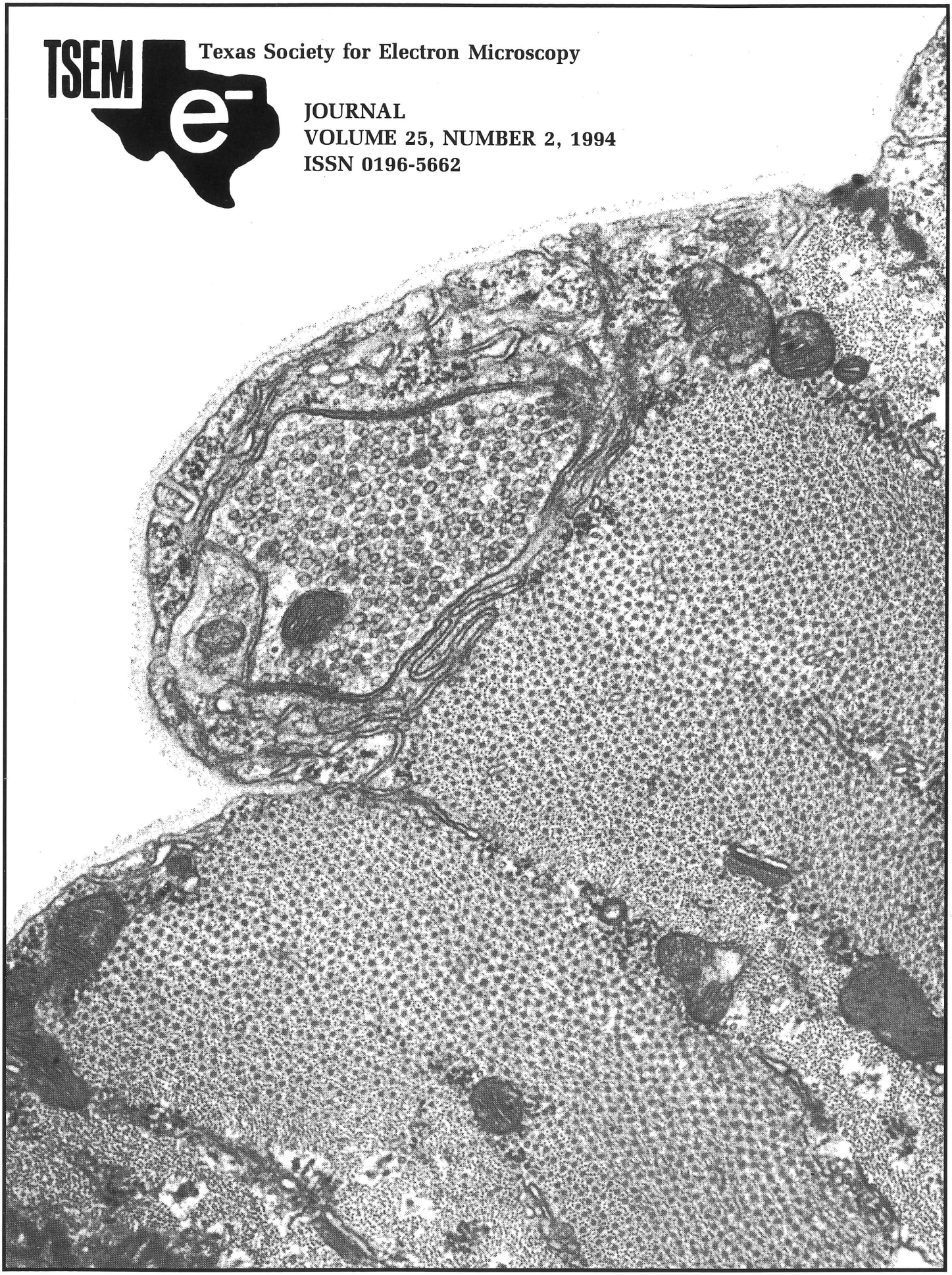
Texas Society for Electron Microscopy

JOURNAL
VOLUME 25, NUMBER 2, 1994
ISSN 0196-5662



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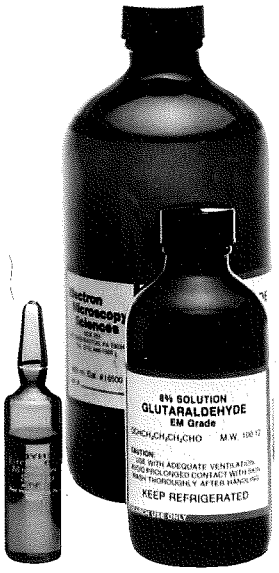

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Electron Microscopy Sciences

Contents

TEXAS SOCIETY FOR ELECTRON MICROSCOPY
JOURNAL
VOLUME 25, NUMBER 2, 1994
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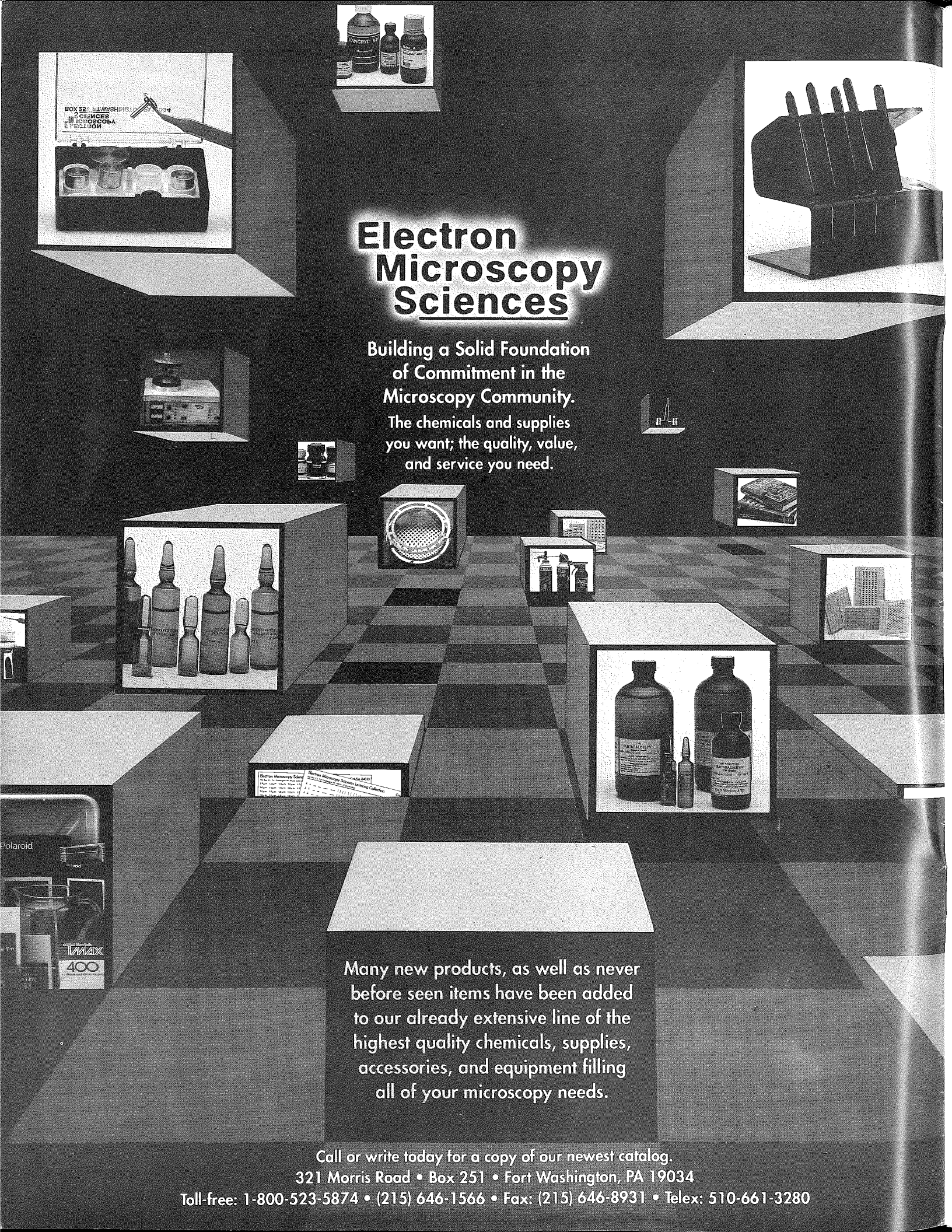
David C. Garrett, Editor
Department of Biological Sciences, University of North Texas, Denton, TX 76203

Texas Society for Electron Microscopy
"For the purpose of dissemination of research with the electron microscope."

ON THE COVER
Nerve passing through muscle fiber surrounding a common oviduct in the female stable fly, *Stomoxys calcitrans* (L.). Note synaptic vesicles, glial structure and mitochondria. Magnification = 108,100X. Photo — Nan Webb Pryor, Food Animal Protection Research Laboratory, United States Department of Agriculture, College Station, TX 77845.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IN MEMORIAM

Daniel K. Roberts
1936-1994

TSEM President 1967-1968

"As third president of TSEM, Dan Roberts was instrumental in getting the society off to a good start. His contacts in the medical research fields allowed us to secure nationally and internationally renowned speakers for our meetings. His untimely passing should not go unnoticed by the society."

— Donald C. Benefiel
Past President TSEM, 1968-1969

Dan Roberts moved to Kansas in 1970. He was chairman of the ob-gyn department and professor of pathology at the University of Kansas School of Medicine - Wichita. He was also chair of the Wesley Medical Center ob-gyn department for 21 years. Believing that research was an important part of medicine, he helped launch the Women's Research Institute in 1984. That institution is dedicated to research on human infertility and other reproductive problems. "His goal in life was to create the best care in the nation - in the world - for women," said his son, Jeff.

— From the *Wichita Eagle*

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President's Message

The distribution of this journal issue will be concurrent with the fall meeting of TSEM in San Antonio. As a San Antonio resident, I welcome you to our city and to the TSEM meeting, which is across the street from The University of Texas Health Science Center at San Antonio. There is always a lot of enthusiasm for a San Antonio meeting. This is the first time that TSEM has met in San Antonio since the 20th anniversary meeting at the Menger Hotel in 1985. I particularly want to thank our program chair, Mitch McCartney of Alcon Labs for his efforts in organizing an outstanding program. The original suggestion was to have a meeting downtown at a hotel on the riverwalk. After obtaining information on costs, a decision was made by the executive council to meet away from downtown. Hopefully, the more affordable hotel accomodations will have a beneficial effect on attendance by some who might be discouraged by higher costs, especially student participants. Being near the health science center and other scientific institutions in northwest San Antonio will enable our vendors to schedule calls with their customers in conjunction with the meeting. And, of course, you can still avail yourself to the downtown riverwalk, Sea World, Fiesta Texas and/or all of the many traditional tourist attractions in San Antonio, such as the Alamo, Brakenridge Park and the zoo.

With the first call for papers, I enclosed a letter from the president, extending a special invitation to non-TSEM members who utilize various microscopic techniques in the San Antonio area and attempted to explain that papers applying all forms of microscopy — not just electon microscopy — are appropriate for presentation. Hopefully, this encouragement will result in recruitment of some new attendees and will enrich our meeting by letting the memberhsip know about some of the exciting research going on in the San Antonio community of biomaterials/biotechnology.

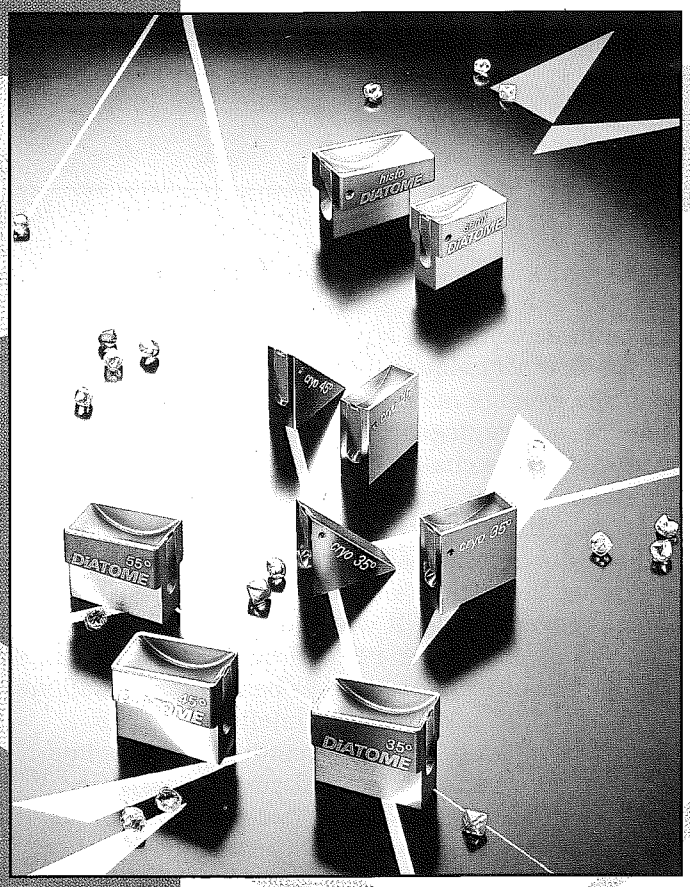
With regard to confusion about the interests embraced by TSEM, I should let you know that the executive council has discussed the possibility of a name change for the society. This would follow suit with name changes [1] by the national society from Electron Microscopy Society of America (EMSA) to Microscopy Society of America (MSA) and [2] by other local societies. The strongest candidate name, to date, seems to be Texas Society for Mircoscopy (TSM). Such a name change would reflect encompassment of all types of microscopy, including all the new techniques (which are developing faster than the average microscopist can track). It would have been advantageous to TSEM to have already made the name change. However, we will be deferring a while longer any vote that would result in a bylaws change. This is based on legal advice, in light of our recent years of trouble and confusion regarding the society's status with the state and federal governments. It was thought that it was all resolved as of October 1993. However, new issues, of which new officers have never been aware, continue to arise. TSEM is extremely grateful to Wayne Sampson of Texas A&M University for his tireless efforts over a period of several years to assist in resolution of these problems. Because of her interaction with the issues as a recent president of TSEM, I have asked Lynn Gray to continue as our point person with the lawyer in College Station. TSEM is grateful to both Dr. Sampson and Dr. Gray for their time, effort, and dedication.

