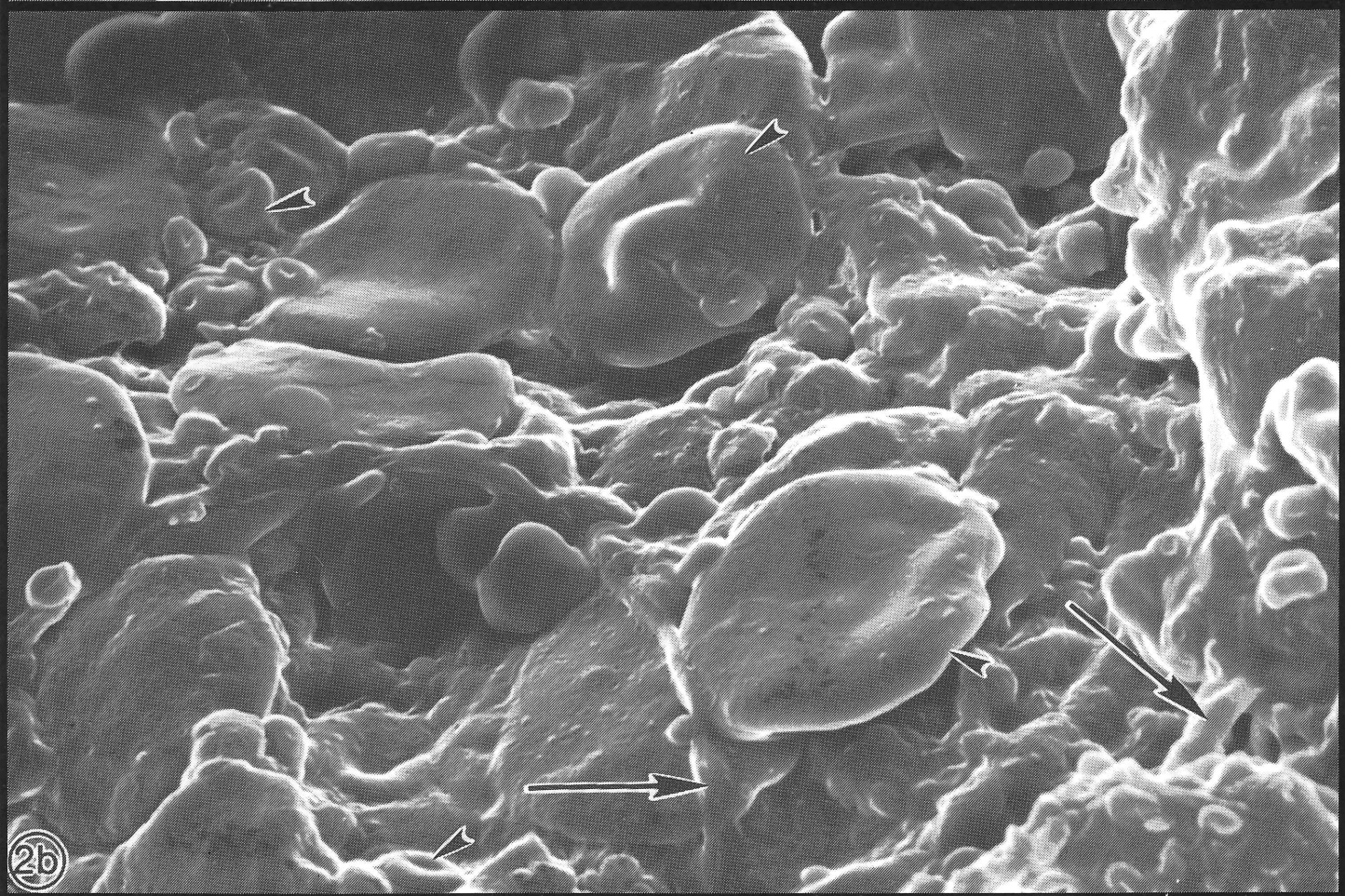


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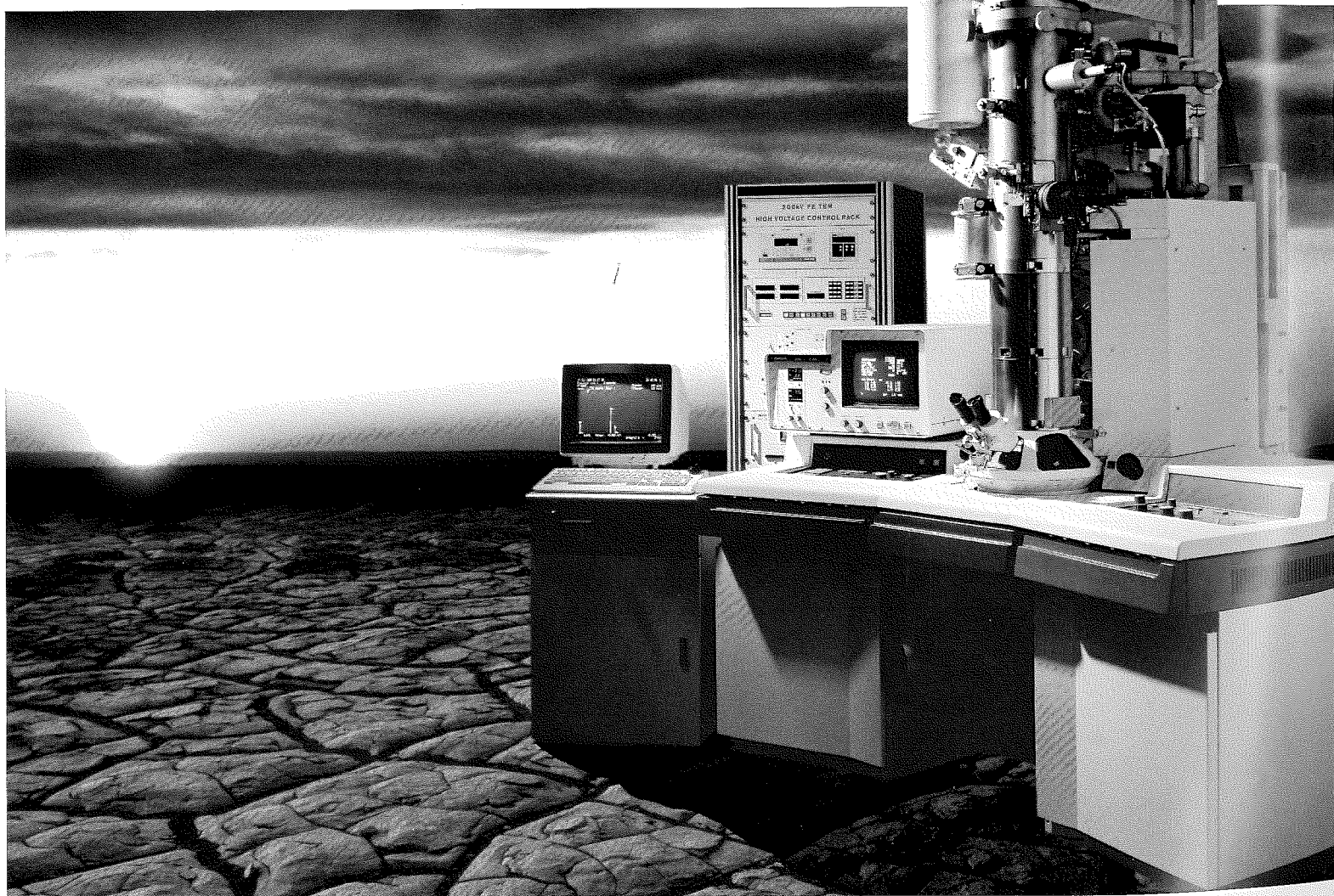
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David C. Garrett, Editor

Department of Biological Sciences, University of North Texas, Denton, TX 76203

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ON THE COVER

Environmental (top) and traditional (bottom) scanning electron micrographs of fresh wheat tortilla dough. Magnification = 980X and 1160X respectively (Arrowheads = starch granules, arrows = gluten network). Cover courtesy of Cassandra McDonough, Texas A&M University, Soil and Crop Sciences Department, College Station, TX 77843

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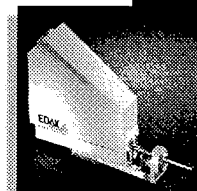
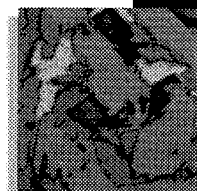
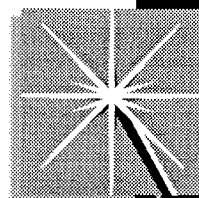
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Hitachi	2 & 24		

President's Message

The Fall meeting in Austin was a successful one - thanks to the planning by President Elect Mitch McCartney who doubled as Program Chairman for that meeting. Those attending the workshop conducted by Malcom Brown had an opportunity to see his research facilities as well as obtain some useful information on atomic and molecular imaging. Our invited speaker, Ms. Cathy Thomas from the Johnson Space Center, gave an interesting talk on "Interplanetary Dust Particles".

The number of papers presented at the past several meetings as well as attendance seems to be minimal or less for the large membership of our organization. Those associated with the medical and dental fields have dropped considerably. I would encourage all of you to become more active in TSEM not only by attending the meetings but by presenting your research findings. I would also encourage you to have your students present their findings at these twice yearly get-togethers as several of you have. The papers and posters that are given at our meetings are equal or better than those at national meetings. It is good to see an increase in the physical science presentations. As you well know the future of TSEM lies in an active membership. Our organization's worthiness and strength depends on you! We can learn a great deal from your techniques and your results if you would only share these with us.

