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MICROPROBE ANALYSIS IN HUMAN PATHOLOGY

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Abstract

This tutorial paper reviews the literature on the application of microprobe analysis to practical problems in diagnostic *human* pathology. The goal is to allow the reader ready access to the literature on specific clinical problems. Specimen preparation and commonly encountered artifacts are also considered. It is concluded that energy dispersive x-ray microanalysis and backscattered electron imaging are at present the most generally useful microprobe techniques for clinical work, and are no longer solely research tools. The findings often have diagnostic, therapeutic, and/or legal implications.

Keywords: Electron microprobe, microprobe analysis, human pathology, scanning electron microscopy, literature review, lung particulates, surgical pathology, human tissue, clinical conditions and diagnosis.

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Introduction

Analytical electron microscopy (AEM) and related microprobe techniques such as ion microscopy and laser microscopy have been widely used in scientific investigation of many types. This *tutorial paper* is designed to review the rapidly expanding body of scientific literature on the application of microprobe techniques (Table 1) solely to problems in *human* pathology. With this goal in mind we have deliberately focused on studies that demonstrate potential for use in diagnostic and clinical pathology, and not on studies that are primarily research oriented.

Also, it is not the purpose of this tutorial paper to discuss articles primarily devoted to secondary electron imaging – the emphasis is on analytical, not morphological, techniques. A review of sample preparation techniques applicable to the analytical study of human tissue is included, as is also a brief discussion of possible artifacts from these techniques.

Instrumentation

Table 1 summarizes several popular and promising techniques for microprobe analysis. Review of the human pathology literature and our experience indicates that the combined use of backscattered electron imaging (BEI) and energy dispersive x-ray microanalysis (EDXA) has proven to be the most useful approach for clinical studies. Typically BEI imaging is used either to identify particulates such as inhaled minerals in tissue sections or to localize histochemical stains (40,90). EDXA is then employed to identify the chemical composition of the mineral. Correlative secondary electron imaging (SEI), transmission electron microscopy (TEM), and/or scanning transmission electron microscopy (STEM) serve to identify the anatomy of the inclusion, and permit correlation with the light microscopic findings.

Thus the analytical (chemical) data obtained with microprobe techniques augment the morphological data obtained with TEM, STEM and SEI. Indeed, it is our bias that *analytical* electron microscopy is more useful for *clinical* work than are purely morphologic techniques such as secondary electron imaging. Secondary electron imaging is, of course, extremely useful in research studies, and is also useful in diagnosis, as has been published in several review articles (2,6,58,59). Nevertheless, as a generalization, we believe that a scanning electron microscope not equipped with BEI and EDXA detectors is less useful in the hospital setting than is an SEM with BEI and EDXA. The reader is referred to several excellent review articles which emphasize

different aspects of this subject (2,4,5,6,8,9,56,57,58,61,62,96,116, 120,141,145,150,167,207,224,243,247,328). For a discussion of the basic instrumentation the reader is also referred to a few useful general references (32,107,126,139,147,254,275).

Selected area electron diffraction (SAED) is an extremely important, yet under-utilized tool for inorganic crystal identification (32,268). Easily performed on most conventional TEMs, SAED involves the analysis of electron diffraction patterns of thin inorganic materials. An aperture-limited (diameter ~1 μm) electron beam is directed at individual particles or selected portions of larger particles, resulting in characteristic diffraction patterns. Much of the application of SAED to date has dealt with the identification of asbestos fibers in lung tissues (72,76,212,246,248,250,320,323). SAED has perhaps its greatest utility as a *complementary* technique to EDXA since many mineral crystals are not classifiable by chemical composition alone. SAED is not without disadvantages, however, as it requires laborious specimen preparation, proper crystal orientation for optimal diffraction results (i.e., a double-tilt stage in the TEM), and finally much time to acquire data on large numbers of particles in a sample.

With the exception of SAED, BEI and EDXA, the other microprobe techniques shown in Table 1 are not used routinely in clinical work. For example, wavelength dispersive spectrometry (WDS) is much more time-consuming than EDXA, having to scan through each element individually. On the other hand, the spectral resolution is much better than EDXA, thus allowing better detection limits for some elements. EDXA, however, is rapid, allowing for the *simultaneous* determination of all elements from Z = 9 (F) to 92 (U). (With a so-called "windowless" detector, lighter elements down to boron, Z = 5, can sometimes be detected with EDXA). Also, the higher beam currents typically used in WDS can seriously damage biological samples. Application of other techniques will be discussed below in **New Techniques**. The intervening sections on preparatory techniques and applications pertain to EDXA, BEI and SAED.

Preparative Techniques

Analytical electron microscopy instrumentation now offers the ability to correlate microscopic anatomy of human tissues with elemental chemical composition. This ability is a significant improvement over older bulk methods of chemical analysis which necessarily disrupt histomorphology. The quality and validity of the morpho-chemical correlation obtained by AEM is, however, highly dependent on the appropriate selection of specimen preparative techniques for the particular problem under consideration.

The route employed to bring any biological tissue from its *in vivo* state to *in situ* form in the microscope is potentially fraught with numerous pitfalls affecting both structural and chemical integrity. Often, a paradoxical trade-off exists between the two. Well-established electron microscopy (EM) preparative techniques which achieve excellent structural preservation (e.g., chemical fixation, osmification, staining) often sacrifice chemical integrity. Yet, techniques suited for chemical preservation (e.g., freeze-fixation) may disrupt subcellular architecture or yield poor ultrastructural images.

The microscopy literature has expanded in the past decade with much effort to delineate and resolve the difficulties associated with various preparative techniques of biological specimens. Several excellent reviews of this work have discussed the evolving available methodologies and their advantages and disadvantages (9,62,120,213,214). In general, no one technique has assumed universal acceptance for all studies in biology and medicine. Instead, the preparative methodology must be thoughtfully tailored to the specific problem under study.

In problems of human pathology, much of the microanalytical work to date has relied upon relatively unsophisticated techniques of specimen preparation. These methods require only minor modifications from routine histologic processing for light or electron microscopy. Since most clinical cases in which AEM has been used have involved foreign material identification and localization – usually non-diffusible, inorganic xenobiotics – these simple techniques have usually been adequate. This fact raises an important point: simple preparative techniques are generally superior to more elaborate ones if they are sufficient to answer the problem under study.

Undoubtedly, greater numbers of pathologists in the future will address more difficult questions in human pathophysiology, using microanalytical techniques to describe the *in vivo* distribution of soluble endogenous elements and molecules. Such work necessarily calls for significant technical improvements in and wider adoption of more elaborate methods of tissue preparation, such as cryotechniques.

In this section, we would like to outline the major preparative techniques employed to date in human pathology and enumerate some common artifacts and contaminants in tissue processing which can hamper proper data interpretation.

Conventional Histologic Sections By far the most widely employed and easiest technique involves the use of routine histologic sections. Formalin-fixed, paraffin-embedded tissue sections have been shown to be particularly helpful in cases where high-resolution ultrastructural elemental localization is deemed unnecessary, thereby avoiding the need for more difficult EM tissue processing. In particular, paraffin-embedded tissue has been demonstrated to be sufficient for cases involving inorganic particulates or insoluble, tightly bound exogenous metals.

