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**PLAINTIFF'S
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Fibrous glass has been given considerable attention as a possible hazard of the general type of asbestos, although current manufacturing practices produce few fibers capable of penetrating into the pulmonary air spaces. A trend toward the production of smaller glass fibers and the emergence of small, respirable, graphite, silicon carbide and other man-made fibers suggests that the problem of the health effects of fibers will increase. These effects are of concern not only to the industrial population, but also to urban dwellers who may breathe fibers from many sources, occupants of buildings with fibrous glass-lined ducts and others who may unknowingly become exposed.

In order to protect the health of the population at risk while at the same time avoiding the penalizing of a valuable economic material which is causing no harm, it is important to learn as much as possible about fibers in materials, in the air and in the body. Fibers occurring in the environment must be detected and identified so that they can be traced to their source. Quantitative estimates of fiber concentration in industrial and community air are needed to establish the relationship, if any, between disease patterns and to determine the effectiveness of control measures. The trace chemical constituents of these fibers are of importance in uncovering the mechanisms of the diseases resulting from exposure.

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mechanism, may have been responsible for the damage. A typical airborne asbestos fiber in a textile plant weighs about 10-10 gram⁽¹⁾. Since most of the fibers deposited in the pulmonary air spaces are smaller than this average size, the most sensitive methods are necessary for identification and gross analysis of fibers found in the lung.

Sample Preparation

Several recent developments in methods of sample preparation enable the analyst to separate the fibers from associated materials and to deposit the material in a suitable receptacle for analysis.

Microsieving. A fraction of a sample containing the respirable fibers may be prepared from a bulk sample by microsieving. The bulk sample material is dispersed in 95 percent ethyl alcohol and placed in a 10-micron sieve in an ultrasonic bath. At fifteen minute intervals sieved portions are removed from the bath and more alcohol is added to the sieve. For one gram of the bulk material, the separation is complete in about four hours. X-ray diffraction analysis of sieved chrysotile asbestos has shown no change in the pattern of the sieved fraction as compared with that of the bulk sample. This technique has been used to demonstrate that there is a greater concentration of nickel and chromium in the finer particle fraction⁽²⁾.

Low-Temperature Dry Ashing. A commercial instrument is now available for the dry ashing of organic matter without volatilizing trace elements. This technique allows the oxidative reactions to proceed in a very gentle manner, thus avoiding any disturbance of the structure of crystalline substances or sintering of ultrafine particles. The technique appears to be promising for the isolation, without alteration, of mineral substances deposited in body tissues. Excited oxygen species produced when molecular oxygen is passed through a high-frequency electromagnetic field attack the sample, which is maintained at about 1 mm Hg pressure in an oxidation chamber from which the exhaust gases are removed by a mechanical vacuum pump (Fig. 1).

The glow region in the RF discharge of oxygen contains 10 to 20 percent of oxygen atoms and about an equivalent amount of electronically excited molecular oxygen⁽³⁾. The rest of the plasma consists of normal ground-state oxygen molecules, some ionized species and a free electron concentration of $1 \times 10^{11} \text{ cm}^{-3}$. A few millimeters away from the glow region, most of the ionic species have disappeared but the "active" species persist and are carried downstream before they deactivate reactively or by collision.

The concentration of oxygen atoms is higher than predicted from the rates of the directly contributing electron-molecule reactions, molecule ion-electron reactions and electronically excited molecular-oxygen dissociation. This high concentration of atomic oxygen is due in large part

Mounting of X-ray Diffraction Samples

The technique of mounting representative portions of dust samples on molecular membrane filters was described by Talvitie and Brewer⁽⁵⁾. A known weight of the finely comminuted sample is suspended in a 250-ml MCA* volumetric flask with the aid of a wetting agent. This mixture is then dispersed by means of a small ultrasonic bath, and an aliquot of the thoroughly agitated suspension, containing a known fraction of the sample, is pipetted out and filtered through a molecular membrane filter. This technique provides a sample of known weight on the filter. Asbestos-containing dust samples are amenable to this procedure after a preliminary dry screening of gross particles through a 200-mesh sieve, followed by grinding fibers to lengths of < 3.5 microns. The membrane filter containing the sample aliquot is mounted in the x-ray diffractometer.