Dr. Joe Dixon, our current Program Chairman has worked hard to make a success for the Spring '96 meeting at College Station. All of the workshops are free to the registered members and guests, so plan to attend one or more of your choice. The corporate members and sponsors would like to visit with each of you at these meetings not only at the Thursday night social but at the breaks during the day. They can bring you up-to-date on their latest products that can help in your researched activities. The attendance at the Friday night banquet seems to be lacking. I would encourage you to attend this also. There is a business meeting held after you've eaten so that you can get the latest information from your executive members to let you know what is going on in TSEM.

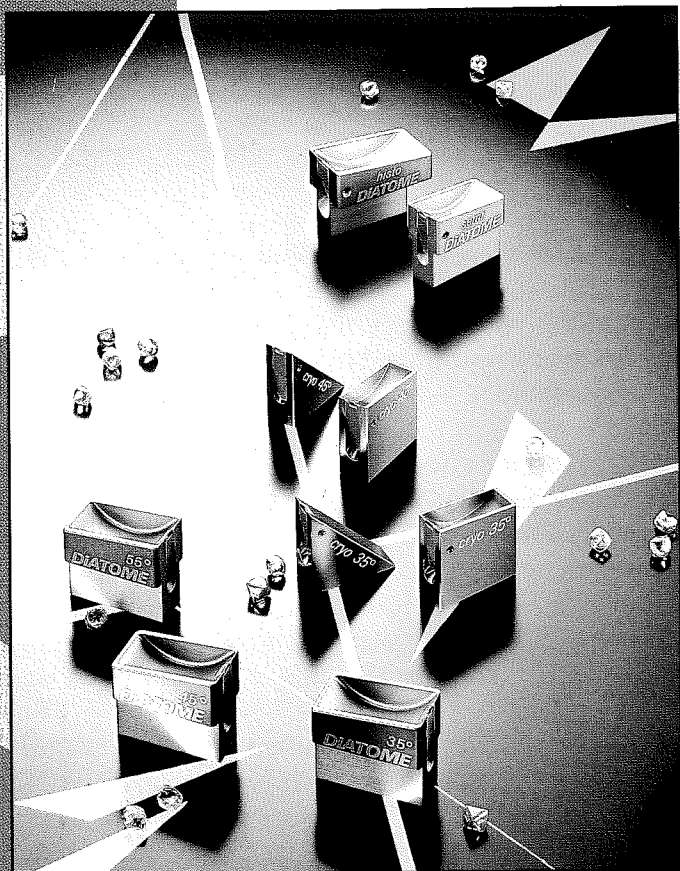
The Fall '96 meeting will be at the Nassau Hilton in Clear Lake, October 24-26. The last meeting we had there in 1991 was a successful one. Start making preparations to attend and to present a paper and/or a poster. Encourage your students to do the same.

Thanks to the efforts of our Journal Editor David Garrett, we have had some published articles in our refereed journal. Consider publishing your findings in the upcoming issues. Also note the new name of the journal, TEXAS JOURNAL OF MICROSCOPY. Encourage your microscopy friends to join our organization and publish their findings in the journal as well.

As my term being your President comes to an end I want to thank the membership for having given me the opportunity. It has been a pleasure working with and for you in this learning experience. All of your officers have been so willing to put forth the extra effort which is required to make it all worthwhile. They have worked very hard! The present and incoming officers are listed in the front of this journal. Become familiar with them not only in name but through visiting with them during the meetings. They all want feedback from the membership as to the positives and negatives of TSEM so they can help to make this a stronger organization. I know that you will give them the needed support as you have in the past. After all, it's only through our communications that this organization will continue to be a great success.

Sincerely,

Louis H. Bragg
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ASSETS ON JANUARY 1, 1995:

Certificate of Deposit No. 1882289323 (Formerly C.D. No. 113515)	\$4,079.37
Checking Account No. 70072962	\$4,319.51
TOTAL	\$8,398.88

CHECKING ACCOUNT RECEIPTS:

Dues	\$3,271.00
Spring 1995: Meeting Registration	\$1,130.00
Workshop	\$265.00
Exhibitors	\$650.00
Donations and Grants	\$1,150.00
Guests	\$50.00
Fall 1995: Meeting Registration	\$860.00
Workshop	\$255.00
Exhibitors	\$450.00
Donations and Grants	\$275.00
Guests	\$130.00
Journal Advertisements: 25:2	\$115.00
26:1	\$1,750.00
26:2	\$1,925.00
Checking Account Interest (Account No. 1882774506)	\$64.47
Checking Account Interest (Account No. 70072962)	\$48.45
Tax Refund	\$858.15
TOTAL	\$13,247.07
Rollover Interest on Certificate of Deposit No. 1882289323	\$278.31