Speaking of changes, another suggested change is to go to only one meeting per year instead of two. At present, the materials science group from UTEP and UNT each find it feasible to attend only one meeting. The Executive Council will discuss the possibility of skipping the fall '95 meeting, which would not require bylaws change. We are also interested in suggestions for future meeting sites. Please pass on to your officers your ideas and feeling regarding name change, meeting changes, or any other changes. A committee comprised of Lynn Gray, Keith Fry and Hal Hawkins is currently doing bylaws review. Their recommendations will be discussed at the October executive council meeting.

Meanwhile, put the spring '95 meeting on your calendar, March 23-25, in Fort Worth. Mitch has another great program planned, and we hope to see you there.

Sincerely,

Nancy K. Rodman Smith
President, 1993-1994



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Treasurer's Report

TEXAS SOCIETY FOR ELECTRON MICROSCOPY
TREASURER'S REPORT
For Period Ending September 1, 1994

ASSETS ON JANUARY 1, 1994:		
Certificate of Deposit No. 113515	$3,890.52	
Checking Account No. 70072962	3,720.75	
TOTAL		**$7,611.27**
CHECKING ACCOUNT RECEIPTS:		
Dues	$3,470.00	
Spring 1994 Meeting Registration	1,140.00	
Workshop	205.00	
Exhibitors	600.00	
Donations and Grants	635.56	
Guest	125.00	
Fall 1994 Meeting Exhibitors	50.00	
Journal Advertisements 24:2	125.00	
25:1	1,125.00	
Miscellaneous	5.02	
Checking Account Interest (Account No. 70072962)	63.54	
TOTAL		**$7,544.12**
Interest, Certificate of Deposit No. 113515	90.09	
EXPENSES:		
Journal, 25:1	$1,949.25	
Other	311.78	
Office Expenses	45.43	
Mailouts	800.00	
Spring 1994 Meeting	1,382.82	
Workshop	265.83	
Student Competition/Travel/Award	430.00	
Invited Speaker	363.00	
Fall 1994 Meeting (deposit)	200.00	
Audiovisual Equipment Purchases:		
Projectors (2), Extra Bulbs, Long Cords	750.00	
Laser Pointer	57.57	
Miscellaneous	65.00	
TOTAL		**$6,620.68**
ASSETS AS OF SEPTEMBER 1, 1994:		
Certificate of Deposit No. 113515	$3,980.61	
Checking Account No. 70072962	4,644.19	
TOTAL		**$8,624.80**

TRANSMISSION ELECTRON MICROSCOPY OF MINERALS IN SOILS

Joe B. Dixon* and G. Norman White

Soil and Crop Sciences Department, Texas A&M University, College Station, Texas 77843-2474

Soils are mixtures of mineral crystals in assorted sizes and shapes (silicates, oxides, and salts), organic matter, living organisms and, in a natural condition, water and gasses. The chemical, physical, and biological properties of soils are greatly influenced by these mineral phases. The solid phases of soils are predominantly crystalline and the use of transmission electron microscopy (TEM) methods can provide important information that may not be available by other methods. TEM can provide an image of the morphological features of small (clay, <2 μm) crystals and reveal their structure by selected area electron diffraction and lattice fringes.

The complexity of soils often requires selective preparative techniques to isolate the components of interest that can be analyzed by a particular TEM method and instrument (Fig. 1). Useful information can often be obtained by careful physical separation of localized phases such as oxide coatings or nodules (e.g. hand picking, scraping, magnetic separation possible combined with dispersion and size fractionation). Yet our common practice is to separate the soil into size fractions and examine the clay fractions by TEM for morphology, electron diffraction, lattice fringes, and chemical composition by energy dispersive spectrometry (EDS). The coarser fractions (silt and sand) are analyzed by scanning electron microscopy methods (1). The objectives of this paper are to briefly discuss the above preparative procedures and to describe the TEM methods employed and the types of data obtained.

MATERIALS AND METHODS

If simple phsyical separation is not adequate, the conventional procedure is to disperse the sample by removing the carbonates with an acid buffer (pH 5.0 NaOAc) and the organic matter by hydrogen peroxide oxidation (Fig. 1). The sample is then saturated with Na by centrifuge washing with a salt solution (2, 3). These preparative procedures are employed to avoid the formation of secondary precipitates that may form on oxidation of organic matter in the presence of calcium or when the pH is raised to promote particle dispersion of mineral particles for size separation. Certain mineral types tend to concentrate in particular size fractions. For example, layer silicates and metal oxides usually are <0.2 μm and quartz and feldspar are more commonly coarser in size. The sand is sieved out and the clay and silt separation is done by a combination of gravity sedimentation and centrifugation based on Stoke's law (2).

The TEM preparations for the clay fractions are by simple drop-mounting of the slightly turbid clay suspension with a disposable pipette on a holey carbon micro-grid (4) on a 200 to 400 mesh Cu grid (Fig. 2). It permits viewing the clay particles in space without obstruction of a continuous film thus providing better contrast for thin particles. Also, crystals in favorable orientation frequently yeild lattice fringes that permit mineral indentification (Fig. 3, 4). Such fringes are poorly visible on a continuous support film. On-the-other-hand, where shadow casting is employed for particle thickness measurement a continuous film is preferred to give uniform topography of the supporting membrane.

Although holey carbon films are widely used for TEM of mineral grains, they do have considerable diversity. Occasionally, they have lumps of excess carbon and surface blemishes that are easily confused with nanometer size mineral grains such as iron and manganese oxides in soils. Recently a special fibrous halloysite called Patch clay has been described (5). It is a clean and uniform material (Fig. 6), has low absorbance in the electron beam (Fig. 7), and can be used effectively for mounting soil clays (6). Also, it is simple to prepare as a mounting medium. The lack of stability of halloysite in the electron beam can be accommodated by mounting it on carbon films with large holes or by working close to metal grid bars when it is applied directly to a metal grid (e.g. Cu 400 or 1000 mesh).

Layer silicates may be investigated for particle shape, size, mass-density contrast, moiré fringes, etc. Metal oxides (Fe, Mn) occurs as clay-size

*Corresponding author.

particles and they are readily investigated by TEM. These oxides are relatively stable in the electron beam, they occur in various orientations, and they often exhibit characteristic twinning (Fig. 5). These features permit more thorough investigation on simple mounts of their electron diffraction patterns and lattice fringes by TEM than many minerals with layered lattices having preferred basal orientation. Investigation of layer to layer changes of platy silicates (micas, etc.) requires mounting the particles in a resin and sectioning with a diamond knife or an ion mill (7) to obtain an edge view of the plates. Graduate students in soil mineralogy at TAMU are instructed with a JEOL 2010 (200 kV) TEM interfaced with an x-ray microanalyzer.

RESULTS AND DISCUSSION

The flow chart in Fig. 1 illustrates how TEM may be conducted in a typical case and the treatments employed for sample preparation. Although TEM is commonly applied to clay fractions it also can be used for imaging coarser fractions even though the particles may be too large. In such cases, the thin edge of a particle may be examined or the particles may be crushed in a mortar (usually wet) and the fragments investigated (Fig. 3). Also, thick particles can be sectioned for TEM.

Small particles are usually mounted on a holey carbon film (Fig. 2). The film shown has MgO particles on it obtained by waving the grid through the smoke created by burning a strip of Mg metal thus giving a convenient reference specimen.

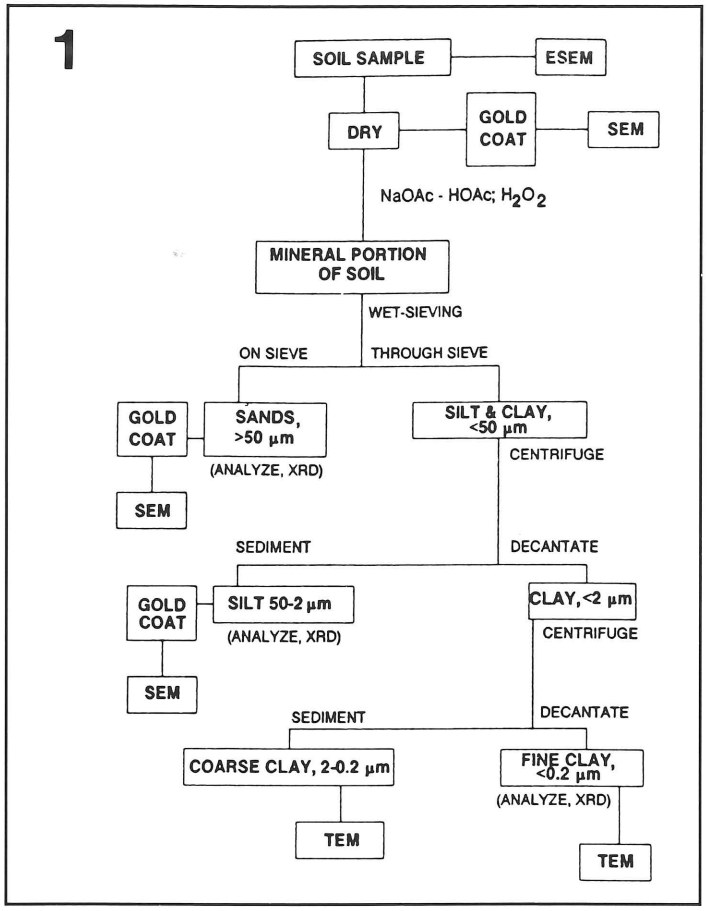
Goethite (αFeOOH) is a common mineral in soils giving them a familiar yellow-brown color and participating in important chemical reactions with P in solution. Thus it is a frequent subject of research. The crystal illustrated in Fig. 3 was a lath-shaped fragment of a reference specimen from Biwabik, Minnesota. It illustrates the (110) lattice fringes at 4.2 Å that are most evident on a thin edge as shown in the enlargement (upper part of Fig. 3). A uniform granularity of the carbon film indicates the lack of astigmastism in the electron beam at the time of film exposure - a convenient property of a holey carbon support film (not shown). Selected area electron diffraction (not shown) can be used to distinguish a single crystal which gives a set of diffraction spots. By contrast, polycrystalline aggregates give ring patterns and amorphous subjects give only weak diffuse rings or none at all. Diffraction patterns can be calibrated and used to identify the crystal phase and crystal orientation in the electon beam based on unit cell dimensions.

TEM often reveals crystals with characteristic morphology that may be useful in tracing their origin. The twin in Fig. 5 is identified as goethite because of the similiarity to other twinned samples of goethite. The composition of such a twin could be confirmed with EDS on the JEOL 2010 and other modern TEMs. Such twins are more common in sediments than soil. This one is from the Cr horizon of an Eastwood soil from Louisiana.

The (110) lattice fringes of an elliptical crystal of goethite from an Eastwood Bt2 soil horizon from Louisiana are shown in Fig. 4. Iron oxides in soils are excellent subjects for TEM investigation because the crystals are small yet stable in the electron beam and they give useful information on the chemical, physical, and pedological properties of soils.

The modern transmission electron microscope is a powerful research and teaching instrument. It provides morphological, structural, and chemical information on crystalline particles composed of only a few unit cells. It reveals crystal imperfections directly that relate to the chemical and physical behavior of the sample even though they are rare in occurrence.