Airborne dusts and fibers collected on a membrane filter are removed by placing the filter in a test tube containing a few drops of a suitable wetting agent and sufficient water to cover it. Immersion of the test tube in the ultrasonic bath for five minutes promotes the removal of the dust and fiber deposit from the filter. After the original filter is removed from the test tube, the resuspended fibrous dust sample is deposited on a second membrane filter which is mounted in the x-ray diffractometer.

Sample Examination

Since fibers are defined by their shape and, generally, some other materials mixed with the fiber have the same chemical and crystallographic characteristics as the fiber, the only certain way of detecting fibers is by visual examination. Furthermore, the only way of being sure of obtaining an analysis of a single fiber from a mixture of other materials is to visually present the fiber to the analytical instrument. It is also possible to identify some fibers if sufficient fine detail can be resolved

Light Microscopy. In the early days of counting dust particles obtained in impinger samples in asbestos textile factories, few fibers were seen, and as a result it was decided to count all particles to give an estimate of overall dustiness. The lack of visible fibers in these samples was due in part to the inefficiency of the impinger in the collection of particles of such small aerodynamic size and in part to the low resolving power of the 10X objective used. As a result of recent studies of the asbestos industry, techniques were evolved for detecting many of the airborne fibers previously missed^(6,7). Membrane filters with a pore size of 0.8 micron are known to have surface collection efficiencies of close to 100 percent for particles down to a few hundredths of a micron. Airborne dust samples collected on these

*Mention of commercial products or concerns does not constitute indorsement by the Public Health Service.

Electron Microscopy. The morphology of certain fibers is sufficiently distinctive to permit positive identification when the fiber is observed with enough resolution to reveal fine structure. For example, the hollow tube down the middle of a chrysotile asbestos fiber distinguishes it from other forms of asbestos and is clearly visible in a 10,000X electron micrograph. Bulk materials may be prepared for electron microscopy in a variety of ways, but the mounting of airborne dust or lung samples is more difficult. An electrostatic precipitator has been developed which is capable of depositing the dust on the flat surface of a copper grid previously coated with formvar⁽⁹⁾. The efficiency of particle collection by this instrument is not size dependent, and the particles are essentially undisturbed by the collection process. However, this device is not suitable for breathing zone samples, and a long sample period is often necessary to obtain a heavy enough deposit. Dust collected on membrane filters used in personal breathing zone samples may be transferred directly to coated grids. A small square slightly larger than the grid is cut from the filter and placed dust side down on a coated grid resting on a support screen in a spot plate. Acetone is added to the plate cavity and allowed to contact the grid and filter from underneath by capillary action. The filter dissolves through the formvar, leaving the dust laying on the grid. Several treatments with clean acetone are necessary to completely dissolve the filter.

Fibers in lungs must first be located before they can be seen by electron microscopy. Since the fibers are very sparse, especially in the lungs of people without occupational exposures, and since the electron microscope field area is so small, direct mounting of a lung specimen is generally not practical. A better method involves concentrating the lung residue by ashing or digestion and locating a fiber by means of a light microscope. The fiber is encased in a collodion film and then transferred to the electron microscope grid.

Analytical Methods

Methods are available to determine either the chemical, or elemental composition or the crystal structure of mixtures of materials containing fibers, pure fiber bundles or single fibers. Their limitations are determined by the amount of sample required and their ability to distinguish the fiber from the associated material.