EXPENSES:

Journal Advertisement: 26:1	\$2,091.80
26:2	\$2,313.14
Office Expenses	\$181.72
Mailouts	\$1,150.00
Spring 1995: Meeting	\$2,799.83
Workshop	\$100.00
Student Competition/Travel	\$286.00
Invited Speaker	\$118.00
Refreshments	\$13.33
Legal Fees	\$260.00
Fall 1995: Meeting	\$2,014.92
Student travel	\$510.00
Miscellaneous	\$455.87
TOTAL	\$12,294.61

ASSETS AS OF DECEMBER 31, 1995	\$9,624.65
Certificate of Deposit No. 1882289323	\$4,079.37
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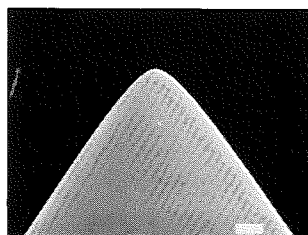
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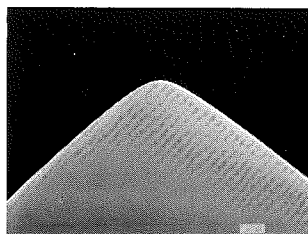
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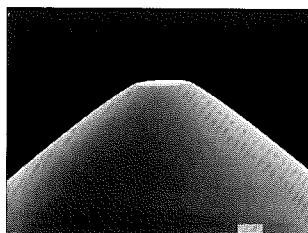
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ROUND TIP



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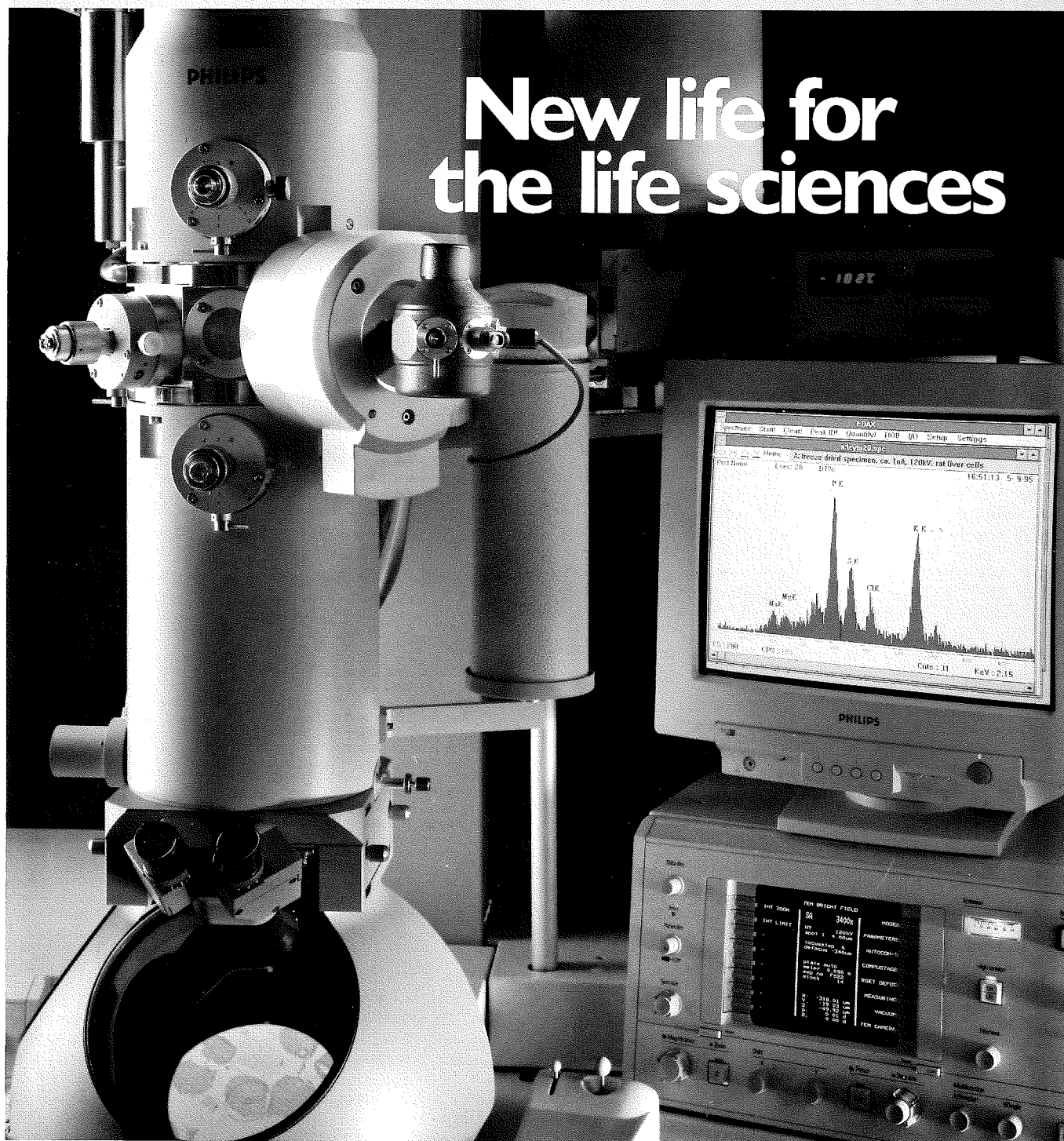
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Abstracts

