

[11] In *1995*, a study concluded that the disposition of all then pending asbestos cases for both personal injury and property damages, if treated in the traditional course of litigation, would require approximately 150 judge years. *See* Jack B. Weinstein, Individual Justice in Mass Tort Litigation 140 (*1995*), citing Thomas Wiliging, History of Asbestos Case Management (Federal Judicial Center staff paper for June *25*, 1990, National Asbestos Conference.).

[12] *Georgine v. Amchem Products*, *Inc*., 157 F.R.D. 246 (E.D.Pa. 1994).

[13] *Amchem Products v. Windsor*, 521 U.S. *591* (1997).

[14] Texas statute: Tex. Civ. Prac. & Rem. Code § 90.001-12; Florida statute: Fla. Stat. § 774.001-209; Ohio statute: ORC Ann. 2307.91-98.

of where a case is filed, choice of law issues may still place limitation on recovery unless a jurisdiction's public policy trumps.

A potential plaintiff's options may also be limited by constitutional factors. For instance, federal law may preempt state law claims. Preemption is grounded in the US Constitution's Supremacy clause which provides that federal law "shall be the Supreme Law of the Land." If federal law occupies an entire field exclusively, then any attempt to apply state law will be deemed fatal. In early 2012, the US Supreme Court held that claims by railroad workers who had been exposed to asbestos-containing brakes and insulation at railroad maintenance facilities were preempted by federal law. Specifically, the 1911 Locomotive Inspection Act (LIA) preempted state law claims that the products were negligently designed. The Court based its ruling on a 1926 interpretation of the LIA in which the Supreme Court held that Congress intent in enacting the act was to "occupy the entire field of regulating locomotive equipment."[15]

Although state law claims made by railroad workers against manufacturers may be preempted under federal law, railroad workers have additional rights and benefits outlined by the Federal Employers Liability Act (FELA). FELA is a series of federal laws passed by Congress in 1908 to improve railroad safety and provide substantial compensation for injured railroad workers and their families. The Act provides that an injured railroad employee may bring an action against his or her employer in state or federal court in any jurisdiction in which that employer transacts business. In 2003, the US Supreme Court upheld judgments involving six former railroad employees seeking compensation for asbestosis and fear of cancer. The Court held that a plaintiff suffering from asbestosis could recover damages for fear of developing cancer and the railroads liability was not reduced by the plaintiff's exposure to asbestos on nonrailroad jobs.[16]

These inconsistencies in the law regarding injuries occurring from asbestos exposure, whether differing by state or occupation or other factors, have created a judicial system in which redress for these often fatal injuries differs dramatically for those suffering from the same asbestos-related diseases.

### 12.2.3 Federal Courts

In 1991, the US Judicial Panel on Multidistrict Litigation created MDL-875 in order to consolidate pending asbestos cases in the US District Court for the Judicial District of Eastern Pennsylvania.[17] This consolidation mechanism was created in an effort to avoid duplicative discovery, ensure consistent pretrial rulings, and conserve the resources of the judiciary and parties involved. By design, cases that are not terminated prior to the conclusion of consolidated pretrial proceedings are remanded to their originating transferor district.

The Judicial Panel on Multidistrict Litigation had first considered and rejected the concept of a consolidation of asbestos cases in 1977 when there were 103 pending federal actions.[18] By 1991, there were over 30,000 such actions.[19] Immediately upon its creation, MDL-875 was flooded with asbestos cases and plaintiffs across the country soon began to view the docket as a "black hole" where cases would remain parked for years with little hope of resolution or trial.[20] By 1995, a study concluded that the disposition of all then pending asbestos cases for both personal injury and property damages, if treated in the traditional course of litigation, would require approximately 150 judge years.[21]

[15] *Kurns v. R.R. Friction Prods. Corp.*, 132 S. Ct. 1261 (2012).

[16] *Norfolk & Western Ry. v. Ayers*, 538 U.S. 135 (2003).

[17] *In re Asbestos Products Liability Litigation*, 771 F. Supp. 415, 423 (D.D.C. 1991).

[18] See footnote 17.

[19] See footnote 17.

[20] See, e.g., Brewster v. A.W. Chesterton Co., 2007 U.S. Dist. LEXIS 29420 (N.D. Cal. Apr. 6, 2007); *In re United States Lines*, 1998 U.S. Dist. LEXIS 10135 (S.D.N.Y. July 8, 1998).

[21] Jack B. Weinstein, Individual Justice in Mass Tort Litigation 140 (*1995*), citing Thomas Wiliging, History of Asbestos Case Management (Federal Judicial Center staff paper for June *25*, 1990, National Asbestos Conference.)

In 2007, with the MDL-875 docket overwhelmed with pending cases, the presiding judge, the Honorable Eduardo C. Robreno, issued Administrative Order 12. This Order made a variety of aggressive demands on plaintiffs aimed at reducing the caseload, including a requirement that each plaintiff submit a medical diagnosing report or opinion in support of their action. It was anticipated that a large number of potential plaintiffs would be unable to present a sufficient medical basis to support their claims. This did not prove to be an accurate assessment of the scope of asbestos-related injuries. From November 2008 through December 2011 alone, 130,500 cases representing 4,509,240 claims were transferred to MDL-875.[22]

The significant increase in asbestos cases since the creation of MDL-875 reflects the continuing uptick in the numbers of Americans diagnosed with asbestos-related diseases annually. In reaction to the skyrocketing number of injuries linked to asbestos exposure, defendants have attempted to use procedural mechanisms to preclude state courts from ruling on the claims brought by their own citizens injured as a result of asbestos exposure.

One of the most utilized mechanisms is the Federal Enclave Clause in the US Constitution which gives the US Congress exclusive authority "over all places purchased by consent of the legislature of the state in which the same shall be, for the erection of forts, magazines, arsenals, dockyards, and other needful things."[23] As a result, a tort committed within a federal enclave gives rise to a federal question and district court jurisdiction.[24] This is particularly relevant in the context of asbestos litigation because a government-owned shipyard has been deemed a federal enclave[25] and asbestos-containing products have been used extensively aboard naval vessels for decades.

Hundreds of thousands of soldiers who served aboard a US Navy vessel have been injured as a result of asbestos exposure. The tight, enclosed spaces aboard these ships, many of which were lined with miles of pipes insulated with asbestos materials, created a deadly combination of frequent and prolonged asbestos exposure. In addition to the pipe insulation materials, Navy vessels utilized turbines, boilers, pumps, valves, and many other pieces of equipment that required asbestos-containing products for their proper use. When these asbestos-containing products are removed, replaced, repaired, or otherwise disturbed, respirable asbestos fibers can be released and breathed in by all those exposed. Traditionally, state courts have had an important interest in protecting their citizens from injury and holding those responsible liable.

However, based on the federal enclave doctrine, defendants have taken full advantage of their ability to remove any claim based on exposure at a government-owned shipyard and have further argued that exposure occurring aboard a Navy vessel docked at a government-owned shipyard during part of the injured party's tour should also be removable based on the federal enclave doctrine.[26]

As of December 2011, there were still approximately 400 asbestos cases being brought in federal district court each year, in addition to the hundreds of cases brought in sates courts throughout the country. Nonetheless, the Judicial Panel on Multidistrict Litigation determined that the backlog of cases had been "largely eliminated" and that cases would no longer be transferred to MDL-875 after January 1, 2012. Without MDL-875, it is likely that a defendant's "threat" to remove a case to federal court will no longer have the same affect of essentially parking the case.

### 12.2.4 Bankruptcy Courts

Asbestos companies are increasingly seeking protection under the Bankruptcy Code. Since

[22] MDL-875 Asbestos Products Liability Litigation, Caseload Statistics, http://www.paed.uscourts.gov/documents/MDL/MDL875/statistics%20MDL-875.jan2012.pdf.

[23] U.S. Const., Art. I, § 8, cl. 17.

[24] *See* 28 U.S.C. § 1331.

[25] *Anderson v. Crown Cork & Seal*, 93 F. Supp. 2d 697 (E.D. Va. 2000).

[26] See footnote 25.

1982, at least 70 companies with asbestos liabilities have sought Chap. 11 bankruptcy protections with others inevitably to follow. A reorganization under Chap. 11 of the Bankruptcy Code is one of the few methods by which a company can isolate its operations from its asbestos liabilities. Although bankruptcy may permit an asbestos defendant to put a permanent end to asbestos litigation against it and resolve liability issues in a coordinated and integrated fashion, it severely reduces compensation to diseased plaintiffs [10]. The first relief for a defendant in asbestos litigation after filing under Chap. 11 is an immediate stay. Section 362 of the Bankruptcy Code, the "automatic stay" provision, enjoins virtually all litigation against the debtor immediately upon the filing of a bankruptcy case.[27] A claims process is established for which both present and future claims are eventually paid at a much reduced value than one would hope to receive in the civil court system. Depending on the bankruptcy, claimants submit information as part of an administrative settlement process that is then processed and paid if the claimant meets the criteria for payment.

In August 1982, Johns-Manville, the largest manufacturer of asbestos-containing products, filed for bankruptcy, and several years later, the Manville Asbestos Disease Compensation Fund (Manville Trust) was created to handle claims filed against Johns-Manville. At the end of 1999, the Manville Trust projected that it would receive almost 500,000 claims over the next four decades. In the year 2000, however, it received 58,600 new claims, an 81 % rise over the prior year, and 2 years after its initial prediction, the Trust revised its projections and estimated that it will receive 1.5–2.5 million future claims.[28]

Unfortunately, the Manville trust's experience is not unique, as many asbestos bankruptcy trusts have had to dramatically lower the amount of money that can be paid to each plaintiff in settlement of his or her claims. Although the claims filed by nonmalignant disease victims have decreased substantially since the early 2000s, mesothelioma claims have surged and are generally far higher than the initial estimates made when the trusts were first created. Due to the combination the increase in mesothelioma claims and the decline in the value of their investments during the worldwide financial crisis of 2008–2009, many trusts have drastically reduced the payouts to claimants on a per-case basis.

## 12.3 Proving the Asbestos Disease Case

### 12.3.1 Knowledge About the Hazards

Under the product liability or premise laws of most states, the plaintiff must prove that the manufacturers either knew or should have known that their asbestos-containing products were hazardous and that the defendants failed to give adequate warnings of those dangers. Establishing this burden can be accomplished in two ways. The plaintiff may prove through oral testimony or documentary evidence that a company was actually aware that its products containing asbestos could cause harm to the users of those products.[29] Alternatively, a plaintiff may prove that, if a company was to have reviewed the scientific and medical literature at the time its products were sold, such an analysis would have revealed that asbestos was known to be dangerous. Under the law of product liability, a manufacturer is presumed to know the hazards of its products, and *Borel* established that a manufacturer is considered to be an expert with regard to the dangers of its products.

[27] See footnote 25.. (noting that the stay is subject to a number of exceptions specified in the statute itself, such as regulatory actions by the government. See 11 U.S.C. § 3 62(b). The bankruptcy court can also terminate the stay, or modify its coverage, upon motion of a party in interest in the bankruptcy case. See 11 U.S. C. § 362(d).)

[28] Insurance Day (September 13, 2001).

[29] Testimony has been repeatedly taken from such industry consultants as Dr. Gerrit W.H. Schepers, former director of the Saranac laboratory, and Dr. Thomas F. Mancuso, the consultant to the Philip Carey Corp., the predecessor of Celotex.

At trial, a plaintiff may establish the company's actual knowledge and corporate misconduct by introducing as exhibits the internal correspondence and memoranda of the companies. Throughout the years, internal documents located in corporate files reveal that many companies not only had actual knowledge of the hazards of asbestos but also took active measures to thwart publication or mention of asbestos-related hazards. In 1935, Sumner Simpson, the president of the asbestos manufacturer Raybestos-Manhattan, wrote to Vandiver Brown, head of Johns-Manville's legal department, telling him, "I think the less said about asbestos, the better off we are." Brown replied, "I quite agree with you that our interests are best served by having asbestosis receive the minimum of publicity" [11]. In the following year, officials from asbestos-manufacturing companies met in New York City to sign a secret agreement to finance animal experiments at the Trudeau Foundation's Saranac Laboratory at Saranac Lake, New York. The intent was to gather data that would support a defense to lawsuits beginning to be brought against asbestos manufacturers. Prior to final publication, the Saranac report made reference to the findings that animals exposed to asbestos developed cancer. The final report, however, published in 1951, deleted all reference to cancer. Furthermore, the revised report was absent in its criticism of the asbestos dust threshold limit value (TLV) and previously published studies linking asbestos with cancer. In 1952, the decision was made not to publish the findings of a Saranac symposium that included discussions of asbestosis and cancer contracted by end users of asbestos products.[30]

This industry code of silence may be shown in a variety of ways. In 1947, members of the Asbestos Textile Institute (ATI) sponsored a study of textile factories by the Industrial Hygiene Foundation. The study found asbestosis in workers, made recommendations about medical exams, and recommended reevaluation of the industry's threshold limit value for asbestos. These findings were never circulated outside the ATI. In the mid-1950s, the Institute rejected funding for cancer studies because "such an investigation would stir up a hornet's nest and put the whole industry under suspicion."[31]

A number of internal documents reveal that the asbestos companies continued to be less than forthcoming with their increasing knowledge of asbestos and disease.[32] As information mounted, so did the industry's fear that the dangers of asbestos would be publicized and adversely impact profits. For example, a once contemplated health and safety booklet was opposed by industry members because "the booklet creates fear in the minds of buyers, users, and workers without justification. These fears would be damaging to the entire industry."[33] The asbestos companies recognized this as an industry-wide problem, and in fact, one such document revealed that the companies understood that "no one company acting independently could adequately or effectively represent an entire industry in dealing with the press and with government officials."[34]

[30] Letter from Frank H. Zimmerman, Director of Safety, National Gypsum Co., to Clifford L. Sheckler, Manager, Occupational Environmental Control, Johns-Manville Corp. (Apr. 17, 1968).

[31] Motley, R., Kearse, A.M., Decades of Deception: Secrets of Lead, Asbestos, and Tobacco, Trial Magazine (October, 1999) (citing Asbestos Textile Institute, Minutes of the Air Hygiene and Manufacturing Committee Meeting (Mar. 7, 1957)).

[32] For example, in 1950, the Quebec Asbestos Mining Association, whose members included Canadian asbestos mining companies, contracted with Saranac to determine whether asbestos caused cancer. A 1952 report, which was never published, showed increased cancer in mice and suggested further study. In 1957, the Canadian association contracted with the Industrial Hygiene Foundation of American to study asbestos and cancer. The resulting report concluded that those with asbestosis had an increased occurrence of lung cancer. Nevertheless, attorneys and doctors hired by the Canadian association recommended those conclusions be omitted from the final report. The 1958 published report concluded that asbestos exposure did not lead to an increased statistical occurrence of lung cancer.

[33] Letter from Frank Zimmerman, Director of Safety, National Gypsum Co., to Clifford L. Sheckler, Manager, Occupational Environmental Control, Johns-Manville Corp. (Apr. 17, 1968).

[34] Swentonic, M.M., Presentation by the Asbestos Information Association/North America to the Asbestos Textile Institute (June 7, 1973).

Against the background of these and other documents that will be introduced at trial, evidence concerning the medical and scientific literature seems to pale in comparison. Yet, with the dwindling number of defendants who conspired to withhold knowledge of the dangers, the role of state-of-the-art case, i.e., what should have been known, is once again of central prominence. This concept holds that every manufacturer had the duty to keep abreast of the medical and scientific literature. The industrial physicians knew as much, if not more, about the hazards of asbestos-containing products than the rest of the scientific community. Thus, a plaintiff may present evidence through a medical historian or a scientist who lived and worked through that period of time that asbestos-related diseases have been recognized throughout the century. As stated by Dr. David Ozonoff, a professor of public health at Boston University and a medical historian:

> The knowledge that exposure to asbestos could cause a serious chronic pulmonary disease called asbestosis was irrefutable and generally accepted by 1930. The suspicion that asbestos could cause cancer of the lung was first voiced in the 1930s, was considered a probable relationship by 1942, and was generally accepted by 1949. Epidemiological studies in the mid-1950s left little room for doubt. The index of suspicion relating asbestos exposure to the rare tumors called mesothelioma was high by *1953*, and by 1960, the full extent of the relationship was being revealed. Exposure to asbestos in the course of work with asbestos-containing products posed the same hazards as exposures in the factory setting; The simple fact that "asbestos was asbestos" was evident from the medical record and confirmed by numerous case reports and studies showing harm to those who worked with asbestos products.[35]

Asbestos companies usually present a medical historian to offer their defense that it was not until the publication of the Selikoff studies in 1964 that they either knew or should have known of the hazards of asbestos to insulation workers. The asbestos companies typically present evidence that the threshold limit value (TLV) for asbestos was established in 1946 as five million particles of asbestos per cubic foot of air and that this standard was not changed until 1969. Additionally, they contend that prior to 1964, it was thought that exposures to asbestos below this TLV were safe and that insulation work usually produced concentrations of atmospheric asbestos less than five million particles per cubic foot of air.

The weakness in this defense is that several of the companies had actual knowledge that the TLV was not reliable as noted above.[36] The medical director of Johns-Manville, Dr. Kenneth Smith, testified that he was aware in the late 1940s and early 1950s that insulation workers were developing asbestosis.[37] Indeed, insulation workers were filing workers' compensation claims against the companies throughout the 1950s and 1960s, and these companies included Fibreboard, Owens-Corning, and Armstrong Contracting and Supply [12]. One corporate executive, after reviewing the number of workers' compensation claims filed by insulators, wrote in 1962 that the list of claims was "rather imposing."[38] Although the state-of-the-art expert for the companies may be able to construct a defense solely based on the medical literature, a review of the corporate documents reveals not only that the companies should have known of the hazards but that they did know that asbestos was causing disease in the workers who were using their products or being exposed on their premises.

