FILE NAME: Avondale (AVD)

DATE: 197o a l·ℕ

DOC#: AVDмнф

DOCUMENT DESCRIPTION: ₩ƨdzℕyl·f ! ℕǔλŎfŜ π LŘŜyűλŦλŎl·ǔλƨƴƨŦ
! ăōŜăűƨă λƴ l dzƳ l·ƴ ¢λăădzŜă

MD-00009164

Identification of Asbestos in Human Tissues

A. M. Langer, Ph.D.; Richard Ashley, B.S.; Victor Baden, Ph.D.; Carl Berkley, M.S.; E. C. Hammond, Dr.S.; Anne D. Mackler, M.S.; C. J. Maggiore, Ph.D.; W. J. Nicholson, Ph.D.; A. N. Rohl, Ph.D.; I. B. Rubin, M.E.; Antonio Sastre, M.S.; and I. J. Selikoff, M.D.

Recent studies have established that occupational exposure to asbestos dust is associated with serious risks of lung scarring and neoplastic disease [1-3] Under certain circumstances, albeit to a lesser degree, similar hazards may exist with indirect occupational exposure,[4 5] with family contact [6 7] and as the result of neighborhood and environmental exposure [8 9] The latter group might include residents within a half mile of an asbestos plant.[6 7]

Although exposure to all fiber varieties have been shown to be associated with disease, there remain differences concerning the relative hazards associated with each asbestos fiber type [10] It has been difficult to determine the quantities or dimensions of any one fiber type needed to produce disease [34] Studies of human exposures to crocidolite,[1 8] chrysotile,[7 11 12] amosite,[14] tremolite,[15] and anthophyllite,[9 113] have been difficult to compare since they reflected different exposures under different conditions

These uncertainties partly result from lack of information defining fiber exposure The asbestos mineral group defined commercially is comprised of five fibrous hydrated silicate minerals [16-18] Although the minerals range in chemical and physical properties, they possess some qualities which permit overlapping usage in thousands of products and applications [19] Fibers often tend to occur together in the same mineral deposit as mixtures [33] All exposures therefore, whether occupational, indirect or environmental, may, to some extent, be "mixed" in nature Essential information about the fiber-disease relationship may be added by analyzing lung tissues of individuals exposed to fibrous dusts However, recovery and identification of asbestos particles from human tissues have been difficult and affected by such factors as the nature of the recovery technique, fiber-type sought, amount of tissue available, level of exposure, analytical technique used in identification, and size-sensitivity of the method of examination

In this report, we review and discuss a number of techniques developed and used in the past with limited success New techniques offer some promise for solution of a number of unanswered questions These will be described and initial results presented

Analysis of Bulk Dusts

A number of instruments have been used in attempts to identify asbestos fibers in human tissues These make use of physical and chemical properties of asbestos minerals The accuracy of the procedures is predicated upon two important assumptions (1) each asbestos mineral possesses a characteristic property by which it may be distinguished from others as well as from other mineral species· (2) asbestos minerals are not altered chemically or physically *in vivo* Initially, those instruments used by physical scientists to characterize large bulk samples of "pure" asbestos mineral were used to analyze tissue extracts It followed logically that the tissue materials analyzed with these "bulk instrumental techniques" were those obtained from individuals *occupationally* exposed to mineral fiber since in such cases, the concentration of mineral fiber in tissue was assumed to be high and relatively large quantities of tissue materials would be available

A. — Extraction of Inorganic Materials from Tissues

A number of techniques have been devised to extract inorganic particles from human tissues These include the formamide method,[20-23] hydrochloric acid digestion [24 25] hot glacial acetic acid [26] alkali digestion,[27 28] oxidation of tissue in electronically activated oxygen [29] hydrogen peroxide digestion and high temperature ashing Most of these were developed to study silicosis, coal miner's pneumoconiosis and other dust diseases of the lungs. Those used to investigate the asbestos problem have produced results of questionable value The stability of asbestos minerals differ from inorganic materials for which the techniques were originally developed thus chrysotile fibers may be reduced to amorphous silica 'ghosts" in extremely weak acids,[18 30 31] the amphibole minerals have different degrees of stability in alkalis and acids [18] Therefore most extraction techniques commonly used in laboratories to study pneumoconioses are too vigorous for the asbestos mineral group Extraction of sub-micron fibers from human tissues by such methods may profoundly change the character of recovered minerals

These observations prompted Nagelschmidt to remark that a good extraction technique for the removal of asbestos fibers from human tissues had yet to be developed [32] It was Nagelschmidt's laboratory which used different techniques on equal aliquots of the same lungs and obtained different mineral extracts with each technique.

Most laboratories rely on oxidation of bulk tissues for recovery of inorganic mineral residues This may involve either

From the Environmental Sciences Laboratory, Mount Sinai School of Medicine of the City University of New York, N.Y.

Reprint requests to Dept. of Community Medicine Mt. Sinai School of Medicine, City University of New York, 5th Ave. and 100th St. New York 10029 (Dr. Langer)

high temperature oxidation of bulk tissue in a muffle furnace or low temperature oxidation of small quantities of tissue in activated oxygen. Both techniques work well with some preference for use of the low temperature technique since the inorganic phases are less likely to be dehydroxylated. Oxidation may be used in conjunction with a wet chemical digestion method if a specific fiber is being sought. For example potassium hydroxide digestion of tissue may be used for chrysotile recovery as the pH of the system (at 5% concentration) is near the isoelectric point of the fiber (10.8).[18] Search for a single fiber type has the disadvantage that the technique may not permit survival of other mineral fibers which may be present and important biologically.

