Occupational Diseases of the Lung

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The number of workers at risk from occupational lung disease is high. Approximately 8 million people, currently alive, have been occupationally exposed to asbestos; millions more have been exposed to coal dust, silica, and other minerals in the mining industry. Workers in the chemical, agricultural, and textile industries are also known to be at risk. A National Institute of Occupational Safety and Health (NIOSH) survey[1] of pulmonary specialists and pathologists, conducted in 1976, indicated that nationwide demand for analytic studies could exceed 10,000 annually. This is probably a conservative estimate and certainly exceeds the current capacity of US analytic laboratories.

This article provides an overview of analytic techniques available to investigate suspected occupational lung disease. The techniques mentioned primarily concern the identification of minerals and particulates within the lung. The identification and quantification of organic compounds such as pesticides, industrial chemicals, and carcinogens in lung tissue and the workplace environment are not considered in detail. The reader is referred to the literature for further information.[2,3]

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Occupational History and Environmental Sampling

The importance of an adequate occupational history cannot be overstressed; all occupations since childhood, however brief, should be elicited. Significant exposure may have occurred for short periods of time many years previously. Most occupational lung diseases have long latency periods; eg, mesothelioma usually has a latency period exceeding 20 years. The history should also elicit information on the specific duties and types of materials handled, because in many cases the hazardous nature of the occupation was not recognized at the time. Information should also be obtained on avocational (ie, hobbies and home interests) and environmental exposure. A history of smoking is also important. Where possible, samples from the worksite should be obtained and compared with materials identified in the tissues.

Medicolegal Aspects of Occupational Lung Disease

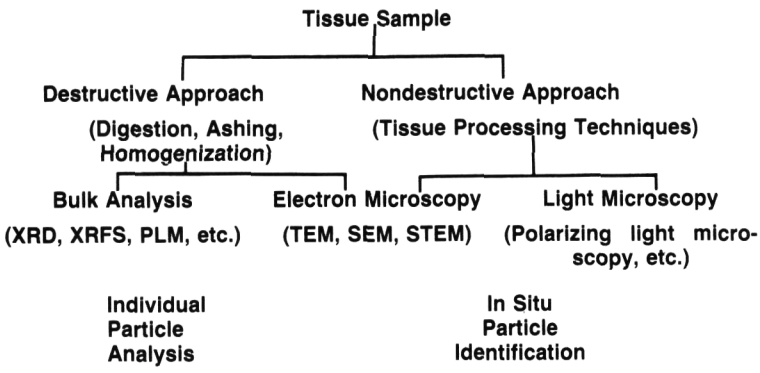
The following points should be borne in mind when submitting or accepting cases of suspected occupational lung disease for analysis. Specimens should be shipped in properly sealed containers to avoid contamination or tampering, and dates of delivery and arrival should be recorded. Laboratory records should document details of procedures and the location and custodial care of specimens and records. Pathologists should have consent and release forms signed by the patients or next of kin, authorizing the release of results. Portions of samples should be retained in remitting laboratories and intact portions should be retained by receiving laboratories for future reference or second opinions. The relative value of destructive versus nondestructive analytic tests should always be weighed.

Fitting the Analytic Technique to the Problem

Choosing an analytic technique depends on many factors, including: (1) the nature, concentration, and distribution of the suspect material; (2) the amount of tissue available and its state of preservation—fresh, fixed, or embedded; (3) the type of information required—qualitative and/or quantitative; (4) the importance of demonstrating a morphologic relationship between the disease and the suspected etiologic agent; and (5) the availability of equipment and expertise.

There are two basic approaches to microanalytic studies of lung tissues: destructive and nondestructive. The destructive approach involves extracting the material from the tissues, whereas the nondestructive approach involves the in situ analysis of particulates within tissue sections. Many analytic techniques lend themselves to both approaches, and these are shown schematically in Fig 1. Each approach has its advantages and disadvantages. The destructive approach best suits quantitative bulk

Fig 1. Schematic Approach to Microanalysis



analyses and should be used in situations where the normal cell constituents would interfere with the analysis. It is highly sensitive and thus is the method of choice for trace element analysis. Bulk analytic techniques are usually more rapid than the nondestructive techniques. The major disadvantage of the destructive approach lies in the inability to correlate the analytic findings with morphologic features. Other disadvantages include the lack of a permanent histologic record (this may be of medicolegal importance) and the relatively large sample size required for analysis (several grams wet weight). The nondestructive approach relies on in situ identification of the particulate and allows direct correlation with the lesion. Additionally, comparison of lesions with adjacent normal tissues provides an internal control system. Other advantages include the ability to use small specimens (eg, a single tissue section) and the preservation of the sample for future reference. Disadvantages include the semiquantitative nature of the techniques, biases due to the nonhomogeneity of the agent sought, and the time-consuming nature of the procedures. Regardless of the approach, it is advisable to use several complementary techniques. For example, most minerals can be adequately identified with a combination of morphology, elemental analysis, and crystallography.

When interpreting the data, important factors to consider are variations in lung particulate content with age, between urban and rural dwellers, and between smokers and nonsmokers. The results of case analyses should be compared with controls in which these variables have been matched as closely as possible.

Autopsy and Biopsy Requirements

For adequate gross or microscopic examination of lung tissue samples it is necessary to fix the inflated lung. At autopsy, one lung should be intratracheally perfused with 10% buffered formalin at 25 to 30 cm H_2O pressure. After fixation for at least 24 hours, the lung can be sliced and the sections examined either by the naked eye or with a low-power stereo microscope. Techniques have also been described for the fixation of surgical biopsy specimens in an inflated state.[4] If microanalytical studies are required or anticipated, it is good practice to freeze a portion of unfixed lung. Fresh tissue should be stored at −70 °C and fixed tissue in glass jars. Plastic containers may interfere with analyses for organic compounds. When fixed tissues are submitted for analysis, it is advisable to save a sample of fixative for subsequent analysis.