INVITED SPEAKER

MICROSCOPY TECHNIQUES USED TO DETERMINE THE MICROSTRUCTURE OF STARCH-BASED FOOD SYSTEMS. C.M. MCDONOUGH and L.W. Rooney, Cereal Quality Lab, Soil and Crop Sciences, Texas A&M University, College Station, TX 77843-2474.

The environmental SEM (ESEM) allows wet or oily samples to be viewed in their natural state. It is ideally suited for the study of food systems, but is under-utilized in food research areas. The ESEM can document the size and shape of the starch granules in cooked products at various stages during processing. Starch functionality has been assessed in many industrial food processing situations, including steaming (steam-flaked sorghum, cous cous), baking (bread, pretzels, wheat and corn tortillas), and frying (tortilla chips, potato chips). The mode of oil entry into fried snack products, such as tortilla chips, potato chips, and extrudates has been documented. Oil circulation within the product, made visible by the electron beam of the ESEM, provides clues about the internal structure, however, when the surface of the food product is coated in a thick layer of oil, other microscopy techniques may be required to ascertain the internal structure. After defatting and vacuum drying, samples can be viewed in the traditional SEM to further document the internal structure. Birefringence is used when needed to validate the extent of gelatinization in a product. The ESEM is a flexible and efficient tool to use in troubleshooting food processing problems and documenting real-time structure of a product during steaming, baking, or frying.

MATERIALS SCIENCES

PLATFORM PRESENTATION—SPRING 1996

ANALYTICAL TRANSMISSION ELECTRON MICROSCOPY OF HIGH COPPER AMALGAM. TEJPAL KAUR HOOGHAN, RUSSELL F. PINIZZOTTO, JOHN H. WATKINS,* AND TORU OKABE.* Materials Science Department, University of North Texas, Denton, TX 76203, *Department of Biomaterials Science, Baylor College of Dentistry, Dallas, TX 75246.

Analytical transmission electron microscopy (TEM) of high Cu dental amalgam has been done in a continuation of our microstructural study of Ag-Sn-Hg ternary amalgams. The structures of Ag dental amalgam have been studied extensively using optical, x-ray and scanning electron microscopy (SEM). Our previous TEM study of a low copper amalgam (Velvalloy), which is the simplest material among the amalgams, revealed the very fine details of the microstructure. Characterization of a more complex amalgam, e.g., high Cu, is the next step in understanding the amalgamation reaction and the effect of alloying elements. The improved properties of high Cu amalgam may be explained by identifying microstructural features with the superior resolution of TEM. Samples of "Tylin," a high Cu amalgam, were prepared with modified wedge mechanical polishing. All phases were identified using selected area diffraction (SAD) and μ diffraction (μ ED) with x-ray energy dispersive spectroscopy (XEDS), and are consistent with the previously reported results. Bright/dark field analysis of unreacted particles shows a microcrystalline structure consisting of mainly Ag_3Sn (γ) with fine precipitates of Cu_2Sn (ϵ). Unlike Velvalloy, it has a matrix only of polycrystalline Ag_2Hg_3 (γ_1) grains. No evidence of HgSn_{7-9} (γ_2) is found in the microstructure. Fine rod shaped precipitates of Cu_6Sn_5 (η') are embedded inside the large Ag_2Hg_3 (γ_1) grains and at the grain boundaries. Occasionally, Ag-Hg (β_1) is also found in the matrix. Reaction layers are not observed around the unreacted alloy particles. Ag-Hg-Sn (β_1) and larger Cu_6Sn_5 (η') grains are found primarily next to the unreacted particles. Relatively smaller Ag_2Hg_3 (γ_1) grains are between γ and γ_1 . Large grains of Cu_6Sn_5 (η') are often found with internal structure. The overall goal of this research is to explain the clinical properties of high Cu, Zn or In containing amalgams based on their microstructures.