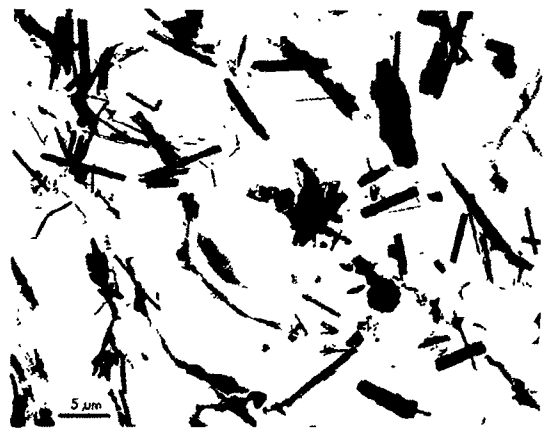
Evaluation of all currently used digestion techniques indicates that no one is suitable for extraction and recovery of *all* fiber types. Some alteration of mineral phases will occur, especially in fibers of small particle size. However, even with these difficulties extractions and analyses of bulk lung residues have been made.

B. X-ray Diffractometry

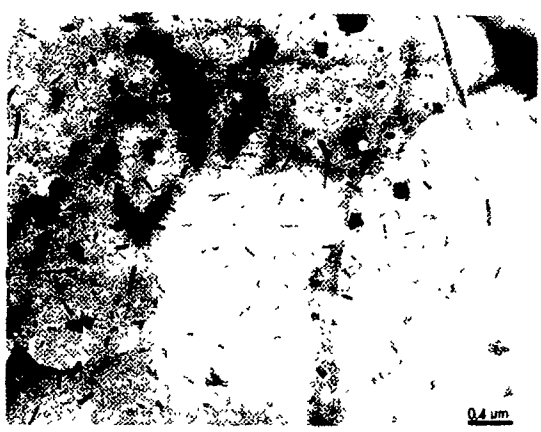
The x-ray powder patterns and crystal structures for individual asbestos minerals have been determined in a number of studies.[35-40] These data have been used primarily with phase identification of settled fibrous dusts[41,42] or for quality control.[43] Some work has been done on tissue extracts with this technique with varying results.[32,44,45] Amphibole asbestos fibers have been identified by this method but in no case has chrysotile been identified even in cases known to have been occupationally exposed to this variety of asbestos.

A number of analyses of lung tissues extracted with the low temperature ashing technique in conjunction with potassium hydroxide digestion have been made. The x-ray diffraction patterns obtained on extracted residues were not characteristic of the asbestos fibers believed to have been present in the tissues on the basis of occupational histories.[46] No successful identification was made on residues obtained from lungs of workmen exposed to chrysotile containing dusts. Some representative x-ray reflections were obtained from samples of dusts from men exposed to amphibole asbestos, e.g., amosite. The technique proved inadequate even when more sensitive powder camera techniques were used.

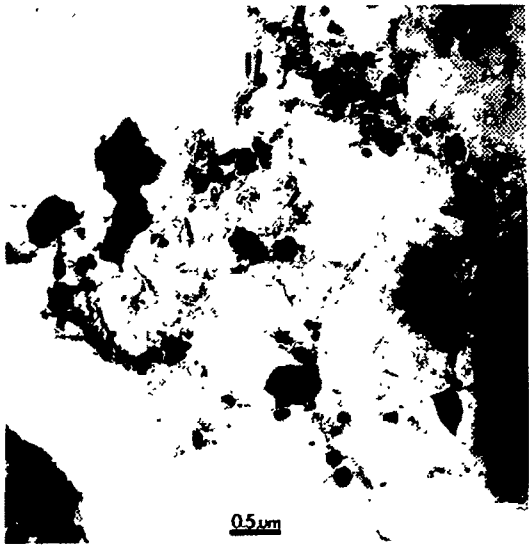
The reasons for lack of success with this technique are the particle population tended to be mixed so the x-ray spectrum obtained from each sample was complex, the x-ray reflections tended to be broad and of low intensity making accurate determination of angles of reflections extremely difficult, inhaled particles tended to be of small size thus producing a "line-broadened" x-ray spectrum[47] with generally poor resolution, the presence of endogenous iron compounds (hemosiderin) tended to induce x-ray fluorescence obscuring



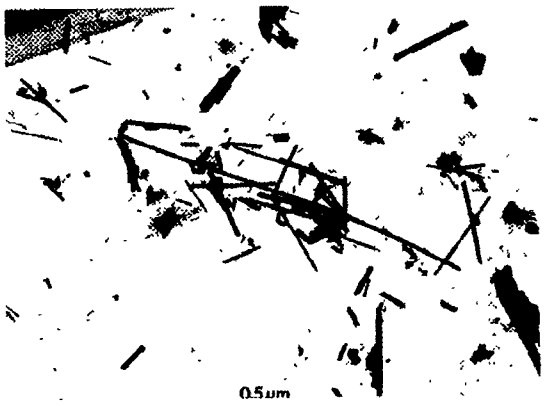
1. Carbon extracted lung tissue specimen obtained from a workman exposed to amosite fiber. Some 50 uncoated fibers and cores of bodies are greater than 5 microns in length. More than twice this number of fibers are present which are less than 5 microns in length. Cause of death: asbestosis.



2. Carbon extracted lung tissue specimen obtained from a man exposed to chrysotile fiber. Exposure resulted from use of asbestos-containing materials in pursuit of a hobby. More than 150 fibers and particles are visible in this field, some are visible by optical microscopy. Cause of death: asbestosis.