Gross examination is the most valuable technique for determining the extent of the following lesions: pigmentation, emphysema, fibrosis, and tumors. If a permanent record is required, 300-μm whole lung paper-mounted sections can be prepared according to the method of Gough et al.[5] An alternative method for examining whole lung sections developed by NIOSH involves freeze-drying whole lungs, x-raying, and cutting thin 300-μm sections for mounting on paper.[6] This technique has several advantages; morphology is preserved, pathologic and radiographic features can be correlated, and the risk of contamination (by fixatives containing metals, asbestos, etc) is minimized, allowing subsequent analytic studies.

The following areas of lung should be sampled for histopathologic evaluation in all cases of suspected pneumoconiosis: pleura with underlying lung, pulmonary parenchyma, large airways, and tracheobronchial lymph nodes. In addition, blocks should be taken from grossly visible lesions and from adjacent, relatively unaffected areas. Specific recommendations have been made by the Pneumoconiosis Committee of the College of American Pathologists for sampling tissues in cases of coal workers' pneumoconiosis and asbestos-related pleuropulmonary disease.[7,8]

Techniques for Extraction of Particles From Tissues

(A) Tissue Digestion—Chemical and Enzyme

Several tissue digestion techniques using chemicals—ie, acids, alkalis, and oxidizing agents such as hydrogen peroxide and sodium hypochlorite (Chlorox®)[9-11]—have been used for the recovery of mineral dusts and other particulates from lung tissues. In situations where these chemicals are likely to destroy or react with the particulate in question, a milder extraction procedure using enzymes is recommended.[12] Filtered Chlorox® is the most widely used method for the extraction of mineral dusts. Again, it should be emphasized that the preferred reagent, chemical or enzyme, is determined by the nature of the particulate in question, and where possible, preliminary tests should be run to determine alteration and/or loss. (For example, strong acids attack silicates and carbonates and are known to leach Mg^{++} from chrysotile asbestos.)

(B) Ashing and Microincineration

If the primary objective is the extraction of an inorganic particulate from the lung for identification, and the sample is small, then ashing may be employed.[13,14] This can be used on both bulk tissues and on tissue sections. The major advantages are its ability to concentrate particulates and the removal of organic coatings which, if present, interfere with the identification and the optical and crystallographic properties of minerals.

High Temperature Ashing (HTA). Ashing of tissue samples is performed

in a muffle furnace or oxygen flow oven at temperatures usually exceeding 500 °C for one to two hours.

Low Temperature Ashing (LTA). Tissue samples are oxidized at temperatures usually less than 100 °C in a reactive oxygen plasma. The process is more controllable than in HTA, and selective etching of organic tissues may be used to expose particulates.

Microincineration. This technique preserves anatomic relationships between the mineral dust and tissue components. Tissue sections are first photographed under brightfield illumination and then ashed. When all the organic material has been oxidized, the section is again photographed, under dark-field illumination, and the two photographs superimposed.[15]

Some compounds may be altered or lost with the ashing techniques because of oxidation and/or volatility. It is important, therefore, to run preliminary tests to ensure that the ashing does not destroy the agent being sought. Another disadvantage of these techniques is that a residue of inorganic material derived from the tissues may remain to complicate the analysis.

After isolation of the agent by one of the above procedures, the particulates can be analyzed in bulk by techniques such as x-ray powder diffraction, infrared spectroscopy, x-ray fluorescence, atomic absorption spectroscopy, neutron activation analysis, mass spectroscopy, and plasma emission spectroscopy. Alternatively, the sample can be mounted on filters or stubs and analyzed by any of the in situ techniques described below. The latter approach allows the characterization of individual particles by morphology, elemental composition, and crystal structure, and this information is essential to adequately characterize cases with mixed dust exposure.

Light Microscopy

Light microscopy of routinely processed tissue sections complemented with stains for connective tissue and iron is sufficient to make the diagnosis in many cases of suspected pneumoconiosis. The distinctive histopathologic features of coal workers' pneumoconiosis[6] and asbestosis[7] have been described. In many cases, however, the exposure is unknown or involves a mixture of dusts and the histopathologic features are not distinctive. These cases require additional procedures.

Special stains have been described for identification of certain inorganic dusts, and these are summarized in Table I. They have variable sensitivities and specificities and before use, the pathologist or technologist should become familiar with their limitations. Polarizing light microscopy (PLM) offers a valuable adjunct for the identification of crystalline material. With a properly equipped petrologic optical microscope, it is possible to gain sufficient information on crystal morphology, color, intensity of birefringence, refractive index, and dispersion characteristics, to be able to positively identify many crystalline minerals and organic fibers.[16,17] Useful information can also be gained by use of phase and interference contrast microscopy. These optical techniques are reliable and rapid but require specialized equipment and an expert knowledge of the properties of crystals. Another drawback is that small particles, less than 0.2 μm in diameter, cannot be resolved. Indeed, the optical properties of crystals cannot be adequately demonstrated if the particles are less than 1.0 μm in diameter. For example, for every asbestos fiber in the lung that can be visualized by light microscopy, there are approximately 10,000 that can only be resolved by electron microscopy.[18] Light microscopy is more valuable for the identification of extracted dust than for in situ identification, as tissue components or tissue reactions (eg, proteinaceous coatings on asbestos fibers) obscure the optical properties. With the average medical laboratory microscope (equipped with crossed polars), it is usually possible to determine only the distribution of dust particles, whether they are opaque or transparent, and the brightness of birefringence, if crystalline (Fig 2). Although these features provide clues to identity and may help to determine the next analytic procedure, they are insufficient for positive identification. Minerals exhibiting strong birefringence include gypsum, talc, mica, tin oxide, calcite, dolomite, hematite, magnesite, and aluminum silicate. Weakly birefringent minerals include crystalline silica and its polymorphs, and crocidolite asbestos.