TRENDS IN SINGLE-EDGED AND DOUBLE-EDGED KNIFE CUTS ON 100% COTTON FABRIC. M. Daniel Matteo and David C. Garrett. Department of Biological Sciences, University of North Texas, TX 76203.

Forensic studies examining fiber damage are useful for determining the source of original textile damage. Cuts can be differentiated from tears, foreign object punctures separated from animal bites and knife cuts from scissor cuts. In this study, certain characteristics were found to be consistent with the particular type of knife used (single- or double-edged). By establishing specific trends, according to knife type, a protocol was established to help in the identification of the type of knife used to stab a 100% cotton T-shirt under controlled conditions.

Single-edged knives tended to produce a "T" or "O" shaped opening with lateral stretching and long fibers at the initial entry point. The terminal end of a single-edged knife stab produced a "V" shape with short fibers. The double-edged knife stab did not ordinarily show a visible initial entry point. There was lateral spread of the fabric along the length of the cut with short fibers and a characteristic "V" shape at both ends of the cut.

Using the protocol established, 15 participants were given minimal training in recognizing the trends exhibited by single- versus double-edged knife stabs and then asked to determine the type of blade used in each of 15 randomly ordered photographs. Each group of photographs consisted of 5 double-edged, 5 single-edged and 5 serrated single-edged stabs. Respondents correctly predicted the blade used 89% of the time. Incorrect responses were then examined in the scanning electron microscope (SEM) for specific trends evident in the failure of individual fibers and fabric. The SEM proved to be a valuable tool to correctly differentiate many of the more obscure cuts.

MICROSTRUCTURAL ASPECTS OF HYPERVELOCITY IMPACT CRATERING AND JETTING IN COPPER. ERICA P. GARCIA, L. E. MURR, E. FERREYRA T., C-S. NIOU, J. M. RIVAS, and STELLA A. QUINONES. Department of Metallurgical and Materials Engineering, The University of Texas at El Paso, El Paso, Texas 79968 USA

There have been numerous investigations concerned with impact cratering and its effects on microstructural evolution. In the past, research has been focused on microstructural changes near and below the crater wall. This study is concerned with the jetting associated with hypervelocity impact cratering and its residual microstructures. Two different copper targets with grain sizes of 38 μm and 763 μm were impacted with an 1100-Al sphere and a soda-lime glass sphere, respectively. The copper targets were impacted at velocities near 6 km/s. The jetting associated with these two craters was very different. The larger grain target exhibited greater plastic flow and a larger rim size. Optical metallography and transmission electron microscopy revealed no significant melt related phenomena in the rim of either crater. The jetting into the rim, and that along the crater wall, were characterized by a flow zone consisting entirely of dynamically recrystallized grains refined by a factor of 10^3 from the undeformed target. Consequently, crater-related jetting is identical to shaped charge jetting, and is a solid-state plastic flow phenomenon. Supported by a NASA-Johnson Space Center Grant NAG-9-481.

OBLIQUE SHOCK LOADING OF COPPER RODS WITH DIFFERENT GRAIN SIZES. J.C. SANCHEZ, L.E. MURR, AND K.P. STAUDHAMMER*. Department of Metallurgical and Materials Engineering, The University of Texas at El Paso, El Paso, TX 79968; *Los Alamos National Laboratory, Los Alamos, NM 87545

Studies involving high strain, high strain rate phenomena such as impact cratering and shaped charges have received a great deal of attention in recent times. The results of such experiments have, for the most part, been analyzed using planar shock wave assumptions. In reality, an oblique shock wave may be a closer approximation. In this study high purity rods in a range of grain sizes, 29 μm , 141 μm and 375 μm , were shock loaded in an oblique cylindrical assembly. The shock loading allowed for peak pressures ranging from 11 to 30 GPa as well as strain from zero to about five percent. Light metallography and transmission electron microscopy revealed deformation twinning as a function of the grain size. In addition to the deformation twinning, microbands were observed coincident with traces of $\{111\}$ planes and intermixed with the twins. The grain size and associated pressure seem to have a direct relationship with microband formation and appearance. The obliquity of the shock wave seems to suppress the critical twinning pressure of copper, estimated to be 20 GPa, since profuse twinning was observed at pressures of only 11 GPa. This research was supported in part by a Patricia Roberts Harris Fellowship (J.C.S.) and a Mr. and Mrs. Macintosh Murchison Endowed Chair (L.E.M.).

MICROSTRUCTURAL FEATURES OF SOME PENETRATOR MATERIALS. CHRISTINE KENNEDY, S. PAPPU, C- S. NIOU, AND L. E. MURR. Department of Metallurgical and Materials Engineering, The University of Texas at El Paso, El Paso, TX 79968.