The sections are cut at 5–10 μm, and picked up onto spectroscopically pure, ultrasmooth carbon planchets. Paraffin tissue sections readily adhere to the carbon substrate after heating at 60°C for 30 minutes, followed by deparaffination in xylene and air drying. The sections are subsequently rotary carbon-coated by vapor-deposition to minimize electron beam charging; for sections five microns thick or less, carbon-coating has been shown to be usually superfluous and may be omitted (9). Also, the thinner the section, the less likely buried particulates will be missed with backscattered electron imaging.

This preparative method permits the microanalytical-pathologist to use routinely fixed, paraffin-embedded tissues for investigating unknown particles discovered by light microscopy (LM). If the particles appear by LM as numerous and relatively homogeneously distributed in the section, it is only necessary to apply this technique to a serial unstained section cut from the paraffin block. In some instances, the paraffin block may not be available, or the particles in question are quite small and few, requiring LM localization prior to SEM study. In these cases tissue section transfer methods have been successfully used to lift the section off the glass slide onto a carbon planchet (6,229). If photomicrographs are taken of particles of interest prior to the

Table 1
ANALYTICAL ELECTRON MICROSCOPY AND MICROPROBE TECHNIQUES

Abbreviation	Technique	Excitation Source	Observed Signal	Comment
TEM	Transmission electron microscopy	Electrons	Transmitted electrons (of primary beam)	High resolution study of structure. Density differences are detected.
SEI	Secondary electron imaging	Electrons	Secondary electrons (ejected from sample)	Three-dimensional surface images with moderate resolultion and excellent depth of field.
SEM	Scanning electron microscopy	Electrons	Usually electrons	*General* term—electron beam is rastered over sample. Often used as synonym for SEI.
STEM	Scanning transmission electron microscopy	Electrons	Transmitted electrons (of primary beam)	Similar to TEM except that the primary beam is rastered over the sample as in SEM.
SAED	Selected area electron diffraction	Electrons	Transmitted, diffracted electrons (of primary beam)	Determination of crystalline structure of inorganic particulates (or organic crystals) in cells and tissues.
BEI	Backscattered electron imaging	Electrons	Backscattered primary beam electrons	Atomic number contrast—e.g., detection of medium to heavy atomic number (Z) particles in tissue.
EDXA	Energy dispersive x-ray analysis	Electrons	Energy of excited x-rays from specific elements within the sample	Simultaneous detection and measurement of most elements (generally $Z \geq 9$).
WDS	Wavelength dispersive spectrometry	Electrons	Wavelength of excited x-rays from the sample	Detection and measurement of most elements (generally $Z \geq 5$). Usually requires flat sample.
TRIX	Total rate imaging with x-rays	Electrons	All energies of x-rays excited from the sample	Atomic number contrast. Identificatin and localization of inorganic particulates in organic matrix.
EELS	Electron energy loss spectrometry	Electrons	Energy absorption of transmitted electrons of primary beam	Detection and measurement of lighter elements (i.e., $Z < 9$) in tissues and cells in very small spatial volume.
SAM	Scanning Auger microprobe	Electrons	Auger electrons from sample	Detection and measurement of lighter elements (i.e., $Z < 9$) in tissues and cells. Surface (< 10 Å) analysis of particles/cells.
CL	Cathodoluminescence	Electrons	Visible photons	Limited applications.
SIMS	Secondary ion mass spectrometry, ion microprobe, microanalyzer or microscope.	Ions	Ions from elements in sample	Analysis and 3-D mapping of elements in particles, cells and tissues at ppm to ppb levels with broad elemental coverage.
LAMMA	Laser microprobe mass analyzer	Laser beam	Ions from elements in sample	Analysis of particles, cells and tissues at ppm to ppb levels with broad elemental coverage. Destructive to sample.
PIXE	Particle or proton induced x-ray emission	Proton beam	Energies of x-rays excited from the sample.	Simultaneous detection and measurement of most elements (generally $Z \geq 13$).

section transfer, specific (inorganic) inclusions can be probed with precision to determine their composition (86,229).

In some rare instances, one may discover the presence of foreign material during the analytical SEM study which had not been appreciated by the prior LM examination. In these cases one would ideally like to re-examine the section by LM to locate and define the histological correlate of the microanalytical finding. In rarer cases yet, one may wish to undertake a more sophisticated mode of microanalysis of the pathologic tissue, such as ion microscopy (IM). In both these situations, a recently described technique of specimen preparation is extremely useful, as it enables correlative light, electron, and ion microscopy of a single histologic section (168,169). In brief, sections are cut onto transparent polyester plastic coverslips. These coverslips permit excellent LM, as well as analytical electron and ion microscopy. Light microscopic examination of the specimen is possible both *before* and *after* microanalytical studies. As this technique is somewhat more tedious and time-consuming than those using serial unstained or lifted stained sections, it should be reserved for cases where precise correlative microscopy is needed and the other techniques do not suffice. A potential advantage is that EDXA may be enhanced after ion microscopy (29).

Epon Sections Numerous studies have used sections of epon-embedded tissues which have been prepared by conventional or modified EM processing (28,34,36,120,122,187,215,259, 263,264,277,285,328). Depending on the tissue and the level of ultrastructural detail desired, osmification and staining with lead or uranium may be omitted, as each of these steps introduces metals into the tissue which can interfere with both imaging and analysis (Table 2). EDXA can be performed on thin sections mounted on grids of copper, nickel, titanium, steel, carbon, or nylon, followed by carbon-coating to minimize charging. The specimens are then analyzed by a conventional TEM or STEM equipped with an EDX detector.

Digestion Techniques Digestion preparative techniques for AEM of inorganic particulates in tissue have been well described by several authors (71,246,250,286). These techniques involve an alkaline digestion step to remove the organic matrix, followed by a concentration step using filtration. The cellulose acetate filter containing the particulates is then transferred to a formvar and carbon coated TEM grid and dissolved slowly by acetone vapor, resulting in the deposition of the inorganic residue directly onto the grid. Both selected area electron diffraction (SAED) and EDXA can then be performed on the particulates to yield complementary data for accurate identification. Replication techniques (140) are an alternative to digestion, and afford better correlation with the histology. The filter can also be mounted on a carbon disc, coated with a suitable conducting film, and examined by SEM (250).

Cryotechniques While freeze-fixation and cryoultramicrotomy are, in principle, ideal methods for the ultrastructural microanalysis of diffusible substances, they currently pose substantial technical difficulties. Ice crystal damage, thawing and ice recrystallization artifacts present real hazards to freeze-fixation, cryosectioning, freeze-drying, and section transfer (17,33,63, 91,148,177,193,254,256,332). Moreover, these techniques are laborious and require a high degree of technical expertise and sophisticated equipment. Accordingly, cryotechnical tissue processing has gained only limited acceptance at the present time among diagnostic microanalytical pathologists.