X-Ray Diffraction. Milligram quantities of chrysotile, amosite and crocidolite asbestos may be determined by X-ray diffraction. Both external⁽¹⁰⁾ and internal^(11,12) standard methods for the analysis of these minerals have been developed. In the latter case the samples are ground dry in a mixer mill to an average fiber length of < 3.5 microns and passed through a 325-mesh U. S. Standard sieve prior to being mixed

This sensitivity permits magnesium analysis of filter halves with deposits so small that light field counts of particles may be made on the other half of the same filter. In the case of samples where the use of this line requires too great a dilution, the magnesium 2026 Å line is used. In either event, the estimated magnesium concentration is converted to the quantity of chrysotile in the total filter sample.

Comparison of this method with the x-ray diffraction procedure has shown a variation of results no greater than ± 10 to 15 percent for a limited number of samples containing a sufficiently large dust deposit for the x-ray technique. The atomic absorption method for magnesium has permitted us to run thousands of personal samples, including many whose total dust weighed less than 0.1 mg. The application, of course, is limited to exposures containing only one source material of the analysis element, namely, chrysotile asbestos in this case. However, where only one etiologic agent containing the analysis element is present in an airborne dust, the technique offers the recognized advantages of high sensitivity, accuracy, specificity and speed common to all atomic absorption methods. The error of the method is $< \pm 5$ percent as determined from the analysis of known samples in our laboratory.

Atomic absorption has also been used to determine several heavy metals in bulk samples of chrysotile, amosite and crocidolite, the asbestos minerals most used in this country. For this purpose, 0.25 g of a bulk material is digested with hydrofluoric acid in a platinum dish for 3 to 4 hours, a procedure which involves a double evaporation to dryness. The final residue is dissolved in 1:1 redistilled hydrochloric acid and double distilled water and then filtered through a Whatman No. 42 paper. After the filter is washed, the solution is concentrated and then finally diluted to 25 ml. A one-half portion of this solution is reserved; the remainder is diluted, as required, for the individual analyses. Ammonium chloride is added to provide a concentration of 20 mg/ml to reduce the interference of iron with chromium. Iron suppresses the chromium response; e.g., 200 ug Fe will suppress the response of 5 ug Cr to that of < 3 ug Cr. Some elements found in asbestos samples are listed in Table II along with their sensitivities (amounts giving one percent absorption) at the stated resonance lines.

Neutron Activation Analysis

This technique is based upon the interaction of neutrons with the stable nuclei of chemical elements. These nuclear reactions are similar to ordinary chemical reactions in that they exhibit a mass change and an energy of activation and they proceed at definite reaction rates, which depend upon the experimental conditions. There are several sources of neutron particles available, but research reactors, with their high thermal neutron flux, are generally used to irradiate samples for trace element analyses.

The theoretical sensitivities of this technique for the determination of trace elements in particles are given in Table IV. As can be seen, an element such as a metal distributed throughout an asbestos fiber in a concentration of a few parts per million would not be detectable. However, concentrated particles of a metal compound contained in or on a fiber bundle could be identified.

Emission Spectroscopy

Emission spectroscopy is the study of the radiations emitted by atoms, ions and a few molecular species after suitable excitation by a thermal or electrical source of energy. These radiations lie in the near infrared, the visible and the ultraviolet regions of the electromagnetic spectrum. Spectrochemical methods of analysis make practical use of these radiations for qualitative and quantitative analytical purposes. The emission spectrograph may be used to analyze more than 70 metallic or metal-like elements in any type of solid or liquid sample.

Most spectrographic analyses in the field of industrial hygiene require the sample to be burned in spectroscopically pure graphite electrodes in a D. C. arc. This source of excitation provides the greatest sensitivity for the detection and determination of trace quantities of the metallic elements. Quantitative methods developed for specific determinations are based upon the employment of optimal excitation conditions and the use of spectroscopic buffer salts which minimize the production of the background or "continuum" and, at the same time, increase the intensity of atomic spectra.