By the 1930s, industrial facilities were aware of the potential hazards associated with the use of asbestos-containing products. In the courtroom, defendants often suggest that knowledge was based on high exposures at manufacturing facilities utilizing raw asbestos. To the contrary, in 1937, a large industrial facility wrote about the hazards associated with asbestos on pipes and boilers and that precautions, including education of workers, needed to be taken with use. The

[35] Ozonoff, D., Report concerning Medical Literature Review (1981).

[36] Hemeon, W.C.L., Report of Preliminary Dust Investigation for Asbestos Textile Institute, Industrial Hygiene Foundation (1947) (unpublished).

[37] Deposition of K. Smith in *Louisville Trust Co. v. Johns-Manville Corp*., No. 174-922, Jefferson Cir. Ct., 7th Div. Ky., April 21, 1976.

[38] Memorandum from W.B. Hofferth to J.E. Zeller, Jan. 17, 1962.

author, Roy S. Bonsib, Chief Safety Inspector for Standard Oil Company (NJ), wrote that asbestos is a hazard as used in pipe and boiler insulation and that such work created dust in excess of the recommended 5 mppcf standard, that removing old insulation created dust in excess of the 5 mppcf standard that was in effect from 1937 to the early 1970s, and, further, recommended various measures to prevent asbestos disease including the necessity to explain and educate the worker why it was important.[39] Importantly, Standard Oil's safety inspector stressed the importance of a key historical industrial hygiene concept, that is, if use of asbestos creates visible dust, a potential hazard exists:

> One common-sense answer is that any atmosphere in which dust is visible to the naked eye is certainly too dusty to be breathed with safety by human beings, and the wise, farsighted human employer will immediately start to decrease the dust content in any atmosphere where dust is visible. After he has eliminated visible dust, there may still remain enough very small invisible dust to cause harm to the health of those who breathe it, but in any event if he has exerted sufficient well-directed effort to remove the visible dust, it is certain that much of the smaller invisible, and probably most harmful dust has also removed.[40]

This publication, a Medico-Safety Survey of Dust Producing Operations, recognized the duty of the industry to protect its employees from health hazards associated with certain activities. The report was to serve as a "guide to operating executives and safety engineers in handling personnel and in providing adequate protection in dusty occupations." Similarly, Union Carbide had an appreciation in the 1930s and 1940s of the hazards associated with the uses of asbestos-containing products. Specifically, in the 1940s, Union Carbide recognized the hazards associated with men removing and applying asbestos-containing insulation in plants. Yet, for many years to follow, Union Carbide would continue to employee and contract with insulators, along with other trades, and not warn them of the dangers.[41]

The concept known as "state of the art" encompasses not only when a company knew or should have known about the hazards associated about asbestos but also when a company knew or should have known about what to do to protect persons from being exposed to asbestos. Warnings and good industrial hygiene, if practiced, may have saved thousands of lives. The need for and the importance of warning information about toxic or cancer-causing chemicals has been a fundamental principle of public health for 100 years. Warnings make possible protective measures by those exposed and provide the support for proper participation in the implementation of the protective measures required [13]. The basic principles of industrial hygiene have been known for many decades [14–16]. The hierarchy of control measures from most desirable to least desirable is as follows:

- Hazard elimination by substitution of less hazardous materials
- Containment of the contaminant at the source of generation through enclosure and/or use of engineering controls
- Use of work practices, such as wetting, which minimize generation of dust
- Use of personal protective equipment such as respirators and protective clothing

In order to adequately protect themselves, workers must have specific knowledge about hazards in the workplace as well as means of control [3]. Warnings, including product labels, must clearly state the specific hazards (e.g., asbestosis, cancer) which may result from exposure. The requirements of warning the workers so they could protect themselves from asbestos were stated in the medical literature as early as 1930 [3].

The use of respirators for protection of workers has long been recognized as generally inadequate under most circumstances and should be

[39] Bonsib, RS, Dust Producing Operations in the Production of Petroleum Products and Associated Activities, A Medico-Safety Survey, Standard Oil Company (N.J.) (1937).

[40] See footnote 39. at pp. 81 (citing Dan Harrington, Chief, Health and Safety Branch, United States Bureau of Mines, (Eng. & Min Jour. March 1937, pp 119–121)).

[41] Deposition of Carl Dernehl in *Frehse v. Anchor Packing Company*, *et al*. (6th Judicial District Ct., Carlton County, MN), March 10, 1989.

used only as a last line of defense. In court, an expert in industrial hygiene would opine that any significant degree of protection, a "respiratory protection program," is necessary and would include proper selection, proper fitting, worker training, routine maintenance, and medical surveillance.

Handling and processing of asbestos products can result in contamination of skin, hair, and work clothing. Asbestos fibers can be transported on a person's clothing and result in human exposure, especially when clothing is shaken or dusted prior to laundering. These exposures are commonly referred to as "take-home exposures" and have resulted in asbestos-related diseases among family members of asbestos workers [17, 18]. Prevention of such exposures requires the use of separate work clothes which are not taken home, separate lockers for storage of work clothes and street clothes, and showering after working with asbestos products.

### 12.3.2 Threshold Diagnosis of an Asbestos-Related Disease

The plaintiff must prove that he or she developed an asbestos-related disease. In order to prove medical causation, plaintiffs' counsel will usually retain an expert medical witness such as a pulmonary specialist, occupational physician, or lung pathologist. The role of the family physician should not be minimized, and even though the treating physician may not be an expert in the diagnosis of asbestos-related disease, it is helpful that the local doctor support the diagnosis. Unless there is no room for debate, the defendants will likely attempt to shift the focus of the trial from the evidence of corporate misconduct to creation of doubt in the jurors' minds about the propriety of the diagnosis. In essence, the victim is on trial as to whether or not they are suffering from an asbestos-related disease.

The cigarette-smoking defense is probably the most powerful argument offered by the asbestos defendants, and it is raised by them in cases ranging from pleural disease to mesothelioma. In asbestosis cases, the defendants argue that cigarette smoke may increase the presence of pleural plaques [19], cause or contribute to interstitial fibrosis, reduce lung volumes ([20], pp 241–254), and impair diffusing capacity ([20], p 241). In lung cancer cases, the defendants argue that cigarettes are a powerful carcinogen, and thus try to establish that smoking is the sole cause of the cancer. Yet, it is well documented in the medical literature that the combination of smoking and exposure to asbestos is a lethal combination and greatly increases a workers chance of developing lung cancer. In 1968, Selikoff et al. noted that the combined exposures of smoking and asbestos resulted in more lung cancers than would be expected from independent exposures. It was found that inhalation of asbestos fibers greatly increased the risk of lung cancer for cigarette smokers. In the group studied, it was calculated that asbestos workers who smoked cigarettes had roughly a 90 times greater risk from men who did not smoke nor were exposed to asbestos [21]. Specifically, as reported in the 1979 Annals of the New York Academy of Sciences:

> Evidence from this study indicates a strong synergistic effect between two type of exposure (asbestos dust and cigarette smoking) in respect to risk of lung cancer.
> Evidence from this study indicates that among asbestos workers, ex-cigarette smokers have substantially lower death rates than cigarette smokers who do not give up the habit. This should be brought forcefully to the attention of present asbestos workers. A young person who is so strongly addicted to cigarette smoking that he cannot break the habit or is unwilling to do so would be particularly well advised not to enter a trade involving exposure to asbestos dust. [22]

To ensure success in a case where pathologic material is available, the plaintiff's counsel should consult with a pulmonary pathologist who has knowledge of asbestos-related diseases to establish the diagnosis and to testify that asbestos was responsible for the disease process. When lung tissue is available, it may not be enough to rely on clinical evidence alone. When sufficient tissue is available, the plaintiff's counsel should utilize the information to be gained from a pathologic examination to the greatest extent possible. The materials should be submitted for microscopic

examination, and pictures should be taken that can then be presented in evidence. Visual evidence of asbestos in lung tissue is extremely persuasive to a jury, and pictures of asbestos bodies coupled with the pathologist's opinion that asbestos was responsible for the disease process may be important factors during jury deliberation. A fiber burden analysis may be helpful in convincing a jury that asbestos caused or contributed to the disease process. The absence of asbestos bodies, however, does not mean that exposure to asbestos was not a contributing factor to the disease process. Specifically, as described in the Helsinki Criteria, a report authored by a multidisciplinary group of pathologists, occupational physicians, and other professionals well versed in asbestos-related diseases:

> Chrysotile fibers do not accumulate within lung tissue to the same extent as amphiboles because of faster clearance rates; therefore, occupational histories (fiber-years of exposure) are probably a better indicator of lung cancer risk from chrysotile than fiber burden analysis. [23][42]

### 12.3.3 Nonmalignant Asbestos-Related Diseases

Varying clinical and pathologic criteria exist for diagnosing asbestos-related diseases. In late 2003, the American Thoracic Society (ATS) updated their 1986 criteria statement giving guidance for the diagnosis and treatment of nonmalignant asbestos-related diseases including asbestosis. In 2004, the ATS published their official statement adopted by the ATS Board of Directors.[43] In it, the ATS stressed the magnitude of the problem in the USA:

> Asbestos has been the largest single cause of occupational cancer in the United States and a significant cause of disease and disability from nonmalignant disease. To this demonstrable burden of asbestos-related disease is added the burden of public concern and fear regarding risk after minimal exposure.
> ***
> One of the most important implications of the diagnosis of nonmalignant asbestos-related disease is that there is a close correlation between the presence of nonmalignant disease and the risk of malignancy, which may arise from exposure levels required to produce nonmalignant disease or mechanisms shared with premalignant processes that lead to cancer.
> ***
> [A] diagnosis of nonmalignant asbestos-related disease does imply a lifelong elevated risk for asbestos related cancer.[44]

As in the 1986 criteria document, the diagnosis of nonmalignant asbestos-related diseases is based on certain criteria. The general concepts, although slightly modified from those in 1986, rest on the same essential criteria:

- Evidence of structural pathology consistent with asbestos-related disease as documented by imaging or histology
- Evidence of causation by asbestos as documented by the occupational and environmental history, markers of exposure (usually pleural plaques), recovery of asbestos bodies, or other means
- Exclusion of alternative plausible causes for the findings[45]

The 2004 criteria add an additional requirement for an impairment assessment if other factors are suggestive of asbestosis. Although the criteria document specifically states its purpose is for clinical diagnosis and not for litigation or adjudication, it will inevitably be discussed in court as was the 1986 criteria document. When asbestosis cases were more prevalent in the courtroom, a lot of bantering centered on an ILO reading of 1/0 versus 1/1. The 2004 criteria continues to recognize the ILO readings and the fact that "a critical distinction is made between films that are suggestive but not presumptively diagnostic (0/1) and those that are presumptively diagnostic but not unequivocal (1/0)." The 2004 criteria still allows for a chest film demonstrating

[42] Tossavainen [23] (This multidisciplinary group of specialist collectively published over 1000 articles on asbestos and associated disorders.)

[43] American Thoracic Society Documents, Official Statement: Diagnosis and Initial Management of Nonmalignant Diseases Related to Asbestos (2004).

[44] See footnote 43.

[45] See footnote 43.

characteristic signs of asbestosis coupled with a history of asbestos exposure to be sufficient for the diagnosis of disease. Although CT scans may be superior to chest films in some instances, and HRCT has replaced conventional CT scans, these additional imaging procedures are not required for the diagnosis of asbestosis.

The results of chest x-rays, CT scans, and pulmonary function tests are all useful in demonstrating to the jury that an asbestos-related disease is present. Nevertheless, if a plaintiff can show asbestos in a plaintiff's lungs in elevated concentrations, the debate over the "courtroom diagnosis" is less likely the center of the defense. As is often stated at trial, the pathologist has the "final word" on the diagnosis of asbestosis. And when there is enough lung tissue available for examination, this observation is certainly true. If a plaintiff's lawyer has tissue available, it is certainly advantageous to have the tissue examined by a pulmonary pathologist. If asbestosis is present, this establishes without question the existence of that disease process; and if lung cancer is coexistent, it is strong, if not conclusive, evidence that the lung cancer was caused by asbestos exposure.

In the absence of a fiber burden analysis, the diagnosis of asbestosis by light microscopy is usually debated only in borderline cases. If asbestosis is severe, multiple asbestos bodies may be found in the presence of diffuse interstitial fibrosis. In questionable cases, however, fibrosis may be present but asbestos bodies may be difficult to identify. In the asbestos trial setting, the debate usually centers around the number of asbestos bodies necessary to support the diagnosis. A 2010 publication by the College of American Pathologists suggests that a minimum of two asbestos bodies must be present in areas of fibrosis before the diagnosis can be established [24]. One of the members of the committee that issued this statement has remarked that "the requirement for two asbestos bodies is probably overcautious" and that "the chance of over diagnosing asbestosis from the observation of only one asbestos body seems very small indeed." ([25], p 260) Although he continues that "the problem is more theoretic than real," ([25], pp 262–263) it unfortunately becomes a matter of frequent dispute in the courtroom. Sometimes only one asbestos body can be identified; in some cases, there are none. In the case of fibrosis alone, one may argue that it is acute asbestosis, but the frequency of such cases is probably rare ([25], p 262). In the courtroom, however, the frequency of the claim is not.

The 2010 publication by the College of American Pathologists, although titled as an "update," has not displaced the official 1982 criteria for the diagnosis of asbestosis published and commissioned by the National Institute for Occupational Safety and Health [26]. Today, this criterion remains the official criteria for diagnosing asbestosis according to NIOSH. Experts in the field still find this criterion to be "the most acceptable method for pathologically diagnosing and grading the disease process of asbestosis."[46]

Defendants will undoubtedly seek to promote the 2010 publication, but it does not carry the same blessing of the "asbestosis committee" of CAP that was involved in the 1982 standard commissioned by NIOSH. Of note, NIOSH was not approached by the authors of the updated criteria in an effort to gain its acceptance or to inquire about its position on modifying the longstanding criteria it commissioned. Suspiciously, certain original authors of the 1982 standard, who are still members of CAP and remain leading national and international experts in the field of pathology, were not notified that a proposed change to the longstanding 1982 criteria was being contemplated by this group and a new committee formed.

There are concerns and criticisms that surround the 2010 publication that must still be addressed. One concern is that it is void of any standard with respect to how much tissue is necessary to evaluate and determine if there are two asbestos bodies per square centimeter. Experts will debate that some sections of the lung will have virtually no asbestos bodies and adjacent sections of lung tissues will show numerous

[46] Declaration of Samuel P. Hammar, M.D., p. 2, (December 13, 2011) in *Medlin v. Cleaver Brooks Boilers, et. al.*, Case No. 2009 CA 008958 A.

asbestos bodies.[47] Unless there is enough sampling to take into account this variability, one is unable to determine the actual number of asbestos bodies to make a pathologic diagnosis of asbestosis. Likewise, experts will argue that there are incidences when no asbestos bodies were identified and yet asbestos fibers were found in great enough concentrations to be classified as asbestosis. The 2010 publication also appears to contradict the published statements of the American Thoracic Society and the European Respiratory Society (ATS/ERS) regarding the importance of known asbestos exposures to the diagnosis to disease, and with such exposures, a diagnosis of idiopathic pulmonary fibrosis should not be considered.

The type of fibrosis necessary to support the diagnosis of asbestosis is also a disputed subject. Although some lung pathologists require a pattern of peribronchiolar fibrosis,[48] it is not always present in cases that are taken to court. Once again, juries are asked to decide issues that are controversial even among the experts, and they are forced to decide whether peribronchiolar fibrosis is necessary to make the diagnosis or whether "its absence in no way mitigates against the diagnosis [13]." In cases that are not so clear-cut, the trial can indeed become a battle of the experts. In nonmalignant claims the propensity of fiber type to cause disease is seldom an issue. The focus is centered on exposure and whether or not there was sufficient dose to cause the disease.

### 12.3.4 Lung Cancer and Mesothelioma

The pathologic diagnosis of asbestosis is not only central to the nonmalignant case but may be significant in relating a lung cancer to asbestos exposure. The problem lies not in those cases where asbestosis is readily diagnosed because most commentators are willing to relate a bronchogenic carcinoma to asbestos if coexisting asbestosis can be found ([25], p 285). If asbestosis cannot be found but there are numerous asbestos bodies, a strong case can also be made that the underlying lung burden is sufficient to have triggered the cancerous process. Many defense experts, however, are unwilling to relate a cancer to asbestos exposure unless all of the diagnostic features of asbestosis are present. This idea has been criticized in a report to the British government, which argued that, because the mechanisms of fibrogenesis and carcinogenesis are separate, there is no good reason why asbestosis must necessarily be present.[49] A better indicator of whether lung cancer can be related to asbestos is an elevated fiber burden, and it has been suggested that a fiber burden in excess of 100,000 per gram of dry lung tissue should be used as a minimum for relating a lung cancer to asbestos [28].

When a patient and potential client's symptoms suggest mesothelioma, physicians will often suggest a number of tests, including x-rays, CT scans, and MRI. These procedures can show any abnormalities within the lungs. Doctors may also order a bronchoscopy, which utilizes a viewing scope to look inside the lungs, and recommend blood work and protein analysis. Nonetheless, there are cases in which a definite mesothelioma diagnosis cannot be made by blood work and imaging studies alone. There are more common diseases, such as benign asbestos-related pleural disease, which can look quite similar on imaging studies to metastatic adenocarcinoma. Thus,

[47] See footnote 46. at 5.

[48] See footnote 41.