TEM continues to serve as an important analytical method for soils investigations largely because the clay particles impact soil behavior greatly. In recent years, TEM has proven essential in characterizing seasonally formed and dissolved iron oxides (ferrihydrite and lepidocrocite) in soils of rice paddies and other wet lands. In turn, these fine iron oxide particles retain P when oxidation prevails and release it when reduction of Fe occurs. Better understanding of Fe oxide behavior has led to pursuit of an improved chemical soil test for plant-available P in paddy soil conditions. Manganese oxide nodules form early during rock weathering and in a pedogenic environment. They have many crystal imperfections that frustrate x-ray diffraction analysis but lattice and structural images are well suited for characterizing these compounds and for relating the findings to weathering and soil forming processes. TEM has proven important again for investigating the wide range of crystal sizes and shapes of kaolinites and halloysites in soils and sediments. Smectites are complex in morphology and structure and TEM is a major source of data on them. Interstratification of small regions of mica among other silicate layers has been shown by lattice fringes of sectioned samples. Mica and its weathering products are major reactants involving K in soils. High resolution TEM has been essential to investigation of K chemistry and mineralogy of soils and clays. Current investigations of smectites flocculated with organic polymers are being strengthened by TEM. Early results reveal a complex arrangement of lattice fringes that help explain the drastic modification of clays when organic complexes form. Some of the results mentioned are yet unpublished and others are discussed in the book edited by Dixon and Weed (8). With the continued improvement TEM instruments and associated instruments such microscopy is likely to continue to serve well the earth and environmental sciences.


1
SOIL SAMPLE
ESEM
DRY
GOLD COAT
SEM
NaOAc - HOAc; H_2O_2
MINERAL PORTION OF SOIL
WET-SIEVING
ON SIEVE
THROUGH SIEVE
GOLD COAT
SANDS, >50 µm
(ANALYZE, XRD)
SEM
SILT & CLAY, <50 µm
CENTRIFUGE
SEDIMENT
DECANTATE
GOLD COAT
SILT 50-2 µm
(ANALYZE, XRD)
SEM
CLAY, <2 µm
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TEM
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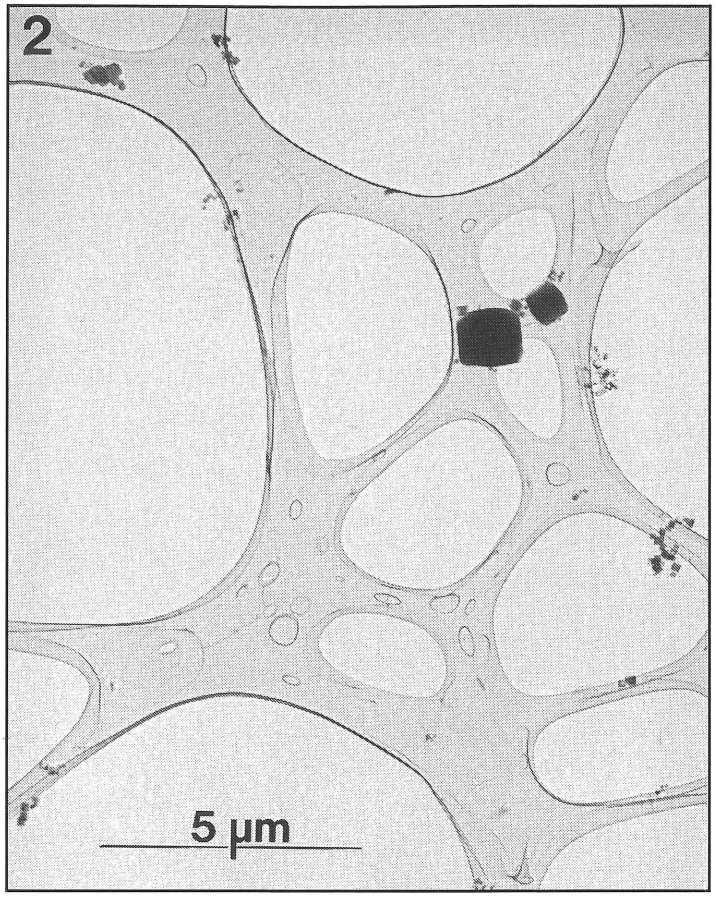
(ANALYZE, XRD)
TEM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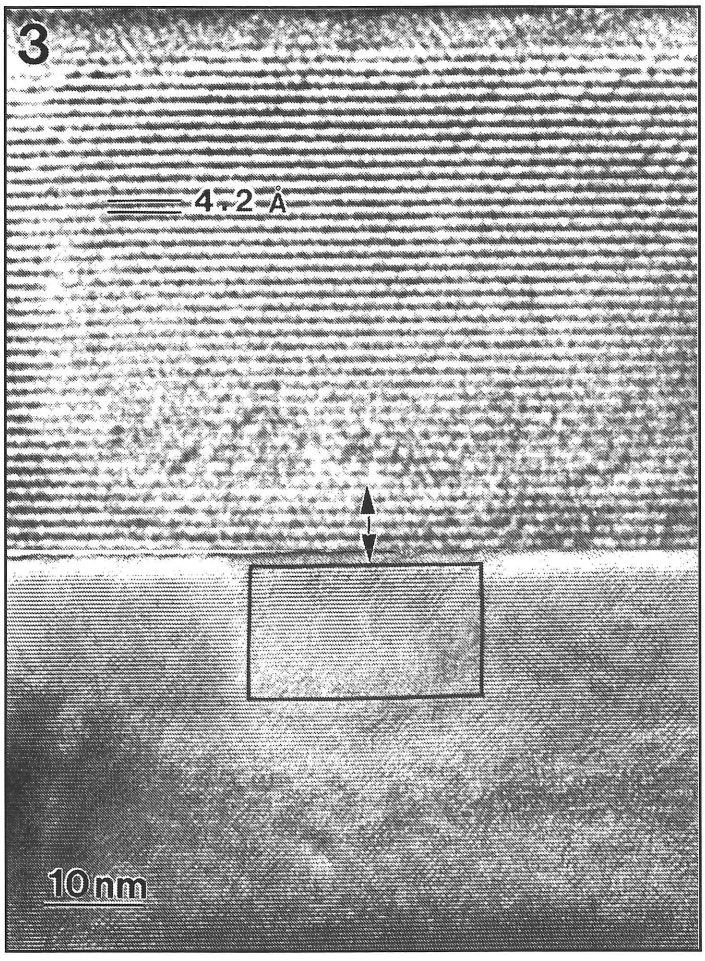
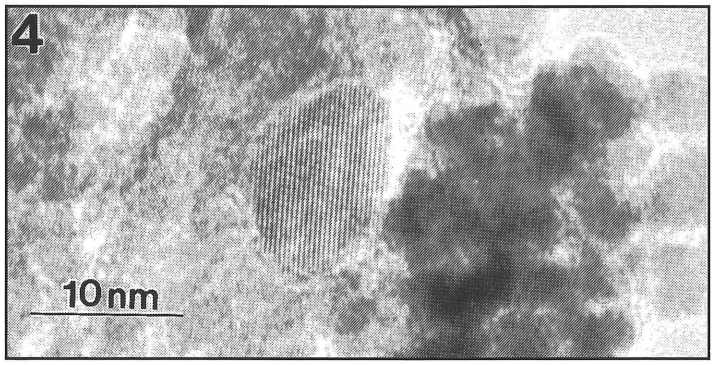
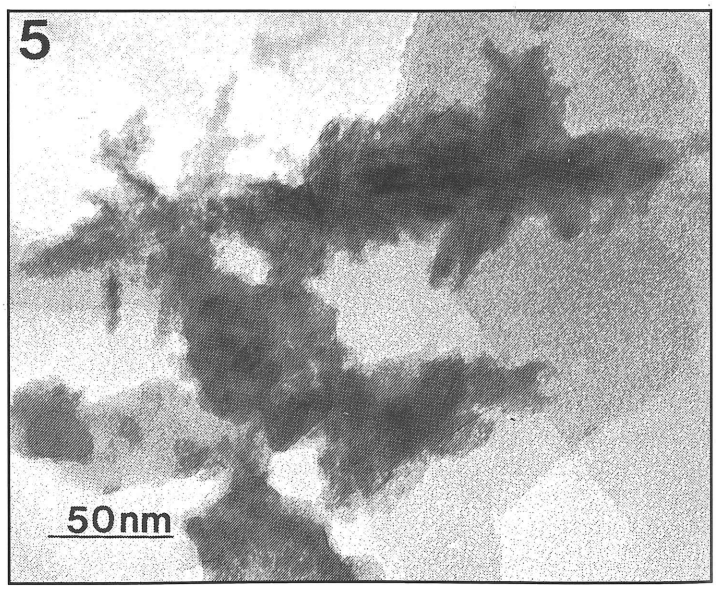
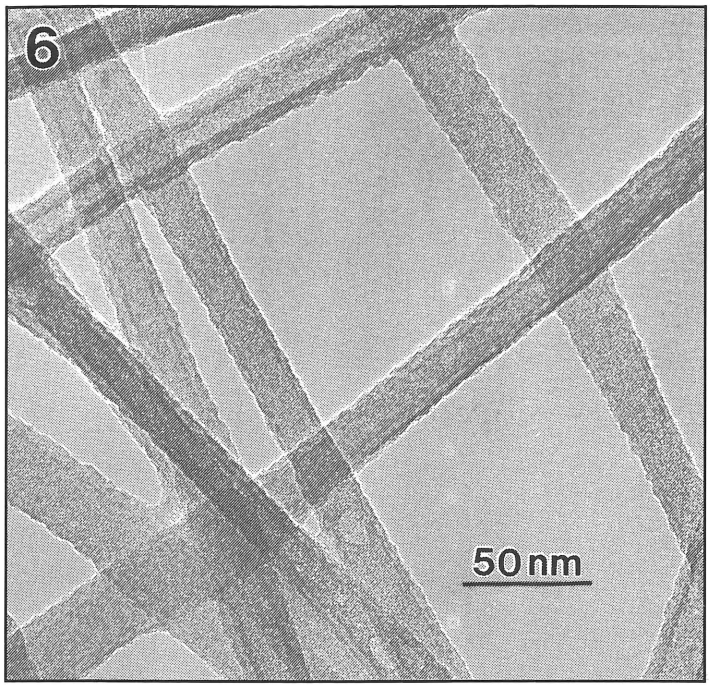
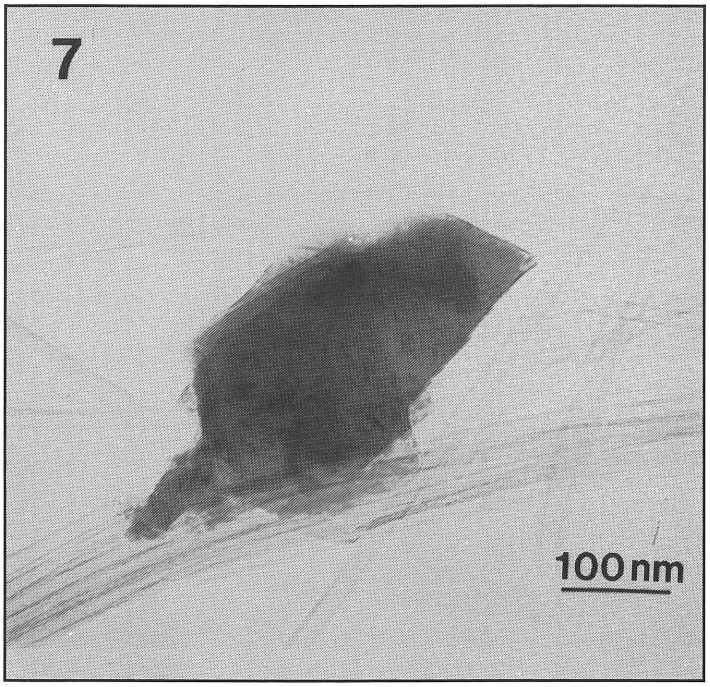
5 µm


3
4.2 Å
10 nm


4
10 nm


5
50 nm





ACKNOWLEDGMENTS

Use of the JEOL 200CX instrument at the Facility for High Resolution Electron Microscopy at Arizona State University, Tempe for Fig. 2 & 3 and use of JEOL 2010 at the Electron Microscopy Center, Texas A&M University, College Station to obtain Fig. 4 & 5 is hereby acknowledged by the first author. Thanks are due Christine Wallace for drafting and word processing. Texas Agricultural Experiment Station Manuscript No. 31380.

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FIGURE LEGENDS

FIGURE 1. Flow chart for electron microscopy investigation of soils.

FIGURE 2. Holey carbon film for mounting small particles.