In the lungs of normal persons and in cases of mineral dust pneumoconiosis, highly birefringent, platy or needle-shaped particles are frequently seen. Subsequent analyses usually reveal that these are ubiquitous silicates of various kinds. They are usually of little pathologic significance (unless present in high concentrations), but are commonly confused with quartz. Such confusion is unnecessary, as quartz is only weakly birefringent and barely discernable in the standard laboratory microscope. It should also be remembered that tissue components such as collagen and exogenous artifacts such as formalin pigment exhibit birefringence and thus may cause diagnostic error.

Table I: Histochemical Detection of Selected Elements

Element	Method	Reference
Aluminum	Morin Method	Feigl[34]
Beryllium	Naphthochrome B	Denz[35]
Calcium	Alizarin Method	McGee-Russell[36]
Copper	Rubeanic Acid Method	Uzman[37]
Nickel	Dimethylglycoxime Reaction	Choman[38]
Iron	Ferrocyanide Reaction	Mallory[39]
Zirconium	Morin Method	Feigl[34]

Electron Microscopy

The interaction of the electron beam with the specimen in both scanning electron microscopy (SEM) and transmission electron microscopy (TEM) generates several different signals including secondary electrons, backscattered electrons, transmitted electrons, diffraction patterns, x-rays, auger electrons, and cathodoluminescence: each provides unique information on the specimen that can be used for particulate identification. Backscattered electron imaging (BEI), x-ray energy dispersive spectrometry

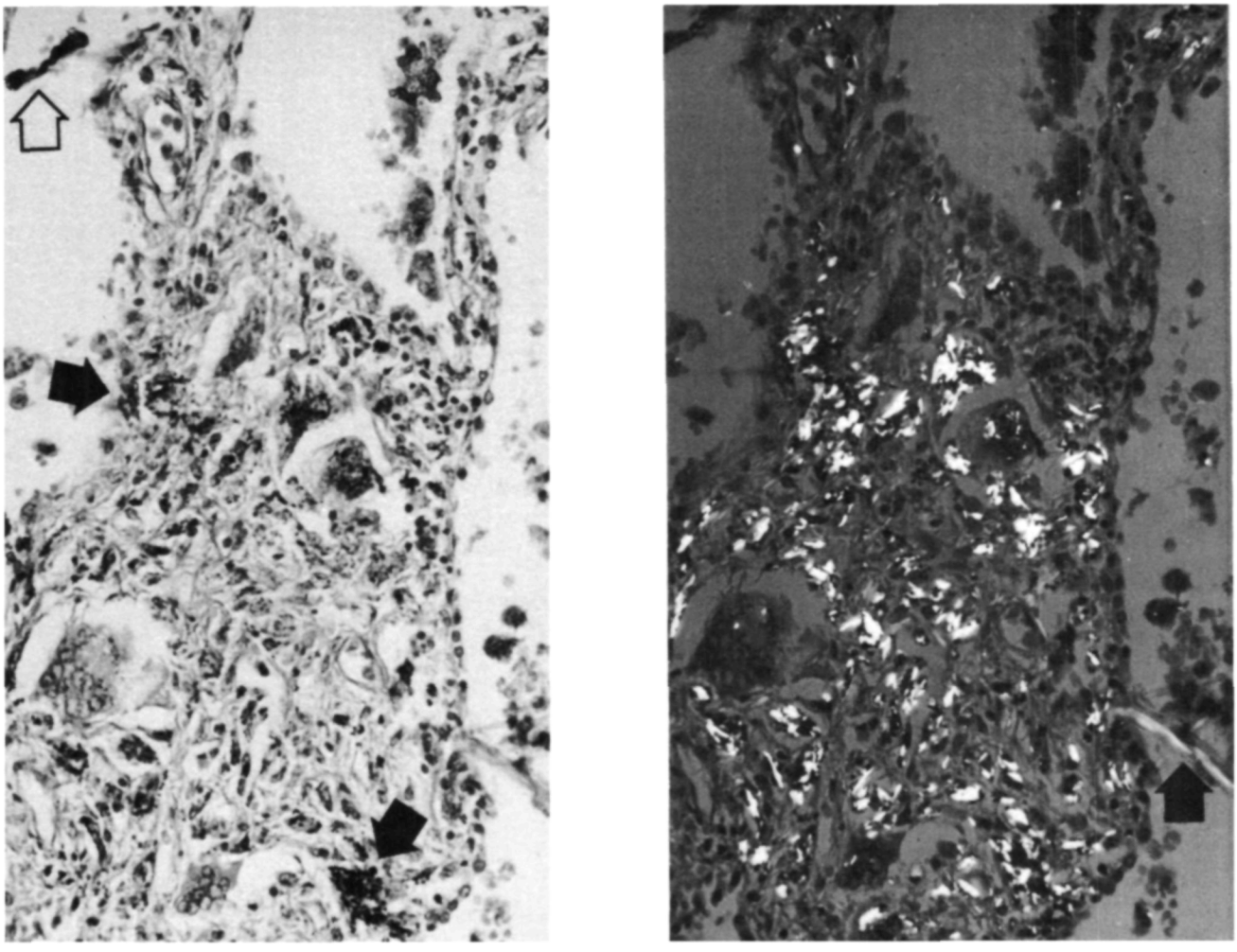


Fig 2. (Left) Light micrograph of pulmonary talc granuloma which is composed of dust, macrophages, giant cells, and fibroblasts. Black, opaque, finely granular, carbonaceous dust particles (solid arrows) are visible; the talc particles are less obvious. A ferruginous body is seen at lower right of field (hollow arrow). Ferruginous bodies are frequently seen in talcosis. (Right) Polarized light micrograph of same field. Numerous brightly birefringent platy particles are apparent within the granuloma. The morphology and intensity of birefringence are suggestive of talc; however, positive identification can only be made with ancillary techniques (eg, XRS and SAED). Note the birefringence of a collagen fiber (arrow) and the lack of birefringence of the ferruginous body (H and E, × 70).