[49] Doll and Peto [27]: "The idea that… asbestos-induced cancers occur only secondary to the fibrosis of asbestosis has sometimes been expressed. The idea originated in the days before the discovery of DNA, when cancers were not thought to result from genetic variation in somatic cells, but from the repair of tissue damage that was macroscopically visible. In light of modern knowledge of carcinogenesis, such an idea does not seem plausible. No threshold for the carcinogenic effect of asbestos has been demonstrated in humans or in laboratory animals and, in the absence of positive evidence for a threshold, we have followed standard scientific practice and assume that none exists. One possible reason for thinking that asbestos induced cancers might be secondary to asbestosis is the high incidence of cancer in the similar condition of cryptogenic fibrosing alveolitis. As, however, the aetiology of this disease is unknown, the argument by analogy does not carry much weight and we have ignored it." p. 32.

biopsies and the special staining techniques are sometimes required in order to make a diagnosis of mesothelioma with the accuracy required for pathologic disputes which materialize in court. The defense in most mesothelioma cases is centered on the difficulty in making a conclusive diagnosis.

In contrast to the landscape of asbestos litigation just 10 years ago, when plaintiffs' attorneys fought to establish the pathologic linkage between exposure and disease, now plaintiffs' counsel must both understand and be able to communicate the nuances of these markers to a jury. Although diagnoses of pleural mesothelioma are still most prevalent, there appears to be increasing diagnoses of peritoneal and testicular mesothelioma. Most pathologists recommend that a minimum of 4 immunohistochemical (IHC) stains be conducted to confirm a diagnosis of mesothelioma as opposed to another condition such as pulmonary adenocarcinoma. The two best positive markers for malignant mesothelioma are CK5/6 and calretinin. The two best negative markers for determining that absence of malignant mesothelioma are CEA and TTF-1.

Even if the full battery of histochemical and immunohistochemical stains is performed, the defendants are usually able to find an "expert" to contest the diagnosis.[50] If the asbestos defendants are unable to present testimony that the tumor is not a mesothelioma, they may argue that a mesothelioma diagnosis cannot be made to a reasonable degree of medical certainty. From a plaintiff's perspective, it is certainly necessary in the mesothelioma case to have all possible diagnostic tests performed on the tumor tissue in order to ensure diagnostic certainty. Although an autopsy of the patient is helpful in order to defeat a potential defense claim that the mesothelioma was a metastasis from some other site, oftentimes an autopsy is not culturally acceptable, or the patient's treatment and diagnosis was undisputable during treatment and did not warrant an autopsy for further confirmation.[51]

A company may argue that the plaintiff is not suffering from an asbestos disease at all and that their ills are caused by something else or that causation cannot be determined and therefore the plaintiff has contracted a spontaneous disease with unknown origin and is labeled as idiopathic. The defense pathologist plays a critical role in this defense. The defendants, however, will engage in tactics of confusion by arguing that there are cases of mesothelioma caused by erionite, therapeutic radiation, certain drugs, and agents that are unknown [29].[52] These claims will be made even in cases where there is a significant occupational exposure to asbestos and where asbestos can be identified in the lung.

Although the defendants cannot dispute that smoking is not a cause of mesothelioma, they argue that cigarette smoke paralyzes the defense mechanisms of the lungs and allows for increased penetration and retention of asbestos fibers that cause mesothelioma.[53] In mesothelioma cases, the defendants argue to possible alternative causes even in the face of strong medical evidence that malignant mesotheliomas of the pleura and peritoneum are extremely rare in persons not exposed to asbestos.[54] Alternative causes of mesothelioma have recently been expanded to include evidence that some people who were inoculated with the polio vaccine contaminated with the SV40 virus are at greater risk of contracting this cancer. The defense of idiopathic or spontaneous mesothelioma is alleged in instances where exposure history is not sufficient to render an opinion based on asbestos exposure, or

[50] Unfortunately, the defendants consistently rely on one or two pathologists, who, in the face of overwhelming evidence favoring the diagnosis, will testify that the tumor is not a mesothelioma.

[51] In one case tried in Virginia federal court, the diagnosis of mesothelioma was confirmed by all pathologists except one who was retained by the defendants. This defense expert contended that the tumor was a metastasis from the thyroid, and the body was exhumed for further analysis. On exhumation, the thyroid gland was found to be free of tumor.

[52] Pelnar [29]. The author is affiliated with the Asbestos Institute.

[53] Cross-examination of Dr. Edwin Holstein in *Kulzer v. Owens-Corning Fiberglas*, No. 87-386T p. *351* (W.D.N.Y., Rochester Div., Apr. 24, 1990).

[54] See footnote 53. at 22616-17.

one's strict criteria will not allow mesothelioma to be the result of asbestos exposure because underlying asbestos markers such as pleural plaques or asbestosis are not present.

In sum, establishing medical causation not only requires that the plaintiff present evidence of an asbestos-related disease but forces the plaintiff to defuse defense arguments that the diagnosis is incorrect and should not be made on the evidence. The plaintiff's counsel must persuade the jury that the defendants have created unreasonable diagnostic criteria so that few, if any, claimants will be able to prove the existence of the disease. The defendants' contentions require that the plaintiff offer a skilled pulmonary or occupational expert to testify and that the plaintiff's counsel become well versed in the medical criteria necessary to establish a diagnosis.

### 12.3.5 Causation and Exposure

Once the diagnosis of mesothelioma is established, it is necessary to prove that exposure to asbestos was the cause. From a practical standpoint, asbestos is the only known cause of mesothelioma that exists in the American workplace.[55] Regardless, the plaintiff must demonstrate that he or she breathed respirable asbestos fibers liberated from the use or in the vicinity of asbestos-containing products. Since exposures to asbestos may have occurred decades earlier, recollection of the use of a specific product by name is extremely difficult. In some instances, a plaintiff or a coworker can establish the name and manufacturer of the asbestos-containing materials used by or in the vicinity of the plaintiff.[56] It is not unusual, however, that many persons who have developed asbestos-related disease cannot actually identify the specific product name because they were exposed from the work of nearby tradesmen and did not have the opportunity to observe product labels.

The nature of asbestos and its ability to permeate a workplace exposed many persons working next to the asbestos trades. In 1963, Drs. Selikoff, Churg, and Hammond, from the Divisions of Thoracic Disease, Department of Medicine, and the Department of Pathology at Mount Sinai Hospital, presented such evidence to the American College of Chest Physicians at an Annual Meeting of the American Medical Association. They noted:

> Asbestos exposure in industry will not be limited to the particular craft that utilize the material. The floating fibers do not respect job classifications. Thus, for example, insulation workers undoubtedly share their exposure with their workmates in other trades; intimate contact with asbestos is possible for electricians, plumbers, sheet-metal workers, steamfitters, laborers, carpenters, boiler makers, and foremen; perhaps even the supervising architect should be included. [30]

Thus, evidence that a particular manufacturer sold its products to a worksite may be admissible in the form of sales records and may raise a presumption that a plaintiff was exposed to this product. A plaintiff may testify that he or she used a specific product over time, and this testimony may be buttressed by the testimony of coworkers that particular products were used. Court decisions concerning product identification evidence have usually required that, before a coworker may testify as to the use of specific products, the coworker must testify that the products were in the general vicinity of the plaintiffs.

One of the best ways to demonstrate that a worker has developed an asbestos-related disease is to obtain a lung tissue specimen, have it analyzed, and demonstrate to the jury the presence of asbestos bodies or fibers. The use of electron microscopy to assess the lung burden of asbestos can be of enormous benefit to the plaintiff in relating a lung cancer or mesothelioma to asbestos exposure. Additionally, an energy-dispersive x-ray analysis that specifically identifies the asbestos fiber type can be used to determine which products may have

[55] In approximately 20 % of cases, the history of asbestos exposure was not taken in the occupational history. However, on further investigation, asbestos exposure can almost always be elicited when preparing the mesothelioma case for trial.

[56] Compare *Roehling v. National Gypsum Co.*, 786 F.2d 1225 (4th Cir. 1986) with *Blackston v. Shook & Fletcher Insulation Co.*, 764 F.2d 480 (11th Cir. *1985*).

contributed to the disease process. For instance, in a mesothelioma case where the plaintiff's lung tissue reveals an elevated asbestos fiber count and the majority of asbestos fibers are amosite, the plaintiff can point as the cause to a defendant whose product contains mostly amosite. By the same token, if the plaintiff worked primarily with chrysotile and tremolite fibers are identified, the plaintiff may be able to implicate as the culprit a defendant who utilized chrysotile in its products.

On the other hand, a defendant may use a fiber analysis to exculpate itself from the case by demonstrating that the fiber type in its products is not present in the plaintiff's lung tissue or that other fiber types, such as crocidolite, are present in excessive quantities. Ninety-five percent of all asbestos used in the USA in insulation products historically was of the chrysotile variety, and the remaining 5 % was mostly amosite. Crocidolite was rarely, if ever, used in insulating materials in the USA [17, 18], and this fiber type was primarily imported into the USA for use in asbestos-cement pipes and certain specialty gaskets [31]. Nevertheless, lung burden analyses often reveal relatively little chrysotile, greater amounts of amosite, and, not infrequently, the presence of crocidolite. Tremolite, a contaminant of chrysotile, is often detected in lung tissue, and it is a marker of previous chrysotile exposure.[57] Chrysotile tends to dissolve in lung tissue and may be removed from the lung, whereas amphiboles, specifically crocidolite, are more durable and are retained. A brief crocidolite exposure may still be evident in lung tissue after decades have passed, whereas heavier chrysotile exposure may be undetected and yet contributed to the disease. As previously noted, the consensus of experts dedicated to research of asbestos-related disease is that occupational histories on exposure is probably a better indicator of lung cancer risk from chrysotile from burden analysis [23].

Differences in the methods of analysis by various laboratories may also result in different findings. Some investigators count all fibers, whereas some do not count those below five microns in length. In one case, lung tissue was evaluated by three investigators. One found an elevated amount of only chrysotile using transmission electron microscopy.[58] Another, also using transmission electron microscopy, identified crocidolite, amosite, tremolite, and chrysotile.[59] Finally, another, using scanning electron microscopy, identified tremolite.[60] The overall lung burden of asbestos also varied from laboratory to laboratory.[61] The variability between laboratories for fiber burden assessments often results in the parties having different investigators perform studies on the same lung tissue.

### 12.3.6 All Forms of Asbestos Are Carcinogenic

Asbestos has been recognized as a human carcinogen for more than 50 years and is the predominant form of asbestos utilized worldwide.[62] Numerous governmental agencies and professional societies have recognized asbestos as a human carcinogen. They include the American Thoracic Society [32], the American Cancer Society,[63] the Environmental Protection

[57] Doll and Peto [27], p. 32.

[58] Report by Dr. Ronald Dodson, Dept. of Cell Biology and Environmental Sciences, University of Texas Health Center at Tyler, May 27, 1988.

[59] Report by Dr. Fred Pooley, Dept. of Mining and Minerals Engineering, University College, Cardiff, Wales, June 23, 1988.

[60] Report by Dr. Victor Roggli, Dept. of Pathology, Duke University Medical Center, Dec. 4, 1987.

[61] Dr. Dodson identified 3,025,082 chrysotile fibers per gram of dry lung tissue; Dr. Pooley identified 7,900,000 chrysotile fibers, 660,000 tremolite fibers, 64,000 crocidolite fibers, and 9,000 amosite fibers per gram of dry lung tissue; and Dr. Roggli found 6,120 fiber per gram of wet lung tissue. Dr. Roggli counted only those fibers whose length exceeded five microns, whereas Dr. Dodson and Dr. Pooley included all fibers in their counts.

[62] Elliot, L., Loomis, D., Hein M.J., Richardson, D, Stayner, L., Lung Cancer Mortality in North Carolina and South Carolina Chrysotile Asbestos Textile Workers, Department of Epidemiology, College of Public Health, University of Nebraska Medical Center (2012).

[63] http://www.cancer.org/Cancer/CancerCauses/OtherCarcinogens/IntheWorkplace/asbestos.

Agency,[64] the National Toxicology Program [33], the Occupational Safety and Health Administration (OSHA) and its research arm the National Institute for Occupational Safety and Health (NIOSH) [34], Centers for Disease Control and Prevention,[65] and the Consumer Products Safety Commission[66] in addition to other established agencies charged with protecting public health. Nevertheless, inside the court room, one of the most hotly contested issues in asbestos litigation is whether chrysotile asbestos can cause mesothelioma ([25], p 289). A number of companies argue that their products could not have caused disease because they contain this "friendly" form of asbestos. They argue in this manner despite that fact that, as noted above, numerous agencies have held to the contrary and 52 countries have banned all forms of asbestos, including chrysotile [35].

Since insulators are developing mesothelioma at an alarming rate and 95 % of all asbestos used in insulating material was chrysotile, it would seem that chrysotile is certainly capable of causing mesothelioma. Nevertheless, certain studies of workers exposed only to chrysotile indicate that chrysotile may be a weaker cause of mesothelioma [36], and the defendants maintain that these studies demonstrate that chrysotile, in and of itself, does not cause mesothelioma at all. Most authorities now accept that amphibole and tremolite are a cause of mesothelioma, and tremolite contaminates most of the chrysotile that has been used in this country.[67] If tremolite can be identified in the lung tissue, it is reasonable to assume that the source of the contamination was the chrysotile asbestos used by the plaintiff.

A few defense experts even contend that amosite is not a cause of mesothelioma and that all mesotheliomas are caused by crocidolite [37]. This position is untenable, and good evidence exists that the likelihood for exposure to crocidolite by most construction or insulation workers is nonexistent. In England, crocidolite was used for insulation purposes, and there is evidence that some British ships have been overhauled in US shipyards. Consequently, in a mesothelioma case arising from an American shipyard exposure, the defendants argue that the potential for crocidolite exposure existed and this exposure is responsible. The defendants will strongly and consistently argue the potency of crocidolite in mesothelioma causation. And if the defendants can convince the jury that crocidolite was the likely cause, the manufacturers may succeed because few, if any, manufacturers utilized crocidolite in their asbestos-containing insulation materials.[68]

Asbestos textile worker cohorts in North and South Carolina exposed to chrysotile asbestos have been studied and published finding increased risk of lung cancer mortality with cumulative asbestos exposure. In 2012, researchers updated their studies as part of their ongoing investigation of the relation between chrysotile asbestos and lung disease in these workers. They concluded that "increased rates of lung cancer were significantly associated with cumulative fibre exposure overall and in both the Carolina asbestos-textile cohorts" [38].

In early 2012, IARC issued its findings in an updated monograph on asbestos:

> There is sufficient evidence in humans for the carcinogenicity of all forms of asbestos (chrysotile, crocidolite, amosite, tremolite, actinolite, and anthophyllite). Asbestos causes mesothelioma and cancer of the lung, larynx, and ovary. Also positive associations have been observed between exposure to all forms of asbestos and cancer of the pharynx, stomach and colorectum. [9]

[64] Environmental Protection Agency, Airborne Asbestos Health Assessment Update, Springfield, VA: NTIS, Report No.: EPA/600/8-84/003F (1986).

[65] http://www.atsdr.cdc.gov/substances/toxsubstance.asp?toxid=4.

[66] Consumer Product Safety Commission, CANCER HAZARD! CPSC Warns about Asbestos in Consumer Products: Safety Alert, Report No.: CPSC Document #5080 (1994).

[67] As stated by Doll and Peto [27]: "It is not practicable to remove tremolite from chrysotile for commercial purposes and any distinction between the effects of chrysotile and tremolite may, therefore, be considered academic, unless supplies of chrysotile can be obtained in which little or no tremolite is present." p. 17.

[68] In answers to interrogatories, all defendants maintain that they did not use crocidolite in their asbestos-containing pipe insulation.

### 12.3.7 No Safe Threshold Level of Exposure

According to the US Congress, "medical science has not established any minimum level of exposure to asbestos fibers which is considered to be safe to individuals exposed to the fibers."[69] This is particularly alarming because it is extremely difficult to know whether microscopic asbestos fibers are in the air we breathe. A single asbestos fiber is, on average, 5,000 times thinner than a human hair and approximately 1.3 million fibers could fit side-by-side in one inch. It is essentially invisible to the naked eye. Scientific experts have testified that there must be at least 30–40 million particles per cubic foot of air in a well-lit environment to enable the human eye to see visible dust. By contrast, in dimly lit areas—such as a smoky, dusty ship compartment illuminated by temporary lighting or a factory floor—asbestos particles may be invisible until exposures reach as high as 100 mppcf.

In order to prove exposure to asbestos fibers, plaintiffs are often asked to recount the conditions under which their alleged exposure occurred. Particularly in cases against friction-product defendants and component-parts defendants whose asbestos-containing products are relatively small in size—such as gaskets and packing—plaintiffs are put on record as to whether the work performed created "dust" and the lack of visible dust can severely diminish the strength of such testimony. However, the lack of visible dust may not be an appropriate indicator of dangerous levels of exposure.

According to the World Health Organization, "there is no safe threshold level of exposure."[70] There is no evidence for a threshold dose of asbestos below which there is no risk [39].

Scientists generally accept that an occupational history of brief or low-level exposure to asbestos should be considered sufficient for mesothelioma to be designated as occupationally related.[71] The Occupational Health and Safety Administration has stated that the cumulative exposure levels below two fibers per cubic centimeter presents an "excess risk of cancer mortality."[72] They concluded, "well-conducted studies demonstrate a substantially increased rate of lung cancer and mesothelioma mortality among workers having low cumulative exposures to asbestos."[73] Again, many agencies and institutions charged with protecting public health have commented that there is no safe level of exposure to asbestos. In a large case-control study conducted in France in 1987, researchers specifically looked at the issue of cumulative exposures and the significance each exposure had on the disease process. The researchers concluded that each exposure contributed to some extent to the mesothelioma [40].

The abundance of empirical evidence regarding the hazards of developing mesothelioma as the result of even low-dose exposure to asbestos has led courts throughout the country to recognize the significance of all exposures and the cumulative affect such exposures have on disease. This recognition has become critical to the causation analysis in terms of proving a particular defendant's asbestos-containing product qualifies as a "substantial factor" in causing the alleged injuries. Thus, each exposure I of itself is significant because it may involve millions of fibers entering the lungs from a single dose.