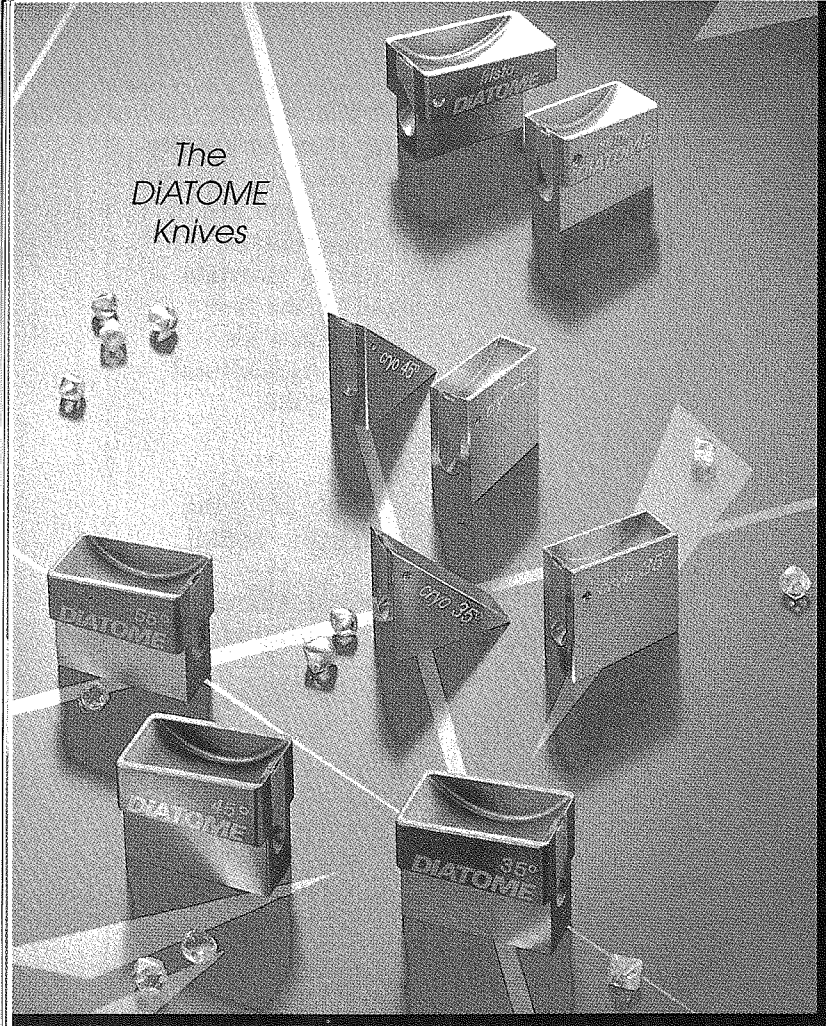
FIGURE 3. Goethite crystal lying on the edge of the support film showing lattice fringes at two magnifications.

FIGURE 4. Small crystal of goethite showing lattice fringes at 4.2 Å in soil clay.

FIGURE 5. Goethite twins from soil clay.

FIGURE 6. Patch clay halloysite fibers: a proposed sample support for TEM.

FIGURE 7. Birnessite particle on fibrous halloysite support.



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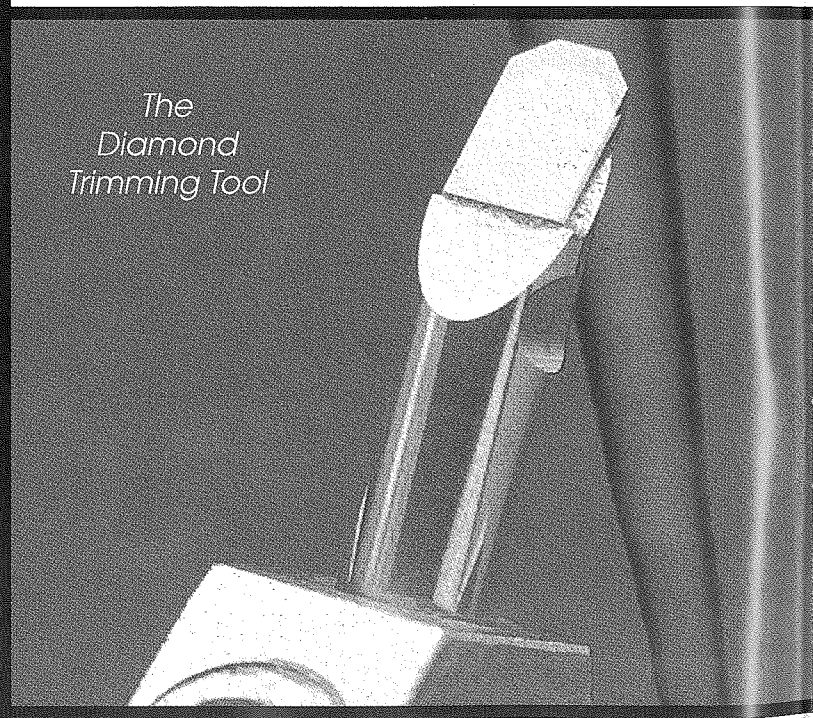

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Letters to the editor are printed as they are received in the order of their arrival. These letters reflect the opinion of the individual TSEM member and do not necessarily reflect the opinions of the editor or the society. The content of the letters should be concerned with the philosophical or operational aspects of the TSEM, the Journal and its contents, academic or national policies as they apply to TSEM and/or its members and electron microscopy in general. Editorial privilege may be evoked to insure that the LETTERS SECTION will neither be used as a political forum nor violate the memberships' trust.

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Micrographs submitted for cover photos should be marked as such. The choice of photographs will be made by the editor. Photograph receipt and/or dispensation will not be acknowledged. Photographs will not be returned. Electron micrographs to be used for cover photos and text fillers are welcome and should be selected with some attention to aesthetic appeal as well as excellence both in technique and in scientific information content.

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The Technical Section will publish TECHNIQUES PAPERS, and HELPFUL HINTS. The TECHNIQUE PAPERS will describe new or improved methods for existing techniques and give examples of the results obtained with methods. The format of the Technique Papers will be the same as that used for regular research reports. HELPFUL HINTS will be in the form of a brief report with an accompanying illustration, if required for clarity. Helpful Hints should embody techniques which will improve or expedite processes and/or procedures used in EM.

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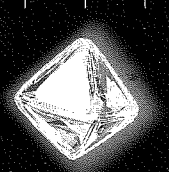

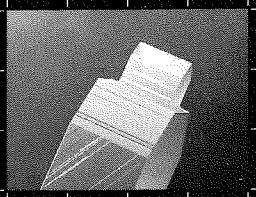

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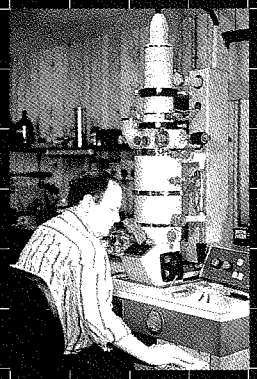

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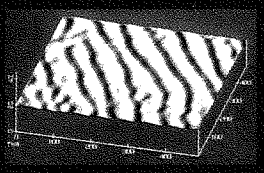

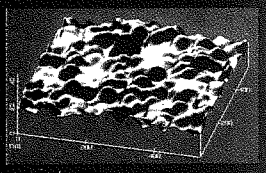

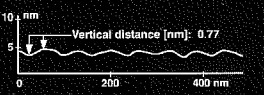

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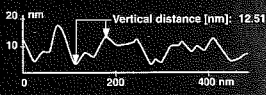

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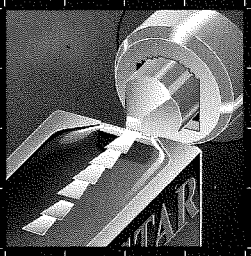

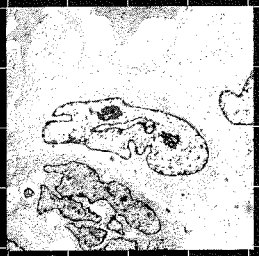

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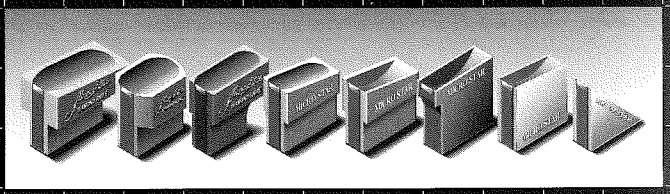
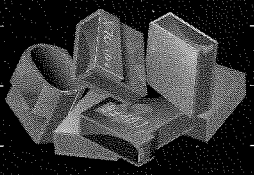
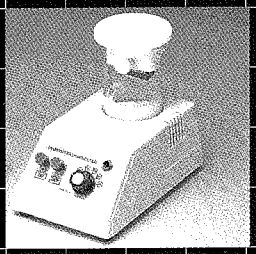
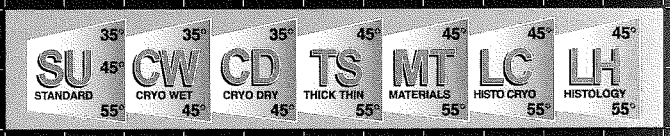






15
TYPE-ANGLE COMBINATIONS



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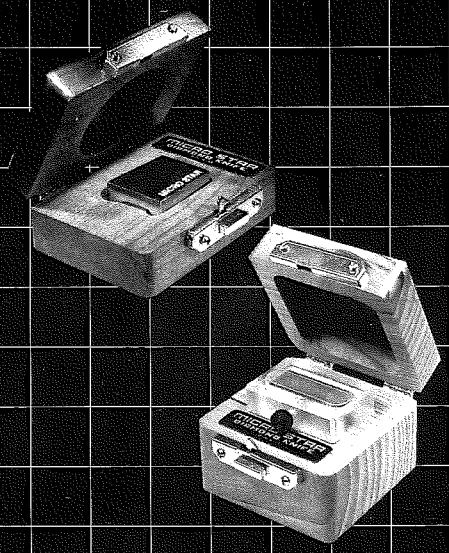

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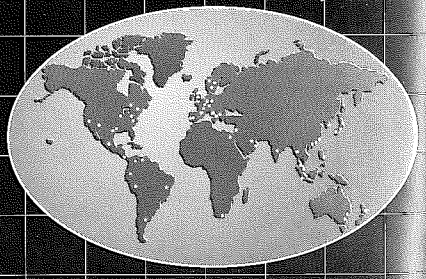

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(3) D. Gabor. Information Theory in Electron Microscopy, in: Quantitative Electron Microscopy. Eds. G.F. Bahr and E. Zeitler (Williams and Wilkins, Baltimore, 1956) 63-68.

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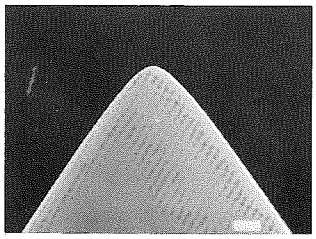
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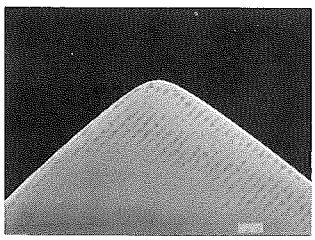
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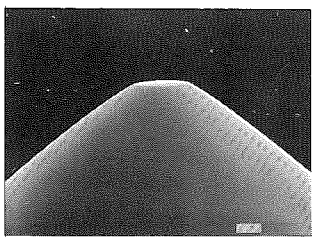
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Abstracts

BIOLOGICAL SCIENCES

POSTER PRESENTATION — FALL 1994

INFECTION OF LEAVES OF SWEET GUM BY THE PARASITIC FUNGUS TUBAKIA DRYINA. S. CLARK AND J. TAYLOR, Dept. of Biology, Stephen F. Austin State University, Nacogdoches TX 75962.

Epifluorescence light microscopy, coupled with scanning electron microscopy and Nomarski differential interference contrast microscopy, was used to examine the infection process of the fungal parasite Tubakia dryina on one of its host plants, sweet gum (Liquidambar styraciflua). From leaves showing the characteristics of red leaf spot disease the pathogen was isolated and grown in pure culture. Leaves of healthy sweet gum seedlings were then inoculated using a suspension of conidia. Plants were maintained in a humid environment to facilitate spore germination. The conidia adhered to the leaf surface and formed septate germ tubes. Germ tubes terminated in small appressoria that appeared to penetrate the leaf surface directly. Artificially inoculated plants were maintained in the greenhouse and exhibited symptoms consistent with red leaf spot observed in nature.

NORMAL AND PATHOLOGIC ANATOMY OF HUMAN PATHOGENIC MONOCYTOTROPIC EHRLICHIAE.

V.L. POPOV, J.W. WEN, V.C. HAN, S-M. CHEN, X-J. YU, H-M. FENG, and D.H. WALKER. Dept. Pathology, The University of Texas Medical Branch, Galveston TX 77555

Ehrlichiae are obligately parasitic intracellular rickettsiae, multiplying in macrophages/monocytes or granulocytes of infected vertebrate hosts to whom they are transmitted by ticks. Most ehrlichiae have veterinary importance but two species - *Ehrlichia sennetsu* (E.s.) and *E. chaffeensis* (E.c.) cause human disease by infection of macrophages. We studied by SEM and TEM multiplication of E.c. (Arkansas strain) and E.s. (Miyayama strain) in the continuous cell lines, DH82, mouse embryo (ME), Vero, BGM and L929. Intracellular distribution of ehrlichial antigens was studied in LR White embedded cells with appropriate poly- and monoclonal antibodies using post-embedding immuno-gold techniques. Both species developed inside intracellular parasitophorous vacuoles (morulae) where they formed two types of spheroid or oval cells: those with a centrally condensed nucleoid and ribosomes, 0.4-0.6 μm diameter - "dense-cored cells" (DC), and larger ones, 0.4-0.6 by 0.7-1.9 μm with uniformly dispersed nucleoid filaments and ribosomes - "reticulate cells" (RC). Some morulae contained a uniform population of E.c. consisting of either RC or DC; others had both of them. DH82 and ME cells were most susceptible to infection with E.c. and had a large quantity of small morulae visible in SEM (1.0-1.5 μm in diameter, each containing from 1 to 5 ehrlichiae), sometimes more than 400 in an ultrathin section. E.s. morulae usually contained single or few RC or DC. RC and DC represent the normal life cycle of ehrlichiae. In addition, large RC were observed having alterations of the cell wall and cytoplasmic membrane structure, abnormalities of protoplast fission and other presumably reversible pathologic phenomena.