3 Carbon extracted lung tissue specimen obtained from a workman exposed to chrysotile fiber Exposure resulted from use of asbestos-containing caulking compound No fibers in this field are visible by light microscopy Cause of death: lung cancer



4 Carbon extracted lung tissue specimen obtained from a workman exposed to asbestos from handling "talc" in a rubber factory Few fibers were observed in his tissues by light microscopy. Cause of death: lung cancer talcosis. A primary malignant neoplasm of the colon was also found at autopsy

small peaks in a heightened background. The two reported cases of success using the x-ray technique[32, 45] were obtained on workmen exposed to the amphibole mineral amosite In addition to the ambiguities which derive from complex mixtures diminished intensities and particle size effects, marked alteration of the particles *in vivo* also limit their identification Biological degradation of asbestos has been reported previously [48–54] The extremely acid-sensitive chrysotile is most readily broken down *in vivo*, reduced in size[50] and depleted in magnesium [48–52] Our observations indicate that even when the occupational history is known the x-ray diffraction technique is inadequate to characterize lung dust burden It is of limited value for amphibole asbestos exposures, especially if fiber types are mixed, and almost valueless if exposure is to chrysotile Although some reports indicate some success in fiber identification, the x-ray technique should be recognized as having limited value

C — Infrared Spectroscopy

The infrared spectra of asbestos minerals have been determined[46, 55, 56]

As with x-ray diffraction, only limited success has been achieved for identification of mineral phases in tissue residues [46, 22] Dusts containing amphibole asbestos minerals yield infrared spectra characteristic of the amphibole mineral *group*, i e , the spectrum obtained is characteristic of the structural array of all minerals in the group rather than one specific member of the group Specific amphibole minerals could not be identified The characteristic spectra of chrysotile obtained on pure mineral specimens were almost identical to the other serpentine mineral phases lizardite and antigorite[56], making them virtually indistinguishable from each other In addition, the infrared spectra of serpentine minerals are remarkably similar to other sheet silicates so that any admixtures of chrysotile with clays or talc for example make fiber identification impossible by infrared spectroscopy alone As in the case of x-ray diffraction alteration of the asbestos fibers *in vivo*, or during recovery from tissue would alter the diagnostic properties Although a high resolution spectrophotometer* was not used in our study, confounding effects existed which lay outside the instrument

design. Complex mineral assemblages, body salts, organic debris and alteration of particles *in vivo* restrict the use of this technique for identification purposes.

D. — Differential Thermal Analysis

The thermal behaviors of various asbestos minerals have been determined[46, 55, 57, 58] for a range of sample and instrumental variables. The amphiboles yield characteristic DTA patterns which are inadequate for unequivocal identification, even in the 'pure' mineral state. Both exothermic and endothermic peaks tend to be small in magnitude and broadened over a long thermal range. Chrysotile produces a well-defined pattern but tends to be extremely sensitive to changes in sample preparation and experimental conditions.[55] Variations in particle size and packing of sample influence the endothermic dehydroxylation peak so that it may shift as much as 100C. The influence of these factors on diffusion of vapor from the site of reaction[59] is well documented. In addition, instrumental variables such as change in heating rate and instrumental sensitivity may alter peak position and configuration of thermal curves.[59]

We had no success characterizing extracted lung dusts using this technique.[46] The major problem was interference of the residual organic phases which produced a series of strong exothermic reactions in the thermal range in which characteristic asbestos reactions occur.

E. — Summary—Bulk Techniques

The use of a bulk instrumental technique to characterize phases in recovered lung dusts presents many difficulties. Even with the most useful technique, x ray diffraction, the results are marginally useful for amphiboles and of no value for chrysotile identification. A number of serious problems are inherent in the analysis: evidence demonstrates that chrysotile alters *in vivo*, thereby losing or changing its physical and chemical characteristics needed for identification; extraction of particles from tissues may lead to extensive alteration of asbestos present; analytical instruments which have routinely achieved acceptable results for pure mineral samples may not produce comparable results on mixed samples or unknowns; the amphibole asbestos minerals tend to overlap in properties making individual phase identification difficult; chrysotile asbestos closely resembles the platy serpentine minerals as well as other sheet silicates, making its identification difficult if not impossible; large quantities of materials are needed for analysis so that only tissues from occupationally exposed individuals are of use and bulk tissue materials are needed for mineral fiber recovery. The results obtained on tissues from individuals exposed to asbestos minerals are generally poor, even when the exposure history is well known.



5. Carbon extracted lung tissue specimen obtained from a man exposed to chrysotile asbestos textiles. This individual handled the materials as a salesman almost 30 years prior to his death. No fibers were visible by light microscopy. Cause of death: pleural mesothelioma.

Identification of Single Particles—Light Microscopy

(a) Asbestos Bodies. — In general, the object most readily noted in lungs of workmen exposed to asbestos dust is the asbestos body. It is readily discernible by light microscopy and is commonly cited as an index of asbestos exposure. The non-specificity of the body has been recognized almost as long as the object itself.[45, 60-65] Asbestos bodies have been used as asbestos markers in numerous studies of groups ranging from occupationally exposed workmen to the general population. Bodies have been counted in sputum, lung smears, lung scrapes, digested bulk tissues and various preparations and modifications of histologic sections.[11, 60-92]

These studies have reported frequencies of asbestos body presences ranging from 0-100%. Evaluation of these findings is impossible because many factors and study designs were not uniform. The studies differed in such variables as age and sex, techniques of material preparation, selection of kinds and amounts of biological specimens examined, lung sites examined; number of specimens examined per case, areas scanned per case, magnifications used in scanning, type of microscope employed, fiber type involved, duration of exposure, relative dustiness of trade and work areas, type of histologic stain; preparation used for digestion and centrifugation of bulk samples. All studies assumed homogeneity of body distribution in the tissue materials.

Some of the above differences may not appear to be obvious drawbacks. For example, asbestos bodies tend to form with different degrees of efficiency in human lungs as a function of fiber type.[93] The same number of asbestos bodies observed in tissues of workmen exposed to different fibers need not indicate similar exposure levels. Different fiber types tend to form aerosols with characteristic size distributions[94] so that "thick" bodies, those easily seen by light microscopy, are more likely to be nucleated on tremolite anthophyllite or amosite. Chrysotile, the most commonly used fiber in the United States, tends to form fibers of small sizes, readily degrades in vivo, and resists formation of

asbestos bodies [92]

(b) Asbestos Fibers. — Lung tissues from 3000 cases from the general population of New York City were examined for asbestos body content [83 95] The optically visible asbestos bodies represented only a small fraction of the total inorganic particles present in lung tissues In these same tissues many inorganic *uncoated fibers* were observed, including many with diameters of less than 1 micron In 1,038 of the 3,000 cases these fibers were observed to be present [95] The occurrence of such thin fibers appears to correlate with asbestos body count.