In 2012, the presiding judge over cases in MDL-875 reconfirmed that each and every exposure evidence "to be sufficiently reliable."[74] Consistent with this position, courts throughout the country have agreed that asbestos diseases are "cumulative" in that many separate exposures can

[69] 20 U.S.C. 3601(a)(3).

[70] World Health Organization, Position on Asbestos, May 5, 2006.

[71] Consensus Report, Asbestos, Asbestosis, and Cancer: The Helsinki Criteria for Diagnosis and Attribution, 23 Scand. J. Work Envtl. Health at 313; National Cancer Institute, *Mesothelioma*: *Questions and Answers*, Cancer Facts (May 13, 2002).

[72] *Excerpts of* OHSA, *Occupational Exposure to Asbestos*, *Tremolite*, *Anthophyllite*, *and Actinolite*, *Final Rules*, 51 Fed. Reg. 22612, at 22619-620 (Jun. 20, 1986).

[73] 51 Fed. Reg. 22621 (June 20, 1986).

[74] *In re Asbestos Prods. Liab. Litig.*, 2012 U.S. Dist. LEXIS 9169, *8 (E.D. Pa. 2012).

each be established as having constituted a substantial factor in causing a plaintiff's asbestos-related disease.[75]

### 12.3.8 Doubt and Controversy

As discussed earlier, as evidence regarding prior knowledge of the hazards of asbestos exposure within the industry continued to mount, defendants in asbestos lawsuits began expanding their strategies to avoid liability. Modeling themselves from the tobacco industry's playbook, the asbestos industry set out to "create" controversy. In the late 1960s, as the health effects of smoking mounted, internal memorandum revealed the tobacco industry's strategy. In their 1969 Smoking and Health Proposal, Brown and Williamson laid out their plan:

> Doubt is our product since it is the last means of competing with the 'body of fact' that exists in the mind of the general public. It is also the means of establishing a controversy.[76]

Not surprisingly, many industry trade associations shared common public relations firms. Throughout the years, various asbestos trade associations sought to counter adverse publicity and "produce some rebuttal."[77] The asbestos industry remains vigilant in its pursuit to create doubt and controversy. One such strategy is to spend large sums of money in an effort to dilute the impact of peer-reviewed scientific studies while simultaneously applying political pressure on the government agencies relying on these studies for the formation of regulatory policy.

For example, since the 1930s, automobile mechanics have been one of the many professions consistently identified as being at an increased risk of developing asbestos-related diseases [41]. Peer-reviewed studies dating back to 1972 have found a scientifically significant percentage of persons working with and around friction products, such as automobile brakes, contracting mesothelioma [42–53]. As far back as 1977, Dr. Irving Selikoff warned that not only were mechanics at risk but people up to 22 m away from where the actual automobile brake repair work is being performed "are exposed to measurable concentrations of asbestos [54]."

Yet, despite the overwhelming empirical evidence developed over decades of scientific study, the automobile industry began pouring money into what has been termed "litigation-generated science." Litigation-generated science is a term inspired by the appearance that industries involved in or anticipating litigation are facilitating the publication of papers and analysis specifically aimed at creating the impression in the minds of jurors that a legitimate scientific controversy exists in circumstances in which the scientific evidence may not support such a controversy. As David Michaels, the US Assistant Secretary of Labor for Occupational Safety and Health, wrote in 2007 regarding the proliferation of papers published on asbestos disease among automobile mechanics: "[M]any of the papers seem to be written for use in litigation, in that they did not include new scientific data, but instead offered conclusions, based on review of previously collected data, on issues likely to arise in litigation like causation or historical exposure levels [41]."

From 2001 to 2006, Ford, General Motors, and Daimler-Chrysler paid two scientific consulting firms more than $23 million to help fight lawsuits brought against them by former workers alleging asbestos exposure from automobile brakes.[78] In addition to facilitating studies and data analysis denying the hazards of exposure to their clients' asbestos-containing products, these firms—ChemRisk and Exponent—paid high-ranking former government regulators whose connections provided the ability to influence

[75] See, e.g.,*Chapin v. A & L Parts, Inc.*, 733N.W.2d 29 (Mich. 2007); Berger v. Amchem Product, 818 N.Y.S. 2d 754, 762 (NY 2006); In re Asbestos Litigation, 911 A.2d 1176 (Del. Super. Ct. 2006); *Rutherford v. Owens-Illinois*,16 Cal. 4th 953, 958 (Cal. 1997).

[76] Smoking and Health Proposal, Brown & Williamson (1969); http://legacy.library.ucsf.edu/tid/rgy93f00.

[77] Minutes, Special Summer Meeting of the Quebec Asbestos Mining Association, August 8–11, 1967.

[78] Pressure at OSHA to Alter Warning, Baltimore Sun, November 20, 2006.

their former agencies. Among those paid by ChemRisk and Exponent were John Henshaw, former head of the Occupational Safety and Health Administration (OSHA), and his daughter. Mr. Henshaw proved to be a strategically powerful ally.

In 2006, OSHA issued a warning informing auto mechanics that the brakes they were working on could contain potentially lethal asbestos fibers. Less than 3 weeks later, Mr. Henshaw both called and emailed[79] his former agency demanding the warning be edited to include industry-financed studies denying any link between asbestos-related disease and brake repair work.[80] The OSHA scientist who authored the advisory bulletin at issue, which cited dozens of scientific studies including several by OSHA itself, refused to include the industry-funded studies and was immediately threatened with a 10-day suspension without pay if the changes demanded by Mr. Henshaw were not made.

To its credit, OSHA ultimately did not retract its warning. However, industry-financed pressure of the type applied by consultants like ChemRisk and Exponent is largely successful in its effort to dilute various asbestos-related publications. Perhaps most egregiously, the US Environmental Protection Agency changed its longstanding publication titled "Guidance for Preventing Asbestos Disease Among Auto Mechanics," commonly referred to as the Gold Book, under pressure from industry-financed groups. Originally published in 1986, a petition was filed in 2003 which claimed that, based on the Gold Book, "jurors inevitably are swayed by the impression that EPA's 'official position' is that friction products are hazardous" and, thus, the publication must be either edited or discontinued [41]. The authors of the petition, who had significant financial links to General Motors and Honeywell,[81] along with pressure from Exponent,[82] were successful in having the Gold Book removed from the EPA website. After 30 years in publication, the 15-page booklet was reduced to a two-page brochure.

Georgia-Pacific Corporation, a manufacturer of joint compound and other asbestos-containing products sued in thousands of asbestos-related lawsuits, is another serial violator of scientific and ethical guidelines. In 2012, the publisher of Inhalation Toxicology, a peer-reviewed medical journal, printed an apology to its readers based on undisclosed conflicts of interest and fabricated funding sources associated with four articles published between 2008 and 2011. This conflict came to light after one of the authors involved testified under oath to at least $6.4 million paid by Georgia-Pacific to a group of "scientists" and consultants to provide papers to respected medical journals. Predictably, these papers argue in support of Georgia-Pacific's litigation position that its asbestos-containing products could not have caused asbestos-related diseases.

Eventually, the following four articles were published in Inhalation Toxicology under the false premise that the work was "supported by a grant from Georgia-Pacific" without any indication of a conflict of interest:

1. Brorby, Sheehan, Berman, Green, Holm, Re-creation of historical chrysotile-containing joint compounds. *Inhalation Toxicology*, 20:1043–1053 (2008).

[79] Stern, E., Memorandum for David Ippolito: Response to proposed suspension, American Federation of Government Employees, Local No. 12, AFL-CIO (Nov 15, 2006); Also reported by Schneider, A., Pressure at OSHA to Alter Warning: Author of advisory on asbestos in brakes faces suspension for refusing to revise it, *The Baltimore Sun* (Nov 20, 2006).

[80] Testimony of Dr. Barry Castleman before the United States Senate Subcommittee on Employment and Workplace Safety of the Committee on Health, Education, Labor and Pensions, March 1, 2007, at 8.

[81] Testimony of Dr. Barry Castleman before the United States Senate Subcommittee on Employment and Workplace Safety of the Committee on Health, Education, Labor and Pensions, March 1, 2007, at 11. According to Castleman, the petition was authored by the law firm Morgan, Lewis & Brocius who refused to disclose their clients to the United Sates Congress or the media. However, an article in *Corporate Counsel* titled "Who Represents America's Biggest Companies" listed Honeywell as a client of the firm. GM is also a client.

[82] Michaels and Monforton [41]; Invoice from Exponent, Inc. dated July 2, 2003 to General Motors, Ford and Daimler-Chrysler included an item listed as: "Prepare Materials to Challenge 1986 EPA."

2. Bernstein, Donaldson, Decker, Gaering, Kunzendorf, Chevalier, Holm, A biopersistence study following exposure to chrysotile asbestos alone or in combination with fine particles. *Inhalation Toxicology*, 20:1009–1028 (2008).
3. Bernstein, Rogers, Sepulveda, Donaldson, Schuler, Gaering, Kunzendorf, Chevalier, Holm, The pathological response and fate in the lung and pleura of chrysotile in combination with find particles compared to amosite asbestos following short-term inhalation exposure: interim results. *Inhalation Toxicology*, 2010, 22(11) 937–962 (2010).
4. Bernstein, Rogers, Sepulveda, Donaldson, Schuler, Gaering, Kunzendorf, Chevalier, Holm, Quantification of the pathological response and fate in the lung and pleura of chrysotile in combination with fine particles compared to amosite-asbestos following short-term inhalation exposure. *Inhalation Toxicology*, 2011:23(7):372–391 (2011).

In reality, there was no grant. The authors of these articles were actually being paid huge sums of money directly from Georgia-Pacific as "litigation consultants" and in other liability-focused capacities. There was no disclosure that one of the authors was a full-time employee of the company. There was no disclosure that another was acting as an expert witness in asbestos cases on behalf of the company. The apology Inhalation Toxicology was ultimately forced to publish included confirmation that the following paragraph would be added to the Declaration of Interest section of each paper:

> Georgia-Pacific has not sold chrysotile-containing joint compounds for more than 30 years, but litigation is pending in which individuals claim exposure to the Company's historic products. The articles listed above report on work that Georgia-Pacific commissioned to address issues that have arisen in that litigation. I, Stewart E. Holm, representing Georgia-Pacific, am an author on all four papers. The other authors are consulting experts retained by or on behalf of Georgia-Pacific to conduct the research and prepare the articles. Dr. Donaldson has been listed as potential testifying expert witness by Georgia-Pacific, and Dr. Bernstein has testified as an expert witness for Georgia-Pacific.

The efforts by Georgia-Pacific and other asbestos defendants to disseminate litigation-generated articles in the guise of unbiased scientific research highlights the depths to which the industry may go in an effort to deny injured workers the redress for their asbestos-related injuries promised by the American Justice system.

Not surprisingly, litigation-driven science and expert testimony offered in the courtroom may be opposite from a company's representations made outside the courtroom. For example, Union Carbide owned and operated a chrysotile mine in King City, California. Their chrysotile asbestos was sold under the brand name "Calidria." In court, Union Carbide takes the position that chrysotile asbestos, including Calidria, does not cause mesothelioma. Yet, in 1985, just before they sold their chrysotile business, Union Carbide issued an MSDS sheet stating the following:

> "Over exposure to Chrysotile Asbestos has caused damage to lungs (asbestosis), lung cancer and mesothelioma of the pleura and peritoneum."[83]

In discovery, the plaintiff's attorney should be vigilant in requesting these internal "medical admissions" made prior to any defense strategy to create doubt and controversy.

## Conclusion

Tragically, the widespread production and use of asbestos continues to cause the loss of human life at a rate of approximately 10,000 deaths a year in the USA alone. Asbestos-related diseases, including mesothelioma, can be extremely painful and debilitating. Today, many doctors, scientists, and cancer specialists are devoted to research in hopes to find a cure or provide better treatment for mesothelioma and other asbestos-related diseases. Without a cure, but filled with hope, qualifying mesothelioma patients often opt for aggressive treatment options that may extend life. For example, an extrapleural pneumonectomy (EEP), a procedure that

[83] Material Safety Data Sheet for Potentially Hazardous Chemicals, Section IV: Health Hazard Data Union Carbide Corporation, November 12, 1985.

involves the removal of the lung and its coverings, has allowed some patients to live longer. Although a patient may survive longer than their initial prognoses suggested, rarely does such treatment allow them to escape the disease's inevitable toll. Other patients participate in clinical trials which test experimental treatments and medications with varying success. Clinical trials bring closer the day a cure may be found, but thus far, being diagnosed with an asbestos-related disease is still a death sentence. If cancers are caught early enough, treatment options may be more effective, and research into early detection may allow patients to seek more treatment options.

Today, there are support groups that allow victims and their families to communicate with others similarly situated. Advocacy groups and public health professionals continue to plead with the industry and governments to cease in the production and sales of asbestos. Yet, the USA and Canada remain outliers in the universe of industrialized nations which has largely banned the production and sales of asbestos-containing products. As a result, cases will continue to be brought for decades to come seeking redress for persons exposed to asbestos. Plaintiffs' counsel will continue to be vigilant in uncovering egregious wrongdoings and corporate misconduct. Defense lawyers will be vigilant in defending their clients. Both plaintiffs' and defense counsel must be well versed and understand the science and medicine that inevitably is played out in the courtroom. But most importantly, each and every victim of an asbestos-related disease deserves to be heard.

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# 13 Medicolegal Aspects of Asbestos-Related Diseases: A Defendant's Attorney's Perspective

Albert H. Parnell

## 13.1 American Asbestos Litigation (1972–2012): A Perspective

### 13.1.1 The Development of Modern Asbestos Litigation in America

The basis for American asbestos litigation was formed during the pivotal 10-year period from 1960 to 1970. A South African physician, Dr. J. C. Wagner, confirmed that malignant pleural mesothelioma was caused by crocidolite asbestos in 1960 [1]. The New York Academy of Sciences led by Dr. Irving Selikoff held a historic, course-changing conference on the biological effects of asbestos in 1964 that was attended by physicians, scientists, and industrial hygienists from around the world [2]. The Restatement, Torts, 2nd was published containing a new section on strict tort liability 402A [3] that significantly changed product liability law and reduced the hurdles that people suing had to overcome to obtain a monetary recovery. These events of the 1960s came together when a Texas jury awarded damages in favor of a worker against manufacturers of asbestos-containing insulation products that was affirmed as *Borel v. Fiberboard* [4] in 1972. These four seemingly disparate and separate events combined in a unique and almost invisible way to create the biggest and most expensive litigation that the USA has ever witnessed. The events started a process that has produced an avalanche of hundreds of thousands of lawsuits in virtually every state of the USA and in the US district courts. The sheer number of cases has threatened to clog the American judicial system and has, in fact, clogged the judicial system in some jurisdictions.

The legal activity in the 40 years since the *Borel* decision has spawned an incredible cottage industry. The asbestos litigation has created immense numbers of jobs for lawyers, for physicians of multiple specialties, and for thousands of people in allied industries and services. It thrives on expert reports from scientific and medical consultants. It has created the need for jury consultants who specialize in asbestos jury selection. Many people from varied professions have acted as expert witnesses in asbestos cases. The asbestos litigation has supported a cadre of supporting industries that include court reporters, industrial hygienists, medical providers of multiple kinds, records providers, and software suppliers. There have been thousands of lawsuits tried, hundreds of thousands of personal injury and property damage claims settled, and billions of dollars distributed among plaintiffs, lawyers, and the cottage industry participants. It has also produced 111 bankruptcies among some of America's once proudest corporate names including Raybestos-Manhattan, Johns-Manville, Owens-Corning,

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T.D. Oury et al. (eds.), *Pathology of Asbestos-Associated Diseases*,
DOI 10.1007/978-3-642-41193-9_13, 

W. R. Grace, Babcock and Wilcox, and the United States Gypsum Company (USG) [5].

### 13.1.2 The Role and Use of Medical and Scientific Witnesses in Modern Asbestos Litigation

The tremendous number of case filings and the influx of cases into state and federal courts have produced a demand for scientific experts of all kinds. It is usual to see a combination of pathologists, oncologists, radiologists, pulmonologists, industrial hygienists, statisticians, mineralogists, and epidemiologists in any typical asbestos disease case. Witnesses in all medical specialties may give testimony about general medicine issues, the health effects of asbestos, anatomy, or the development of the medical knowledge about asbestos. The medical specialists also may give specific medical testimony. Pulmonologists generally act as medical examiners and offer opinions about the health of individual plaintiffs. Radiologists, particularly B-readers, are used for a variety of tasks but often are the first, through screenings, to suggest that a person has asbestosis, cancer, or pleural changes. Pathologists are the arbiters of the presence or absence of asbestosis and cancer when sufficient tissue is present. These experts, some more expert than others, offer opinions on questions relating to diagnosis, causation, risk assessment, prognosis, and life expectancy. The expert witnesses testify about specific aspects of the total case, either affirming or refuting asbestos as a causative agent in the production of disease in a specific person.

The medical and scientific opinions that each witness is allowed to offer are governed by the trial judge's interpretation of the law that applies. The courts in each state and the courts of the federal court system have each developed specific rules of evidence that govern the evidence in every trial. The respective rules of evidence determine what is and is not relevant. Each judge determines how the rules of evidence are going to be applied in each case. The judge determines what documents may be introduced, the subject matter that each witness may testify about, and the scope of any expert's testimony. Each judge determines the substance of each witness' testimony. The rules of evidence and procedure are, in fact, different in each state court and the federal courts. The courts in each jurisdiction, state and federal, make little effort to be consistent.