Mineral substances may be ground and then analyzed directly by spectrographic procedures. However, some of the metallic impurities in such minerals as chrysotile, amosite or crocidolite types of asbestos may be quite low in concentration or may not be homogeneously dispersed throughout the fibrous matrix. In such cases, the analyst will be required to digest the mineral by means of the hydrofluoric acid procedure to reduce the bulk of the mineral diluent and to provide a solution of the silica-free residue. The resulting sample solution is diluted or concentrated, as required, for the analysis of the elements of interest. Sample solutions are measured precisely into the craters of graphite electrodes with micropipets. After the addition of an internal standard solution, the electrode is dried at 105 C, cooled and burned in the D.C. arc - just as any other solid sample.

The technique is sensitive to approximately 0.01 microgram of cobalt, nickel, vanadium, chromium and other elements of similar volatility contained in a 2-milligram charge of the sample residue introduced into the electrode crater.

Conclusion

Virtually all available methods applicable to the detection, identification and analysis of fibers have been brought to bear on the problem of evaluating health hazards resulting from exposure to fibers and the discovery of the mechanisms of the diseases involved. Frequently, due to limitations in sample size and the necessity of dealing with samples of fibers mixed with other materials, indirect sources of data have had to be combined to obtain an overall characterization of the fiber. Work is proceeding, however, in several laboratories to develop new and improved methods.

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14. Langer, A. M.: Personal communication (1968).

Table II

Elements Determined by Atomic Absorption
Spectrophotometry Applied to HF Digests
of Asbestos Minerals

<u>Element</u>	<u>Resonance Line, Å</u>	<u>Sensitivity,* µg/ml (1% Absorption)</u>	
		<u>1 X Scale</u>	<u>3 X Scale</u>
Co	2407	-	0.2
Cr	3579	0.4	0.15
Fe	3720	1.0	-
Fe	2483	0.15	-
Mn	2795	0.1	-
Ni	2320	0.4	0.15

*Determined in authors' laboratory.

Table IV

Sensitivity of Electron Microprobe Analyzer

<u>Element</u>	<u>Min. Detect. Mass, g.</u>	<u>Min. Detect. % in 1μ Particle</u>	<u>Spectrometer</u>
Be	1×10^{-14}	20.0	Grating
Ca	8×10^{-16}	0.1	ADP-LiF
Co	4×10^{-16}	0.03	LiF
Cr	5×10^{-16}	0.03	LiF
Fe	4×10^{-16}	0.03	LiF
Mn	5×10^{-16}	0.03	LiF
Ni	2×10^{-16}	0.04	LiF
Pb	5×10^{-14}	0.01	LiF-ADP