Recently, much interest has been generated in the use of Ta, Ta-W and W-HfC for penetrator applications, such as shaped charges and explosively formed penetrators (EFPs). These materials have high density and exhibit high-temperature properties. Optical and transmission electron microscopy (TEM) have been employed to observe distinct microstructural features of annealed and shock-loaded Ta and Ta-2.5W, as well as an experimental, mechanically alloyed W-HfC. Some observed features include twinning in shock-loaded Ta and Ta-2.5W, and the dispersion of non-coherent HfC particles within the W matrix. This work was supported by the U.S. Army, ARDEC, Picatinny Arsenal, NJ under contract DAAA21-94-C-0059.

MORPHOLOGY OF WATER DISPERSIBLE SAMPLES FROM AGRICULTURAL SOILS OF THE SOUTHEASTERN UNITED STATES. G. N. WHITE AND J. B. DIXON, Dept. of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843-2474.

As a part of the regional investigation on water dispersible clays, the sand fractions of 33 regional samples which had been water dispersed only were examined to determine the morphology of the undispersed clays, organic material and interesting features of the sand fraction.

Organic material appeared showing some features of the plants from which they were formed, as thin sheets or filaments and as pollen grains. The organic material did not appear to be the cement holding the aggregates.

Many of the soils had aggregates of clay-sized material. These aggregates often appeared in a compact form suggesting that dispersion had failed. Other clay material stayed in the sand fraction as coatings of sand particles. Some clay was also present in the sand fraction as lithorelicts.

Quartz was found in a variety of morphologies. Plant opals were also common. Volcanic glass was found in soils derived from volcanoes. Other minerals observed were kyanite, ilmenite, gibbsite, TiO_2 , CaCO_3 , apatite, Fe oxides, kaolinite, mica and serpentine.

UNREACTIVE ORGANIC MATERIAL FROM A HIGHLY WEATHERED BRAZILIAN ALFISOL. S. R. TEIXEIRA, G. N. WHITE and J. B. DIXON, Soil and Crop Sciences Dept., Texas A&M University, College Station, TX 77843-2474

The morphology and composition of a hard black material obtained from the sand fraction of one tropical soil (Alfisol) was studied by x-ray diffraction, and optical and scanning electron microscopy (SEM). The sample was collected from the B22 horizon (100 to 160 cm depth) in a sandy clay loam dark red (2.5 YR 4/6) soil in Presidente Prudente County, São Paulo State, Brazil. This material which at first glance can be confused with tourmaline contains grains with tunnels, like bamboo, with variable diameter ($< 15 \mu\text{m}$) in two perpendicular directions. Iron oxide (hematite) and quartz grains were observed by optical microscopy inside these tunnels and confirmed by SEM/EDS (energy dispersive spectroscopy). The black material was determined by EDS to be composed of organic matter (carbon, oxygen, sodium and calcium). The x-ray data is characteristic of non-crystalline material with a broad peak near $21^\circ 2\theta$. The grains have a density near that of hydrogen peroxide and they are not reactive to it. They were identified as partially decomposed organic material. S. R. Teixeira is a visiting researcher partially supported by CAPES-Brazil.

KAOLIN MINERALOGY OF A RED BRAZILIAN OXISOL. S. R. TEIXEIRA, J. B. DIXON and G. N. WHITE, Soil and Crop Sciences Dept., Texas A&M University, College Station, TX 77843-2474

The mineralogy of one Oxisol from Presidente Prudente County, São Paulo State, Brazil, was investigated. The sample was collected in the B23 horizon (80 to 150 cm depth) in a loamy sand red (2.5 YR 4/8) soil and was analyzed by x-ray diffraction, and scanning and transmission electron microscopy (SEM and TEM). The x-ray diffraction patterns of the fine and coarse clays indicate that kaolinite, iron oxides and quartz are the principal phases present in this soil. The presence of the halloysite is indicated by the presence of the 0.44 nm x-ray peak and by TEM analyses. The microscopy results show the presence of titanium oxide (anatase), stacks of kaolinite flakes, plates of hexagonal shape and thick laths. Beside the lath shapes some curved grains and layer separation were observed too by the TEM suggesting halloysite. It was observed that this material is very unstable under the electron beam. This very kaolinitic soil clay has the unusual combination of well formed thick kaolinite crystals and curved disorderly particles that resemble halloysite. S. R. Teixeira is a visiting researcher partially supported by CAPES-Brazil.

MATERIALS SCIENCES

POSTER PRESENTATION—SPRING 1996

MORPHOLOGY OF SYNTHETIC APATITES. B. Sutter¹, L.R. Hossner¹ and D.W. Ming². ¹Dept. of Soil and Crop Sci., Texas A&M University, College Station, TX 77843. ²NASA, Johnson Space Center, Houston, TX 77058.