It is important for the physician or medical expert to understand that there are many variables that can determine if, when, and what the expert may say. The judges and the attorneys have a role in determining what experts and others may say long before the actual trial begins. The judge has an important role because testimony is governed by the rules of evidence that apply to the specific case as applied and interpreted by that judge. The judges, even in the same states, do not always apply the rules equally, evenly, or consistently from county to county or even courtroom to courtroom. The lawyers also have rules to follow. Every jurisdiction requires some level of disclosure about the case before the case is tried so everyone knows the case. It is each lawyer's responsibility, depending on state laws, to comply with the disclosure requirements of the state or the court. These discovery requirements usually require the lawyers to disclose in advance of the trial the names of all potential witnesses, the area or areas of expertise of each witness, and the limits of each witness' testimony. These disclosures usually have to be made within a specific time frame before trial. Lawyers in cases may not adequately anticipate testimony of witnesses and fail to timely notify the opposing counsel. The trial judge may exclude a witness or portions of a witness' testimony when the lawyers fail to comply with discovery orders. Therefore, the expert opinions of one expert may be allowed in a courtroom in Delaware and the same testimony disallowed in Iowa. The testimony in an asbestos case on both exposure and diagnosis may be far different in Delaware than in Iowa.

### 13.1.3 The Modern Asbestos Trial Procedure

The courtroom is the operating room of the tort litigation hospital. The judge presides over the

trial (operation). The trial in the tort litigation hospital is led by at least two or more competing lawyers, each of whom differ significantly in their respective philosophy about the desired course of the trial and the desired end result. The judge, unlike the two lawyers, is usually a passive observer. The judge intervenes only when one of the trial lawyers presents a plan for the trial that is opposed and objected to by one of the other lawyers or is adverse to the rules of the court that the judge is applying. One or more of the competing trial lawyers may propose a course of conduct or trial procedure that is experimental or that at least has not been adopted by multiple members of the profession. The judge may have to exercise discretion based upon the best available information. Usually the lawyers representing the plaintiff can be paid only if the trial is a success from the standpoint of that lawyer. That lawyer can receive no fee, a small fee, or a large fee depending on the value of the result. The lawyers representing the defendants are paid irrespective of the result. The jurors in the case determine whether the trial is or is not a success, depending on the perspective of the viewer.

### 13.1.4 The Makeup and Role of the Jury in the Modern Asbestos Trial

The American jury system is a highly competitive and leveraged advocacy system that depends, in part, on the skill and diligence of the lawyers presenting the case. Each case is different. There is no universal right. There is no universal result. Two different or twelve different juries in the same or different jurisdictions can reach disparate and vastly different conclusions based on what many observers consider to be essentially the same facts. The facts vary, to some degree, in every case. The lawyers and witnesses are different in each case. The juries are vastly different in each city and from court to court, jurisdiction to jurisdiction, and state to state. The jury is a product of geographic location and every location is different. There are gender, race, ethnic, political, religious, and educational differences in every locality and every jury. Thus, a jury in El Paso may be markedly different from a jury in Boston. The differences abound in every conceivable way.

The lawyers and the jurors are central to the American jury system, and it is important to understand the roles of the lawyers and the jurors in order to understand the role of the scientist in the American tort system. The lawyers are advocates. Each lawyer presents an opinion, a view. Theoretically, the lawyer presents the best possible facts for that opinion or view. The other lawyer advocates an opposite or different view. The trial, though a search for truth, is a search for truth within the context of the facts presented in each trial by the advocate lawyers through their witnesses. There is no universal truth because every case is different. There is only the truth of the case under consideration. Each lawyer, the plaintiff and the defendant, is proposing the truth that he or she advocates. Each lawyer may see some or all portions of the truth differently from the other. Therefore, each lawyer seeks to find jurors who are more likely than others to see the truth from that lawyer's perspective.

Prospective jurors are almost universally randomly selected from voting lists for the chance to be selected to serve on a specific case. At the moment of their initial selection, they are the home of many gender, racial, religious, educational, and ethical opinions, bias, and prejudices, known or unknown. The lawyers question a subset of these opinionated educated or uneducated prospective jurors. Each of the lawyer advocates has a profile of what he or she perceives is the "best" and "worst" juror for the specific facts of the case. The opposing lawyers can remove or eliminate a specific number of the potential jurors from the panel when the process is complete. Trials are heard and determined by either 6 or 12 jurors depending on the jurisdiction. Assuming a 12-juror panel, it is typical to start with a panel of 24 prospective jurors when 12 jurors will ultimately be selected. Each lawyer then gets to remove the 6 jurors each believes is the 6 "worst" jurors from each lawyer's perspective. The remaining 12 are the jurors. The selected juries in most jurisdictions are racially, sexually, and ethnically diverse,

have approximately a high school education, and have incomes in the lower third to half of incomes on a national average.

### 13.1.5 The Medical Witness and the Jury

The medical and scientific witness has to understand that the evidence has to persuade the jury to a given point or conclusion. The lawyers present medical and scientific evidence to this jury in accordance with the rules of evidence of the jurisdiction as interpreted by the sitting judge. The jury's ultimate verdict is based on the evidence that is presented in that courtroom, by those witnesses, and by those advocates. The jury cannot seek additional information on its own from the Internet or the local library. The jury cannot seek outside information to determine methodically whether the science it hears is representative of the science presented at medical seminars.

The "truth" of the medical science presented to any specific jury depends on many factors. The most expert doctor in a field may not offer an opinion. The most expert of doctors in a specific field may not want to be a part of the judicial process for any number of reasons. The medical and scientific witnesses may have their own separate agenda in testifying. The witnesses may be professional witnesses who make all or most of their incomes from the courtroom. Since the cases have been in existence for 40 years and many of the witnesses have been used hundreds of times, it is not unusual for an expert witness to have charged millions in fees over time. The evidence that the jury hears may be less than optimum. The scientist who committed to attend the trial may not come because of schedule changes and may not be available at the date or time of the scheduled trial. All expert witnesses are not universally persuasive. Many witnesses, who have inferior credentials, make better witnesses, communicate with jurors better, and are more persuasive than a witness with a superior background. In short, there are many witness factors that govern the result in a trial.

### 13.1.6 The Legal Requirements of Medical and Scientific Testimony

The introduction of medical evidence at a trial in the US district courts is governed in general by the Federal Rules of Evidence, 701–705 [6]. Most state courts have similar rules but there is variance from federal court to federal court and from state court to state court. Scientific testimony is generally allowed where the witness is an expert in the field of the prospective testimony, the evidence the witness is about to offer is reliable, the evidence is obtained in a usual and customary way, and the testimony will aid the jury in reaching a decision. These general rules govern all cases involving asbestos and disease.

The science and medical witness must testify within the parameters of the proposed specialty in order for the jury to consider the evidence. The proposed evidence must pass a number of evidentiary considerations before the jury hears it. In all US district courts and in some state courts, the judge acts as a gatekeeper to screen out evidence that is ruled to be "junk science" or unsupported by the evidence. Appellate courts in *Daubert* [7], *Havner* [8]; and similar cases have set rules governing the threshold standards that proposed scientific testimony must pass before the testimony can be presented to the jury. The threshold standards are not "litmus" test requirements but are considerations that judges must use in considering whether to admit or rule out specific questions or scientific positions or data.

### 13.1.7 The Role of the Medical or Scientific Witness at Trial

There are a number of different kinds of cases involving asbestos. The most numerous and most typical is the personal injury tort suit. The plaintiff in the tort suit claims that he or she suffers from an injury that was caused by exposure to asbestos. Each case is different. The chief medical questions are whether the person has an asbestos-related injury or whether the person's alleged asbestos disease was caused by a specific or several specific

exposures. These causation questions involve an analysis of the type of exposure and the length of exposure. The causation answer may depend on the asbestos fiber type involved in the exposure. The answer may also involve an analysis of principles of epidemiology, pulmonology, oncology, and pathology. The jury may resolve the causation question by reliance on the credibility of one witness and one witness only. In most states the jury can rely on one witness and reject the testimony of every other witness who testifies. However, most lawyers are reluctant to present only one witness in an asbestos case. Consequently, the trial usually involves the testimony of multiple scientific witnesses from multiple specialties.

As an example, the exposure in a specific case may be weak, peripheral, or in an occupation that is not traditionally associated with asbestos disease. An industrial hygienist may testify about length and severity of exposures to this particular person with a conclusion that there is or is not sufficient exposure to cause the disease or insufficient exposure to a specific product or premises to have participated in the cause of the disease. Witnesses from other specialties may be used to make the exposure evidence of the industrial hygienist more or less credible. A radiologist may support high or low exposure through the presence or absence of radiographic evidence of plaques or asbestosis. The pulmonologist may support or refute the same propositions through an interpretation of pulmonary function tests (PFFs), x-ray readings, and the physical examination. The pathologist may support or reject exposure assessments through the presence or absence of plaques and asbestosis, the presence or absence of quantities of asbestos bodies, or the presence or absence of quantities of uncoated fibers. All disciplines come together in most cases to complement or confound the facts offered to the jury for belief.

### 13.1.8 The Role of the Pathologist at Trial

The pathologist is a very important witness in the trial of the appropriate asbestos case. The importance of the pathologist is enhanced because the pathologist examines actual tissue as opposed to a radiologist who examines shadows or the pulmonologist who interprets PFTs. Most lawyers believe that jurors attribute greater weight to the testimony of the pathologist. The belief centers on the thought that the pathologist is seen as having touched the inside or soul of the patient. Lawyers believe that jurors see the pathologist as the doctor who resolves diagnostic disputes. Most jurors understand that it is the pathologist who will likely determine their fate if they are in the hospital facing cancer surgery. It is because of this thought process that lawyers seek to expand the potential testimony of the pathologist at trial.

The lawyer in the asbestos trial is trying to convince the jury of a point of view or concept. The pathologist is a natural trial partner with the lawyer because the pathologist is, by nature, a visual person. The pathologist in everyday practice utilizes a number of diagnostic procedures that are visual in nature to affirm or reject a diagnosis. The pathologist physically examines the tissue under the microscope and can, if necessary, demonstrate that process in the courtroom. The pathologist can take pictures or photomicrographs of the tissue and display them in court. The pathologist often stains the tissue as an aid to diagnosis, and the pictures of the stains can be used to aid the lawyer in persuading the jury. The pathologist can order and supervise digestion studies and explain them in a way no other doctor can. The pathologist's photomicrographs can illustrate emphysema, idiopathic fibrosis, the presence or absence of asbestos bodies, and multiple other findings that cannot be demonstrated to juries to the same degree by other specialties.

The fundamental principle that a pathologist is credible in the eyes of the jury because he or she touches the tissue is very important in the trial process. The assumption of this concept allows the lawyer to elevate and extend the trial value of the pathologist. The lawyer seeks to capitalize on this credibility by attempting to get the pathologist to testify in areas that extend beyond the actual diagnosis or lack of diagnosis. The credibility acts as a springboard for other testimony. The concept is that if the pathologist can

define disease, then a priori, the pathologist must be an expert on the dose required to produce the disease, how the dose was acquired, how the dose acted on the tissue and the cell structure, the relationship for determining causation, and the epidemiology of the disease.

## 13.2 The Asbestosis Case: The Defense Position

### 13.2.1 The Asbestosis Case in 2012?

The defense of an asbestosis case in 2012 rests on questioning whether the diagnosis of asbestosis is a correct diagnosis. The chief trigger for the question rests on the principle that asbestosis is a dose response disease. No one questions the fact that workers in particular occupations had the chance for very high doses of asbestos in the 1940s, 1950s, 1960s, and even the 1970s. However, most experts believe that OSHA lowered the regulations regarding doses of asbestos in 1972 and that those regulations reduced exposures to asbestos in the workplace. The question? If the dose of asbestos has steadily decreased since 1972, can workers exposed to post-1972 levels of asbestos develop asbestosis? Virtually all of the asbestosis reported by numerous authors in the 1960s and 1970s, but principally, the cohorts reported by Irving Selikoff and colleagues, resulted from high and significant exposures to asbestos in the 1930s, 1940s, and 1950s. Many scientists and reporters demonstrated high peak dust count exposures to shipyard workers [9], some as high as 500 f/cc [10]. These exposures were significantly higher than the levels mandated by OSHA in 1972. The exposure picture began to change with the creation of OSHA and the promulgation of OSHA and EPA asbestos exposure standards, beginning in 1971 [11]. The OSHA standard has steadily diminished from 5 to 0.1 f/cc. The steady decrease in exposure that has been forced on industrial and occupational settings since 1971 suggests that workers exposed after 1971 are either less likely to develop asbestosis or, if asbestosis is found, it takes longer to develop and is generally less severe. Consequently, the diagnosis requires more scrutiny as the legal and scientific community faces patients and plaintiffs whose majority exposure is post-OSHA.

### 13.2.2 The Modern Litigation Process as a Reason to Defend the Diagnosis of Asbestosis

The history of asbestos litigation is that there was an outpouring of new asbestosis cases filed during the late 1990s and in the early years of 2000. There are valid social reasons to question the individual diagnosis of asbestosis in cases that appeared during this period. The history of asbestos disease demonstrates that cases of asbestos-related disease were discovered and diagnosed differently in the years prior to the asbestos litigation explosion. In the 1950s, 1960s, and 1970s, most of the plaintiffs with serious pulmonary conditions were exposed to asbestos during the heavy exposure years of the 1930s, 1940s, 1950s, and 1960s. They had worked in occupational trades that we now know were rife with heavy asbestos exposures. They saw their doctors because they were symptomatic and sought medical care. Those patients became plaintiffs as they gravitated to the litigation after they had received a diagnosis.

The usual asbestosis plaintiff of today reaches the asbestos litigation in a far different way. They have not been exposed to the same levels of asbestos as plaintiffs in prior years. They are not symptomatic. They have never missed any time from work. They have never sought medical treatment of any kind, much less for asbestosis. Many unions in the 1980s and 1990s and many law firms in the 1990s and 2000 began a process of x-ray screening of large numbers of people. Law firms advertised screening in local newspapers for any individual who has an x-ray taken. The x-rays are obviously taken without a history, not in response to a complaint of a symptom, and offer a very temporal view of the subject. Any person who has an abnormal x-ray is a potential plaintiff based on that x-ray interpretation and nothing more. Most of the plaintiffs who enter modern asbestos litigation with a nonmalignant disease are discovered through screening of some nature. It is unlikely that any have pulmonary function studies or

pathology. Consequently, most modern asbestosis plaintiffs discover that they might have a diagnosis of asbestosis when they receive a letter from a union official, lawyer, or physician stating that there has been a suspicious finding on their x-rays.

The screening produces a plaintiff, a lawsuit, and a response. Once the screening has produced an x-ray, the plaintiff's lawyer typically sends the plaintiff to a physician selected by that lawyer or law firm. The doctor physically reviews the plaintiff. The doctor may or may not generate x-rays and/or pulmonary function studies. The usual post-1972 asbestos-exposed plaintiffs have a supporting screening x-ray reading of 1/0 on the ILO Scale (the most minimal positive finding). The PFTs are either normal or show a restrictive or obstructive component or some other process. Since the PFTs are usually normal or nondiagnostic, the overwhelming majority of nonmalignant plaintiffs carry a diagnosis of asbestosis based on the original screening x-ray.

The plaintiff is then seen by physicians chosen by the defendants. The defense physicians almost always disagree with the findings of the physicians selected by the plaintiffs. The radiologist chosen by the defense invariably reads the screening film as normal or attributes any change to something other than asbestos. The pulmonologist selected by the defense does not report physical examination findings consistent with asbestosis. The defense PFTs are either normal or show obstructive disease. Thus, the usual clinical argument is between the pulmonologist and the radiologist without the benefit of the pathologist.

### 13.2.3 The Pathology of Asbestosis and Critical Standards

One of the severe difficulties in defending the disputed asbestosis case is the absence of a single standard to judge asbestosis. All of the medical experts who testify at trial recognize the seminal work of the College of American Pathologists [12] and the American Thoracic Society [13]. These standards, years later, still serve as the base point for the clinical and pathologic diagnosis of asbestos-related diseases in the courtroom. The pathologic standard of the College of American Pathologists is more rigidly and universally applied than the suggestions of the American Thoracic Society standard, but they are only applicable in the rare case where pathology exists. The ATS standard is subject to significantly more interpretation in the clinical and courtroom setting than the suggestions of the College of American Pathologists. Many of the scientists at trial impose personal standards for the clinical diagnosis of asbestosis. They recognize the ATS standard as a starting point, but they quickly either substitute or offer their own personal judgment about why the ATS applies or does not apply to their diagnosis or opinion.

The defense advocates a strict application of objective standards to make the diagnosis of asbestosis and would prefer a requirement of a lung tissue sample in every case. The litigation over asbestosis would likely stop or diminish significantly if lung tissue was required or if there was one objective standard to judge the presence or absence of asbestosis. However, the nature of the development of the case dictates that there will not have been a pathologist in the clinical history. Therefore, there are rarely tissue samples in the modern asbestosis case and no pathologic opinion. There is no concrete standard to apply. The diagnosis is subjective. Even the ATS standard does not require any rigid application. The result is that a jury determines which of the competing subjective views is most persuasive.

The defense always seeks pathology and the opinion of the pathologist. The defense finds that having an opinion of a pathologist is very helpful in resolving the subjective disputes between the clinicians about whether a patient does or does not have asbestosis. The pathologic diagnosis, when available, can be used in a number of ways to either support or refute the clinical diagnosis of one or more of the physicians.

### 13.2.4 The Defense Position on Asbestosis and Coexisting Ailments

The clinical argument for and against asbestosis is clouded by the presence of confounding factors in the diagnosis, the presence of coexistent disease with symptoms similar to those exhibited by

asbestosis, and the presence of major scientific differences between the published studies on asbestosis. The American Thoracic Society has noted a major problem in the differential diagnosis for asbestosis when the patient suffers from multiple disease processes.