While the light microscope appears to be adequate for the search for asbestos in human lungs, there is much evidence to suggest that this is not so Even after light microscopy indicates the presence of "fibers" and "bodies", important questions remain unanswered What are the uncoated fibers, *specifically*, what lies at the core of "asbestos bodies"? Light microscopy furnishes information about the morphology of a particle, its color, relative crystallinity if polarized light optics are employed, and the indices of refraction if immersion oils are used This may provide information for fiber identification were one certain that the particle had not altered *in vivo* More important, the major drawback in using the light microscope to search for and identify asbestos in human lungs is the fact that it is size and resolution limited Identification of particles is based upon correlation with standard asbestos fibers, the optical properties of which were determined on much larger fibers, fibers recovered from tissues tend to be much smaller and the optical properties associated with larger fibers are infrequently observed in smaller ones Optical microscopy should be considered preliminary because size and resolution limitations allow only narrow perspective of the total lung burden of particles The number of light-visible fibers in human tissue is limited compared to smaller, sub-light microscopic fibers. Observation of the prevalence of asbestos bodies with the optical microscope may or may not indicate the burden of asbestos fiber The search for and identification of fibers must be made on the sub-light microscopic level

Identification of Single Particles—Electron Beam Instruments

A great number of sub light microscopic asbestos fibers occur in lungs of workmen exposed to aerosols containing asbestos [53 94 96 97 98] The small fibers appear to be present in amounts estimated from an equal amount to more than *1,000 times greater than those fibers visible by light microscopy* This is of importance in studying exposure in the general population among whom there appear to be *primarily* sub-light microscopic fibers in lungs,[95 98 99] a finding understandable in light of recent work demonstrating sub-light microscopic chrysotile fibers as significant constituents in urban air samples [101] The search for asbestos fiber should be made by a method which permits comparison on all exposure levels and for all fiber types. Electron beam instruments may be employed for such purposes

To examine specimens on the sub-light microscopic level, tissues should be prepared so they are compatible with the technique. Several methods currently available for this are (1) direct micromanipulation of objects from tissues on to appropriate substrates (2) digestion of bulk tissue, (3) carbon extraction of particles from histologic sections

(a) Tissue Preparation. (1) The micromanipulator has been used to remove and transfer objects from histologic sections to appropriate substrates for examination by electron beam instruments [51 52] This technique requires localization of objects with a light microscope removal of the tissue matrix by low-temperature oxidation, placement of the ashed slide on the micromanipulator stage and use of a mechanical manipulation system to remove selected objects from the section. The use of the light microscope in the search for these objects precludes, by definition, sub-light microscopic particles from analysis Asbestos bodies so removed tend to be nucleated on amphibole fibers [52 73] Uncoated fibers are only rarely removed from tissues by this method, (2) Occasionally bulk tissue is digested by the alkali method or ashed in air or activated oxygen and the residues examined by electron beam instruments.[46 52 95 99 101] One procedure involves several cycles of washing and centrifugation (alkali digestion technique) and both require homogeneous dispersal of the residue by sonification in an aqueous medium and pipetting the homogenate on to electron microscope grids The technique is excellent for tissues in which low concentrations of asbestos dusts are present. The major drawbacks are the requirement for bulk tissue samples and the partial destruction by alkali of some amphiboles. (3) A technique has been developed which permits using normal histologic sections in the search for asbestos particles [53 93 96–98 102] This method is called the carbon extraction technique and consists of a procedure in which the tissue is ashed on a glass slide, impregnated with a water-soluble plastic removed from the slide, a layer of carbon deposited on the tissue surface, and the plastic dissolved with hot water leaving a floating carbon film with relic tissue and particles [53 98]

The importance of the carbon extraction technique cannot be overstated It may be used for all tissue materials, even in cases where only slides are available all exposure levels may be examined in this manner by electron beam instruments (general population, or low intensity exposure cases, require *much* scanning time), the carbon provides a stable medium and supporting film for electron beam examination of all particles, the same section may be transferred to the various beam instruments for determining the range of physical and chemical parameters required for characterization.

(b) Transmission Electron Microscopy — The examination of tissue preparations by transmission electron microscopy makes visible all fiber in the tissues and the morphological characteristics by which they may be identified Chrysotile generally consists of broken open, often curved, "bundles" of unit fibrils or of isolated fibrils These fibrils are 200-400Å in diameter and are made up of an electron translucent center (a capillary), electron dense "walls" (crystalline structure containing the capillary) and an electron translucent

amorphous edge which increases in thickness with time of exposure to the electron beam. The relative dimensions of the units may vary. These features have been described in the mineralogical[101–105] and medical literature[52, 94–99]. Occasionally chrysotile may be present in tissue as clumps[98] but more frequently as individual fibrils, especially in individuals not occupationally exposed to the fiber[96–99, 101]. Chrysotile that has been vigorously treated, such as in hammer- or ball-milling, tends to deform readily under an electron beam, suggesting that the crystalline structure at the edges of the fibrils have been disrupted[55]. The morphological characteristics of fibrils observed in human tissue vary to some degree, yet they are distinctive enough for unique identification[97, 99].

At first glance the overall morphological characteristics of the amphibole asbestos fibers are remarkably similar, tending to be electron dense and either straight or bent. Occasionally under high magnification "cross-hatching" produced by diffraction contrast may be observed along the fiber's width. These 'bands' may occasionally be observed to move up and down the fiber as the intensity of the electron beam is varied on the surface of the sample. These effects are likely caused by thermal stress and change in convergance of the electron beam. It is possible to differentiate the amphibole asbestos minerals morphologically[94]. Fibers are measured across their width and a width distribution is determined. Each amphibole type apparently possesses a relatively unique width distribution which may be used as an identification tool. Current studies in the Environmental Sciences Laboratory suggest that this may not be as straightforward as previously indicated. Also in many nonoccupationally exposed cases the number of such fibers observed in tissues is small so that the usefulness of differential width distributions is restricted. Also, mixed fiber exposures, apparently more the rule than the exception, make the results equivocal.