A noteworthy application of the cryotechniques to human pathology is the study of physiologic ions in human skeletal muscle and myocardium (97,98,280,325–327). In these studies, the investigations employed rapid freezing, followed by *conventional* cryostat thick sectioning at −20°C. The sections were placed directly onto carbon planchets and subsequently freeze-dried prior to AEM. As these studies attempted microanalysis at the cellular −not subcellular−level, this simplified cryotechnique sufficed.

Fluids Extremely accurate techniques have recently been described for quantitative elemental analysis of biological fluids using AEM (42,175,176,236–238). In all such methods, the analysis of microvolume (100 picoliter) samples can be performed after a series of steps designed to produce uniform evaporation and evenly distributed, small (<1 μm) crystals. These analytical methods have proven extremely useful for microdroplet studies in basic renal physiology, as well as in the study of other biological fluids. Relatively few studies to date, however, have analyzed normal or pathologic human fluid (67,105,236–238,292). This area holds a great deal of potential for further investigation in human pathology.

Whole Mounts A variety of normal and pathologic human tissues have been analyzed by air-dried, whole-mount preparations. Hair, fingernails, urinary stones, and suspected environmental pollutants can be mounted directly onto spectroscopically pure carbon planchets using conductive carbon paste (10,161, 163,252,253,271). Erythrocytes, platelets, and sperm cells have likewise been analyzed as whole-mounts, on TEM grids or SEM mounts, requiring no adhesive material (61,62,80,105,178,328, 329). Such simple techniques have often been shown to be adequate when compared to other techniques such as wet chemical or frozen preparations.

Artifacts Any preparative technique can potentially introduce artifactual changes in the specimen which seriously impair proper data interpretation. Several authors have described these artifacts (9,61,62,110,114,213,223,254,256), and some of their major sources and interpretational problems will be reviewed here.

Preparative Artifacts Wet chemical preparative techniques are notorious for causing losses, redistribution, and additions of diffusible substances. All wet processing steps, but particularly fixation, have been well demonstrated to result in significant leaching of endogenous diffusible elements. Organic compounds may also be lost during dehydration and embedding steps. Diffusible elements may then redistribute themselves down concentration gradients, become preferentially bound to other tissue structures, and may eventually appear concentrated in artifactual locations. The formation of osmium-calcium precipitates in conventionally EM processed tissue is such an example. Moreover, phase transformations of endogenous substances can occur during wet techniques, amorphous calcium phosphate may become crystalline hydroxyapatite during conventional processing. (214).

Finally, exposure of the tissue to a variety of histologic solutions necessarily causes the introduction of extraneous elements and contaminants into the specimens. Mercurial fixatives such as Zenker's formalin add mercury and chromium, cacodylate-buffered glutaraldehyde introduces arsenic and sodium, and osmium tetroxide post-fixation, of course, deposits osmium. Moreover, numerous common stains contain a host of heavy elements as normal constituents. Examples include iron in Prussian blue, aluminum in hematoxylin, bromine in eosin, copper in luxol fast blue and in Alcian blue, as well as silver, lead, and uranium

Table 2
Important Spectral Interferences in Medical EDXA

Element	Symbol	Z	Found in	Interfering Lines and their Energies (keV)*	
Fluorine	F	9	Teflon	K_α	0.68
Iron	Fe	26	Tissue, grids	L_α	0.70
Copper	Cu	29	Grid, Stain	L_α	0.93
Zinc	Zn	30	Grid	L_α	1.01
Sodium	Na	11	Tissue, Minerals, Buffers	K_α	1.04
Magnesium	Mg	12	Tissue, Minerals	K_α	1.25
Arsenic	As	33	Cacodylate Buffer	L_α	1.28
Bromine	Br	35	Stain	L_α	1.48
Aluminum	Al	13	Stain, Tissue, Minerals	K_α	1.49
Osmium	Os	76	Fixative	M_α	1.91
Phosphorus	P	15	Tissue, Buffer	K_α	2.01
Phosphorus	P	15	Tissue, Buffer	K_α	2.01
Platinum	Pt	78	Coating	M_α	2.05
Sulfur	S	16	Tissue	K_α	2.31
Lead	Pb	82	Stain	M_α	2.35
Ruthenium	Ru	44	Stain	L_α	2.56
Chlorine	Cl	17	Epoxy resin, tissue	K_α	2.62
Ruthenium	Ru	44	Stain	L_β	2.68
Uranium	U	92	Stain	M_α	3.17
Potassium	K	19	Tissue	K_α	3.31
Potassium	K	19	Tissue	K_β	3.59
Calcium	Ca	20	Tissue, Buffers	K_α	3.69

*(The values stated are weighted averages usually used by manufacturers in calculating the MLK line displays on the X-ray spectrometer cathode ray tube. The x-ray intensities are usually given (126) assuming the following relative weights: $K \neq L$, $K_\alpha \cong 100$, $K_\beta \cong 10$, $L_\alpha \cong 100$, $L_{\beta_1} \cong 70$, $L_{\beta_2} \cong 20$, $L_\gamma \cong 10$. Silicon escape peaks (energy of peak minus 1.74 keV) and doublets (twice the peak keV) are not shown here but are potential problems, particularly for elements present in high concentration such as in pure mineral inclusions).

staining. Contaminants from the histology laboratory may also occur from poorly washed glassware, exposure to dust particles, and the use of tap water. We therefore recommend the use of distilled and/or deionized water for the preparation of all appropriate chemicals and fixatives and all cleaning of glassware. During the course of our work, for example, we have found lead as a common contaminant in stained paraffin sections from biopsies obtained from outside hospitals. The addition of such exogenous elements to the specimen can severely compromise the AEM study, both in terms of imaging and x-ray data interpretation. Histologic stains can also *remove* elements—we have found that calcium and phosphorus are leached from undecalcified bone sections stained for aluminum (132,188).

Cryotechnical processing is likewise susceptible to artifactual problems, particularly those of ice crystal damage, thawing, and ice recrystallization. Such phenomena can result in the gross disruption of histomorphology and the serious translocations of endogeneous diffusible elements. Likewise, contamination during tissue transfer steps, freeze-drying and carbon coating is always a potential threat to chemical integrity.

For both chemically treated and cryoprepared tissues, a specimen substrate should be chosen which will not emit x-rays that overlap with elements under study. Likewise, coating the specimen with heavy metals to reduce charging should be avoided; rotary carbon-coating is usually adequate, and importantly does not compromise BEI or x-ray detection. Often no coating whatsoever is needed if 5 micron or thinner sections are examined by BEI.