Asbestosis rarely exists in a vacuum. Fibrosis rarely exists in a vacuum. Asbestosis and fibrosis coexist in most cases with emphysema, chronic obstructive pulmonary disease, asthma, heart problems, and multiple other ailments. The presence of coexisting ailments makes it difficult for all parties to evaluate the cause of the fibrosis because of the effect of the coexisting disease. Pulmonary function studies are always in conflict and are inconclusive in most cases. The existence of other diseases and drugs often provides a logical explanation for the presence of fibrosis of any kind. Therefore, a social history of cigarette smoking or a clinical history of heart disease and the use of some drugs sheds light on the question whether asbestos is the cause of the fibrosis in question.

The doctors agree in some cases that fibrosis is present. The question in those cases is whether the fibrosis is asbestosis. The conclusion is complicated for a number of reasons. First, the diagnosis is confounded if there is a history of significant cigarette smoking. There have been a number of articles suggesting that cigarette smoking produces the same types of small irregular opacities produced by asbestos and other dusts [14]. Second, there are often questions about the true occupational history of any significant asbestos exposure. There are many questions raised about whether a person can develop asbestosis at current occupational levels. Third, the diagnosis is confounded by the high incidence of idiopathic interstitial fibrosis. The existence of significant numbers of idiopathic fibrosis in the absence of documented exposure to occupational levels of asbestos forces the consideration that idiopathic fibrosis should be a part of the differential diagnosis.

The origin of the fibrosis and whether it is asbestosis, something else, or idiopathic fibrosis is important in the litigated case. Putting aside the issue of whether there is actual exposure to asbestos, many of the plaintiffs have a unique exposure to many substances and minerals in their occupational life. The defense urges the physician to consider the other causes of fibrosis to critically review whether the fibrosis is diffuse. The fibrosis could be focal, a response to some other stimuli in the particular plaintiff: idiopathic fibrosis, usual interstitial pneumonia, fibrosing alveolitis, a tumor, or some other factor.

### 13.2.5 The Defense of Lung Cancer Cases

The defense attorney in the defense of an alleged asbestos-related lung cancer case examines evidence from seven areas of our knowledge about asbestos and lung cancer to formulate whether a defense to the case is plausible: (1) Is there evidence that the carcinoma is of lung origin or is it a metastasis from another organ? (2) The general knowledge and agreement that cigarette smoking is bad for human health and causes cancer. (3) What was the extent and duration of the cigarette smoking history of the plaintiff? (4) Does the plaintiff have underlying asbestosis? (5) The strength or lack of strength of the association between this plaintiff's asbestos exposure and the development of lung cancer. (6) Does the plaintiff have exposures to other carcinogens that would explain the presence of the lung cancer? (7) Does fiber-type exposure offer any defense to the causation issue?

### 13.2.6 Did the Carcinoma Originate in the Lung?

The threshold question for the defense lawyer in an alleged lung cancer case is the origin of the cancer. Is it a lung primary or a metastatic tumor from another organ? The pathologist plays the significant role in making the determination and is generally better at assisting the lawyer than any other specialty. The first problem facing the defense attorney is that the clinicians are not

necessarily interested in the question of what toxic agent caused the cancer. The physician's job for them is treatment. The issue of whether the tumor is metastatic is important in determining whether there is a course of treatment that will cure the cancer. However, because there is often insufficient information to determine if it is a metastatic tumor, it is not always of primary importance to the clinician.

The clinician or clinicians have taken a history. They have made a physical examination. X-rays have been reviewed. There may or may not have been an operation in which tissue was removed for analysis. Clinical, not legal, considerations are paramount. Consequently, there may be little or no tumor tissue available for review. Autopsy is not performed in most cases so that there is some conjecture as to whether the tumor was a lung primary.

The treating pathologist's opinion of the origin of the tumor is extremely important in cases where there is lung tissue. The jury usually sees the treating pathologist as an impartial participant who is above the fray. The treating pathologist was on the scene before the lawyers, was not visited by lawyers, and in theory, made an independent analysis. Therefore, the opinions of the treating physicians, and particularly the treating pathologist, are analyzed by both sides. The lawyers who feel they have the most to gain usually attempt to solicit treating physicians for the case.

The plaintiff and the defense often hire an independent records review physician to look at all of the medical records, operative reports, x-rays, autopsy materials, and other reports to reach an independent conclusion as to the likelihood that the tumor originated elsewhere and was caused by some other agent. The defense radiologist will review the x-rays, magnetic resonance images (MRIs), and computed tomography (CT) scans to determine if there is any chance that the tumor originated in another source. The defense pathologist will review the records and the tumor to determine whether the cell type is capable of being a metastatic tumor. When the evidence points to another site of origin, the attorney will pursue that line of defense.

### 13.2.7 The General Knowledge About Cigarette Smoking and Human Health

The defense in the disputed lung cancer case assumes that the jury has become aware that cigarette smoking is dangerous to human health. It seeks to capitalize on the recent publicity about cigarette smoking, the ever-present warnings on tobacco products and some advertising, and the constant news references to the various tobacco lawsuits as a basis from which to develop information that the jury can process about cigarette smoking. Using that as a basis, the defense develops evidence that Oscar Auerbach and Alton Ochsner, two physicians, led science into study of the effect of cigarette smoking on human health in the mid-1950s [15, 16]. It shows that Sir Richard Doll and Julian Peto followed with their epic study of British physicians in 1955 [17]. The defense presents evidence that the Surgeon General of the USA originally published its landmark piece on cigarette smoking in 1965 [17], followed with a number of updated studies [14, 18], and has just completed its most recent tome on cigarette smoking and women [19].

These studies are a formidable weapon for the defense lawyer in a lung cancer case because they provide ample scientific data and information about the health effects of cigarette smoking. It builds upon the jury's existing knowledge. It supplies them with specific information that cigarette smoking and tobacco use are responsible for more than 30 % of all cancer deaths, including cancers of the lung, larynx, oral cavity, pharynx, pancreas, kidney, bladder, and cervix. The Surgeon General reports that smoking is responsible for approximately 85 % of the lung cancer cases in the USA [20]. The most recent Surgeon General's report documents the alarming increasing rates of cancer in females who smoke.

The defense relies on the presentation of data from Selikoff. It is hard to overestimate the effect that cigarette smoking has played in the health of the American worker who was employed between 1916 and 1965. Eighty-three percent of the insulation workers in Selikoff's 17,800 insulator

study were present or former cigarette smokers [21]. Other studies of the American and North American worker show similar figures for the same or similar workers. Consequently, there is a strong suggestion, if not presumption, that cigarette smoking is at least a factor in almost every lung cancer case. The defense can use Selikoff's data to show that virtually every lung cancer in his asbestos-exposed cohort had a history of present or past cigarette, pipe, or cigar smoking [21].

The defense will present evidence that there is extensive information supporting the significantly increased risk to lung cancer for all cell types in present and former cigarette smokers. The latest information from the American Cancer Society indicates that smoking males have a lung cancer relative risk of 22.36 which is increased from a relative risk of 11.35 reported in the September 1982 study [19]. The findings of this new study of 1.2 million men and women indicate that mortality risks among smokers have increased substantially for most of the eight major cancer sites and are causally associated with cigarette smoking [19].

It continues to be clear that cigarette smoking is a significant factor in all cases of lung cancer and that lung cancer is rare in the absence of smoking despite the presence of other risk factors.

### 13.2.8 The Plaintiff's Smoking History, Knowledge of Danger, and History of Following the Advice of Physicians

The analysis and defense of the lung cancer case is complicated. There are many factors about the case that have to be discovered, assessed, and evaluated. The factors come from many sources including the plaintiff, the plaintiff's family, the medical records, and the tissue, if any exists. When taken together, they form the basis for the personal defense of the lung cancer case.

There are two cornerstones of the defense of lung cancer cases in asbestos-exposed individuals: (1) the strong causative link between cigarette smoking and lung cancer and (2) the extent and duration of the smoking history of the plaintiff. I have previously cited materials that suggest that virtually all asbestos-exposed lung cancer plaintiffs have a smoking history. It is important for several reasons to prove as extensive a smoking history as possible. The extent and duration of the smoking history is important socially as well as scientifically in an evaluation of the factors that jurors take into consideration when they make decisions about liability. The more the plaintiff smoked, the more likely it is that the jury will consider cigarette smoking the total cause or the principal cause of the disease. The older the plaintiff, the more likely that the smoking started early in life. The older the plaintiff, the more likely that unfiltered cigarettes were smoked. The older the plaintiff, the longer the smoking habit lasted. The more smoking, the more likely that smoking was the cause.

There appears to be an underlying factor in jury decisions in lung cancer cases that is secondary to the amount of smoking. Juries judge knowledge and culpability. The juries seem to penalize people who do not take care of themselves irrespective of other factors. The establishment of the true number of pack-years of smoking enhances the probability that smoking caused the cancer. The establishment of the true number of pack-years smoked is also an indication to the jury of the number of years that the plaintiff avoided warnings and failed to exercise responsible conduct.

It is difficult to obtain an accurate measure of any individual's true smoking history. Every internist or pulmonologist will attest to the difficulty in getting an accurate account of a person's smoking history. No one keeps a smoking diary. The patient never kept accurate accounts of the smoking amounts that may have varied substantially over the lifetime. Every quantification is based on the memory of ancient history. The plaintiff may have ceased smoking or cut down on consumption at various parts of the smoking years. The plaintiff may have smoked a combination of filtered and unfiltered cigarettes. The plaintiff may have smoked cigars and pipes during a period of the complete history. There are additional confounding factors. Litigants and non-litigants have social reasons for reducing their smoking history. Plaintiffs in litigation are schooled by their lawyers and are quite likely to

increase their remuneration as their smoking history decreases.

Lawyers understand that the plaintiff may have a reason to inaccurately quantify a smoking history. Lawyers understand that the physician currently treating the patient at the time of the litigation either may not have treated the patient before the litigation or may not have time to go back through records to see if there is conflicting information about smoking. Therefore, the search for information about smoking history does not stop with the plaintiff and the current physician. The extent and duration of the cigarette smoking history of the plaintiff has to be established by combining information from several different sources. The plaintiff, if alive, has to testify under oath and answer sworn questions about the cigarette smoking history. The relatives and friends of both living and deceased plaintiffs must testify as to their memories of the smoking habit. Lawyers and their staff review references in past medical records that predate the start of litigation to determine if a different smoking history was given before the lawsuit started. Lawyers search for conflicting information to and between the patient and their physicians. It is the composite picture that is important.

The inaccuracies in smoking also affect the science. All risk assessments are correlated to the amount of smoking that is or has been experienced by the cohort. The researcher develops information about a deceased cohort member from the next of kin who either does or does not have reliable information on the deceased's smoking history. Therefore, the inaccurate classification of smoking may also inaccurately report the risk of developing any specific disease. There is also no way to measure the amount or the effect of sidestream smoke on the outcome.

The defense tries to demonstrate the influence that cigarette smoking has had on the body of the plaintiff in order to try to establish cigarette smoking as the dominant problem. The defense makes every attempt to engage the expertise of every medical specialty. The radiologist is utilized to visually demonstrate to the jury the presence of findings associated directly with cigarette smoking (emphysema, flattened diaphragms, and added translucency) or indirectly related to cigarette smoking (congestive heart failure, vessel disease, etc.). The pulmonologist is employed to explain physical examinations consistent with cigarette smoking (wheezes) and the presence of obstructive lung disease evidence by PFTs. The pulmonologist may also reinforce the x-ray finding testimony of the radiologist. The pathologist will be called to testify about the tissue. The testimony will cover the presence or absence of emphysema, the cell type, the appearance, the location, the cancer origin, and the cancer development.

Cessation of smoking is also a factor for several reasons. First, if the record reveals that a plaintiff failed to cease smoking after warnings by the attending physician, juries have a tendency to blame the plaintiff. If the plaintiff stopped smoking, the jury will evaluate the length of time between the smoking cessation and the development of the cancer. The defense will present evidence of the cell doubling times of the specific cell types in an effort to offset the psychological effect that smoking cessation has on causation issues. In general, juries are less likely to penalize the plaintiff as more years have passed since the plaintiff stopped smoking.

### 13.2.9 Does the Plaintiff Have Underlying Asbestosis?

The most critical asbestos issue for the defense is the presence or absence of asbestosis in the plaintiff [22]. Virtually all physicians and scientists who testify in asbestos cases agree that where there is confirmed underlying asbestosis, asbestos is considered to at least be a contributing cause of the tumor. Therefore, where asbestosis is present, the only defenses available to a specific defendant are exposure issues.

The defense actively and aggressively pursues the presence or absence of an asbestosis diagnosis in lung cancer cases to determine whether there is a plausible defense. The defense relies heavily on the opinions of the pathologist about the presence or absence of asbestosis or pleural plaques in the available tissue. The presence of pleural plaques is not scientifically associated with the presence of asbestosis or the cause of lung cancer, but its presence makes it emotionally more difficult for

the jury to discount a contribution by asbestos exposure. If the pathologic diagnosis is against the existence of asbestosis, that defense is pursued. Both plaintiffs' lawyers and defense lawyers have considerably more difficulty in the absence of sufficient tissue for pathologic diagnosis or a disagreement between qualified pathologists. The same difficulties in the diagnosis discussed in the section on asbestosis apply to this controversy.

The defense asserts that the presence of underlying asbestosis is required before medical causation can be attributed to asbestos. The defense engages that position from the considerable debate in the scientific community about whether there is any attributable risk to lung cancer from exposure to asbestos in the absence of underlying asbestosis. The question is whether the lung cancer develops from exposure alone or from the asbestosis. Medical science and epidemiology have been unable to completely differentiate between the rates at which, if any, lung cancers occur in asbestos-exposed individuals who do not have underlying asbestosis. The seminal studies of Selikoff, Hammond, and Seidman [23–25], did not report the numbers of their cohorts with lung cancer who also had underlying asbestosis. These authors confounded the problem by defining their nonsmoking population as "never smoked regularly." Their study included people who did smoke cigarettes but who did not meet their definition of regular smoking. It is clear that at least one study by Selikoff's colleagues at Mt. Sinai found underlying pathologic asbestosis in every case of lung cancer in their series [26].

### 13.2.10 The Strength or Lack of Strength in the Association Between Asbestos Exposure and the Development of Lung Cancer

The lawyers must assess the medical and scientific data regarding studies that demonstrate a link between asbestos exposure and the existence of lung cancer. It is clear that there are a number of articles that do associate the presence of asbestos with the occurrence of lung cancer. However, there is clearly a debate among medical people about the strength of the association and the incidences where that association takes place. The defense witnesses testify about the differences between the various articles and the existence of what they consider to be flaws in the methodology.

1. Does the plaintiff have exposures to other lung cancer carcinogens that would explain the presence of the lung cancer?

In some cases, the plaintiff clearly had sufficient exposures to ambient asbestos to amply connect the asbestos exposure with the lung cancer. In other cases, the exposure to asbestos is less precise or less substantial. The defense lawyer examines every aspect of the plaintiff's life and the lives of parents and siblings to attempt to discover other exposures to toxic agents that are associated with lung cancer to determine if the exposures are causative. That evidence is presented by expert witnesses if the exposure to other carcinogens is sufficient to produce doubt.

The defense can be used that the plaintiff had exposures to lung cancer-causing agents sufficient to be the sole cause of the cancer or if the other exposures acted in combination with cigarette smoking to produce the lung cancer. The analysis of the success of the defense under these circumstances is very similar to the analysis of the success of the defense in the presence of cigarette smoking. The jurors have to be convinced that the lung cancer was caused, in whole or in part, by an agent other than asbestos. The asbestos defendant, depending on the occupational and environmental setting of the plaintiff's workplace, implicates all of the other workplace and occupational contaminants that have caused lung cancer in animals or humans. These other agents include ionizing radiation, arsenic, and a host of chemicals and other agents.

2. Does fiber-type exposure offer any defense to the causation issue?

The chrysotile issue is very prominent in the lung cancer case as well as the mesothelioma case. The defendant who manufactured a product from chrysotile or who had chrysotile products on the premises uses the defense to attempt

to convince the jurors that chrysotile did not produce the lung cancer or was less likely to produce the lung cancer. The defense attempts to demonstrate the concept that chrysotile is less likely to reach the lungs and that once in the lungs is removed much more rapidly than amphiboles. The ultimate position is that the chrysotile is much less likely to produce the underlying asbestosis because of chemical and physical factors.

## 13.3 The Defense of Pleural and Peritoneal Mesothelioma Cases

### 13.3.1 The Changing Course of Mesothelioma Cases from 1977 to 2012

The quality and number of defenses of pleural mesothelioma cases have changed significantly in every respect from the early litigation of 1977 to the 2012 cases. The changes have occurred because everything about the litigation has changed. The nature and work processes of both the current plaintiffs and the current defendants have changed. The length and intensity of exposures of the current plaintiffs to the products and premises of current defendants has changed. The number of the mesothelioma cases has multiplied. The ratio by sex of the patients has changed. The occupations have changed. Many of the changes are directly related to the advances in medicine and diagnosis but not all of the changes can be explained in this fashion. The changes encompass many aspects of societal change and reflect the changing landscape of the litigation in 2012. It is a view of contrasts.

#### 13.3.1.1 The Numbers of Pleural Mesotheliomas Have Changed

The number of diagnosed pleural and peritoneal mesotheliomas has changed significantly from the beginning of the litigation. The lawyers involved in the early asbestos litigation in the 1970s and portions of the 1980s rarely saw a pleural mesothelioma and even more rarely saw a peritoneal mesothelioma. In the 1970s and 1980s, pathologists often disagreed on the diagnosis of pleural and peritoneal mesothelioma. All of this has changed in 2012. In 2012 mesotheliomas rule and dictate the case disposals in almost every jurisdiction. As the latency period has increased, there has become a steady increase in the number of mesotheliomas diagnosed in the USA and the number of mesothelioma cases filed. There are more cases because people are living longer and because they have not succumbed at an earlier age to some other disease.