It appears that, on a morphological basis, chrysotile may be differentiated from all other inorganic silicate fibers. The amphibole fibers may be identified on a specific fiber type basis only in cases where exposure is "pure." Such exposures are generally rare so that only the amphibole group may be identified usually, not the fiber species.

(c) Selected Area Electron Diffraction. — Most transmission electron microscopes today are equipped with controls which make possible change in instrument geometry so that diffracted electrons may be brought into focus on the image screen. Crystalline materials scatter electrons in patterns representative of their crystal structure and interplanar spacings. Single crystal Laue geometry (fixed film and crystal) applies so that both dimension and angle characteristics may be measured directly[106]. From each asbestos fiber type a characteristic single crystal pattern may be obtained[97]. This technique permits more exact identification of fibers when morphological characteristics are not definitive.

Chrysotile yields a unique diffraction pattern and may be identified on this basis as well as by its morphology[107, 108]. Diffraction studies of fibers in tissue materials have demonstrated the importance of the technique as a tool for identification of chrysotile[97–99]. One report recently proposed that it is possible to differentiate among amphibole asbestos types with the diffraction technique[109]. However, these observations have recently been criticized because several patterns appear which are not accounted for by the mineral's space group symmetry[110].

Recent work has shown that reflections obtained by the electron diffraction method indexed according to known space groups for the amphibole minerals, show heretofore unsuspected structural modifications[110]. Therefore, the amphibole mineral group possesses structural characteristics which make the mineral *group* but *not the individual members of the group*, identifiable.

(d) Electron Microprobe Analysis. — The electron microprobe analyzer makes possible x-ray spectrochemical analysis of small particles under an electron beam. The imaging system is a limited depth of field scanning electron microscope with spatial resolution, using secondary electrons, of about 0.2 microns. X-ray spectrometers[111, 112] are equipped with crystals which serve as dispersive x-ray analyzers. The system may detect elements down to and including atomic number 5 (boron) and achieve a concentration sensitivity of 50-100 ppm depending on instrumental and sample conditions.

We have analyzed asbestos bodies and fibers by means of the electron microprobe[43, 51–53]. The possibility of successful analysis of fibrous cores of asbestos bodies and uncoated fibers was predicated upon chemical differentiation of the asbestos fiber types. The chemical composition of single fibers was determined on the probe. Asbestos fiber types, especially amphiboles, could be chemically differentiated and identified on a single fiber basis[49, 51, 52]. A number of interesting observations have been made. chrysotile fibers are not stable in the biological environment and tend to lose magnesium; single amphibole fibers may be identified on a chemical basis. amphibole fibers which occupy the cores of asbestos bodies are not degraded chemically whereas uncoated fibers appear to have lost some magnesium from their structures[53].

It is possible to chemically characterize and identify uncoated asbestos fibers in human tissues. Success has also been obtained on fibrous cores of asbestos bodies only when the fiber protrudes from the coating material. Individual species of the amphibole fibers may be differentiated with this technique.

(e) Summary—Electron Beam Instruments. • Preparation techniques are now available which make possible examination of any human tissues in electron beam instruments. With the carbon extraction technique, gross histologic structures are recognizable and inorganic particles preserved in their original anatomical sites. No physical or chemical artifacts are created during tissue preparation. This preparation forms a stable matrix for the materials and allows long time examination of particles under an electron beam. The mounting of the preparation on an electron microscope grid permits its transference into any beam instrument and

allows multiple analyses to be performed by one or several instruments

Asbestos fibers may be identified on the basis of morphological, structural and chemical criteria Chrysotile may be identified on the basis of morphology and selected area diffraction pattern The amphibole minerals, when occuring as single fibers, need chemical characterization as well for unique identification

Some Observations

Our laboratory has now examined tissues from some 250 individuals with exposures to asbestos (occupational to 'general population") with electron beam instruments The tissue preparation and identification techniques have included all those described in this presentation

In all cases where the occupational history indicated asbestos exposure, fibers were observed in the lung tissues Some of these were large enough to be seen by light microscopy However, most fibers present were well below the resolution of the light microscope This was observed in workmen occupationally exposed to amphibole fibers as well as chrysotile In the latter case however, the small fibers (fibrils in many cases) were present in numbers about 100-1 000x that of the larger fibers This size distribution existed in the tissue since the carbon extraction technique was used in tissue preparation. Extrapulmonary tissues have been examined, e.g. spleen, colon, pancreas, liver Primarily sub-light microscopic fibers were present

In the New York City cases from the 'general population" chrysotile fibers were found in the lung tissues of 76 of 126 (about 60%) cases † The amount of tissue examined in this study was extremely small Larger amounts would surely have increased the positive occurrences Here too, small fibers and fibrils of chrysotile predominate in the particle population Because of utilization of a bulk tissue digestion technique, it was assumed that this might be an artifact size distribution produced during extraction However this size particle was observed to predominate in relic tissues prepared by the carbon extraction technique in another study.[18] In a number of cases, fibers have been found which could *only* be detected by electron microscopy However even in these cases, there exists some uncertainty as to whether the size distribution reflects biological degradation or the original inhaled aerosol The prominent fiber in these cases is chrysotile

Small amphibole fibers less than 0 1 microns in width have been observed in the lungs of workmen occupationally exposed to amosite dusts The proportion of sub-light microscopic fibers to light visible fibers ranges from about 2 to 100 times and is apparently a function of the anatomical site in the lung.‡ There are few published data suggesting that the majority of small fibers were produced by biological degradation The biological effects observed in tissues in which these fibers occur have often been attributed to the associated larger particles The concomitant sub-light microscopic particles, once demonstrated, cannot be discounted as a major pathogenic agent in individuals with disease Figures 1-5