(EDS), x-ray wave length dispersive spectrometry (WDS), and selected area electron diffraction (SAED) are the most commonly employed modes both for the in situ identification of mineral and metal dusts in tissues and for the analysis of tissue extracts. The various modes provide complementary information on morphology, microchemistry, and crystal structure. The recent development of scanning-transmission electron microscopy (TEMSCAN) allows sequential examination of the sample in all modes.

Tissue Preparation for Microanalysis

Sample preparation should be kept as simple as possible to minimize contamination and to avoid sample loss. These problems can be minimized by familiarity with the techniques involved and the parallel processing of positive control samples. Conventional methods of tissue preparation for TEM[19-21] and SEM[22] are appropriate in most instances, although some fixation methods may produce elemental loss due to solubility or physical loss of particulate.[23] Avoidance of specimen contamination must be given primary consideration when preparing tissue samples for x-ray microanalysis, irrespective of the method employed. Reagent-grade chemicals and high purity filtered water should be used when making up buffers, fixatives, etc. Prior to use, reagents should be filtered through a 0.2-μm membrane filter to remove all fibers or other particulate contamination. Only acid-cleaned glassware, which has been rinsed several times with filtered high purity water, should be used when mixing reagents. Ideally, all work on the sample, including preparation of reagents, should be carried out in areas with high-efficiency particulate air (HEPA) filtration where up to 99.9% of all particulate matter can be removed. Laminar flow biological safety cabinets exhausted to the outside of the building (Class 11B) are well suited for this purpose.

Scanning Electron Microscopy

The SEM can provide valuable information on the surface features and dimensions of dust particles and, combined with BEI and x-ray spectrometry (XRS), has been successfully used to identify and characterize mineral inclusions in tissue sections.[1,24]

The secondary electron imaging (SEI) mode provides information on tissue morphology and pathologic lesions; the BEI mode allows visual separation of particles from the tissue background for subsequent chemical characterization by x-ray analysis (Fig 3). SEM requires relatively simple preparation procedures and can be used to study fresh and fixed tissues as well as sections of tissues embedded in plastic or paraffin. It is particularly useful where only microscopic slides are available. With the coverslip removed, the tissue section can be directly observed in the SEM. If the elemental composition of the glass slide interferes with subsequent microanalysis, the section can be removed and remounted on a stub.[25]

Backscattered Electron Imaging

The BEI mode in SEM or transmission-scanning electron microscopy (TEMSCAN) has proven useful for the in situ identification of mineral particles in tissues.[1,24] The scattering of incident beam electrons is directly proportional to the atomic number of the sample. Thus, particles composed of high Z elements scatter more electrons and produce a brighter image than the surrounding low Z tissue matrix. As most metals and minerals have higher average Z than tissues (with the notable exception of beryllium) the particulates can be readily located in situ. Once located, the particles are further analyzed by high magnification SEI or XRS.

X-ray Spectrometry

The interaction of the incident electron beam with the specimen generates x-rays, which have energies and wavelengths characteristic of the elements excited. As the electron beam can be focused to less than 1 μm in diameter, the elemental composition of small particles can be determined in situ. The x-rays can be analyzed by either wavelength dispersive spectrometry (WDS) or by energy dispersive spectrometry (EDS). WDS systems are more sensitive and can detect elements of lower atomic number (eg, beryllium). However, they are able to analyze only one element at a time. Thus, WDS is time-consuming and of little use for the detection of unknown elements. EDS is less sensitive to trace elements and will not distinguish elements below sodium in the periodic table, but it does produce multi-elemental profiles in a short period of time, and is therefore the method of choice when the chemical composition of the particulate is not known. Although the intensities of the characteristic x-ray emissions are proportional to the concentration of elements in the sample, quantitative analysis of thin sections has not yet been established.