THE INFECTION PROCESS IN OAK LEAF BLISTER DISEASE. D. BIRDWELL AND J. TAYLOR, Dept. of Biology, Stephen F. Austin State Univ., Nacogdoches TX 75962.

Nomarski differential interference contrast microscopy, epifluorescence light microscopy, and scanning electron microscopy were utilized to observe the parasitic fungus Taphrina caerulescens on the host water oak (Quercus nigra). Isolation of ascospores from infected leaves exhibiting symptoms of oak leaf blister was performed and the pathogen was grown in pure culture. Leaves of healthy water oak seedlings were inoculated and conditions favorable for infection were maintained. Germination of spores, development of mycelial hyphae, and indirect penetration of host tissue were observed.

LIGHT MICROSCOPY OF THE STRUCTURE OF A GEL.

M. Sun, G.A. Griess, and P. Serwer, Department of Biochemistry, The University of Texas Health Science Center at San Antonio, San Antonio, TX, 78284-7760

The fibrous network of those agarose gels typically used for electrophoresis is too fine to observe by light microscopy. In the present study, light microscopy, apparently for the first time, reveals the fibrous network of an agarose gel that has a micrometer-scale fibrous network. The agarose used is a derivatized, irradiated agarose previously shown by electron microscopy to have the following characteristic: When a 1.5% gel is cast at 30°C, but not at 10°C, fibrous bundles of agarose chains partition to form wider fibers that, in turn, form a network that has micrometer-sized and larger pores. When observed by phase-contrast light microscopy, gels cast at 30°C have a clearly defined fibrous network not present for gels cast at 0°C. The temperature-dependency of the network observed by light microscopy indicates that this network is the same as the micrometer-scale network observed by electron microscopy. For the network observed by light microscopy, the mean chord length varies inversely with the agarose concentration. The least concentrated gel observed has a concentration of 0.3%. Slight pressure on a 0.4% gel caused microfracture of the gel, thereby producing pores of a dimension between 5 and 50 μm. These pores, freely permeable to latex spheres, will be useful for determining the hydrodynamic damping of intrapore, single-particle motion by gel fibers.

EFFECT OF MONENSIN FOR IMMUNOCYTOCHEMISTRY ON MATRIX METALLOPROTEINASE 3. H.NAKAYA, A.M.TRAN, D.L.COCHRAN, Dept. Periodontics, Univ. of Texas Health Science Center at San Antonio, San Antonio TX 78284.

The matrix metalloproteinases are considered to play an important role in the destruction of connective tissue in periodontal disease. The monovalent ionophore, monensin, has been used in a procedure to immunolocalize metalloproteinases in cultured fibroblasts. Specifically this study investigated the effect of monensin on immunocytochemistry of matrix metalloproteinase 3 (MMP-3) by comparing immunolocalization and gene expression in human periodontal ligament (PDL) cells. Cultured PDL cells were treated with or without IL-1β (1000pg/ml) for 24h. Monensin (5uM) was added to half of the PDL cells for the last 3h of the incubation period. Immunolocalization of MMP-3 was observed by the avidin biotin peroxidase complex method. Gene expression of MMP-3 was observed by reverse transcriptase polymerase chain reaction (RT-PCR). Immunolocalization of MMP-3 in PDL cells was observed when cells were treated with monensin. By RT-PCR, monensin did not alter the MMP-3 gene expression which was stimulated in the presence of IL-1β. There was a positive correlation between the number of MMP-3 producing cells and the amount of MMP-3 mRNA. Our results suggest that monensin treatment for immunocytochemistry on PDL cells is an effective method for visualizing MMP-3 without affecting MMP-3 message synthesis.

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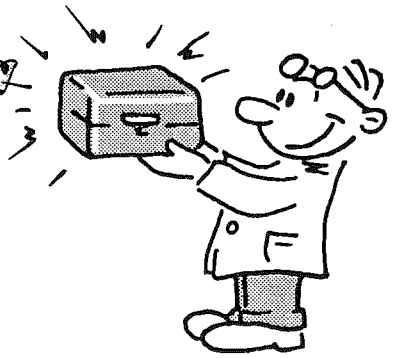

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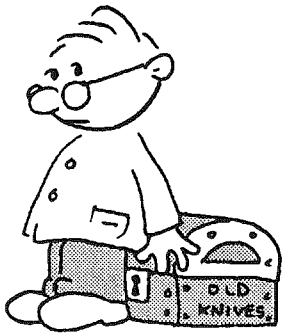

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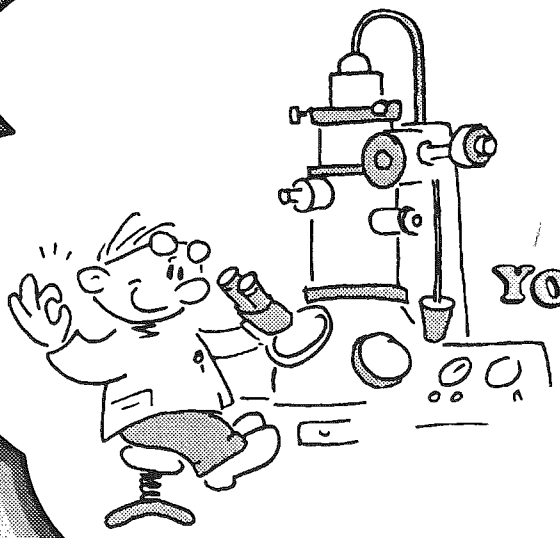

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STAPHYLOCOCCUS EPIDERMIDIS SLIME DETECTED *IN SITU* BY A LECTIN-BIOTIN ASSAY USING TEM. B.A. Sanford, M.A. Ramsay and M.M. Miller. Depts. Microbiology and Pathology, University of Texas Health Science Center, San Antonio, Texas 78284-7758

A lectin-biotin assay was developed for use in the detection of slime produced by *S. epidermidis* in *in situ* biofilm. Mature biofilm was formed on PVC disks using a combined chemostat-modified Robbins device (MRD) model system in which disks were exposed to a free-flow of staphylococci, grown in chemically defined medium, for six days. Adherent cocci were removed from multiple disks and quantified. Representative disks were fixed and processed for SEM. Fixed specimens were also: 1) stained with ruthenium red, 2) reacted overnight with biotin-labeled lectins (WGA, succinyl-WGA, Con A, or APA) followed by treatment with gold-labeled extravidin, or 3) reacted with antibodies against *S. epidermidis* RP62A capsular polysaccharide/adhesin (PS/A) using an immunogold procedure. These treated specimens were then post-fixed, dehydrated, infiltrated with resin, sectioned, stained with uranyl acetate-lead citrate, and examined by TEM. WGA and S-WGA reacted with cell-associated and exocellular slime only. In contrast, Con A, APA, and anti-PS/A reacted with the bacterial cell surface but not with either cell-associated or exocellular slime. WGA and S-WGA, which have binding specificities for N-acetylglucosamine, demonstrated that the slime matrix material produced in *in situ* mature biofilm was distributed between cells, as well as in the interface formed between the cocci and the surface of the PVC polymer. These results indicate the usefulness of the WGA (and S-WGA) lectin as a specific marker for detection of the presence and distribution of slime matrix material in *S. epidermidis* biofilm.

A S.E.M. EXAMINATION OF IDIOBLASTS IN THE STEM OF *NYMPHAEA*. M. PENDLETON, S. KINDT, and S. MEOLA. U. S. Dept. of Agriculture, Agricultural Research Service, Food Animal Protection Research Lab, 2881 F & B Road, College Station, TX. 77845.

Many genera of the perennial aquatic herb *Nymphaea* exhibit branched idioblasts in stems and leaves. These idioblasts have been described in previous studies utilizing light microscopy. This study employs scanning electron microscopy to provide superior depth of field and magnification to observe these idioblasts in the air chambers of *Nymphaea* stems. Numerous cubical or rhombohedral crystals of calcium oxalate are embedded in the walls of the idioblasts. Several

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variations in the shape of these idioblasts have been observed. Within the air chamber of the *Nymphaea* observed, these idioblasts were derived from parenchyma cells which became elongated on one or more sides toward the air chamber or other small intercellular space. These elongations produced arm-like projections which form the stellate idioblast.

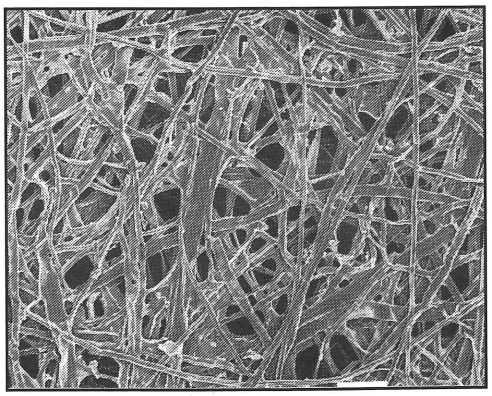
Sections of mature *Nymphaea* stems two cm in length were critical point dried in a Denton Vacuum critical point dryer model DCP-1 using liquid CO_2 following dehydration in alcohol. Each dry section was mounted with colloidal silver paint on aluminum pin stubs for examination with a Cambridge Stereoscan 200 scanning electron microscope operating at 15 KeV, working distances of 12-16 mm, and magnifications of 15 to 4,500 X.

EVALUATION OF FOCUSED ION-BEAM CROSS-SECTIONING OF RESIN-DENTIN INTERFACES. B. VAN MEERBEEK[1], D. SCHRAUB[2], L.J. CONN JR.[1], E.S. DUKE[1], F. GHAFGHAICHI[3], [1]Clinical Research Facility, University of Texas Health Science Center at San Antonio TX 78284; [2]Sony Microelectronics, San Antonio TX 78245; [3]Accurel Systems, Austin TX 78731.

Recently, focused ion-beam (FIB) etching, commonly used as a cross-sectioning technique for analysis of semiconducter devices, has been applied to biological tissues to expose their ultrastructure for examination. It was the aim of this investigation to evaluate the practical utility of FIB to cross-section resin-dentin interfaces in order to assess the bonding efficiency of resin adhesive systems that are currently used in routine dental practice to restore teeth. Basically, the underlying bonding mechanism involves an initial demineralization of the dentin surface with an acid etchant, exposing a collagen fibril arrangement with interfibrillar microporosities that subsequently become impregnated by low-viscosity monomers. The reported "clean" FIB-cutting without inducing detectable damage beyond 100 Å to fixed dehydrated tissues was thought to reveal indisputable information about the resin saturation degree of the collagen scaffold. A major concern using this adhesive technique possibly leaving an uninfiltrated collagenous band as a weak link in the resin-dentin bond by incomplete resin infiltration is currently raised and has so far not been resolved by alternative cross-sectioning techniques. Two representative commercially available adhesives were bonded to dentin of recently extracted teeth to evaluate the FIB-cutting capability. After appropriate fixation and dehydration of the resin-bonded dentin samples, cross-sections through the resin-dentin interface were made using the Seiko SMI-8100 Focused Ion Beam System and were subsequently examined in a field-emission Jeol 6400F SEM at 5 kV. Results indicate possible artifact production at the cross-sectioned interface hiding its actual ultrastructure, probably due to a heat-effect with possible recrystallization. Further studies of FIB are needed to optimize its usefulness for resin-dentin interface examinations and other biological tissue applications.

ANSWER TO "WHAT IS IT"

from TSEM JOURNAL 25:1



The micrograph published on the back cover of Volume 25, Number 1, 1994 was an SEM view of a tea bag filter. The filter forms the bag which surrounds the tea and prevents the tea leaves from entering into the brewed tea solution. The micrograph was produced as a part of a forensic study (See TSEM Journal, Vol 25:31) in which Melissa Tennant and I investigated the many components involved in the packaging of tea. We specifically examined tea packages produced in Celyon and marketed in the U.S. in attractive small boxes made of balsa wood. These have been marketed in Texas under several different labels; we investigated the brand "Terra Teas", variety "English Breakfast."

Micrograph — H.J. Arnott, University of Texas at Arlington.

BIOLOGICAL SCIENCES

PLATFORM PRESENTATION — FALL 1994

THE INITIAL DEVELOPMENT OF CALCIUM OXALATE CRYSTALS IN THE LEAVES OF *VITIS MUSTANGENSIS* AND *V. LABRUSCA*. HOWARD J. ARNOTT, LINDA E. LOPEZ AND MARY ALICE WEBB[1], Department of Biology and Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019 and [1]Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907.