The relationships of fiber size and type in association with human disease are complex Arbitrary simple assumptions or conclusions based on light microscopy that one specific fiber type or size is responsible for disease are unwarranted

*The infrared spectrophotometer used was a Perkin Elmer Model 21 equipped with NaCl optics

†Completed study in the Environmental Sciences Laboratory to be published

‡Completed study ESL - to be published

References

1 Selikoff IJ et al Asbestosis and neoplasia, *Amer J Med* 42 487 1967

2 Enticknap JB and Smither WJ Peritoneal tumors in asbestosis *Brit J Indust Med* 21 20 1964

3 Selikoff IJ Hammond EC and Chung J Asbestos exposure, smoking and neoplasia *JAMA* 204 20, 1968

4 Harner PC Asbestos hazards in naval shipyards *Ann Occup Hyg* 11 135 1968

5 McEwen J et al Mesothelioma in Scotland *Brit Med Jour* 4 575, 1970

6 Newhouse M and Thompson H Mesothelioma of pleura and peritoneum following exposure to asbestos in the London area *Brit J Indust Med* 22 261, 1965

7 Lieben J and Pistawka H Mesothelioma and asbestos exposure *Arch Environ Health* 14 599 1967

8 Wagner JC Sleggs CA and Marchand P Diffuse pleural mesothelioma and asbestos exposure in North Western Cape Province *Brit J Indust Med* 17 260 1960

9 Kivilioto R Pleural calcification as a roentgenologic sign of non-occupational endemic anthophyllite asbestosis *Acta Radiolog* 194 (1) 1960

10 A symposium held in Cardiff, Wales, 7-9 April 1970 concerned with the biological effects of asbestos There was much discussion at this meeting concerning the relative biological hazards associated with the different asbestos mineral fibers Opinions ranged from 'all are active biologically' to only some fibers are active Properties of single fiber types were also discussed including fiber length trace metals associated with the fiber surface hydrocarbons etc No agreement was reached as to the importance of each of these properties biologically

11 Dreessen WC, Dallavalle JM Edwards TI Miller JW and Sayers RR A Study of Asbestosis in the Asbestos Textile Industry USPHS Public Health Bull no 241, August 1938

12 Doll R Mortality from lung cancer in asbestos workers *Brit J Indust Med* 12 139 1955

14 Selikoff IJ, Hammond EC and Seidman H Asbestos disease associated with insulation work in shipyards in the United States Intl Symp on Safety and Health in Shipyards and Ship Repairing Helsinki August, 1971, in Press

15 Kleinfeld M et al Mortality among talc miners and millers of New York State, *Arch Environ Hlth* 14 663 1967

16 Hendrix MW The geology occurrences and major uses of asbestos *Ann NY Acad Sci* 132 12 1965

17 Gaze R The physical and molecular structure of asbestos *Ann NY Acad Sci* 132 23 1965

18 Speil S and Leineweber J Asbestos minerals in modern technology *Environ Res* 2 166 1969

19 Rosato DV Asbestos its Industrial Applications Rheinhold Pub Co 214 1959

20 Thomas K and Stegemann H Formamide methods of tissue digestion of Silicotic lungs *Beitrage Silik Forsch* 28 1, 1954

21 Lorentz H Embrodt HJ and Klosterkotter W Grain size and mineral content of lung dust of coal miners compared with mine dust Inhaled Particles and Vapours 2nd Intl Symp Davies E Pergamon Press 381, 1967

22 Ulrich J and Jurk A Fate of submicronic inhaled dust in the lungs Inhaled Particles and Vapours 2nd Intl Symp Davies Ed Pergamon Press 439 1967

23 Stober W Embrodt HJ and Klosterkotter W Quantitative studies in dust retention in animal and human lungs after chronic

inhalation Inhaled Particles and Vapours 2nd Intl Symp Davies Ed, Pergamon Press 409 1967

24 Cartwright J Airborne dust in coal mines The particle size selection characteristics of dust sampling instruments Inhaled Particles and Vapours 2nd Intl Symp Davies Ed, Pergamon Press 393 1967

25 Morris TG et al Comparison of dust retention in specific pathogen free and standard rats Inhaled Particles and Vapours 2nd Intl Symp Davies Ed Pergamon Press 205 1967

26 Bergmann J The determination of coal in formalin fixed pneumoconiotic lungs a new method of tissue digestion using glacial acetic acid *Analyt Chem* **38** 441 1966

27 Rivers D et al Dust content radiology and pathology in simple pneumoconiosis of coal workers Part 1 General observations Part 2 Detailed analysis of the data *Brit J Industr Med* **17** 87 1960

28 Rossiter CE et al Dust content radiology and pathology in simple pneumoconiosis of coal workers Inhaled Particles and Vapours 2nd Intl Symp Davies Ed Pergamon Press 319 1967

29 Berkles C Chung J and Selikoff IJ Detection and localization of mineral fibers in tissue, *Ann NY Acad Sci* **132** 48 1965

30 Nagy B and Bates TF Stability of chrysotile asbestos *Amer Mineral* **37** 1055 1952

31 Hargreaves A and Taylor WH An x-ray examination of decomposition products of chrysotile (asbestos) and serpentine, *Mineral Mag* **27** 204, 1956

32 Nagelschmidt G Some observations on the dust content and composition in lungs with asbestosis made during work on coal miners pneumoconiosis *Ann NY Acad Sci* **132** 64 1965

33 Bowles O Asbestos *USBM Bull* **403** 92 1937

34 Gilson JC Wyers memorial lecture Health hazards of asbestos Recent studies on its biological effects *Trans Soc Occup Med* **16** 62 1966

35 Ernst WG Amphiboles crystal chemistry phase relations and occurrence New York Springer Verlag 125 1968

36 Timbrell V Characteristics of the UICC standard reference sample of asbestos Pneumoconiosis Proc of the Intl Conf, Joh, 1969 HA Shapiro Ed Oxford University Press Capetown 28 1970

37 Jagodzinski H and Kunze G Die rollchenstructur des chrysotils I Neues Jahrbuch für Mineral Nos 4 5 95 1951

38 Jagodzinski H and Kunze G Die rollchenstructur des chrysotils II Neues Jahrbuch für Mineral No 6 113 1954

39 Selfridge GC An x-ray and optical investigation of the serpentine minerals Ames *Mineral* **21** 463 1936

40 Whittaker EJW and Zussman J The characterization of serpentine minerals by x-ray diffraction *Mineral Mag* **31** 107, 1956.