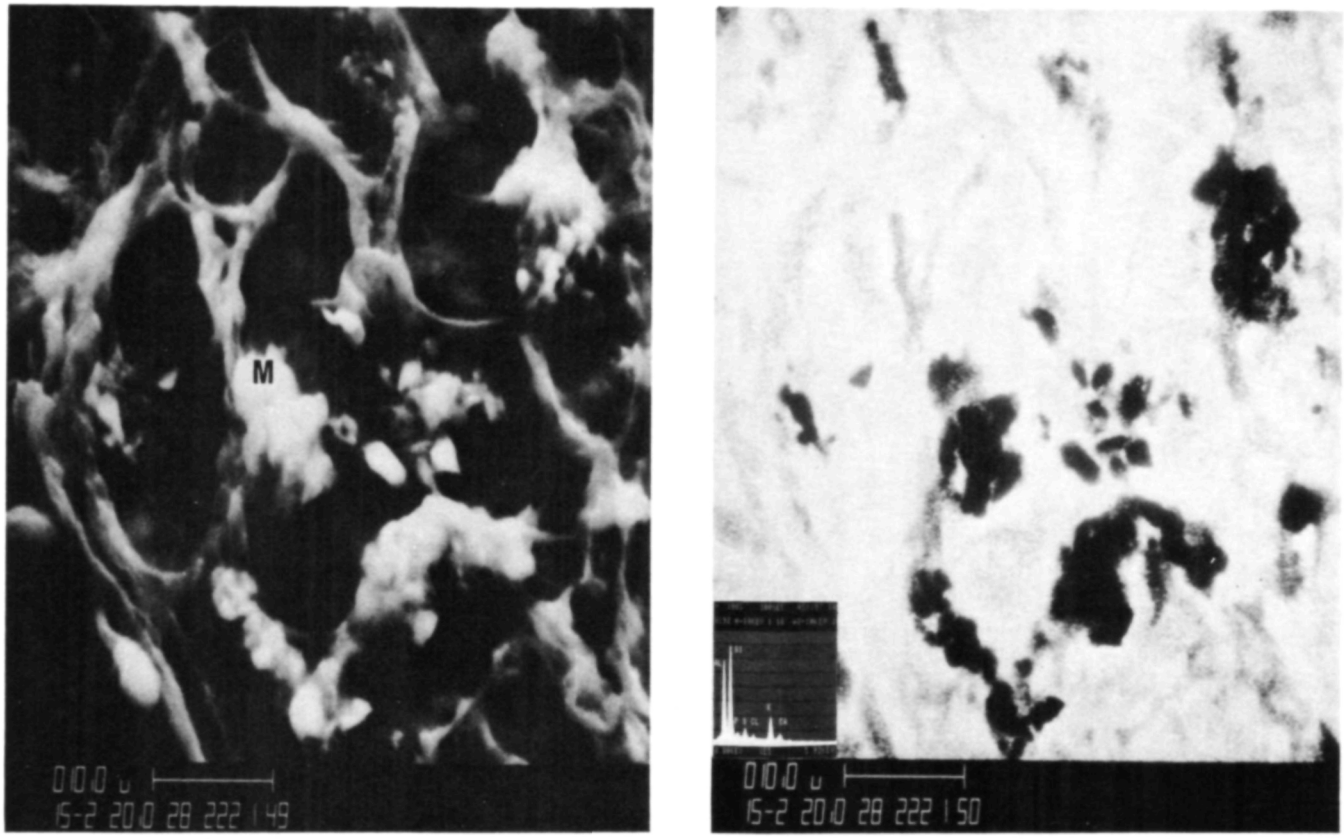


Fig 3. Case of a logger working on Mount St. Helens, May 18, 1980. Suffered massive ash exposure and died ten days later from extensive burns. Autopsy material was processed for routine light microscopy and a serial 5 μm thick section was mounted on a carbon planchet, deparaffinized, and examined in the scanning electron microscope. (Left) Secondary electron image (SEI) showing surface features of alveoli in which there are macrophages (M). (Right) Negative backscattered electron image (BEI) showing dust particles (black) within macrophages. With BEI, the image intensity is directly proportional to atomic number (Z). The mineral particles are clearly delineated from the surrounding tissues due to differences in average Zs. Once identified by BEI the mineral inclusions can be further characterized by XRS. Inset*: XRS of representative particle. The majority of the volcanic ash particles were silicates of the plagioclase class of minerals. Free crystalline silica accounted for approximately 5% of the particles.*

Scanning Transmission Electron Microscopy

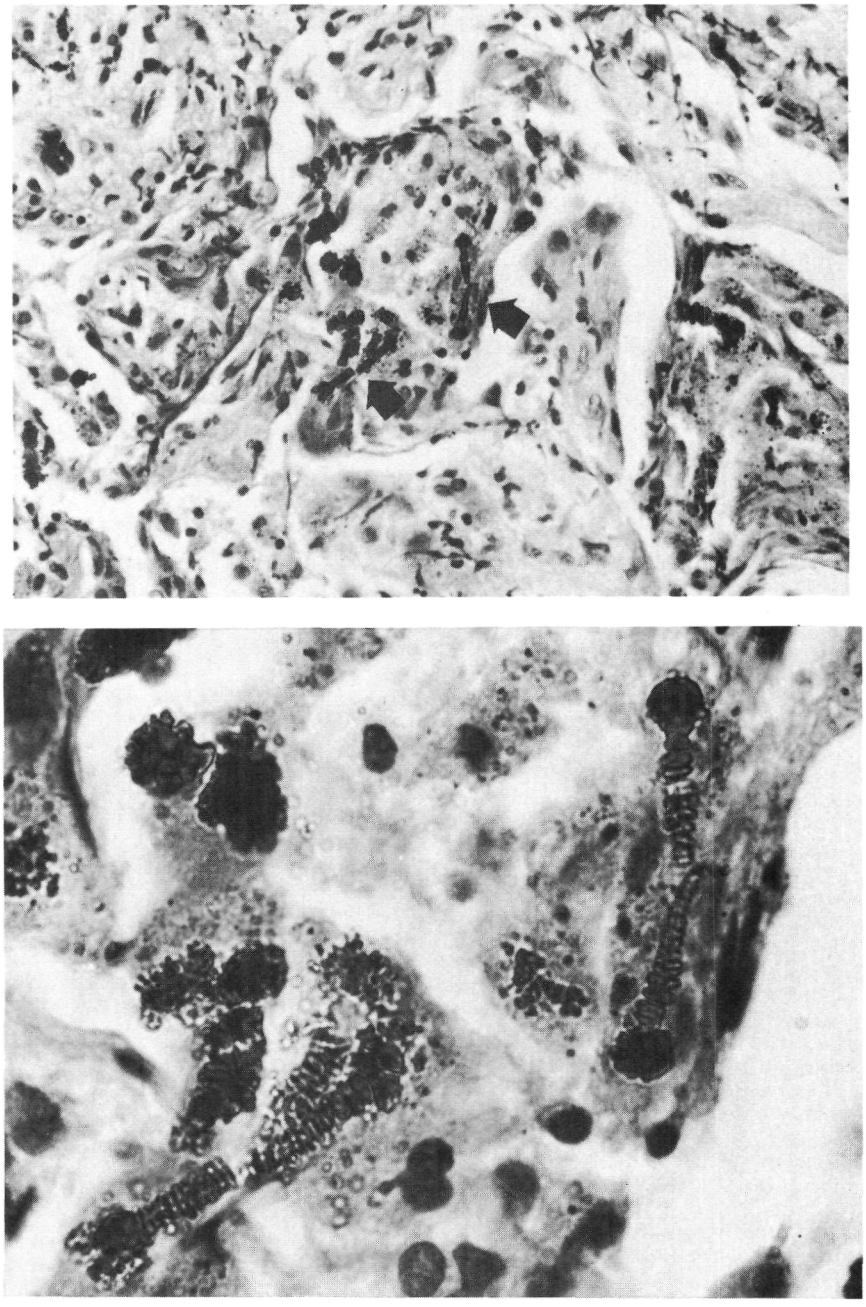
The addition of scanning transmission electron microscopy (STEM) to SEM or TEM permits examination of thicker sections with transmitted electrons. This is necessary because thin sectioning of tissues containing hard mineral inclusions commonly results in the loss of particulate, tears, and damage to the knife. With STEM, sections up to 500 nm in thickness can be examined, thus producing greater contrast than TEM sections and a greater signal for microanalysis. The technique allows correlation of particulate with cellular or subcellular architecture.