X-ray spectral peak interferences in EDXA must be appreciated, as erroneous conclusions can be drawn from data, where artifactual additions have occurred. The major confounding overlaps that we have encountered are shown in Table 2. Usually it is possible to document the presence of high atomic number elements by noting the presence of their higher energy x-ray lines. However, it may be impossible to ascertain the presence of a low concentration of a low atomic number element in the presence of high atomic number material. For example, a low concentration of aluminum ($Z = 13$, $K_\alpha = 1.49$ keV) cannot

Table 3

ANALYTICAL ELECTRON MICROSCOPY IN HUMAN PATHOLOGY–A GENERAL OVERVIEW OF APPLICATIONS

I. Identification of xenobiotics (foreign material)

A. Particulates
- –Mineral pneumoconioses, especially asbestosis
- –Unexplained particulates
- –Unexplained granulomas

B. Drugs, metals and other elements
- –Unexplained pigments (e.g. amalgam tattoo)
- –Iodinated drugs
- –Toxic elements (e.g. arsenic, lead)

II. Identification of endogenous substances

A. Particulates
- –Renal stones and other calcifications
- –Unusual crystals

B. Diffusible ions
- –Cystic fibrosis
- –Cell injury (e.g. myocardial infarction)

III. Stains
- –Enzyme histochemistry

IV. Forensic medicine

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be detected reliably in a conventional paraffin section stained with hematoxylin and eosin because the eosin may add bromine ($Z = 35$, $L_{\alpha,\beta} = 1.48$ keV). Numerous other spectral interferences exist in EDXA (200). These overlaps should be anticipated prior to specimen preparation so that appropriate modifications, such as the omission of osmification or staining, can be made to avoid them altogether.

Finally, if one anticipates a future need for chemical or microanalytical studies in a given clinical case, it is always wise to *snap-freeze* a portion of the tissue and to *hold* an aliquot of tissue in appropriately buffered formalin or glutaraldehyde. By so doing, an individualized technique of specimen preparation can subsequently be tailored to the problem under consideration if serious artifactual problems occur with routinely processed tissue.

Instrument Artifacts In this tutorial paper it is not possible to review this complex subject in depth. The reader is referred to other articles which provide detailed information (106,139). With regard to BEI imaging, we wish to stress that BEI not only provides atomic number/density contrast, but also is sensitive to topography (119). Ideally one should use a flat and smooth specimen; since this is not normally the case with biological specimens, great care must be exercised in reaching conclusions about the meaning of the contrast seen in the images (119).

With regard to EDXA, it is important to stress that artifactual or background peaks for metals in the instrument such as iron, nickel and chromium are common problems in many analytical electron microscopes. Lines for silicon and sulfur may derive from contaminating diffusion pump oil, "O" rings and instrument parts. Chlorine present in the embedding medium (e.g. Epon) obviously interferes with tissue values. These problems necessitate multiple controls–e.g., in addition to obtaining a spectrum from a feature of interest, it is important to obtain (using identical instrument parameters) the spectrum from tissue without the feature of interest and from the support film devoid of tissue. Only by comparing *all three* spectra can reasonable qualitative conclusions be reached. Use of X-ray dot maps correlated with the LM or STEM image can be another way to distinguish true from artifactual localization (40,274).

Beam damage problems including mass loss and mass gain are far more difficult to control. It is clear that elemental losses can be significant, especially for volatile elements such as mercury (87) or vanadium. Use of low beam currents minimizes these losses but may not provide enough x-ray signal. Cooling the specimen to liquid nitrogen or liquid helium temperatures is the best way to minimize mass loss. Unfortunately, cold stages are still not widely available.

Applications

The applications of electron probe microanalysis are varied and cross the ill-defined boundaries between pathology, physiology, and anatomy. In this section we focus on studies which illustrate the usefulness of this technique in diagnostic pathology, though reference to some articles showing the range of pathology research investigations will be made. Thus, we have attempted to be comprehensive in our review of the diagnostic human pathology literature, and eclectic in our review of the relevant research literature. A brief overview of the vast literature on pulmonary asbestosis is included.

Analytical electron microscopy (AEM) studies in pathology can be roughly grouped into several broad categories (Table 3). We will describe here investigations centering on analyses of (1) xenobiotic particulates, (2) metal deposits, (3) endogeneous particulates, (4) soluble ions, and (5) materials in forensic science. These comments are summarized and amplified by the references in Tables 4 through 8.

Xenobiotic Particulates

Lung The field of characterization of xenobiotic particulates in various human tissues has widely utilized the technique of electron probe microanalysis in the past decade. The vast majority of these studies have concerned inhaled particles present in lung tissue. Numerous cases of various types of pneumoconioses have been examined and indeed this represents the most common diagnostic role of AEM. The identification and characterization of intrapulmonary deposits is important not only in determining a patient's diagnosis, but also as a source of evidence for medicolegal situations. Accurate identification of the material involved may allow recognition of sources of exposure and lead to measures to reduce exposure and prevent the possibility of harm to others.

Pulmonary disease resulting from the inhalation and retention of inorganic particles has been recognized for many years.

Table 4
Foreign Material in Human Tissue Documented by EDXA

Xenobiotic	Selected References
Aluminum	7,14,19,36,45,64,144,227,228,262, 285,291,309
Antimony	14
Arsenic	105
Asbestos	4,9,14,36,73,74,75,76,83,92,94,172, 246,249,250,311
Barium	14,169,189,248
Bismuth	14
Bromine	14
Cadmium	14
Cerium	14
Chromium	14,19,291
Clay	36
Cobalt	19
Copper	14,66,108,253
Gold	14,19,31,120–122,317,326,331
Gunshot residue	195,279,299,318
Gypsum	36
Kaolinite	50,68,138,174
Lanthanum	14
Lead	14,19,24,53,239,291
Mercury	14,53,159,202
Mica	36,138,174,260,291
Nickel	14
Osmium	14
Quartz	14,36,85,111,138,260,291,311
Selenium	53,134,202
Silica	4,9,14,19,26,36,85,88,89,111,112, 192,204,225,230,278,291,293,311
Silicates (various types)	4,7,9,11,14,19,26,36,50,51,53,85,88, 111,128,129,138,186,192,217,226, 235,257,276,291,295,311
Silicon	259,319
Silver	14,19,38,146,202,241
Steel	216,290
Talc	11,13,19,26,35,43,47,48,51,53,124, 138,142,143,174,190,209,291,296, 297,310,311
Tantalum	14
Technetium	14
Teflon	261
Thorium	44,135,206,220
Tin	14,290
Titanium	14,36,101,187,221,291
Tungsten	14,291
Vanadium	14
Zeolite bodies	251,269
Zinc	14
Zirconium	7,14,203

This varied group of diseases includes asbestosis, coal workers pneumoconiosis (164), silicosis and many more (77). The determination of the pulmonary dust burden is primarily, but not exclusively, directed at identification of the mineral content (219, 235, 284). Electron probe microanalysis has been useful with a variety of dust constituents identified and implicated in the disease process (Table 4 and 5). Specific agents identified through the use of AEM include: silica and a variety of silicates (36,41,47,50,51,85,88,100,111,112,138,230,257,276,278,293); talc dust (19,35,48,51,53,124,174,209,310); aerosolized particles of aluminum (19,64,144,185); titanium pigments (187,221); zeolite bodies (251,269); aluminum silicates found in cat litter (217); mercuric chloride (53); iron (128); abrasives from sandpaper (226); cobalt, chromium, iron and nickel from welding sources (12,277); and sand particles containing silica and iron (295) (found in the lung of an Egyptian mummy with pneumoconiosis). In many of these cases AEM was used to confirm a known or suspected particulate exposure, but in others the microanalysis provided identification of a previously unsuspected dust constituent.