In the leaves of *Vitis mustangensis* and *V. labrusca* there are numerous raphide idioblasts, each containing from 200-500 elongate needle-shaped crystals of calcium oxalate monohydrate. Each individual crystal is surrounded by a crystal chamber membrane and the entire complement of crystals is embedded in an organic matrix contained in the vacuole. We isolate the crystal bundles and their matrix (that is, the contents of the crystal vacuole) from native and cultivated species of *Vitis*. The individual raphide crystals are between 50 and 100 μm in length and average about 1.0 μm in thickness. One end of each raphide is pointed, the other end is bidentate; such a configuration is characteristic for twin crystals. Early developmental stages of *Vitis* raphides, herein termed *crystal initials*, are reported here for the first time. Crystal initials are often less than 1 μm in length and substantially less in thickness. In shape, crystal initials fall into two general categories, those that are flat and unlike the mature raphide twins and those that appear to be short miniature examples of mature raphide twins. The latter have pointed and a bidentate ends characteristic for mature raphide twins. However, the flat crystal initials have two sharp pointed ends and do not exhibit a bidendation at either end. By careful means it is possible to make "spread preparations" which are circumscribed in such a way that the number of crystal initials per individual bundle can be determined using polarization light microscopy or SEM. In leaves of the species investigated, an average of 23 (range 6 to 51) initials are associated with each raphide bundle. When viewed in SEM preparations the crystal initials often appear to be situated on the periphery of the crystal packet.

COMPARATIVE MICRO MORPHOLOGICAL STUDIES ON THE SEED COAT OF SEVEN SPECIES OF *ALBIZIA* THROUGH SCANNING ELECTRON MICROSCOPY. Nabarun Ghosh[1], Don W. Smith[1] and A. Chatterjee[2]. [1]Dept. of Biological Sciences, University of North Texas, Denton, TX 76201. [2]CAS, Dept. of Botany, University of Calcutta, India.

This investigation was undertaken to find out the taxonomic interrelationship of seven species of *Albizia* (Mimosoiideae-Leguminosae) and to standardize a procedure for identifying the seeds utilizing Scanning Electron Microscopy (SEM) on the seed coat of these species. Seeds of a particular species are often less variable than the other organs. The simple microscopic and stereo-microscopic examinations revealed the color and nature of seed surfaces including pleurograms, fissures, and hilum at lower magnifications. The SEM study revealed the specific ornamentation pattern of each type of seed sample. The ornamentation including undulations, reticulations and rugae could be observed under SEM at various magnifications. Characteristic crackings were evident in *Albizia falcararia, A. lebbeck* and *A. odoratissima*. Crackings are connected with the seed pleurogram and respond to humidity inside and outside the seed by stretching and shrinking. Dehydration of the seeds at maturation and absorption of water during germination probably also takes place through these cracks. The cracking patterns of these three species of *Albizia* are similar, but, each species exhibited it's own characteristic size and shape of plates outlined by the cracks. By seed coat ornamentations and cracking patterns seeds of all these species could be easily identified.

ELECTRON MICROSCOPIC ANALYSIS OF THE EFFECT OF DEXAMETHASONE ON CULTURED HUMAN LAMINA CRIBROSA CELLS. D. CANTU-CROUCH, M.D. MCCARTNEY, S. BROWDER AND A.F. CLARK, Alcon Laboratories, Inc., Fort Worth, Texas 76134

Glaucoma is a sight threatening disease characterized by a rise in intraocular pressure and a progressive loss of visual fields. The loss of visual fields is due to axonal degeneration which is associated with changes in a supporting structure within the optic nerve known as the lamina cribrosa (LC). The rise in intraocular pressure is due glaucomatous damage to the trabecular meshwork (TM) which decreases aqueous humor outflow. The administration of glucocorticoids can induce ocular hypertension and a disease very similar to glaucoma. Previous studies from our laboratory have shown morphological changes in TM cells in response to glucocorticoids. The purpose of the present study was to investigate whether glucocorticoids would also induce morphological changes in cultured human LC cells. Cultured human LC cells were grown to confluence on Thermanox® coverslips and then treated for 14 days ± the glucocorticoid dexamethasone (DEX) before being examined using freeze-fracture and standard TEM methods. Freeze-fracture analysis indicated that both control and DEX cells had a similar density and distribution of intramembrane particles. Small punctate gap junctions between cells were infrequent for both treated and controls cells. Vesicle fusion sites were present on control cells but were found in much greater numbers on DEX treated cells. Cross sections of cells showed that DEX treated cells had increased amounts of extracellular matrix material and linear stacking of rough endoplasmic reticulum. The DEX-induced morphological changes in LC cells shown in the present study are similar to those described previously for cultured TM cells. These findings support biochemical data suggesting a similarity between TM and LC cells.

CONTINUED ON NEXT COLUMN ►

TECHNIQUES USED IN ENTOMOPALYNOLOGICAL RESEARCH Gretchen D. Jones*[1], Juan D. Lopez, Jr.[1], Pete D. Lingren[1], and Vaughn M. Bryant, Jr.[2]
[1]USDA-Areawide Pest Management Research Unit (AWPMRU), [2]Palynology Laboratory

Pollen identification is a useful tool in the study of migration, foraging resources, and feeding habits of lepidopteran adults. Sample preparation and microscopy techniques for entomopalynological research vary considerably. However, there has not been a report of pollen analyses of the crop (part of the esophagus in which food is stored preparatory to digestion). We examined the possibility of pollen occurrence in the crop of eight male Helicoverpa zea, captured in sex pheromone traps in the spring. Of the eight crops examined, seven contained pollen. Quercus sp., oak, pollen had the highest frequency of occurrence. Most samples contained three different taxa. A total of 11 taxa were identified from the eight samples. Advantages of this new technique include: use of light microscopy for pollen examination and maneuverability of the pollen grain to reveal diagnostic characteristics. Disadvantages include: length of preparation and examination time, and lack of resolution. This preliminary evaluation of the crop as a source of pollen for ecological studies indicates that it has promise; however, additional research is needed to verify its usefulness.

THE CALCIUM OXALATE CRYSTAL COMPLEMENT IN THE LEAVES OF THE VIRGINIA CREEPER (*PARTHENOCISSUS QUINQUEFOLIA*). HOWARD J. ARNOTT AND AMY J. JEFFREYS. The Department of Biology and Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019.

Using heretofore described isolation techniques, raphides, druses and prismatic forms of calcium oxalate crystals were isolated from Virginia Creeper leaves. All of the crystals are intensely birefringent as seen under polarized light. The isolated raphide crystals consist of bundles of several hundred individual needle-like crystals embedded in a well developed matrix. The matrix is internally differentiated, with two or more layers being visible. Two dense terminal spheres are often seen at each end of the matrix. In many cases the matrix may be 2-4 times the length of the crystals, indicating that the crystal idioblasts (cells) that manufacture these crystals are long and narrow with length/width ratios from 1.8 to 12. The crystals range between 30 and 95 μm in length; each individual has a sharply pointed and bidentate end suggesting twins; in many it was possible to demonstrate independent extinction of each twin. The druses are multi-interpenetrant twin crystals which range from 10 to 30 μm in diameter. Druses vary from a small number of interpenetrants to those with many interpenetrating crystals. Prismatic crystals are rare but often show brilliant colors when viewed with polarized light. Powder x-ray diffraction indicates that only one crystalline species, calcium oxalate monohydrate (whewellite) (COM), is present despite the different forms of crystals seen. This is consistent with the brilliant colors displayed by all the crystals, a characteristic of COM crystals. Examination of intact raphide bundles showed many raphide initials associated with the surface and the ends of the mature crystals within the matrix. Expanded raphide bundles exhibited a relatively large number of raphide initials. The number observed was considerably greater than the average of 23 determined for *Vitis*.

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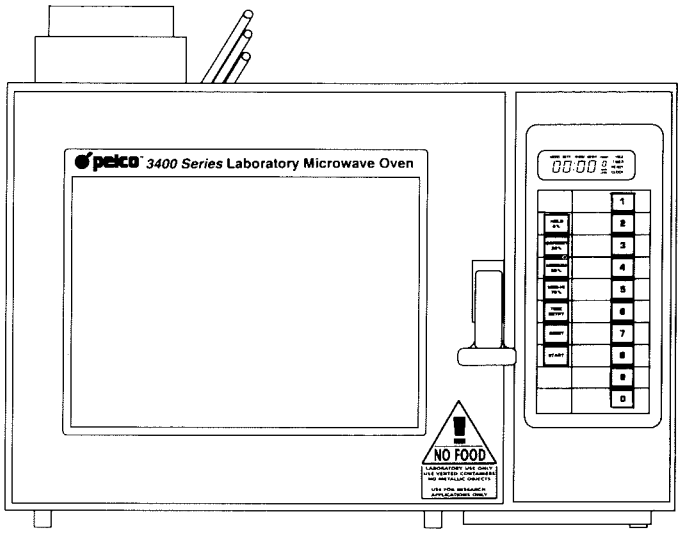

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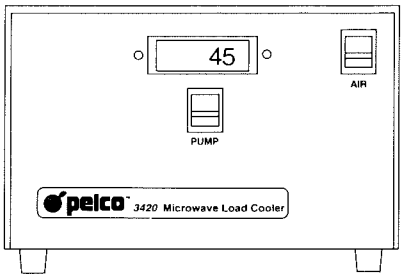

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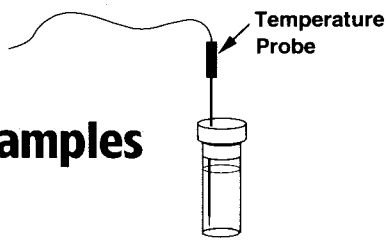

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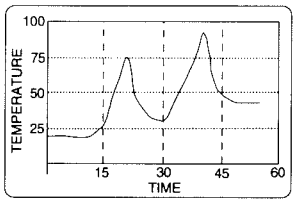

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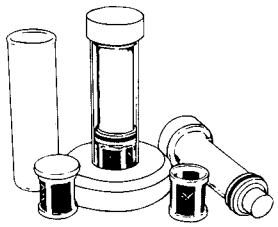

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ORGANIZATION OF HAIR CELL STEREOCILIA IN RODENTS AND ECHOLOCATING BATS. R. HALLWORTH* & N.K.R. SMITH+, Depts. of *Otolaryngology and +Cell and Structural Biology, University of Texas Health Science Center, San Antonio TX 78284.

Hair cells, the receptor cells of the inner ear, possess on their apical surfaces bundles of fine sensory projections called stereocilia. The mammalian cochlea has two kinds of hair cells. Inner hair cells (IHCs) have bundles of linearly-ordered stereocilia. Outer hair cells (OHCs) have bundles arranged in a 'V' pattern. Stereocilia are thought to contribute significant stiffness to the organ of Corti and may therefore influence the vibration pattern of the basilar membrane. The arrangements of stereocilia bundles are known to vary with hair cell type, species and cochlea place. However, these patterns have not been systematically described for any species.

Using a scanning electron microscope (JEOL JSM-35), we have examined stereocilia configurations of inner and outer hair cells in two unrelated species, the Mongolian gerbil (*Meriones unguiculatus*), a typical rodent, and the mustache bat (*Pteronotus parnellii*), an echolocating bat. Parameters measured were the dimensions of the stereociliary bundles, the number of rows of stereocilia and the number of cilia per row, the magnitude of the included angle in the OHC 'V' and the relative positions of OHC bundles with respect to each other. Parameters were examined as a function of cochlea place, which in turn governs the frequency to which the hair cell is most sensitive (the apex is most sensitive to low frequencies, while the base is most sensitive to high frequencies). The map of frequency to cochlear place is known for both species. Some parameters varied systematically with cochlea place. For example, OHC included angle was maximal apically in the bat, decreased and then increased as the location moved basally, and finally decreased at the base. In the gerbil, two maxima of included angle were observed, one mid-apical, the other mid-basal. However, no clear relation of any parameter with frequency was observed in either species. The included angle was observed to be a systematic function of cell size in both species. These findings diminish the possibility that hair cell stereociliary bundle geometry plays a significant role in cochlea tuning.

Supported by NIH(NIDCD).

DIGITAL ALTERNATIVES TO THE DARKROOM. R. Anderhalt, Philips Electronic Instruments, 85 McKee Drive, Mahwah, NJ 07430

To document and report results involving electron microscopy has historically involved the preparation of negatives and photographic prints in the darkroom. Today, more and more researchers are expressing a desire to eliminate much or all of the darkroom work in order to improve their working environment, their efficiency, or to minimize the disposal of photo-chemical wastes. The most obvious and viable alternative to the darkroom would be some form of digital image capture combined with a digital print. At this time, the digital process can not quite match the photographic print in the rendering of very fine image detail, but it offers many advantages that make digital imaging increasingly acceptable for the routine work in many EM facilities. Some of the digital advantages are continuous contrast control, midtone (or gamma) correction, digital dodge and burn functions, and digital filters.

Common and relatively cost-effective ways to capture digital images in the TEM would include at least the following: (1) the "grabbing" of images from a TV camera; (2) grabbing images with higher resolution, slow-scan cameras with resolutions of at least 1024x1024 with eight or more bits; or (3) scanning the already processed negative with an image scanner. Any of these techniques can be coupled with a digital printer.

The process of scanning and printing the negative will be demonstrated and is a hybrid analog/digital approach. This approach offers the highest final image quality and represents the only means to incorporate the image archive contained in hundreds or thousands of negatives from past years into a digital image database management system. The negative is still the highest resolution and most cost-effective image media available for the TEM.