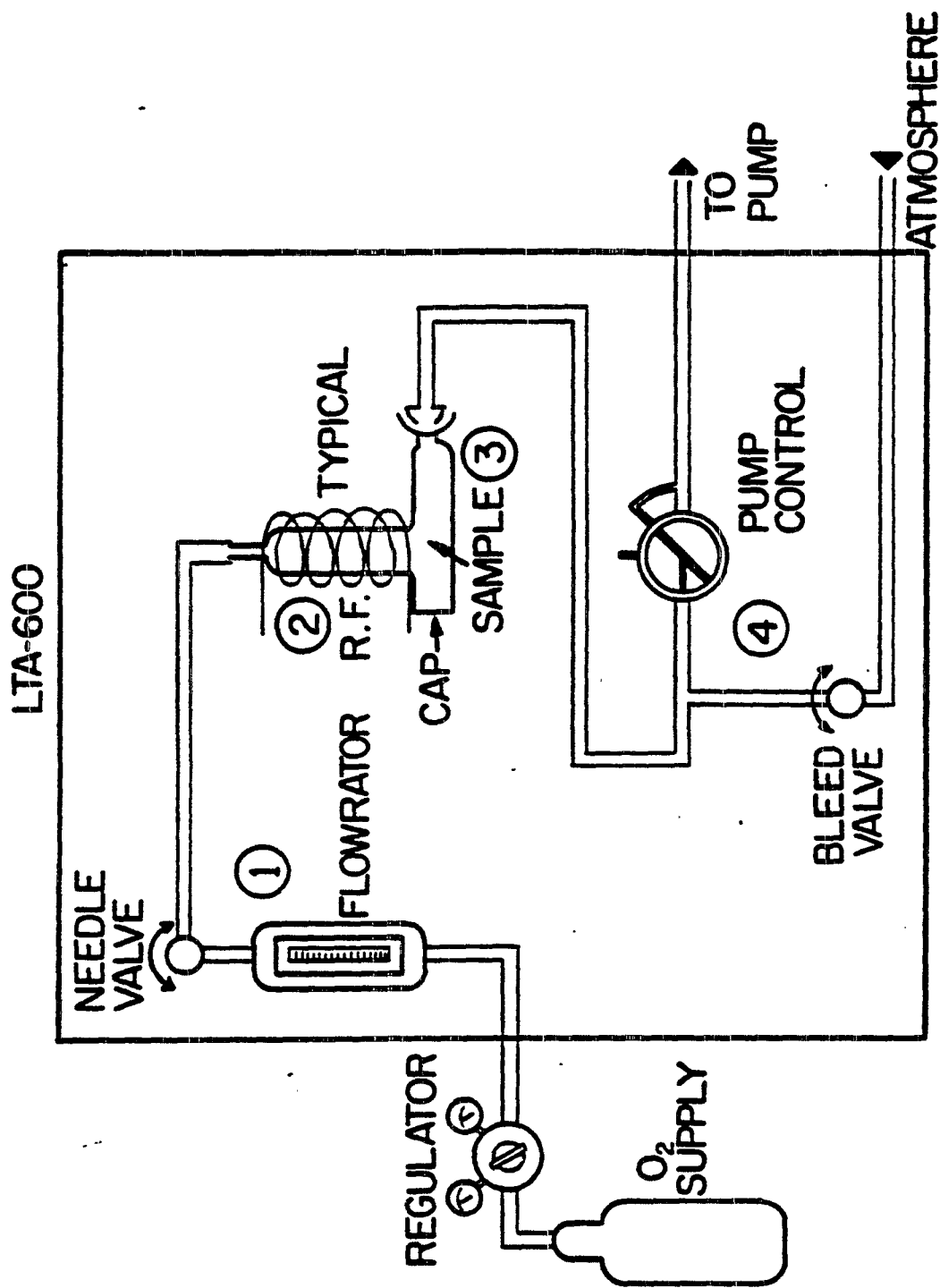


Figure 1.
Low-temperature dry ashing apparatus.

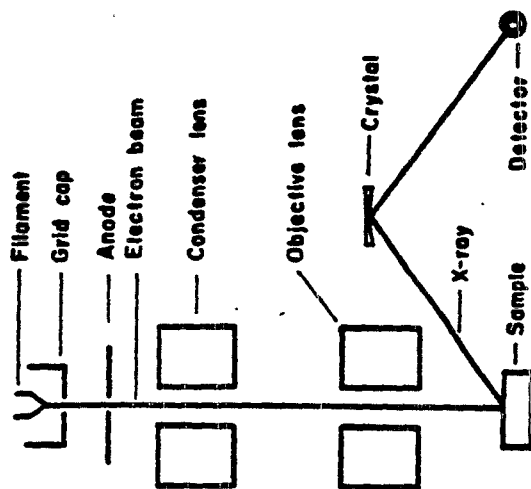


Figure 3.
Electron microprobe schematic.

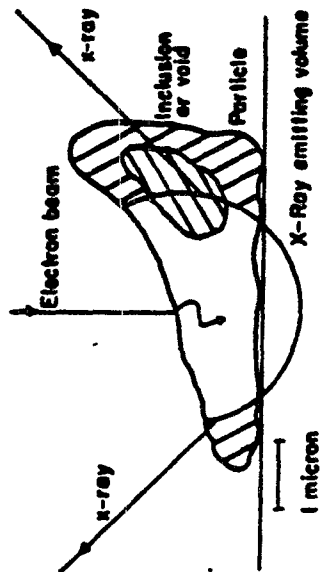


Figure 5 .
Electron microprobe analysis of a single particle.