Two major problems with this type of study are: the paucity of published data on particulates in *normal* human lung (14,291), and the time and tedium required to probe and count statistically significant numbers of particles by hand. Automated image analysers (which collect morphologic *and* chemical data simultaneously) will solve both problems during the next few years (153).

Electron probe microanalysis has been extensively applied to the study of the nature of the cores of ferruginous bodies in the human lung. In addition to being useful in the analysis of the chemical composition of asbestos fibers in patients with known or suspected exposure (49,83,94,118,130,170,246), this technique has also been used to identify low concentrations of bodies with asbestos cores and even lower concentrations of bodies with nonasbestos cores in the general population (73–75). In one case, AEM led to the recognition of the presence of amosite fibers coated with crystalline particles shown to be calcium oxalate instead of the more typical iron-containing coating (92). The use of EDXA allows one to differentiate between subdivisions of chemical types of asbestos fibers (75,172), and this may permit the identification of the sources of asbestos exposure as well as provide important evidence for use in litigation based on personal injury.

Table 5
INORGANIC PARTICULATES IDENTIFIED IN HUMAN LUNG

Fibers	Ref	Non-fibrous Particulates	Ref	Exogenous Metals and Elements	Ref
Actinolite	137	Alpha-quartz	14,19,36,138	Aluminum oxide	14,19,64,144, 291,309
		Amorphous carbon	73,185	Antimony	14
Amosite	73,74,137,231, 246	Andalousite	36	Barium sulfate	14,19,248,288
				Beryllium oxide	4,116,155,205, 233
Anthophyllite	73,74,231	Chlorite	36		
Attapulgite	70	Cordierite	36	Bismuth	14
Chrysotile	75,173,251	Cristobalite	36,138	Bromine	14
Crocidolite	73,74,231	Diatomaceous earth	73,173,248	Cadmium	14
Feldspar	70	Fly ash	125	Cadmium oxide	288
Fiberglass	173,248,288	Graphite	113,288	Calcium oxide	36
Illite	70	Illite	36,137	Cerium	14
Kaolinite	70	Kaolinite	50,138,216	Chromium	14,19,216,291
Pyroxene	70	Leucite	36,257	Cobalt	19
Rutile	70,269	Melanophlogite	36	Copper	14
Sericite	36,138	Micas	36	Ferric oxide	14,19,36,311
Sillimanite	36	Montmorillonite	36	Gold	14,19
Talc	70,231	Pyrophyllite	36,138	Gypsum	36,70
Tremolite	74,231,251	Talc	35,36,124,209, 310	Lanthanum	14
Zeolite	251,269	Tridymite	138	Lead	14,19,291
		Vermiculite	36	Manganese	14
		Zircon	4	Manganese oxide	36
				Mercury	14
				Nickel	14,19,216
				Osmium	14
				Silver	14,19
				Tantalum	14
				Technetium	14
				Tin oxide	14,288,291
				Titanium oxide	14,19,221,288, 291
		Endogenous Particles	**Ref**		
		Calcium carbonate	234,248,321	Tungsten carbide	14,19,288,291
		Calcium oxalate	170,248	Vanadium	14
		Calcium phosphate	65,321	Zinc	14
		Hemosiderin	138,183,248	Zirconium	14

Asbestos consists of a group of mineral species, which includes serpentine and amphibole varieties. Chrysotile is the sole member of the serpentine group of asbestiform minerals, and comprises 90% of the asbestos consumed in this country. The amphiboles include the two commercially useful forms, amosite and crocidolite, and the non-commercial forms—actinolite, anthopyllite, and tremolite. These various types of asbestos can be conclusively identified by the combination of EDXA and SAED techniques (210). Analytical electron microscopy of lung tissues has provided some interesting and somewhat surprising facts with respect to asbestiform minerals. Firstly, virtually all typical ferruginous bodies isolated from human lungs contain an asbestos core, and hence may properly be called asbestos bodies (72–74). Secondly, the vast majority of asbestos bodies contain commercial amphibole (i.e., amosite or crocidolite) cores, although such fibers only account for 10% or less of the asbestos consumed. Thirdly, many asbestos bodies isolated from the lungs of women (but not men) from the general population contain anthophyllite and tremolite cores (74), which are known contaminants of commercial talc (209). Fourthly, analysis of autopsy lung tissues of chrysotile miners demonstrate that the quantity of tremolite equals or surpasses that of chrysotile, although the former accounts for only about 1% of the mined chrysotile ore (69,258). With respect to asbestos related diseases—asbestosis, mesothelioma, lung cancer, and parietal pleural plaques—analytical electron microscopy will continue to provide information which will supplement that provided by epidemiologic studies (84,208).

In other studies involving lung tissue, EDXA has shown usefulness in the identification of pulmonary granulomas caused by microcrystalline emboli of talc from the intravenous administration of drugs intended for oral use (190) and pulmonary vessel foreign body vasculitis in an infant with pulmonary hypertension (43). This technique has also been utilized in investigations of inclusions in alveolar macrophages from the lungs of cigarette smokers (50,68,194,273) and those exposed to occupational particulate air pollution (12,129).

Table 6
Human Tissues and Fluids Studied by EDXA

Tissue	Selected References
Arteries	131,165
Blood	292
Bone	45,109,135,287
Bone marrow	44,105
Brain (neurons)	227,228
Bronchoalveolar lavage	22,183,185,186,192,235
Chromosomes	60
Colonic epithelium	301
Cornea	136,303
Endometrium	127
Fallopian tube	21
Fingernails	108,252,255
Follicular fluid	66
Gallstones	300,324
Glial cells	39
Hair	10,108,196,253,271,272
Heart	280
Kidney	54,202,263,285,317
Liver	211,220,281
Lung	1,2,4,5,7,9,11,13,14,15,19,20,35,36, 43,47,48,50–53,64,68–76,83,85,88, 89,92,94,100,101,111,112,124,128, 129,138,144,172,174,186,187,190, 192,216,217,221,225,226,230,232, 234,246,248–251,257,260,267, 269,276,277,278,291,293,295
Lymphocytes	330
Meninges	206
Nasal mucosa	302
Oral Tissues	53,133,134
Ovary	143
Platelets	80,329
Prostate secretions	238
Renal stones	65,161,162,163,179,245
Rheumatic disease crystals (joint crystals)	86,104
Saliva	236,237
Seminal fluid	238
Skeletal muscle	97,98,197,198,199,282,326,327
Skin	24,26,31,38,108,146,159,202,203, 204,241,270,286,297,304
Sperm	61,63
Sputum	94,124,129,234
Stomach	142
Sweat	236,237
Synovial membranes	104,121,122
Thyroid	244
Urine	236,237

Skin Electron microprobe studies on the skin have recently been reviewed by Forslind (108). Various types of inorganic material may produce granulomas of the skin. These must be distinguished from similar appearing lesions such as the non-caseating granulomas which are a manifestation of sarcoidosis or an infection with mycobacteria or spirochetes. Electron probe microanalysis of skin biopsy specimens can be valuable in the identification of foreign materials such as silica (26,204), zirconium (203), or talc (26,297) which may produce these lesions.