Mesothelioma was a rare disease in 1977. The asbestos defense lawyer practicing in 1977 rarely saw a mesothelioma case in a year. There was a significant controversy and disagreement between pathologists about whether the disease existed and how to correctly define diagnosis. Many of the stains and diagnostic tools that exist today did not exist in the 1970s or at least were not as well understood. Many of the early cases were not properly diagnosed, and many of the cases did not get to lawyers because the disease and asbestos were not as commonly associated as they are in 2012.

The current understanding of the disease and the sheer numbers has provoked an incredible interest and need to understand the numbers associated with the disease. Obviously the principal focus is the patient and his/her treatment and well-being. However, the increase in filings, trials, and settlement of mesothelioma cases has created a legal and financial need to project and determine the numbers of cases and dollars which will be associated with the treatment of patients and the impact of the legal system and industry. Most pathologists in 2012 are aware of pleural mesothelioma and its association with asbestos exposure. There are improved diagnostic techniques that increase the chances of a correct diagnosis. Further, every physician, not just pathologists, knows, at a minimum, that asbestos has some potential connection with many, if not most, mesotheliomas. Most of the diagnosed cases of mesothelioma are referred to attorneys. When the physician does not directly refer a case of mesothelioma, the patient who turns to the Internet to find out more about the disease and

treatment cannot escape the proliferation of lawyer Internet websites promising monetary resolution and monetary recovery. It is estimated that the new pleural mesotheliomas will cost in the area of $3 billion annually through 2015.

The presence of increased numbers of bankruptcy trusts and legal needs to project has resulted in multiple studies and projects designed to project numbers. Although there is disagreement, one common projection often repeated is that there will be about 2,000 cases of newly diagnosed mesotheliomas in the USA until about 2020 decreasing over time to a "background" rate around 2040 [27].

Nearly every diagnosed case of mesothelioma in 2012 and later will be investigated by a lawyer. The disease itself is more widely known than in the past. It is hard to watch television on any given day without seeing an "ad" for mesothelioma cases. Virtually every doctor knows about mesothelioma and the mesothelioma litigation. Improved diagnostic techniques increase the opportunity for a correct diagnosis. When the doctor does not directly refer the patient to a lawyer for a case of mesothelioma, the patient turns to the Internet to find out more about the disease and immediately sees hundreds if not thousands of Internet ads for lawyers or placed by lawyers. It is estimated that the mesothelioma litigation will produce between three and five billion a year in settlement and costs. The defense of mesothelioma cases has become the biggest challenge for the asbestos defense lawyer and defendants in the twenty-first century.

#### 13.3.1.2 The Defendants Are Different

Today's defendants are different from yesterday's defendants. The traditional pipe covering manufacturers of amosite and crocidolite products are virtually gone from present litigation. The absence of these manufacturers from the lawsuits does not mean that their products did not cause or contribute to the current development of disease. It means that as the traditional pipe covering defendants have filed for bankruptcy, lawyers have sought to replace them with new, nontraditional defendants. The plaintiff of today has to prove that the disease was caused in whole or in part by a defendant that still has assets to get any money. This requirement has caused more than one tenuous allegation of exposure.

The new defendants did not make pipe covering products that produced massive exposures similar to the pipe covering and cement exposures of the past. These new defendants made different products. Many of the products contained less asbestos on a percentage basis than pipe covering and cement products. Many used exclusively chrysotile asbestos. These defendants made joint compound, floor tile, and roofing materials. They made gaskets. They made brake linings. They made encapsulated products. They are premises owners who did not make any products but had asbestos on their premises. The defenses for these defendants are substantially different from the defenses that could have been urged by the pipe covering defendants because the exposure levels have been significantly lowered from earlier exposures. In addition, these defendants are sued at different levels than the traditional defendants. Instead of a virtual exposure to every person and product, the difference in the plaintiffs means that they were exposed to different products in different ways than previous defendants.

#### 13.3.1.3 The Exposure Levels Are Different

The traditional exposures of insulators, pipefitters, sheet metal workers, and other direct users of asbestos-containing products in the shipyards and powerhouses before 1972 were extremely high. The exposures were often uncontrolled and consisted of exposure to amosite, crocidolite, and mixed asbestos exposures. It was very difficult for the traditional pipe-covering manufacturers to advance any defenses based upon low exposure or asbestos fiber type.

The new, nontraditional defendants almost always have plausible arguments that their products did not release any (much) asbestos to the ambient air or that exposures were controlled on their premises. Many of the products of the current defendants release little or insignificant amounts of asbestos to the ambient air. They are gaskets, friction products, and like products.

Often, if they release asbestos, they release ambient asbestos at level orders of magnitude lower than that associated with pipe covering, block, and cement materials. In many instances, the releases of asbestos are at levels well within OSHA requirements or at levels that are near or below the normal ambient air levels and at levels that even the most liberal investigator believes would not cause asbestosis or pleural plaques. Most of these products were made almost exclusively of chrysotile asbestos raising fiber type and fiber burden questions that were never investigated, written about, or discussed in 1977.

#### 13.3.1.4 The Plaintiffs Either Have Different Occupations or Were Not Occupationally Exposed

The current plaintiffs are different than the occupations of earlier plaintiffs. While there is still an occasional insulator, insulator helper, pipefitter, or boilermaker, the reality is that most of the current plaintiffs have occupations that were not traditional asbestos exposure occupations of the 1940s, 1950s, and 1960s. Many of the current plaintiffs are not and never have been involved in the traditional asbestos-exposed populations. The current plaintiffs often never directly used asbestos themselves, or if they did, it was more casual than occupational. When they directly used asbestos, they did so on a fleeting basis. Many do not have an independent idea of if, where, or how they were exposed. Even the current plaintiffs who have jobs that carry such traditional occupational titles as insulator, pipefitter, and sheet metal worker have never knowingly worked with or removed asbestos. The current plaintiffs are also building occupants, homemakers, and children. In most cases, it is hard to suggest that their doses are anywhere near the doses upon which past epidemiology is based.

#### 13.3.1.5 The Sex of the Plaintiff is Different

The early cases of mesothelioma were almost all men. Men were employed as insulators and in other direct asbestos trades. This is no longer the case. A significant number of the current plaintiffs are female. They are family members of former employees in the asbestos insulation industry and allied trades. They are workers who became involved in industry after 1972. They are homemakers who have little or no known extensive exposure to asbestos.

### 13.3.2 The Diagnosis of Pleural Mesothelioma in the Defense Case

The first threshold in the defense of the pleural mesothelioma case is to confirm or dispute the diagnosis of mesothelioma. Almost every current plaintiff has had a complete thoracic and pathologic workup. The plaintiff has undergone a radical pleuropneumonectomy in some cases. The clinicians have already made all the judgments between adenocarcinoma, metastatic tumor, benign pleural fibrosis, and other tumors and processes. There are some differences of opinion between pathologists but disagreements have become rare. It is only occasionally that there fails to be complete agreement. Consequently, disagreement in diagnosis ceases to be a major defense issue.

### 13.3.3 Asbestos Causation as a Defense to Pleural Mesothelioma Cases

Most modern cases do not have clear and distinct exposure to high levels of asbestos. Plaintiffs no longer worked exclusively with insulation products nor did they always work in jobs that are traditionally known for heavy asbestos exposure. These plaintiffs often did not work in industrial settings. Exact exposures to asbestos are not easy to find, isolate, and classify. There is often little or no documentation of the level or duration of the alleged asbestos exposures. The absence of clean and precise knowledge of asbestos exposure enhances the defense of asbestos as the culprit in the development of the tumor. Therefore, there is less compelling evidence that asbestos is or was the cause of the plaintiff's tumor.

The fact that all mesotheliomas are not caused by asbestos coupled with the lower and less certain exposure creates doubt as to whether this product or this exposure is the real cause in each specific case. It is clear that many substances and agents have been identified in producing mesotheliomas in animals and humans [28]. Spirtas has written that approximately 80 % of the pleural mesotheliomas in men and 20 % of the pleural mesotheliomas in women are conclusively related to asbestos exposure [29]. Other authors have given similar numbers [30, 31], although there are some authors who believe that all pleural mesotheliomas are caused by asbestos [32]. There are studies that demonstrate that there are non-asbestos agents and other occupational associations that are implicated in the cause of mesothelioma [33–35]. Scarring of the pleura, chronic inflammation, chemical carcinogens, viruses, hereditary predisposition [36], chronic empyema, and therapeutic pneumothorax [28] have been implicated in mesothelioma. Chemical carcinogens [37], genetic factors [38], and therapeutic irradiation [39–41], have also been implicated. These other potential and proven causes of mesothelioma offer jurors alternative causes in asbestos cases.

### 13.3.4 Fiber-Type Defenses in Mesothelioma Cases

The vast majority of defendants in cases in 2012 made, sold, or used products that were composed of Canadian chrysotile. It is unusual to find defendants who used amphiboles in their products. All modern defendants, even those with amphibole products, attempt to defend causation based on lack of exposure evidence as well as based on the fiber type of exposure. The reduced, limited, or ambient exposures to asbestos in current cases usually dictate that the plaintiff will not have underlying clinical pleural plaques or any medical consensus as to the presence of asbestosis. The judgment on causation will have to come from the mere presence of the tumor without tissue confirmation. The connection must come from the presence of the tumor and anecdotal evidence of sufficient exposure to cause disease.

The substantial controversy among the most esteemed asbestos authors on whether, and the extent to which, chrysotile asbestos causes malignant pleural mesothelioma provides a healthy fact issue in any mesothelioma case. The controversy is twofold: Does chrysotile cause mesothelioma? If so, what is the threshold level of exposure required?

It is a given that amphibole asbestos is the principal or main cause of pleural mesothelioma [42, 43], and, as late as 1978, Chris Wagner reported his opinion that all of the cases of asbestos-related mesothelioma were caused by exposure to crocidolite [42]. There seems to be no dispute that the amphiboles, crocidolite and amosite, have a more significant association with pleural mesothelioma than does chrysotile [44–46]. In fact, some recent studies suggest that the toxicity ratio between the fibers is 500× for crocidolite, 100× for amosite, and 1 for chrysotile [47]. There have been several theories advanced to explain the lower relative risk for mesothelioma for chrysotile than for amosite and crocidolite. Some suggest that the shorter lung biopersistence of chrysotile is a factor [48]. The evidence is clear that the half-life of the amphiboles in the lung is consistently [49] significantly longer than that of chrysotile. The chrysotile fiber changes more in the lung than do the amphiboles [50]. The increased presence in the lung may provide a longer and more important time for the amphiboles to be in contact with target cells to create DNA changes. It seems that chrysotile is, at most, a weak carcinogen for mesothelioma [45]. Some authors have questioned whether chrysotile causes mesothelioma or any increased health risk at today's occupational exposure levels [51–52].

The developments in the knowledge about chrysotile and its relation to mesothelioma present a new dynamic for the trial of mesothelioma cases where the sole or principal exposure is to chrysotile asbestos.

### 13.3.5 Fiber Burden in the Defense of the Pleural Mesothelioma Case

Scientific studies by Roggli, Warnock, Churg, and others since 1977 have shed considerable light on the amount of exposure to various fiber types of asbestos that is required to produce pleural mesothelioma. These same studies have provided information on fiber burden and fiber-type comparisons between asbestos-related diseases. Fiber burden studies and their techniques have been identified [53]. The important points from a litigation standpoint center on those that suggest a presence or absence of a causal relationship between exposure to asbestos and pleural mesothelioma from a fiber standpoint. It is clear that individuals who live in urban populations carry a substantial lung burden of asbestos without developing any asbestos-related disease [49]. Churg has suggested that the general population of Vancouver (Canada) has as many as 40 million fibers of chrysotile, 40 million of tremolite, and 400,000 fibers of amosite or crocidolite in each pair of dried lungs weighing approximately 40 g [49]. Roggli and his colleagues have studied the lungs of occupationally and nonoccupationally exposed people. They have determined that there is a background incidence among their cohort that would be classified as having fewer than 20 asbestos bodies (ABs) per gram of wet lung [54]. Further, Churg and Mossman report on some of Churg's earlier published data that demonstrate lung burden differential for amphiboles versus chrysotile for both mesothelioma and asbestosis [55]. Churg compared lungs from shipyard workers exposed to amosite and mixed dusts with those of chrysotile miners and millers. His results showed that mesothelioma occurred in the amosite-exposed shipyard workers at fiber burdens, on average, almost 220 times less than the average fiber burden of the lungs of the chrysotile miners and millers. He also found that asbestosis occurred in the amosite-exposed shipyard workers at fiber burdens, on average, almost 17 times less than the average fiber burden of the lungs of the chrysotile miners and millers.

The defense lawyer has several options in using the fiber burden studies as a defense to the claim that the mesothelioma was caused by asbestos. First, a fiber burden study that showed the absence of asbestos or asbestos below the established background levels for the laboratory lends weight to the argument that the mesothelioma was not caused by asbestos. This result follows whether the product was an amphibole or chrysotile. In fact, it may argue more strongly for amphibole products because the amphiboles stay in the lung significantly longer than chrysotile. The results also may aid the manufacturer of the chrysotile products if either no chrysotile is found in the lung fiber burden and/or amphiboles are found.

### 13.3.6 Retrospective Exposure Assessments in the Defense of Mesothelioma, Lung Cancer, and Asbestosis Cases

The advances in knowledge about levels of exposure and the onset of disease allow the lawyer to use other scientific methods to support or attack the proposition that any asbestos disease is related to a specific exposure, series of exposures, or specific product use. Retrospective exposure assessments (REAS) use scientific methods and facts to recreate the exposure to a lifetime of agents, a specific agent or product, or a comparison between products and exposures. REAS are particularly helpful in asbestos litigation in mesothelioma causation cases and in nonmalignancy cases where there is a clinical dispute about whether there is asbestosis and there is a question about the sufficiency of the exposure.

The purpose of REAS is to define the limits of a past exposure. It begins with identifying who is or was exposed. The method looks at the agent under investigation. It attempts to quantify the types of exposure to the products by using a number of approaches including actual records or exposure levels doing similar jobs in similar ways. It looks at the era in which the exposure took place and the impact that the evolution of

occupational health standards may have had on exposure. It incorporates preexisting scientific and medical reports that examine past exposures, exposure levels, and the development of disease. REAS develop a model for assessing the data in a scientific way. Finally, the method reports a quantitative description of the exposure and real-time assessments of the amount of exposure. REAS are traditionally and often used in the industrial hygiene profession.

There are several examples that illustrate the use of REAS in litigated matters. Assume an alleged chrysotile exposure to a 46-year-old woman with pleural mesothelioma who alleges that her only exposure to asbestos was when she was with her father when he was changing brakes on his car 6 times in her early childhood. There is substantial data on the amount of dust generated during a brake change and the length of time of the average brake change. The industrial hygienist calculates this exposure as a time weighed average (TWA) and determines, as an example, that the combined exposure was between a range of .003 and .001 fiber/cc-years. Both of these exposure levels are in the range of ambient exposure and the lawyer would use this comparison to urge the lack of asbestos causation. In another example, assume that an REA of a pipefitter with mesothelioma had a combined occupational exposure of 150 f/cc-years. The pipefitter had 147 f/cc-years of amosite exposure and 3 f/cc-years of chrysotile exposure. The defense will ask the jury to compare the exposures and determine that the chrysotile exposure was not a *substantial factor* in the production of the disease. The defense lawyer might also argue that exposure levels of 3 f/cc-years of chrysotile would not exceed the threshold for mesothelioma causation [56].

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# Erratum to: Pathology of Asbestos-Associated Diseases, Third Edition

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**Erratum to:**
Appendix in: T.D. Oury et al. (eds.), *Pathology of Asbestos-Associated Diseases*,
DOI 10.1007/978-3-642-41193-9

Figures 1 and 2 were inadvertently reversed. Figure 1 goes with the legend on page 342 whereas Figure 2 goes with the legend on page 340.

The updated original online version for this chapter can be found at
DOI 10.1007/978-3-642-41193-9

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# Appendix: Tissue Digestion Techniques

Victor L. Roggli

## 1.1 Method A

The digestion procedure used by the author [1] is a modification of the sodium hypochlorite digestion technique described by Williams et al. [2]. The details of the procedure are as follows:

**Materials:**

0.4-μm pore size, 25-mm-diameter Nuclepore® filters
Nuclepore® filtering apparatus, including cylindrical funnel (10 cc), fritted glass filter support, and 250-cc side-arm flask
Vacuum source, vacuum tubing, trap
20-cc plastic screw-top scintillation counter glass vials
Aliquot mixer for blood tubes (Miles Laboratories)
Two-sided sticky tape
Scalpel handle, clean scalpel blades
Forceps (coarse and fine tip)
25-mm-diameter rubber "O"-rings for filters
Pasteur pipettes with rubber bulbs
Rectangular plastic weighing dishes
Analytical balance

**Reagents** (all reagents prefiltered through 0.4-μm pore size filter):

5.25 % sodium hypochlorite solution (commercial bleach)
8.0 % oxalic acid solution
Absolute ethanol
Deionized water
Chloroform (caution: cannot filter through Nuclepore filter, as it will dissolve the filter!)

**Methods:**

Step 1: Selected specimen (up to about 0.3 g) is weighed wet in plastic dish on an analytical balance after gently blotting excess fluid with a paper towel.

Step 2: Tissue is minced into 1- or 2-mm cubes within plastic dish using fresh scalpel blade and coarse-tip forceps.

Step 3: Two Pasteur pipettes full of filtered sodium hypochlorite solution are added to plastic dish, and tissue in hypochlorite solution is carefully transferred into a 20-cc plastic screw-top scintillation counter vial.