Transmission Electron Microscopy

Transmission electron microscopy (TEM) has a high resolution and depth of focus and is the most useful tool for studying the morphology (including aspect ratios) of submicroscopic particles and the ultrastructural tissue changes induced by dusts. Limitations of TEM include the small sample size (less than 3 mm in diameter), fracturing, and loss of particles during sectioning. Elaborate and complicated preparative procedures for TEM also present a disadvantage. Regardless of these technical limitations, TEM equipped with STEM, XES, and SAED is the most powerful tool available today for the simultaneous morphologic, microchemical, and microcrystalline identification of mineral inclusions in tissues or tissue extracts.

Selected Area Electron Diffraction

Selected area electron diffraction (SAED) provides information on the crystal structure of mineral particles. As most minerals have distinct diffraction patterns, it is an important identification technique. The diffraction patterns obtained should be compared with reference standards. The technique is time-consuming and is subject to limitations such as overlapping patterns produced by structural defects and twinning, loss of crystallinity, instrument variabilities, and errors in interpretation.[26,27] Large particles may not produce a pattern and prolonged exposure of a particle to the electron beam may contaminate the surface and obscure the diffraction pattern. It is therefore advisable to perform SAED before x-ray analysis. These limitations are not severe, however, provided the operator has suitable equipment and a thorough knowledge of the principles of electron diffraction. Moreover, SAED is the only method available for determining crystal structure with the electron microscope. SAED is commonly used for the identification of asbestos minerals (Fig 4). While SAED patterns for chrysotile asbestos are distinctive, the SAED patterns of the amphiboles can only be used to identify the group as a whole, not the individual minerals within the group.[26,28] Thus, identification of amphibole asbestos minerals requires additional procedures, such as x-ray analysis, for definitive identification.



*Figs 4A-G. Case of a middle-aged woman with interstitial pulmonary disease. Her occupational history included a ten-year period recycling fiber bags, a job she described as very dusty. Portions of a lung biopsy were prepared for light microscopy and particulate was extracted from the tissues by low temperature plasma ashing and Chlorox digestion for subsequent examination by SEM and TEM. (A)*Top. *Light micrograph of lung biopsy shows interstitial fibrosis with several asbestos bodies (arrows), (H and E, original magnification × 240) (B)*Bottom. *High-power view showing asbestos bodies and fragmented asbestos bodies. (H & E × 620) These light micrographic changes indicate asbestosis.*

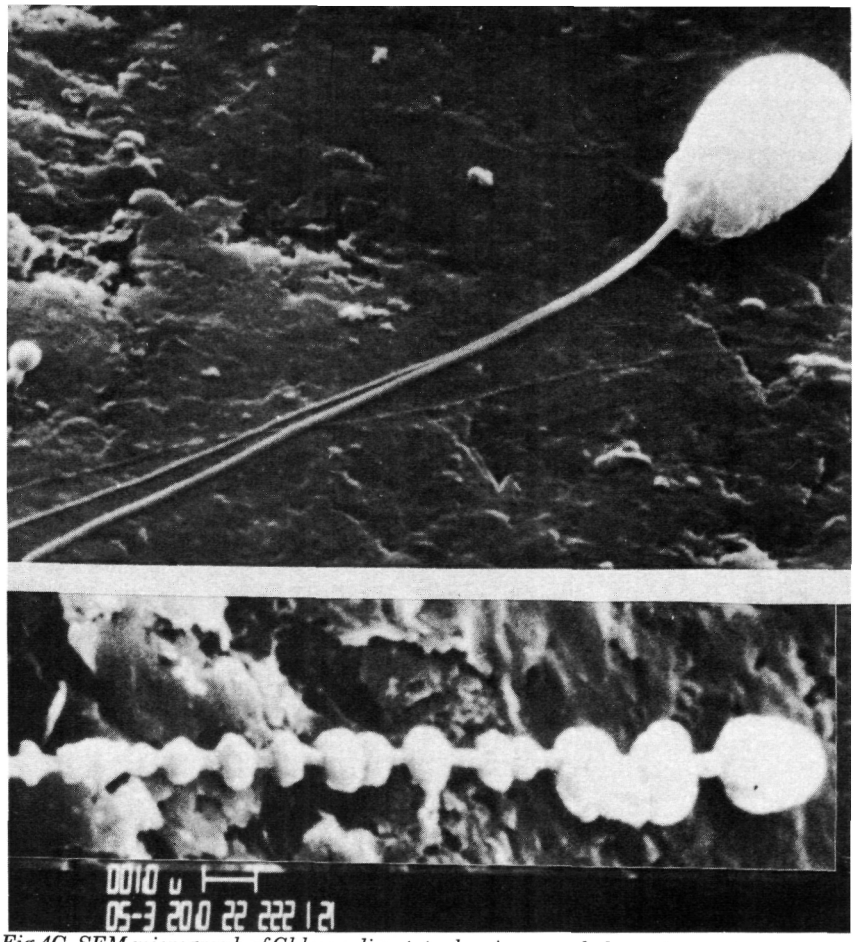


Fig 4C. SEM micrograph of Chlorox digestate showing morphology of two asbestos bodies. The body at top of the field is only partially coated and the bare portion of the fiber is fragmenting.

X-ray Fluorescence Spectrometry

X-ray fluorescence is an emission spectrometric technique used in the qualitative and quantitative analysis of elements from sodium to uranium. The technique is precise and sensitive when several elements require quantitative analysis, and it can be used to analyze thick tissue sections or homogenized tissue pellets. In routine clinical applications this technique is not efficacious, as it is relatively insensitive for elements of low atomic number and requires prolonged analysis for samples of unknown composition.