Other Tissues AEM can be applied to investigations of foreign material in any human tissue though the number of cases of other tissues is limited at this time. For example, EDXA can also be valuable in the analysis of intraocular foreign material (66,318) in cases both with documented trauma and with no known trauma. This could be an important application of this technique in medico-legal work and particularly in the field of industrial accidents. More unusual studies have demonstrated the presence of talc particles in ovarian tissue (143) and kaolin, talc, and a variety of silicates in stomach tumors (142). What role these foreign materials play in disease processes is unknown but now that they have been identified further investigation can follow.

Surgical Implants A variety of foreign materials are placed into human tissues in surgical procedures. Orthopedic surgery sometimes involves the placement of devices such as pins, plates, hinges and prosthetic joints. Silastic and silicon surgical implants also have considerable usage. These implanted foreign materials may affect the surrounding tissues and AEM can be useful in investigations of the tissue reaction as well as in foreign material identification. Tissues surrounding surgical implants of polydimethylsiloxane (silastic) have been noted to have a granulomatous foreign body reaction with multinucleated giant cells and refractile and/or birefringent crystals. EDXA was utilized in one study of this phenomenon and the crystals were revealed to be a magnesium silicate, probably talc, and most likely represented contamination from the implantation procedure (296). In other studies the presence of silicon in the tissues surrounding breast implants was investigated with the finding of free silicon in the fibrous capsule around the implants (259,319). The diagnostic application of the identification of this type of foreign material was demonstrated in the identification of a foreign body granuloma caused by polytef (Teflon) paste (261). This lesion

Table 7
Clinical Conditions Studied by EDXA

	Selected References		Selected References
Acute alveolar damage	14	Minocycline induced pigmentation	264
Adhesive arachnoiditis	206	Myocardial ischemia	280
Alzheimer's disease	227	Myopathies (various types)	97,98,197,199
Amalgam tattoos	134	Parasites	21
Amiodarone induced pigmentation	304	Parkinson's disease	97
Amyotrophic lateral sclerosis	228	Parkinsonism–dementia of Guam	228
Argyria	38,146,241	Plumbosis cutis	24
Arsenic toxicity	105	Pneumoconioses	2,4,5,9,11,13,14,15,35,36,47,48,52, 53,64,85,88,89,100,101,111,112,124, 128,138,174,186,187,192,216,217, 221,225,226,230,251,257,260,269, 276,278,293,295,309,310,311
Arterial disease	131,165		
Asbestosis	4,9,14,36,73,74,75,76,83,92,94,172, 249,250,311		
Calcite sputum lith	234	Pneumocystis carinii	1
Chrysiasis	31,82	Pneumonia	14
Cysticercosis	21	Polytef granuloma	261
Cystic fibrosis	108,237,252,255	Pulmonary alveolar microlithiasis	158,232
Dermatomyositis	199	Pulmonary alveolar proteinosis	201
Desquamative interstitial pneumonia	14,19,144,277	Pulmonary Aspergillus niger fungus ball	170
Duchenne muscular dystrophy	197,198,199,329	Pulmonary vessel vasculitis	43
Gallstones	300,324	Renal calculi	65,161,162,163,179,245
Gold nephropathy	317	Renal osteodystrophy	45,287
Green hairs	108,253	Rheumatic disease	86,104
Hypersensitivity pneumonitis	14,51	Salmonella	242
Hyperthyroidism	244	Sarcoid	7,14,51
Intra-ocular foreign body	66,318	Secondary bilary cirrhosis	281
Lead poisoning	239	Skin granulomata	26,202,203,204,297
Leukemias	44,330	Thorium induced tumors	44,135,220
Malakoplakia	270	Wilson's disease	136,303
Mercury pigmentation	159		

presented 17 months after laryngeal polytef injection and mimicked a cold thyroid nodule. EDXA of the foreign material present in the granuloma and of a sample of polytef paste produced identical spectra both with a strong single peak for fluorine, not surprising since Teflon is a polymer of tetrafluoroethylene. This case demonstrated how EDXA may be helpful in the support of a suspected foreign material etiology–based on a clinical history.

Metals and other elements

Electron probe microanalysis has been used in a variety of clinical cases for the identification and localization of deposits of metals in human tissues. Many of these investigations are primarily based in research. For example, following increasing evidence that aluminum toxicity may be involved in the disturbances of mineral metabolism and subsequent bone disease in patients with chronic renal failure on regular hemodialysis, x-ray microanalytical studies were performed and aluminum was detected in bone biopsies from such patients (45,287). Similarly, aluminum accumulations have been located in neurons of patients with dialysis dementia (116,117) and in neurofibrillary tangle-containing neurons in brain tissue from patients with Alzheimer's disease and amyotrophic lateral sclerosis–Parkinson's dementia syndrome in Guam (227–228). Though obviously these studies are of importance in the process of investigating the

Table 8
Specific Clinical Conditions in Which EDXA May Be Diagnostic

Condition	Selected References
Asbestosis	4,9,36,73,74,75,76,83,92,94,172,249,250,311
Cystic fibrosis	108,236,252,255
Mineral pneumoconioses	2,4,5,9,11,13,14,15,35,36,47,48,52,53,64,85,88,89,100,101,111,112,124,128,138,174,186,187,192,216,217,221,225,226,230,251,257,260,269,276,278,291,293,295,309,310,311
Renal stones	65,161,162,163,179,245
Unexplained granulomas	7,26,64,190,203,204,261,297
Unexplained pigments or deposits	24,38,134,146,241,253

etiology of these disorders, they do not appear to be clinically useful for diagnosis at this time. Another application of analytical electron microscopy has been to demonstrate the presence of copper within Descemet's membrane of the peripheral part of the cornea from patients known to have Wilson's disease (the pathognomonic finding commonly known as the Kayser-Fleischer ring) (136,303). Though these studies were performed as investigatory research, the identification of copper would be diagnostically useful in some situations. Barham and Tarara (28) have shown this to be true in the case of liver biopsies from patients with Wilson's disease.

EDXA has been shown to be clinically useful in other identifications of deposits. For example, this method has been used to locate aluminum in dense deposits within the glomerular basement membrane of patients with renal failure (262,285). Deposits of thorium dioxide in bone marrow (44), liver cells (220) and in a sarcoma of bone (135) have been positively identified in patients who had previously received injection of Thorotrast for diagnostic purposes. Further, thorium has been identified in the central nervous system of a patient who had undergone Thorotrast myelography (206). Arsenic has been detected in the bone marrow of a patient with suspected arsenic-induced peripheral blood and bone marrow abnormalities (105). Detection of lead deposits in the gingiva is possible and can be utilized in making the diagnosis of lead poisoning (239).