Practical Aspects of Polarized Light Microscopy. D.C.N. CHAN, Department of Restorative Dentistry, UTHSC San Antonio, San Antonio, TX 78284.

Polarized light microscopy (PLM) has the advantage of assessing quantitatively and qualitatively the carious process. It allows the researcher to characterize, and evaluate sound enamel, carious enamel and experimentally treated carious enamel. The simplicity and usefulness of PLM in the study of enamel formation, structure, demineralization and remineralization is unsurpassed. This presentation will focus on the theory of operation and practical uses of PLM. Previous PLM study indicated that 20 applications of PREMA™ compound resulted in a seemingly highly mineralized dense outer enamel layer. The purpose of this study was to observe enamel microabraded surfaces under the PLM after various applications of PREMA™. The loss of enamel in manual vs mechanical method was also compared. 24 intact, freshly extracted maxillary incisors were randomly divided into 4 groups: 1) 5X; 2) 10X; 3) 15X and 4) 20X. Each group is further subdivided into manual (A) or mechanical (B) application. Round cavity preparations (~3mm) were made in the mesial or distal half on the facial surface and filled with Duralay™ to serve as reference point. In addition, 2 matrix band strips were fastened to the line angles with cyanoacrylic adhesive, half overlapping the Duralay™. The space between the two strips was at least as wide as the mandrel tip fitted in a 10:1 gear reduction handpiece. The compound was applied for 5 sec, rinsed with tap water for 10 sec, air dried and applications repeated. Manual applications were performed with a PREMA™ Hand Applicator. After the prescribed applications, the teeth were sectioned mesiodistally with an Isomet precision saw. Enamel loss were measured by computer image analysis. No difference is observed between manual and mechanical technique ($t_{0.975}$, $p>0.05$). The sections were further polished down to approximately 100 μm thickness or less with a series of aluminum oxide grits and examined using PLM. None of the sections showed the highly mineralized region. This observation was also confirmed radiographically. The dark superficial region reported before probably is an artifact due to the edge effect of thicker enamel sections.

THE DISEASE CYCLE OF *GAEUMANNOMYCES GRAMINIS* ON *CYNODON DACTYLON*. HOWARD J. ARNOTT AND MARY SUSAN HUFFINE, Department of Biology and Center for Electron Microscopy, University of Texas at Arlington, Arlington, TX 76019.

Light and scanning electron microscopy were used to study the disease cycle of *Gaeumannomyces graminis* variety *graminis* on *Cynodon dactylon* in vitro at the ultrastructural level. Seedlings and fungal inoculum were grown together in sterile chambers, maintained at room temperature, then fixed and prepared for microscopy at varying ages. Fungal hyphae grew from the inoculum square and contacted the seedlings. Following the inoculation stage of infection the mycelium grew and spread on the epidermal surface of all parts of the host and formed infection structures (appressoria) on the surfaces of the roots, stem, coleoptile, and leaves. Epidermal cells of the root, stem, and coleoptile were directly penetrated by distinctive forms of appressoria. After penetrating the surface of the root and stem, hyphae established infection within the cortex. Lignitubers were formed by the host cells in the cortical tissue. Lignitubers were tested for the presence of lignin using phloroglucinol. In all cases they tested negative. The eventual colonization of the seedlings occurred when hyphae progressed into the vascular tissue, sometimes almost filling various components of the vascular system. The death of the seedlings occurs when the stellar elements deteriorate.

CONVERSION OF *WANGIELLA DERMATITIDIS* THIN-WALLED YEASTS TO PHASE I YEAST CELLS: A MORPHOMETRIC AND STEREOLOGICAL STUDY. D.C. Garrett and A. S. Kester, Department of Biological Sciences, University of North Texas, Denton, TX 76203.

Morphometric and stereological analyses of the conversion of thin-walled yeast cells to thick-walled phase I yeast cells of *Wangiella dermatitidis* were conducted over 15 days of growth. Log phase growth persisted for 48 - 60 hrs and there was no loss in viability up to 15 days. Average cell volume dropped from 23 μm^3 on days 1 and 2 to 18 μm^3 on days 3 and 4, then increased to 38 μm^3 by day 15. Wide confidence limits for measurements of cell volume at each point indicated a very heterogenous population. Wall thickness increased throughout the growth period with actual thickness increasing from 0.04 μm to 0.30 μm and the volume fraction (Vv) from 0.10 to 0.24. Mitochondria and nuclei together made up less than 8 % of the cell volume (Vv mitochondria, 0.025 and Vv nuclei, 0.05). Both declined over time, as a result of increased cell volume from isotropic growth, although nuclei also may have become smaller. Glycogen was always present and increased to about 18% of the cell volume by day 4 but then decreased to 10% by day 15. Lipid was absent in early log phase cells but made up about 40% of the cell volume by day 15; and is presumably synthesized at the expense of glycogen and from residual sugar. Vacuoles, evident in early log cells (Vv 0.10 to 0.20), declined rapidly and were rare after 8 days.

BLEPHAROPLAST ARCHITECTURE OF THE MIDSTAGE SPERMATID OF LORENTZIELLA IMBRICATA (MITT.) BROTH. ANN E. RUSHING, Department of Biology, Baylor University, Waco, TX 76798.

The blepharoplast of the midstage spermatid of the moss Lorentziella imbricata is comprised of a 4-layered multilayered structure (MLS) and two dimorphic basal bodies that are staggered in position above the MLS. The upper layer of the MLS is the microtubular spline. The lower three layers form the lamellar strip (LS). The spline varies in width from 19-21 microtubules at its widest point. It retains nearly its maximum width throughout at least one-half gyre around the developing cell and then decreases in width to about 10-13 microtubules as it completes its first gyre. The anterior basal body (ABB) is approximately 1.6-1.8 μm in length and is situated to the right of the spline's midline just above the spline's closed aperture. The posterior basal body (PBB), about 6.0-6.5 μm in length, actually curves in front of the ABB in the anteriormost region of the blepharoplast. A divergent spline microtubule is associated posteriorly with the PBB in some but not all cells. The LS is approximately rhomboidal in shape, about 2.0-2.4 μm long and 0.6-0.7 μm wide. It is wider than the spline both anteriorly and on the right-hand margin. Although extensions of the LS beyond the width of the spline have been reported in a number of bryophytes, this type of extension, on the right-hand side, is not common. The overall aspect of the mid-stage spermatid in L. imbricata is similar to that seen in the few other mosses that have been studied but the architecture of the spermatid is different from both liverworts and hornworts. In comparison to Funaria hygrometrica, the most closely related moss (in current taxonomic schemes) for which data are available, L. imbricata has a wider spline and an LS of quite different shape but is similar in characteristics of the basal bodies.

A COMPARISON OF AFM, SEM AND OPTICAL MICROSCOPE IMAGES OF VLC GLASS IONOMER BONDED TO HUMAN DENTIN. G. T. Knight, Department of Restorative Dentistry, The University of Texas Health Science Center, San Antonio, TX 78284-7890.

Currently, the most commonly used method of imaging the interface of a dental material with tooth structure is the Scanning Electron Microscope. Using this system, specimens must be desiccated and sputter coated prior to imaging. These procedures contribute to debonding or an alteration of the bond between the restorative material and the tooth. The atomic Force Microscope (AFM) images a specimen that has been highly polished without desiccation or sputter coating which ensures an intact bonded surface. This study examined the interface between a light-cured glass ionomer restorative material (Fuji II LC; GC Corporation; Tokyo, Japan) and a freshly prepared dentin surface to determine the effectiveness of AFM as an imaging technique. A 3x6 mm cervical class 5 cavity preparation was made on the facial surface of freshly extracted teeth. Each preparation was placed approximately 1.5 mm into dentin using a #56 carbide bur in an ultraspeed handpiece with water spray. The preparations were treated with GC Dentin Conditioner (GC Corporation) for 20 seconds, rinsed for 15 seconds and subjected to light air flow to remove excess moisture. Fuji II LC was mixed according to manufacturer's directions, applied into each preparation using an applicator syringe and light cured for 60 seconds. The teeth were sectioned longitudinally on a hard tissue microtome to produce two sections through each restoration. The sections were imbedded in an autopolymerizing resin and polished on a polishing machine. Imaging of the intact glass ionomer/dentin interface was accomplished using an Atomic Force Microscope. Images were obtained at 7,000x, 30,000x and 152,000x magnification. Results of atomic force microscope imaging of the Fuji II LC light-cured glass ionomer/dentin interface reveal that Atomic Force Microscopy presents a viable option for imaging the resin dentin interface at a molecular level.

CONFIRMATION BY SCANNING ELECTRON MICROSCOPY OF CECAL MUCOSAL EPITHELIUM COLONIZATION IN NEWLY HATCHED CHICKS TREATED WITH A CHARACTERIZED CONTINUOUS FLOW COMPETITIVE EXCLUSION CULTURE OF AVIAN CECAL BACTERIA. R.E. Droleskey, D.E. Corrier, D.J. Nisbet, and J.R. DeLoach. USDA/ARS, Food Animal Protection Laboratory, 2881 F&B Road, College Station, TX 77845

Early colonization of chick ceca by indigenous microflora has been proven to aid significantly in the prevention of chick ceca colonization by enteric pathogens such as *Salmonella*. Postulated mechanisms for the protective effects of indigenous microflora include the competition for binding sites on the cecal mucosal epithelium between microflora and pathogens. To determine if the previously documented protective effects resulting from the administration of a continuous flow (CF) culture of indigenous microflora was due in part to the early establishment of cecal microflora colonization of the cecal mucosal epithelium, ceca from three day old chicks administered a CF culture on day-of-hatch, and ceca from uninoculated control birds, were examined by scanning electron microscopy. Extensive colonization was noted in the ceca of CF treated chicks, with large colonies of bacteria predominately located within and between adjacent cecal crypts. In contrast, cecal crypts from control chicks were relatively uncolonized by bacteria, with only thin strands of a fibrous material containing bacteria and digesta evident. Individual and clumped bacteria bound to the mucosal epithelium were observed in both CF treated and control chicks. Colonization by the CF culture bacteria was accompanied by an increase in the concentrations of volatile fatty acids, especially propionic acid, in the cecal contents with respect to controls.

NANOMETER-SCALE SURFACE FEATURES OF OTOCONIA OBSERVED WITH THE ATOMIC FORCE MICROSCOPE. R. HALLWORTH, M.L. WIEDERHOLD, J.B. CAMPBELL, & P.S. STEYGER, Dept. of Otolaryngology, University of Texas Health Science Center, San Antonio TX 78284, and Southwest Research Institute, San Antonio TX 78228.

Otoconia are protein-mineral complexes, in the form of crystallized calcium carbonate, that are found in the inner ear of terrestrial vertebrates. They act as mass loading for the gravimetric and linear acceleration-sensing organs, the utricle and saccule. In amphibia, otoconia from the utricle and saccule have different crystal types (calcite and aragonite) and different morphologies (barrel-shaped in the utricle; mainly prismatic in the saccule).

The nature of the interaction between protein and mineral in otoconia is unknown. From electron diffraction studies, utricular otoconia are thought to be composed of small (100 nm or less) perfect microcrystals between which protein is intercalated. We have examined otoconia from the Japanese red-bellied newt (*Cynops pyrrhogaster*) using an atomic force microscope (Digital Instruments Nanoscope II using SiN and epitaxial Si tips). The use of the newt preparation allowed us to compare otoconia of different morphologies and mineral phase. Otoconia were dissectedby aspiration from the animal after decapitation, rinsed in distilled water, air dried on metal-coated glass coverslips and were examined in air. The surfaces of both otoconial polymorphs consisted of small (approx. 50 nm) round elements that were usually tiled but sometimes overlapped. Despite their different gross morphologies, little difference was seen in element size and form between the otoconial types, although in some utricular otoconia, the elements were irregular in shape or rectangular. The elements may correspond to the microcrystals suggested by the electron diffraction studies. Implications for the method of otoconial synthesis are discussed.

Supported by NIH(NIDCD), NASA and the VA Medical Research Fund.

MATERIALS SCIENCES

PLATFORM PRESENTATION — FALL 1994

A STREAMLINED PROCEDURE FOR ACQUISITION AND MEASUREMENT OF ELECTRON DIFFRACTION PATTERNS. D.C. DUFNER AND J.M. EHRMAN*, Electron Microscopy Center, Texas A&M University, College Station, TX 77843. *Mount Allison University, Sackville, New Brunswick E0A 3C0 Canada.

Electron diffraction (ED) is one of the most widely used techniques in the characterization of materials in the TEM. There are a number of computer programs used for measuring, indexing, and simulating electron diffraction patterns (EDPs), but many are stand-alone programs and require significant user interaction.

In this work, we have been working on a more streamlined approach in acquiring, saving, and measuring EDPs on a PC-based computer system. A TV camera on a TEM is used to collect EDPs in the form of either live images or images recorded on a videocassette tape. When a pattern is selected, it is saved, and a macro sequence calls up ImagePro Plus (Media Cybernetics, Silver Spring, MD) to measure the pattern. ImagePro Plus is used to determine the centroid positions of the transmitted and diffracted beam spots, and a set of d-spacings is calculated from the measured distances between the spots. A window can be fixed in a noninteractive mode or specified interactively by the user to select the number of spots to be measured.