X-ray Diffraction

X-ray diffraction (XRD) is a powerful method for the qualitative and quantitative identification of crystalline particulates, as every species of mineral has a unique x-ray powder pattern.[29] Two types of XRD methods can be used to study dust samples: x-ray powder photography and x-ray diffractometry. The former is a qualitative technique employed only for identification purposes; the latter is both qualitative and quantitative.[27] Identification of patterns is made by either manual or computerized comparison with reference standards.[27,29] As tissue components interfere with the generation of diffraction patterns, minerals must be extracted from tissues before analysis. Since XRD does not destroy the particles, other techniques can be applied subsequently. It is mostly used for mineral identification, but it has potential application for the identification of organic compounds such as DDT, lindane, and chlordane.[29] It is useful for the identification of minerals that have similar or identical elemental composition (eg, chrysotile asbestos and talc) and for the identification and quantification of polymorphs (eg, quartz, tridymite, and crystobalite). Some of the minerals for which XRD analytic methods have been developed include talc, zinc oxide, zirconium oxide, chrysotile asbestos, fibrous tremolite, and quartz.[27,29] The major drawbacks to XRD include interference from other crystalline materials, the effects of particle size distribution in the test and reference samples, and the variations in reflection intensity for the same mineral derived from different geologic sources.[27,29]

Automated Image/X-ray Analysis

Automated image/x-ray analysis is a relatively new technique useful for the rapid physical and chemical characterization of large numbers of particles or inclusions.[30,31] A typical system consists of a computer-based

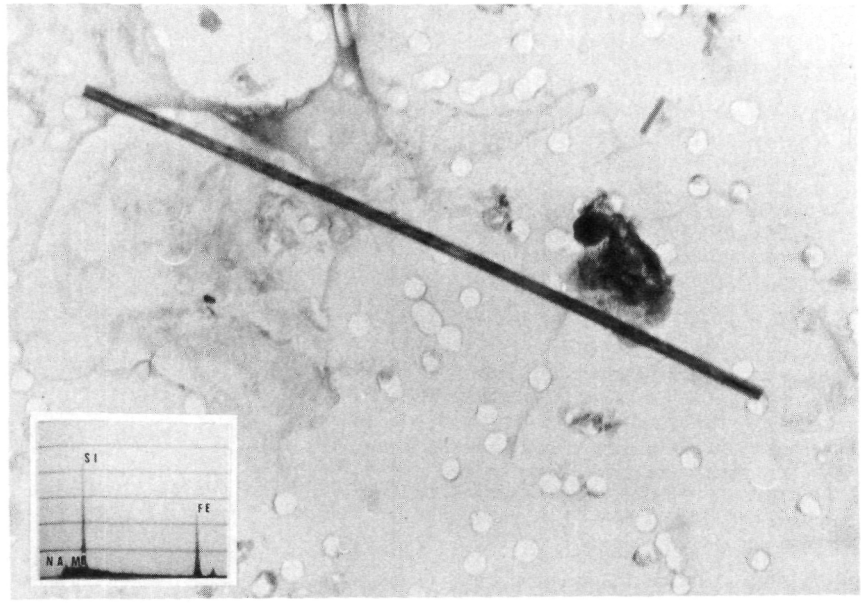


Fig 4D. TEM micrograph of plasma-ashed sample showing bare fibers. The majority of fibers were long, thin, and straight, measuring less than 0.2 μm in diameter and had aspect ratios greater than 10:1. Inset: *XRS confirms that this is crocidolite.*

Table II: Other Methods Available for Study of Pneumoconiosis

Method	Principle	Information	Reference
Laser Raman Spectroscopy (LRS)	Laser beam exites the sample and Raman scattered light is detected. Rapid, sensitive detection limits to picograms. Disadvantages are fluorescence, heating, and photolytic reactions.	Elemental, Molecular, Structural	Etz[40] Abraham[41]
Auger Electron Spectroscopy AES or Scanning Auger Microscopy (SAM)	Auger electrons produced by electron bombardment are detected. Good sensitivity for lower Z elements. Heavier elements produce complex spectra useful for studying particulate surfaces as signal is limited to top 10-15 Å.	Elemental, Binding energies, Molecular	Schaffner[42] Janssen[43]
Electron Energy Loss Spectroscopy (EELS)	Inelastically scattered electrons produced through the inner shell excitation by the electron beam are detected. Technique is more sensitive to the lower Z elements.	Chemical, Elemental bonding	Colliex[44]
Ion Microprobe Analysis (IMA) and Secondary Ion Mass Spectrometry (SIMS)	Uses ion beam to sputter the sample. Ions sputtered are collected by an electrode and analyzed by a mass spectrometer. Sensitive to all elements in periodic table to picogram range. Analysis time is short. Sample is etched away.	Elemental, Isotopic	McCrone[45] Scheifers[46]
Electron Spectroscopy for Chemical Analysis (ESCA)	Photoelectrons emitted by x-ray excitation are detected.	Elemental, Chemical bonding	McCrone[45]
Proton Induced X-ray Emission (PIXE)	Charged particle beam of protons induces characteristic x-rays. The detection limit is 1 to 10 ppm.	Elemental	Bartsch[47]

image analyzer and XES interfaced to an SEM or TEMSCAN. In practice, the system can automatically scan a microscopic field, detecting individual features, determining their physical parameters (ie, size, shape, area, volume), and then generate an elemental spectrum by XRS analysis. The operator may restrict the features analyzed and classified by setting threshold limits based on size, shape, and/or elemental composition. Classification may be based on user-defined classes or on dimensional-elemental data previously acquired from standards and stored on computer tape as a reference library. This technique is particularly valuable for the analysis of particles extracted from the lungs of patients with mixed mineral dust exposure and allows for the separation of potential etiologic agents by morphology and elemental composition.