EDXA has also demonstrated its usefulness in the identification of elemental deposits in the skin. Granules of silver, sulfur and selenium have been found in skin biopsies from patients with occupational argyria (38) and silver and sulfur particles in skin biopsies from a patient with generalized argyria (146). Lead has been located in skin biopsies from a patient shown to have plumbosis cutis (24), and mercury pigmentation from industrial exposure has been documented (159). We and others have consistently found silver, tin and, curiously, selenium in amalgam tattoos (53,134). As selenium is not normally a component of dental amalgam, we assume it is adsorbed *in vivo*. Gold has not only been located in the skin of patients who had received long-term chrysotherapy for rheumatoid arthritis (31), but also in the kidney (317), skeletal muscle (326), and synovial membranes (121,122). Our own work with correlative light microscopy and scanning electron microscopy with EDXA led to the positive identification of yellow, refractile particles seen in ordinary bright field light microscopy of kidney, heart, liver, spleen and lung sections as containing gold. This type of application of analytical electron microscopy obviously has a role in diagnostic pathology.

Endogeneous Particulates

In addition to the usefulness of AEM in the identification of xenobiotics, this technique has been shown to be helpful in the characterization of endogenous particulates such as crystals and calculi (154). Electron probe microanalysis of crystalline deposits is relatively simple, quick, accurate and essentially nondestructive. In this manner one can detect, map and roughly quantitate the composition (for elements with Z greater than 9) of individually viewed crystals. EDXA can detect small crystals or particulates which are not detected by bulk x-ray diffraction as shown by Kim in the case of human renal stones (161,162,163).

Urinary stones, or renal calculi, are a common human ailment. It is clinically important to identify the constituents of a calculus because various components have different etiologies and treatments. Several AEM studies of calculi have been performed (65,161,162,163,179,245) and have demonstrated that this technique facilitates the precise identification of the crystalline constituents. Similarly, AEM has been utilized for the determination of the structure and composition of gallstones (300,324); for the detection and characterization of crystals present in intra-articular and periarticular tissues from patients with rheumatic diseases (86,104), for Schaumann bodies in sarcoid granulomas (51), and for structural and elemental analysis of crystalloids in salivary duct cysts (294).

Various types of calcifications have been examined with microanalytical techniques. The majority of these studies are investigatory in nature (27,78–80,131,165,171) and generally not useful for the practicing clinician. However, there may be clinical settings in which it is useful to determine the structure and elemental composition of lesions such as presumed metastatic calcifications (316), pulmonary alveolar microliths (158,232), or calcite sputum liths (234). Occasionally small clear crystals are noted in giant cells in pulmonary sarcoidosis, raising the question of a pneumoconiosis. In this setting AEM can be useful to show that these calcium carbonate crystals only give a peak for calcium by EDXA, and thus are not likely an inhaled mineral such as quartz. Schaumann bodies are also different in that they give peaks for calcium and phosphorus (51,321).

Diffusible Ions and Drugs

Analytical electron microscopy has been recognized as an important research tool for use in investigations of disease processes. The study of the localization of various soluble electrolytes is limited by methodology requiring procedures such as snap-freezing and vacuum-drying, but nonetheless, AEM has been applied to the study of diffusible elements in human pathophysiology. In this manner, qualitative and quantitative data concerning sulfur, phosphorus, potassium and chlorine in freeze-dried sections of muscle from patients with rheumatoid arthritis have been obtained, with significantly lower sulfur concentrations found in type IIA and IIB muscle fibers as compared to healthy controls (326). Other investigations have examined the composition of muscle sections from patients with Duchenne

muscular dystrophy (197,198,199), Parkinson's disease (97), dermatomyositis (199), and a variety of other conditions affecting skeletal muscle (95,98,199). This application of AEM shows great promise for future diagnostic use. Pathologic chemical alterations may likely precede changes in histomorphology in these diseases of unknown etiology (that is, degenerative or metabolic). Other AEM studies have looked at the distribution of calcium, iodine and phosphorus in human thyroid glands (244); the structure of collagen, mineral and ground substance in human cortical bone (109); and the composition of fluids such as sweat, saliva and urine (236,237). Snap-frozen kidney biopsies may be studied by EDXA in future clinical studies of renal diseases, particularly tubular disorders (177).

Several studies utilizing EDXA have investigated ion shifts in cell injury, particularly ischemic injury to myocardial cells (18,218,222,280). Other investigations have examined accumulation of calcium in the cytosol of cells injured by either ischemia or direct membrane damage (306,308), mitochondrial deposits in sideroblastic anemia (307), and the possible role of ion shifts in cell reproduction in normal and cancer cells. While the majority of these studies are of research interest only, a study of human myocardial tissues (280) demonstrated detection of myocardial ischemic injury from elevations of the Na/K ratio as determined by EDXA. This technique proved itself to be simple and fast and, indeed, shows promise as a means for diagnosing early myocardial damage, for example in medicolegal autopsies. It is well known that diagnostic ion shifts occur long before demonstrable histologic changes.

One interesting diagnostic application of x-ray microanalysis is based on the examination of electrolytes in nails. Cystic fibrosis patients have higher concentrations of sodium, potassium and chlorine in their nails as detected by x-ray microanalysis (252) and these results suggest a diagnostic efficiency of this procedure, but the technique appears to be clinically useful in screening for CF, and could reduce the need for the more difficult to perform sweat test. Fingernails from children in remote areas can even be mailed in to a central laboratory removing the necessity of taking the children to a medical center properly prepared to perform accurate sweat tests.

Although most drugs used in human medicine are not detectable by EDXA due to their organic composition, iodinated drugs such as amiodarone and/or their iodinated metabolites can be detected in human tissues (304), thus permitting better understanding of the pharmacology. Other iodinated molecules including x-ray contrast agents and other heavy metal-containing drugs (e.g., platinum, bismuth, antimony) can be identified as well (18,218,313). Barium can also be readily identified (168,169,189).

Forensic Science

Scanning electron microscopy and x-ray microanalysis have been used in the practice of forensic science for several years. Applications have included matching of broken surfaces (279), analysis of small amounts of unusual alloys (279), identification of gunshot residues (195,279,299), identification of weapons by bullet fragments (299), and the identification of human head hairs (271,272). Many other materials in the field of forensics have been examined by microanalysis but the subject will not be dealt with in depth here. The reader is referred to original work in the area for further information (25,195,271,279,283,298, 299,322).

New Techniques

A revolution in the technology of microanalytical instrumentation has made available several new techniques for the characterization and identification of xenobiotics in cells and tissues. Each technique has its own unique advantages and limitations with which one must be familiar in order to select the most appropriate technique for any particular analytical problem. These new techniques are discussed and are outlined in Table 1.