41 Crable JV and Knott MJ Application of x ray diffraction to the determination of chrysotile in bulk or settled dusts, *Amer Ind Hyg Assn J* **27** 383 1966

42 Crable JV, and Knott MJ Quantitative x ray diffraction analysis of cocciolite and amosite in bulk or settled dust samples, *Amer Ind Hyg Assn J* **27** 449, 1966

43 Badollet MS and McGourty JP Identification of minerals associated with asbestos by x ray diffraction patterns *Trans Can Min and Metall Bull* **51** 169, 1958

44 Nagelschmidt G Personal communication Work described in laboratory of Safety Mines Research Establishment Sheffield London 1967

45 Sundius N and Bygden A Der Staubinhalt einer Asbestosis Lunge und die Beschaffenheit der sogenannten Asbestosis-Körperchen, *Arch Gewerbepath Gewerbehyg* **8** *Zh*, 1938

46 Berkley C Langer AM and Baden V Instrumental Analysis of inspired fibrous pulmonary particulates *NY Acad Sci Trans* **30** 331 1967

47 Cullity BD Elements of x-ray diffraction Addison-Wesley Pub Co Reading, Mass 514, 1967

48 Morgan A and Holmes A Neutron activation techniques in investigations of the composition and biological effects of asbestos Pneumoconiosis Proc of the Intl Conf Joh, HA Shapiro, Ed Oxford Univ Press Capetown 52 1970

49 Langer AM Rubin I and Selikoff IJ Electron Microprobe analysis of asbestos bodies Proc of the Pneumoconiosis Conf, Joh HA Shapiro, Ed Oxford Univ Press Capetown 57 1970

50 Suzuki Y, and Churg J Structure and development of the asbestos body *Amer J Path* **55** 79 1969

51 Langer AM Electron microprobe analysis (study of asbestos fibers and bodies from lung tissue) Lab Diag of Diseases caused by Toxic Agents, FW Sunderman and Sunderman FW, Eds WH Green and Co St Louis 126 1970

52 Langer AM, Rubin I Selikoff IJ Chemical characterization of asbestos body cores by electron microprobe analysis *Histochem and Cytochem* **20** 723 1972

53 Langer AM, et al Chemical characterization of uncoated asbestos fibers from the lungs of asbestos workers by electron microprobe analysis *J Histochem and Cytochem* **20** 735, 1972

54 Beattie J, and Knox FJ Studies of mineral content and particle size distribution in the lungs of asbestos textile workers Davies, CN Ed Inhaled Particles and Vapours, Pergamon Press Inc, NY 419, 1961

55 Martinez E Chrysotile asbestos relationship of the surface and thermal properties to the crystal structure *Transactions*, **69** 414 1966

56 Brindley GW and Zussman J Infrared absorption data for serpentine minerals, *Amer Mineral* **44** 785, 1959

57 Brindley GW, and Zussman J A structural study of the thermal transformation of serpentine minerals to forsterite *Amer Mineral*, **42** 461, 1957

58 Faust GT and Fahey JJ The serpentine group minerals US *Geol Surv Prof Paper* **384-A** 92 1962

59 Langer AM and Kerr PF Evaluation of kaolinite and quartz differential thermal curves with a new high temperature cell, *Amer Mineral* **52** 509, 1967

60 Cooke WE Asbestos dust and certain bodies found in pulmonary asbestosis *Brit Med J* 2 578 1929

61 Williams E 'Curious bodies' found in the lungs of coal-workers *Lancet* **2** 541 1933

62 Schiller F Adsorptionserscheinungen an Stauben in vivo Pseudo asbestose und Schaumankörperchen Arch Gewerbepath *Gewerbehyg* **14** 664 1956

63 von Rütiner JR Uber Asbestose und Pseudoasbestosekörperchen Schweiz Z Allg *Path*, **15** 628 1952.

64 Undjian MD, Gross P and DeTreville RT Ferruginous bodies in human lungs *Arch Env Health* **17** 327, 1968

65 Gross P Cralley LJ, and deTreville RTP Asbestos' bodies Their non-specificity *J Amer Indust Hyg Assoc*, **28** 541 1967

66 Stewart MJ, and Haddow AE Peculiar bodies in pulmonary asbestosis *J Path Bact* **32** 172 1929

67 Simson FW and Strachan AS Asbestos bodies in sputum *J Path Bact* **34** 1 1931

68 Fulton WB et al Asbestosis Part III Special Bulletin No 42 Penn Dept of Labor and Industry Harrisburg Penna 1935

69 Page RC Sputum in pulmonary asbestosis *Amer J Med Sci* **189(1)** 44 1935

70 Horai Z et al Asbestos bodies in sputum samples, *J Nara Med Assoc* **12** 387 1961

71 Planteydt HT Asbestos bodies in shipyard workers *Med T Genessk* **108** 49 1964

72 Sluis-Cremer GG Asbestosis in South Africa *Ann NY Acad Sci* **132** 215 1965

73 Stumphius J and Meyer PB Asbestos bodies and mesothelioma *Ann Occ Hygiene* **11** 283, 1968

74 Thomson JG Kaschula R and MacDonald R Asbestos as a modern urban hazard *South African Med J* **37** 77, 1963

75 Cauna D Totten RS and Gross P Asbestos bodies at autopsy *JAMA*, **192** 371 1965

76 Anjilvel Ldy and Thurlbeck WM Incidence of asbestos bodies at random necropsies in Montreal *Can Med Assoc J*, **95** 1179 1966