Step 4: An additional aliquot of hypochlorite solution from a Pasteur pipette is used to rinse the weighing dish, and this is added to the vial. Two more aliquots (total of 10 cc) of hypochlorite solution are added directly to the vial.

Step 5: The vial is labeled for identification and placed on an aliquot mixer with double sticky tape (Fig. 1). Digestion proceeds until tissue fragments are no longer visible to the naked eye (usually 20–25 min for a 0.3-g sample however, more time may be required for severely fibrotic or deparaffinized specimens).

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T.D. Oury et al. (eds.), *Pathology of Asbestos-Associated Diseases*,
DOI 10.1007/978-3-642-41193-9, © Springer-Verlag Berlin Heidelberg 2014

Step 6: The digested suspension is transferred into the glass cylinder of the assembled filtration apparatus (Fig. 1). It is best to add no more than about 25 % of the suspension to the filter at any one time.

Step 7: As the filtration slows, aliquots of 8.0 % oxalic acid, absolute ethanol, and fresh hypochlorite solution may be added to the filter surface with a Pasteur pipette to reduce buildup of any organic residues. An aliquot of deionized water should be added between additions of oxalic acid, ethanol, or hypochlorite solution to prevent crystal deposition on the filter surface.

Step 8: After the final portion of the suspension has passed through the filter and a final rinse with oxalic acid, ethanol, and hypochlorite solution has been effected, then a final aliquot of absolute ethanol is washed through the filter.

Step 9: The filter is transferred from the filtering apparatus onto the surface of a glass slide using fine-tip forceps. The periphery of the filter should be attached to the surface of the slide with small, torn portions of white, lightly adhesive tape (to prevent folding and buckling of the filter when chloroform is added).

Step 10: After the filter has completely dried, chloroform is added dropwise to the filter surface with a Pasteur pipette until the filter is covered and cleared. The tape securing the edges of the filter can now be removed with fine-tip forceps before the chloroform dries.

Step 11: After the chloroform dries, a coverslip can be added to the slide in a suitable mounting medium (e.g., Permount), and the slide is now ready for viewing by light microscopy.

Step 9a: If one wishes to examine the filter by scanning electron microscopy (SEM) rather than light microscopy, the filter can be mounted on a 25-mm carbon disc with colloidal graphite and sputter coated with gold, platinum, or carbon (Fig. 1) [3]. If the examiner wishes to employ transmission electron microscopy, then a small portion of the filter can be cut out and transferred onto a TEM grid and the filter material removed by chloroform using the Jaffe wick technique [4, 5].

**Notes regarding digestion procedure:**

In selecting tissue for digestion, one should avoid areas of tumor, congestion, or consolidation as much as possible. These would affect the denominator in calculations of asbestos fiber or body concentrations and thus would tend to falsely lower the calculated value. The author prefers formalin-fixed tissue, although fresh lung tissue works just as well. An adequate sample is at minimum an open lung biopsy, lobectomy, or pneumonectomy. Autopsy tissue is even better.

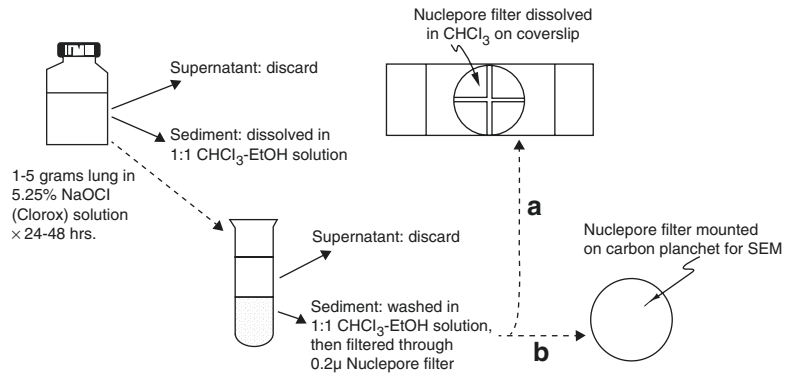


**Fig. 1** Method A: Technique for extracting mineral fibers and other inorganic particulates from lung tissue. The tissue is first digested in sodium hypochlorite solution (commercial bleach) and the residue collected on a Nuclepore® filter. The filter may be mounted for light, scanning electron, or transmission electron microscopy. See text for details

Transbronchial biopsies are inadequate to give meaningful results [6]. For lobectomy or pneumonectomy specimens, the author generally prepares two or three filters, and for autopsy cases, four filters (one from the upper and lower lobes of each lung). One filter is typically examined by SEM for asbestos bodies and uncoated fibers; the rest are examined by light microscopy for asbestos body content.

In some cases, only paraffin-embedded lung tissue is available. In such cases, a portion is selected from the block, deparaffinized in xylene, and rehydrated through absolute and 95 % ethanol. The usual times for deparaffinizing tissue are doubled to maximize paraffin removal, since residual paraffin clogs the pores of the filter, obscuring fibers and bodies. The wet weight is obtained from the specimen in 95 % ethanol. A correction factor has to be applied to deparaffinized specimens because of lipids removed at the time the tissue was originally processed [6] (see below).

There may be some variability in Steps 6 and 7 (filtration) depending upon the individual sample. Some samples pass readily through the filter and require little rinsing with oxalic acid, ethanol, or hypochlorite solution to remove residues. Other cases may sharply decrease their rate of filtration and require considerable effort to remove organic residues. Sometimes ethanol most readily restores the filtration rate, while in other cases oxalic acid is more effective. The use of warm water rinses helps to reduce crystal accumulation on the filter surface. If there is systematic slowing of filtration on multiple samples, this may be due to clogging of the fritted glass filter support. This can be rinsed with acetone or hot double deionized water to remove such residues.

## 1.2 Method B

In some cases, the asbestos body content is too low to obtain an accurate estimate from a 0.3-g sample. If an accurate quantification is desirable in such cases, then the original method of Smith and Naylor [7] employing a larger sample size may be preferable. The details of the procedure are as follows:

**Materials:**

0.4-μm pore size, 25-mm-diameter Nuclepore® filters
Nuclepore® filtering apparatus, including cylindrical funnel (10 cc), fritted glass filter support, and 250-cc side-arm flask
Vacuum source, vacuum tubing, and trap
300-cc glass jar with lid
Scalpel handle, clean scalpel blades
Forceps (coarse and fine tip)
25-mm-diameter rubber "O"-rings for filters
Pasteur pipettes with rubber bulbs
50-cc screw-cap conical centrifuge tubes
Rectangular plastic weighing dishes
Analytical balance
Desktop centrifuge

**Reagents** (filtration optional)

5.25 % sodium hypochlorite solution (commercial bleach)
Chloroform
95 % ethanol
50 % ethanol

**Methods:**

Step 1: Selected specimen (approximately 5 g) is weighed wet in plastic dish on an analytical balance after gently blotting excess fluid with a paper towel.

Step 2: Tissue is minced into 2- or 3-mm cubes within plastic dish using fresh scalpel blade and coarse-tip forceps.

Step 3: Tissue is transferred from the dish into glass jar with scalpel blade. The dish is rinsed with a 2-cc aliquot (one Pasteur pipette full) of sodium hypochlorite solution which is added to the jar. About 250 cc of hypochlorite solution is then added to the jar (approximately 50-cc hypochlorite solution per gram of tissue).

Step 4: The glass jar is allowed to sit for several days to allow time for the tissue to digest and for the asbestos bodies to settle to the bottom (Fig. 2) [8]

Step 5: The supernatant is removed by gentle aspiration using a Pasteur pipette attached to the vacuum system, being careful not to disturb the sediment at the bottom of the jar.

Step 6: A 20-cc aliquot of chloroform is added to the jar to suspend the asbestos bodies embedded in the sticky layer on the bottom of the jar. After swirling the chloroform to dissolve these residues, a 20-cc aliquot of 50 % ethanol is added to the chloroform suspension. The ethanol chloroform mixture is then transferred to a 50-cc screw-cap conical centrifuge tube.

Step 7: The centrifuge tube is labeled for identification and placed in a tabletop centrifuge. A tube from another sample or a tube filled with water is used as a counterbalance. The specimen is centrifuged at 200 g for 10–15 min.

Step 8: The supernatant is removed by gentle aspiration using a Pasteur pipette attached to the vacuum system, being careful to remove pigment and lipid residues at the chloroform-ethanol interface and leaving approximately 5 cc of chloroform and sediment at the bottom of the tube (chloroform is heavier than ethanol and settles to bottom).

Step 9: If the sediment remaining after Step 8 is black, then an additional 20-cc aliquot of chloroform and 20 cc of 50 % ethanol may be added to the centrifuge tube and Steps 7 and 8 repeated. Otherwise, about 15 cc of 95 % ethanol are added to the sediment and residual chloroform.

Step 10: The sediment is suspended in the 95 % ethanol, using vigorous shaking or a vortex mixer, if necessary. This suspension is then transferred into the glass cylinder of the assembled filtration apparatus (Fig. 2), and the sediment collected on the filter surface.

Step 11: The filter is transferred from the filtering apparatus onto the surface of a glass slide using fine-tip forceps. The slide is then prepared for examination by light microscopy as described in Steps 9–11 under Method A (see above).

**Notes regarding digestion procedure:**
Tissue selection guidelines are similar to those outlined for Method A above. Generally, a lower lobe sample abutting the pleura is used.

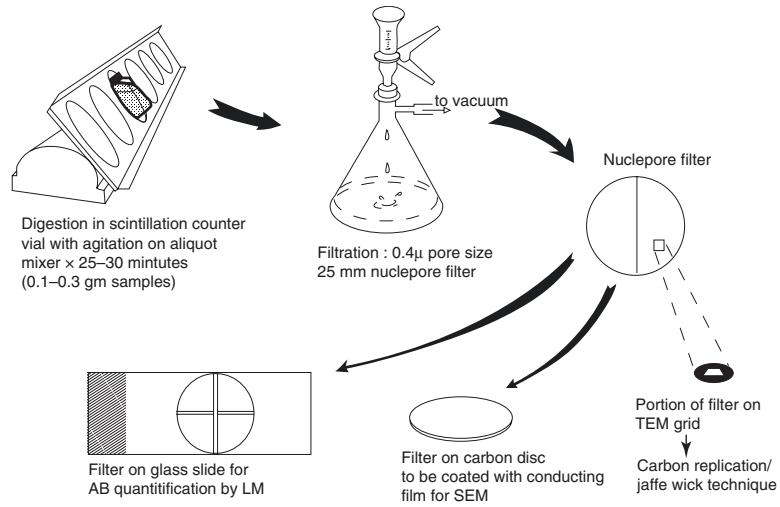


**Fig. 2** Method B: Technique for extracting asbestos bodies from lung tissue. The tissue is first digested in sodium hypochlorite solution (commercial bleach), followed by a centrifugation step to separate the inorganic particulates from inorganic carbon and undigested lipid residues, the latter remaining at the chloroform-ethanol interface. The residue is recovered on a Nuclepore® filter, which may then be mounted on a glass slide for examination by light microscopy. See text for details (Reprinted from Ref. [8], with permission)

The values obtained for asbestos bodies per gram of wet lung using Method B are generally quite comparable to those obtained with Method A. In a study of ten cases in which both methods were used to quantify the asbestos body content, the average ratio of the results obtained by Method B to those obtained by Method A was 1.1 (range, 0.3–3.5) [9]. Although filters prepared by Method B can also be examined by analytical electron microscopy, the author does not recommend this because there is evidence of a significant uncoated fiber loss at the chloroform-ethanol interface during the centrifugation step (unpublished observations). Others have also reported fiber losses with each sequential centrifugation step [10].

It is important to emphasize the necessity for maintaining scrupulously clean glassware. Studies have shown that asbestos bodies and fibers adhere to glassware surfaces and may be removed with difficulty [11, 12]. Such loss of fibers or bodies may give a falsely low count. Of greater concern, however, is the contamination of glassware with carry-over of bodies or fibers from a case with a heavy burden to a case with very low tissue asbestos content [13]. A new scalpel blade, centrifuge tube, or glass vial should be used for each case. The cylindrical glass funnel and the glass jars are carefully cleaned with warm soapy water and a scrub brush between cases, rinsing with copious amounts of deionized water. Cleaning with acetone in an ultrasonicator is recommended after cases with a particularly heavy asbestos burden.

Filtration of reagents is optional for Method B because asbestos bodies derive only from biological systems and do not contaminate these reagents. (Uncoated fibers, on the other hand, may contaminate many different reagents and give falsely elevated fiber counts by electron microscopy. This is especially problematic for small chrysotile fibers, which are ubiquitous. Reagent blanks should be prepared and examined to control for this possibility whenever electron microscopic techniques are employed for asbestos fiber quantification.)

## 1.3 Bronchoalveolar Lavage Fluid

In some circumstances, it may be of interest to analyze the asbestos content of bronchoalveolar lavage fluid (BALF). This procedure is typically performed by digesting the BALF pellet in sodium hypochlorite solution and collecting the residue on a Nuclepore® filter. For this purpose, we typically use a 13-mm filter, which further concentrates the specimen since this filter has only 25 % of the area of the 25-mm filter used for lung digests. The filter may be mounted on a glass slide for asbestos body quantification by light microscopy or on a carbon planchet for examination in the scanning electron microscope. Counting rules are the same as for lung digest samples (see below). The results may be reported per ml of BALF or per million ($10^6$) cells [13].

## 1.4 Counting Rules and Calculations

The morphologic features of asbestos bodies are described and illustrated in detail in Chap. 3. Asbestos bodies, which have thin translucent cores, are to be distinguished from pseudoasbestos bodies, which have broad yellow sheet silicate or black cores. The author enumerates the true asbestos bodies and pseudoasbestos (non-asbestos ferruginous) bodies separately. Except in rare cases, true asbestos bodies are more numerous than pseudoasbestos bodies. Indeed, asbestos bodies are identified in more than 90 % of cases, whereas pseudoasbestos bodies are observed in the author's experience in about 23 % of cases. Identification of asbestos bodies by scanning electron microscopy is dependent upon morphologic features in combination with elemental composition as determined by EDXA, since one cannot appreciate the additional information regarding the color of the core fiber that is available from light microscopic observations.

Fibers are defined as particles with a length-to-diameter (aspect) ratio of at least 3:1 and roughly parallel sides. The author does not count particles with aspect ratio less than 3:1 or with

sides that are nonparallel or excessively irregular. Clumps of fibers are seldom encountered with the digestion procedures described above. Both asbestos and non-asbestos fibers are counted together (total uncoated fiber count). Although there is considerable morphologic overlap, most of the fibers that are 10 μm or greater in length with aspect ratio greater than 10:1 are asbestos, whereas non-asbestos mineral fibers tend to be shorter than 10 μm in length and have aspect ratios less than 10:1. Asbestos fibers are distinguished from non-asbestos mineral fibers on the basis of their morphology and elemental composition as determined by energy dispersive spectrometry (see Chap. 11). Asbestos fibers must also be distinguished from crystals that may form on the filter surface, which often have pointed ends. Such crystals are not included in the total fiber count.

Quantification of the asbestos body content of a lung tissue sample requires a determination of the numbers of bodies per unit area of filter surface. This can usually be accomplished by counting the number of bodies in a portion of the filter of known area and multiplying this value by the total effective surface area. The author usually counts the number of bodies in two perpendicular strips at a magnification of 400× (Figs. 1 and 2). In cases with a low asbestos body burden, the entire filter surface may need to be counted to obtain accurate results. In quantifying the asbestos body and uncoated fiber burdens by scanning electron microscopy, the author counts 200 fibers or 100 consecutive 1000× fields, whichever comes first. The latter amounts to approximately 1 % of the surface area of the filter. The total numbers of bodies or fibers on the filter can then be determined by multiplying the number of bodies or fibers per $mm^2$ of surface area by the total effective surface area of the filter. Determination of asbestos body or fiber concentration can then be accomplished by dividing the numbers per filter by the amount of wet tissue digested in preparing that particular filter. For paraffin blocks, the value must be multiplied by 0.7 for the results to be comparable to wet fixed lung tissue [9] (see above). Some investigators prefer to report their results in terms of bodies or fibers per gram of dried lung. In this circumstance, a portion of lung adjacent to the one actually digested should be weighed wet and then dried to constant weight in a 60–70 °C oven. Then the asbestos body or fiber concentration per gram of wet lung can be multiplied by the wet-to-dry weight ratio, yielding an asbestos body or fiber concentration per gram of dried lung (see also Chaps. 3 and 11).

## 1.5 Sample Calculations

**Example 1 (light microscopy)**

Sample weight: 0.308 g

Total effective filter area = $\pi r^2 = \pi$ (10.5 mm) $^2$ = 346 $mm^2$

Asbestos bodies counted in two perpendicular strips of filter (see Figs. 1 and 2) = 423

Area of two perpendicular strips = 2 × 21 mm × 0.42 mm (empirically determined diameter of one 400× field) = 17.6 $mm^2$

Asbestos bodies (AB) per $mm^2$ = 24

Therefore,

$$AB/\mathrm{g} = \frac{\left(24/mm^2\right)\left(346 mm^2\right)}{0.308\mathrm{g}} = 27{,}000\, AB/\mathrm{g}$$

**Example 2 (scanning electron microscopy)**

Sample weight = 0.299 g

Uncoated fibers counted in 100 fields = 35

Total area of 100 fields = 2.3714 $mm^2$

Effective area of filter = 346 $mm^2$ (see above)

Fibers/$mm^2$ = 14.76

$$Fibers/\ filter = \left(14.76\ fibers/mm^2\right)\left(346\, mm^2\right) = 5{,}110$$

Fibers/g = (5,110 fibers/filter)/0.299 g = 17,100 fibers/g

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DOI 10.1007/978-3-642-41193-9, © Springer-Verlag Berlin Heidelberg 2014

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