Total Rate Imaging with X-rays (TRIX) can be accomplished on an SEM or STEM equipped with EDXA. TRIX images are similar to BEI in that they exhibit atomic number contrast. However, these are x-ray images and therefore "see" deeper into tissue, revealing particulates which might be missed by BEI (149,240). Also, TRIX imaging is less sensitive to the organic (tissue) matrix topography than BEI. Since the image is formed with the x-ray detector, x-ray dot maps for specific elements correlate better with the TRIX image than with BEI. However, BEI is much faster than TRIX and has better resolution. Accordingly, we view TRIX as a technique *complementary* to BEI. At present, TRIX is of research interest only, BEI being entirely adequate for current clinical studies. Nevertheless, we believe that as TRIX imaging becomes better known and as the image acquisition time diminishes with better technology (240), it may be used in studies of mineral pneumoconioses. We found that quartz and aluminum silicates are readily and clearly detected by TRIX.

In contrast to TRIX, Electron Energy Loss Spectroscopy (EELS) detectors are not widely available and the technique is more difficult than EDXA (99,110,151). However, EELS has been used to detect beryllium in human pulmonary berylliosis (93,155,205). With EELS it may be possible to study the subcellular metabolism of many organic compounds including drugs and hormones by tagging them with fluorine (81,99).

Scanning Auger microscopy (SAM) (152) requires a high vacuum and a very clean sample. To date there have been very few studies with human tissues. We were able to show that black inclusions in human alveolar macrophages contained carbon using SAM (185).

Boyde and Reid have recently described a simple, inexpensive cathodoluminescence (CL) detector which can easily be installed on any SEM (46). This group has used CL to study mineralized tissues including human teeth. An important future possible application of CL is the study of the ultrastructural location of antibodies tagged with fluorescent dyes. Presently the main problem impeding this work is the development of dyes which remain stable under electron beam bombardment.

Two additional techniques are secondary ion mass spectrometry (SIMS) (3,55,103,115,160,181,182,184,289,305) and laser microprobe mass analysis (LAMMA) (123,156,157,166,183,265). These techniques determine chemical composition of the sample by ionizing the constituent atoms which can then be analyzed by mass spectrometry. SIMS instruments produce secondary ions from the sample by bombarding it with a primary ion beam; LAMMA produces ions from the sample by vaporizing a portion of it with a laser beam. Both the microanalytical versions of SIMS (the ion microprobe and ion microscope) and the LAMMA have part per million sensitivity for most elements and their isotopes, and have lateral spatial resolutions of about one micron. These techniques are applicable to the study of human tissues as shown by studies such as Abraham's demonstration of the

presence of beryllium in pulmonary granulomas using SIMS (7,20,23,233). Because of their greater sensitity, SIMS and LAMMA should theoretically be better than EDXA for studies of aluminum localization in neurons and bone (115,266,312). Also of great interest is the laser-Raman microprobe, which provides Raman spectral data (37). This instrument has also been used to identify organics such as polymers in human tissues (17,102). Finally the proton microprobe or PIXE (particle or proton induced x-ray emission) shows great promise for quantitative studies on low levels of potentially toxic metals such as lead and copper (191,314,315). While the spatial resolution is at present not equal to that of the electron or ion probes, the sensitivity may be better for some elements. Forslind (108) has used PIXE to analyse trace metal contaminants in human hair, and Bartsch and colleagues (30) have studied trace metal distributions in human lung. Lindh (180) has used a nuclear microprobe to study heavy metal distributions in human bone.

Conclusions

1. Microprobe techniques—especially the AEM techniques of BEI and EDXA—are no longer solely research tools. The findings often have diagnostic, therapeutic and/or legal implications. Some of the most commonly encountered specific clinical conditions in which EDXA findings may be diagnostic are listed in Table 8.
2. AEM technology is evolving very rapidly—as are related techniques such as SIMS and LAMMA.
3. As cryotechniques improve, studies of ion distributions in health and disease will provide new insights into human pathophysiology.
4. As microprobe techniques improve it will be increasingly possible to identify organics and compounds, not just elements and isotopes.
5. It is difficult but important to correlate the results obtained with these new techniques both with each other and with older, established techniques such as light microscopy.

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Discussion with Reviewers

B.F. Trump: In the section on "Preparative Artifacts," you state that exposure of tissues to various histologic solutions introduces contaminants in the specimens. Although this is indeed the case, could not the identification of such be used as an advantage rather than a disadvantage?

Authors: Yes. The use of metals as specific stains for different carbohydrates is just one example (see Spicer SS, Schulte BA, Shelburne JD: Carbohydrate cytochemistry by transmission and scanning electron microscopy. Scanning Electron Microscopy 1983; IV:1827–1834, and text-references 40 and 90).

B.F. Trump: Has there been any published information on the amount of sodium found in cacodylate-buffered glutaraldehyde as measured by AEM?

Authors: We are not aware of such studies. We speculate that the retention and addition of sodium would vary considerably with different tissues and even with different subcellular organelles. One approach to this question might be the use of a sodium isotope imaged with ion microscopy.

B.F. Trump: With the microprobe techniques described, data acquisition, analysis etc. is accomplished with computers. Currently, different types of configurations and computers are used, depending upon the manufacturer, with the pathologist having no choice in the matter. Since a pathology laboratory (clinical and research) may have any number of other types of instrumentation, in addition to that used for AEM, which requires the use of computers, is it feasible, in order to keep costs down, to use only one or two computers? If so, is it possible to write software programs which will handle all instrumentation and, even more importantly, will such eventually be available commercially?

Authors: Yes. One example of this approach is the LeMont image analysis system which uses the computer associated with an x-ray spectrometer to perform multiple image measurements. While it is theoretically possible to write software programs to handle all instrumentation, it is probably not practical in most settings.

B.F. Trump: In line with the above questions, what is the best currently available commercial software for microprobe analysis (EDXA)?

Authors: Changes in commercial software are occurring rapidly. All of the major manufacturers provide adequate software for qualitative and quantitative microprobe analysis.

B.F. Trump: With regard to cryotechniques, what is the currently acceptable method for freezing, cryomicrotomy, transfer and analysis of pathological specimens when diffusible elements need to be analyzed?

Authors: Pathological specimens should be treated with the same techniques used for physiological specimen preparations. A recent superb review article by T.A. Hall and B.L. Gupta has just appeared in the Journal of Microscopy ("The Application of EDXS to the Biological Sciences," Vol. 136, pp. 193–208, 1984.) The major problem is not the choice of technology, but rather finding funds and trained personnel to handle pathological specimens with the same care that has been devoted to problems in biology.

B.F. Trump: In a diagnostic pathology laboratory which is also heavily research-oriented, of the wide range and availability of analytical instruments currently manufactured, which one or ones do you consider to be of the most importance?

Authors: It is our bias that a scanning electron microscope equipped with secondary, backscattered and transmission electron detectors and an energy dispersive x-ray detector is probably the most generally useful analytical instrument for most diagnostic pathology laboratories. The large sample stage available on most scanning electron microscopes is often of great importance in being able to *adequately sample* complex tissues such as the human lung.

B.F. Trump: Due to the high costs of buying, maintaining, etc. these instruments, do you recommend regional centers for AEM much as HVEM centers have been established where investigators may either go to analyze specimens themselves or have someone else analyze their specimens on a per cost basis?

Authors: Yes, we do recommend regional centers for AEM and agree that there is a need for such centers in North America.