This procedure has also been used to measure EDPs scanned from contact prints, provided that a suitable calibration pattern is included to calibrate the measurement facilities in the program. The program is very useful for analyzing a large number of EDPs obtained from well-populated sample grids such as those containing thin films and dispersed particulate materials.

CONTINUED ON NEXT COLUMN ►

TRANSMISSION ELECTRON MICROSCOPY OF Ag-Sn-Cu DENTAL AMALGAMS. T. K. Hooghan, R.F. Pinizzotto, J. H. Watkins, Center for Materials Characterization, University of North Texas, Denton, Tx 76203

The strengthening effects of Cu and Zn additions to dental amalgams are not fully understood because there is incomplete knowledge of the microstructure. The traditional techniques of X-ray diffraction (XRD) and scanning electron microscopy (SEM) both lack the necessary special resolution and leave ambiguities in amalgams phase identification . A problem with XRD is that the γ–Ag_3Sn and β_1-Ag_3Hg_2 phases have the same principle X-ray peaks. TEM has only been attempted once before on amalgams because of sample preparation difficulties. We have successfully used the "wedge technique", developed for semiconductor TEM, to prepare TEM amalgam samples. A combination of microdiffraction , x-ray energy Dispersive spectroscopy and selected area diffraction have been used for phase identification . The phase confirmed in low-Cu "Velvalloy" are γ, γ_1-Ag_2Hg_3, η– Cu_6Sn_5, β_1, and γ_2-$HgSn_9$ while high Cu "Tytin" contains γ, γ_1 and η only. The goal of this research is to characterize the micro structures of these amalgams to elucidate their strengthening mechanisms.

Stoichiometry and deposition-temperature dependence of microstructural and electric properties of BST thin films, G. Wang, P. Peña, Y.G. Rho, E.G. Jacobs, R.F. Pinizzotto, and S. Bilodeau*. Center for Materials Characterization, UNT, Denton,TX 76203. *ATMI, Danbury, CT.

The effect of stoichiometry and deposition temperature on the microstructures and electrical properties of barium strontium titanate (BST) thin films were studied. BST was deposited on platinum (Pt/ZrO_2) coated substrates using MOCVD. One set of samples was deposited using various group II (Br, Sr) to Ti ratios ranging from 0.658 to 1.022. Another set of samples was deposited at temperatures between 550°C to 700°C. The samples were characterized using conventional plane view TEM.

A perovskite phase and an amorphous phase were observed in all of the samples. The average grain size of the BST was not affected by the group II/Ti ratio, ranging about 22 to 30 nm. The volume fraction of the amorphous phase decreased with decreasing Ti content. The dielectric constant and the capacitance per area of the BST films increase as the group II /Ti ratio increased to 1.

The perovskite phase and the amorphous phase have also been observed for various deposition temperatures. The average grain size of the perovskite phase increased from 15- 26 nm as the temperature increased. Qualitatively, the amount of the amorphous phase decreased with increasing deposition temperature. The dielectric constant was found to increase, and the leakage was found to decrease with increasing deposition temperature. In conclusion, the dielectric constant of BST thin films increases upon a decrease in the volume fraction of the amorphous phase. Such decrease of the amorphous phase can be caused by varying stoichiometry or deposition temperature.

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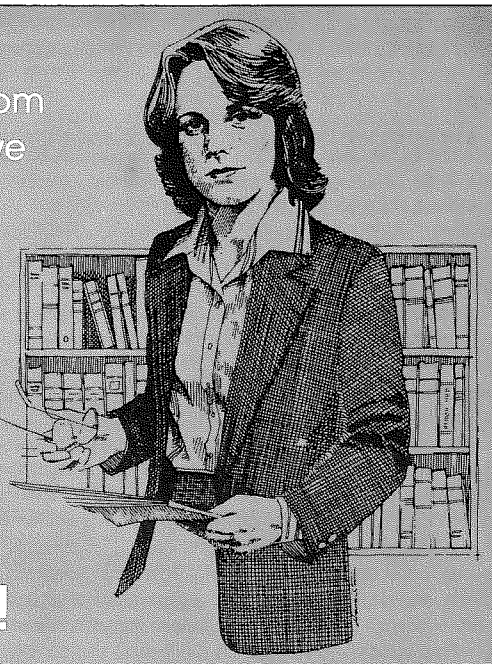

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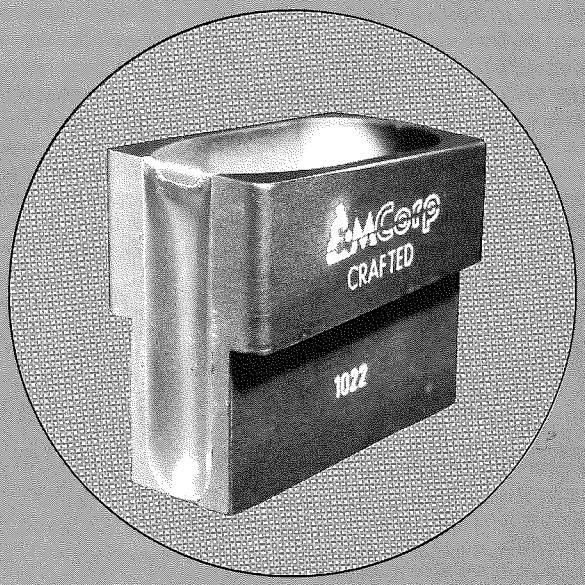

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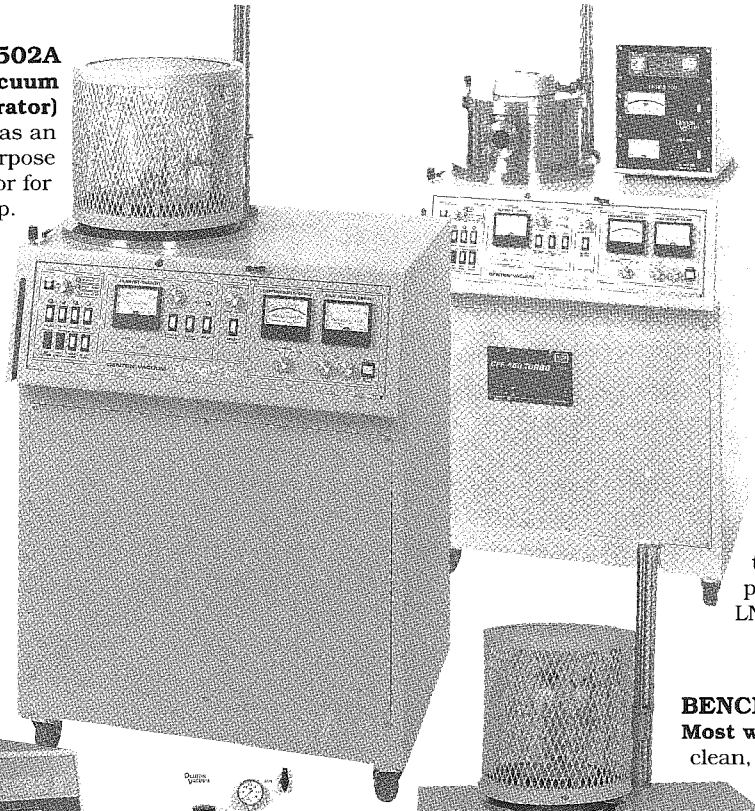

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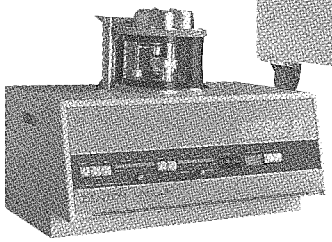

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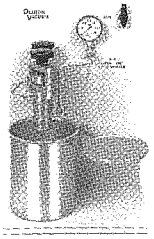

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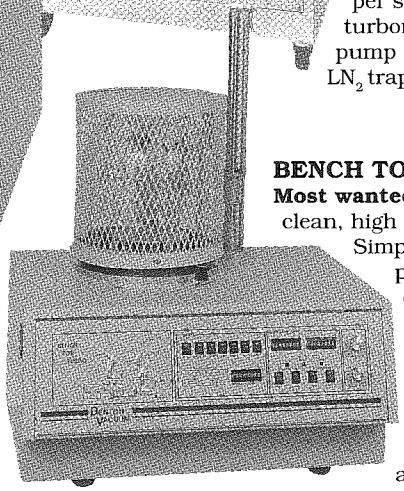

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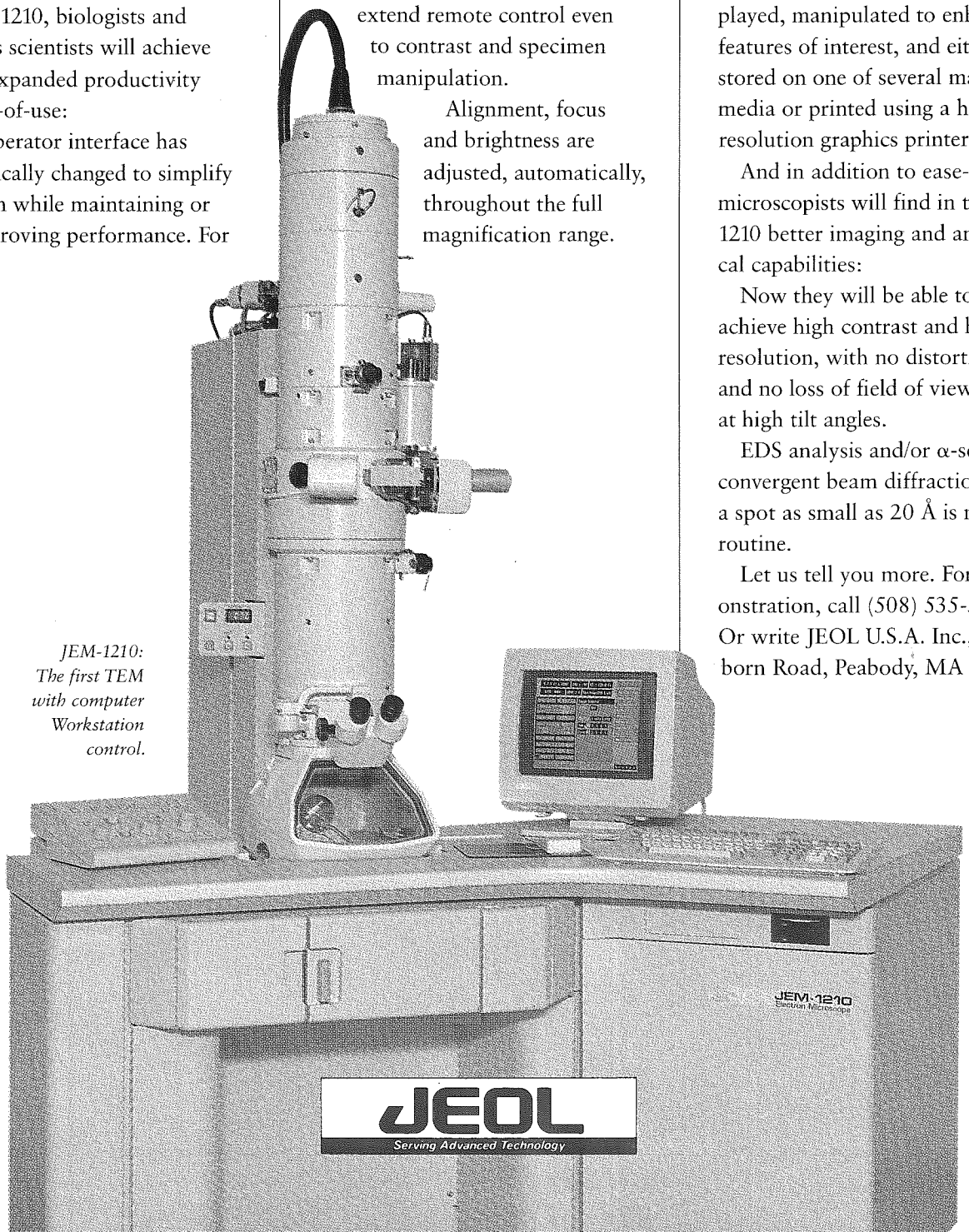

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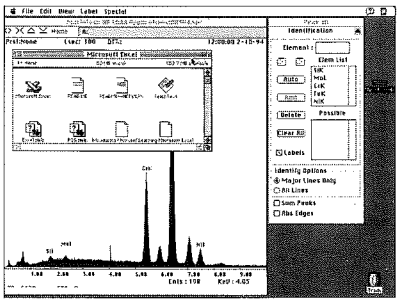

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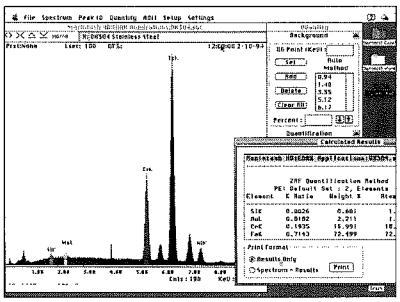

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Photo by Howard J. Arnott, Dept. of Biology, University of Texas at Arlington, TX. Bar = 1μm.