Other Techniques

Many techniques are applied to the analysis of particles in tissue sections or tissue extracts. Most provide elemental information, but some, such as laser Raman spectroscopy, provide molecular information that can be used to identify specific minerals, metals, and organic materials. These techniques are summarized in Table II. Two of the techniques may be of value for the identification of beryllium and its compounds in tissue sections; these are secondary ion mass spectroscopy (SIMS) and laser Raman spectroscopy (LRS). Very few techniques exist for the identification of dust in living tissues. The recent development of magnetopneumography is exciting in this respect. Several mineral dusts, such as those generated in welding, asbestos, and coal mining, contain a quantifiable ferrimagnetic fraction.[32,33] The technique is sensitive to picogram values. It can also provide information on spatial distribution and clearance rates for dust in living subjects and experimental animals.[32,33]

Fig 4(E) SAED of one of these fibers showing amphibole pattern.

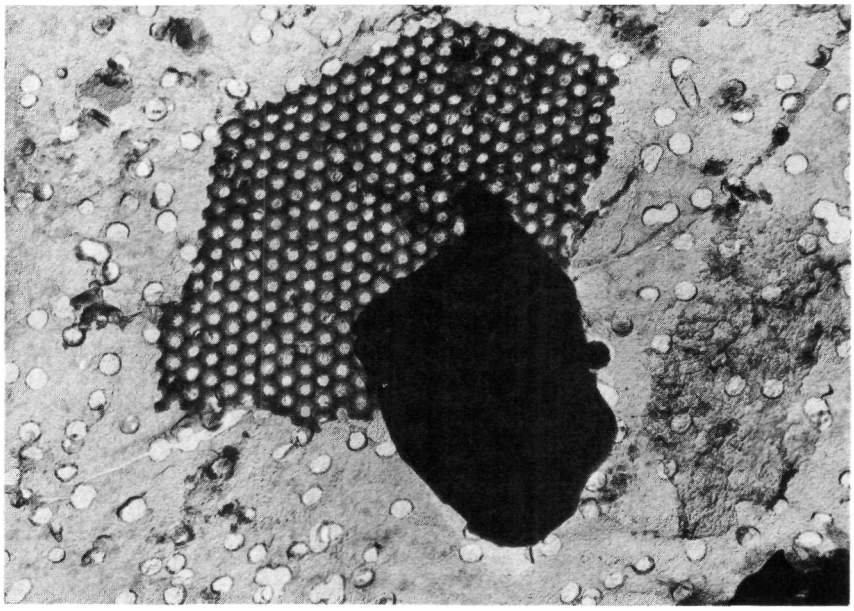


Fig 4. TEM micrograph of plasma-ashed sample showing ferrestrated diatomaceous material.

Summary and Conclusions

The simplest and most applicable techniques should always be selected for any particular diagnostic problem in occupational lung disease. Tissues should be sampled from areas of obvious disease and from relatively normal areas. Well-matched controls should be used where possible. Many cases of pneumoconiosis can be adequately diagnosed by light microscopy. Microanalytic procedures are best for determining the type and degree of exposure and for delineating dose-response relationships. More than one technique is necessary for the positive identification of particles. The combination of morphology, elemental analysis, and crystallography is usually sufficient to identify most minerals and metals. Transmission electron microscopy, combined with energy dispersive x-ray analysis and selected area electron diffraction, is the most widely used technique. This approach is not readily applicable to organic dust pneumoconiosis. In these cases, light microscopy combined with polarizing light microscopy is best. New, sensitive in situ techniques that provide molecular information, such as Raman spectroscopy, and in vivo techniques, such as magnetopneumography, will find increasing application in the diagnosis of pneumoconiosis.

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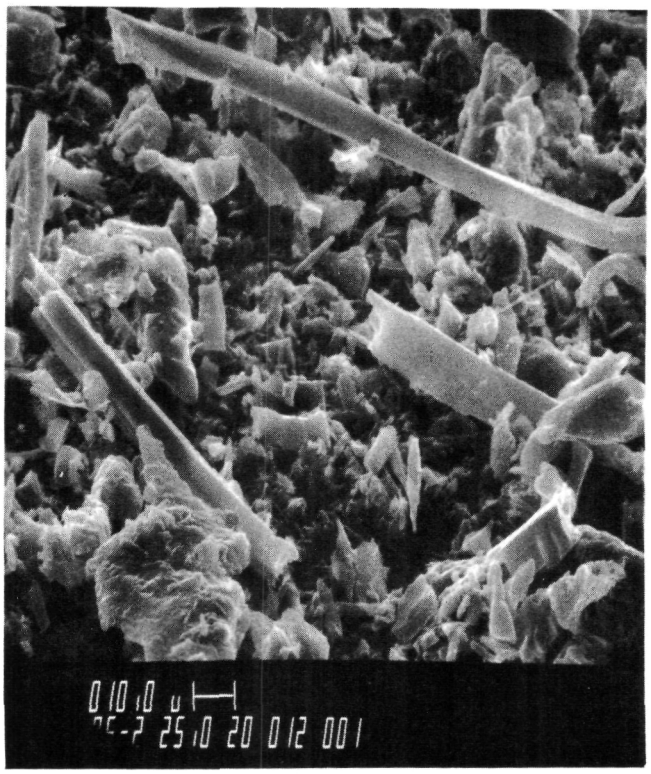

Fig 4(G) SEM of material removed from bagging used at workplace showing numerous plate-like particles and a few fibers. XRS and SAED analysis demonstrated primarily talc with lesser amounts of tremolite and occasional chrysotile asbestos fibers.

Comment on Figs 4A-G: The microanalytic studies supported the light microscopic diagnosis of asbestosis. Exposure appears to have been predominantly to crocidolite. In this case, analysis of the workplace environment did not show a correlation between the dust in the lungs and that in the workplace. Diseases such as asbestosis have latency periods in excess of ten years, thus it is quite likely that the dust analyzed from the workplace was not representative of past exposure. Alternatively, exposure was from another, cryptic source.

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