77 Thomson JG, and Graves WM Asbestos as an urban air contaminant, *Arch Path* **81** 458, 1966.

78 Ghezzi I, Molteni G, and Puccetti U Asbestos bodies in lungs of inhabitants of Milan, *Med Lavoro* **58** No. 3 223 1967

79 Roberts GH Asbestos bodies in lungs at necropsy, *J Clin Path*, **20** 570, 1967

80 Ashcroft T Asbestos bodies in routine autopsies on Tyneside *Brit Med J* 1 614 1968.

81 Gibson AAM et al Epidemiology of mesothelioma in Scotland Proc of 2nd Intl Conf on Biological Effects of Asbestos Dresden Meeting, 21-25 April, 1968, Paper 69

82 Auvl I and Champeix J Results of asbestos exposure in France Pneumoconiosis Intl Conf Johannesburg H A Shapiro Ed, Pergamon Press Capetown 104 1970

83 Baden V and Schwartz J Demonstration of asbestos bodies Comparison of available techniques Proc of 2nd Intl Conf on Biological Effects of Asbestos, Dresden Meeting 21-25 April 1968 Paper 6

84 Prášlack A and Sacks M Asbestos bodies in human lung smears, *Israel J Med Sci* 4 223 1968

85 Pearcock PR Lung smears for asbestos bodies, *Europ J Cancer* 5 155 1969

86 Appell JM and Bhathal PS *Asbestos Path* 1 327 1969

87 Elmes PC McCaughey WT and Wade OL Diffuse mesothelioma of the pleura and asbestos *Brit Med J* 1 350 1965

88 Hounsham DOB Biopsy views in mesothelioma Attempts to identify asbestos *Annal NY Acad Sci* 132 647 1965

89 Meurman LO Asbestos bodies and pleura plaques in a Finnish series Acta Path *Microb Scand Suppl* 181 1102 1966

90 Goldstein B and Rendall REB Ferruginous bodies Pneumoconiosis Intl Conf Joh, H A Shapiro Ed Pergamon Press Capetown 40 1970

91 Berger PI Über die Asbestosiskörperchen Virchow, *Arch Path Anat* 290 280 1933

92 Gait C Data presented in Symposium on the tissue response to asbestos 7-9 April 1970, Cardiff Wales 1970

93 Pooley FD Asbestos bodies their formation composition and character *Envir Res* 5 363, 1972

94 Timbrell D Pooley FD and Wagner C Characteristics of respirable asbestos fibers Pneumoconiosis Proc of the Intl Conf Joh, H A Shapiro Ed, Oxford Univ Press Capetown 120 1970

95 Langer AM, et al Inorganic fibers, including chrysotile, in lungs at autopsy preliminary report Inhaled Particles III Proc Intl Brit Occ Hyg Soc Symp London 1970 W H Walton Ed Unwin Bros Ltd, London 2 683 1971

96 Pooley F et al The detection of asbestos in tissues Pneumoconiosis Proc of the Intl Conf Joh 1969, H A Shapiro Ed, Oxford Univ Press Capetown 108 1970

97 Langer AM Pooley FD Identification of single asbestos fibers in human tissue Proc of the Intl Conf on the Bio Effects of Asbestos Lyon France 2-6 October 1972 Pr No 19 1972

98 Pooley FD Electron microscope characteristics of inhaled chrysotile asbestos fiber *Brit J Ind Med* 29 146 1972

99 Langer AM Selikoff IJ and Sastre A Chrysotile asbestos in the lungs of persons in New York City, *Arch Env Health* 23 348, 1971

100 Selikoff IJ Nicholson WJ and Langer AM Asbestos air pollution, *Arch Env Health* 25 1 1972

101 Langer AM and Selikoff IJ Chrysotile asbestos in the lungs of residents in New York City Proc 2nd Intl Clean Air Congress Wash, D C Dec 1970 H M Englund and W T Beery Eds Acad Press New York 161 165 1971

102 Henderson WJ et al Talc and carcinoma of the ovary and cervix *J Obstet and Gynecol (Brit Commonwealth)* 78 266 1971

103 Clinton RA Huggins CW and Shell HR Hollow chrysotile fibers *Amer Min* 51 500, 1966

104 Bates TF Comer JJ Further observations on the morphology of chrysotile and halloysite Proc Sixth Natl Conf Clays and Clay Mins NAS-NRC 237 1959

105 Yada K Study of chrysotile asbestos by a high resolution electron microscope, *Acta Cryst* 23 704 1967

106 McConnell JDC Electron microscopy and electron diffraction Physical Methods in Determinative Mineralogy, J Zussman, Ed Acad Press NY 514 1967

107 Zvyagin BB Electron Diffraction Analysis of Clay Mineral Structures *Trans S Lyse* Plenum Press NY 364 1967

108 Zussman L Brindley GW and Comer JJ Electron diffraction studies of serpentine minerals, *Am Min* 42 133, 1957

109 Skidmore MI Talbot JH and Rendall REG Electron diffraction patterns of U I C C Asbestos Samples *Env Res* 4 141, 1971

110 Pooley FD Personal communication, April 1972, work in progress, Department of Mineral Exploitation University of South Wales and Monmouthshire Cardiff

111 Birks LS Electron probe microanalysis Monograph of Anal Chem and its applications *Interscience Publ NY* 17 253 1963

112 Andersen CA An introduction to the electron probe microanalyzer and its application to biochemistry Methods of Biochem Anal D Glick Ed *Interscience Publ NY* 15 147 1967

113 Meurman LO Kivilunto R and Hakama M Mortality and morbidity of employees of anthophyllite asbestos in mines of Finland Intl Conf Biolog Effects of Asbestos Pr 31 Lyon, Oct 2-6 1972