Research is being conducted to develop synthetic apatites as a component of a plant growth medium that will provide sufficient nutrients for crops as part of NASA's Advanced Life Support System. The objective of this study was to determine the morphologies and sizes of a synthetic apatite and four synthetic apatites, each containing one of four metals (Fe, Mn, Cu, and Zn). The results obtained in this study will assist in providing information on the mechanism of metal release during synthetic apatite dissolution.

X-ray diffraction analysis confirmed the apatite structure. Metal concentrations were determined by instrumental neutron activation analysis to be 1.2 wt. % Fe, 0.05 wt. % Mn, 0.005 wt. % Cu and 0.04 wt. % Zn. A JEOL 2010 transmission electron microscope (200kV, mag 40K \times 1.0M) was utilized to study crystal size and morphology.

All apatite crystals had lath-like morphology as well as the appearance of laths fused together. All apatite crystals possessed multiple circular and elongated pits. The apatite laths were found to be as long as 110 nm and as wide as 25 nm. The size of the fused laths were difficult to measure because they possessed no definite shape. The elongated pits were as long as 22 nm and as wide as 5 nm. Many of the pits were circular and had diameters up to 6 nm. The Fe containing apatites had pit sizes that were typically smaller (e.g., largest observed pit 10 nm \times 4.5 nm) than the pits found on the other apatites. Because the Fe-apatites had smaller pits, the morphology had a smoother appearance than the other apatites. The higher Fe concentration apparently had an effect on apatite morphology.

Some of the apatite laths had notches on the sides and ends. When the crystal stopped growing, the pits on the sides and ends did not close and notches were formed. It has been well documented that mineral dissolution sites occur at edges. Pits and notches may provide edges that could serve as sites for apatite dissolution.

(409) 845-3682

URIC ACID DIHYDRATE IN ATMOSPHERIC DUST COLLECTED IN NIGER, WEST AFRICA. L.R. Drees and A. Manu, Texas A&M University, College Station, TX 77843

Dust traps were installed in Niger, West Africa to monitor dust infall as a possible nutrient renewal vector to soils of the Sahel. On occasion, dust samples contained extremely high water soluble K levels. The high K values were correlated with samples containing small quantities of white, sand-sized particles. These particles ranged from 17 to 27 g kg^{-1} water soluble K. Additional investigations utilizing X-ray diffraction, optical microscopy, microprobe, and scanning electron microscopy identified this material as uric acid dihydrate. Bird urine contains a high proportion of uric acid, uric acid dihydrate, as well as water soluble cations. The initial material is composed of spheres 2-10 μm in diameter. Upon wetting, the spheres quickly transform to euhedral, bladed to lenticular crystals of uric acid dihydrate. The white part (Urine) of bird droppings is generally viscous, mucoid and composed mostly of uric acid and other urates. This is the mechanism that birds use to eliminate waste products of nitrogen metabolism. The spherical form apparently allows for easy passage of uric acid crystals through the renal duct. The mechanism of transformation (recrystallization) from disordered spheres to ordered crystals is not clear. Open bucket dust traps provide a convenient perch for birds, with their excrements being incorporated into the dust. Such inputs may adversely influence the measured chemical properties of dust samples. Caution needs to be exercised when making interpretations of chemical analyses when the potential for outside contamination exists.

(Modified from Agronomy Abstracts, 1991)

L.R. Drees, (409)845-3669

BIOLOGICAL SCIENCES

PLATFORM PRESENTATION—SPRING 1996

INFECTION OF ROSE LEAVES BY THE PATHOGENIC FUNGUS *PESTALOTIA MICROSPORA*. V. DHEVAN and J. TAYLOR, Dept. of Biology, Stephen F. Austin State University, Nacogdoches, Texas 75962.

Pestalotia microspora was observed on infected leaves of rose obtained from a local garden. Culturing on potato dextrose agar and reinfecting plants in the greenhouse with the fungus resulted in spore formation on the leaves. Studies were conducted with *Rosa hybrida*, cv. La Reine. New spore formation was noticed within 7-10 days after inoculation. Investigation of the structure and mode of penetration of the fungus was conducted with both scanning and transmission electron microscopy. Fungal germ tubes terminated in penetration structures called appressoria. TEM was used to observe the growth of the hyphae and ultrastructural changes in host cells.

PYCNOTHYRIUM ULTRASTRUCTURE IN *TUBAKIA DRYINA*. J. TAYLOR, Dept. of Biology, Stephen F. Austin State University, Nacogdoches, Texas 75962.

Tubakia dryina, a fungal leaf parasite of numerous forest trees, produced fruiting bodies called pycnothyria on infected sweet gum leaves. These structures were excised and prepared for transmission electron microscopy using standard chemical fixation techniques. The pycnothyrium was umbrella-like in appearance. Its surface layer, the scutellum, was composed of thick walled prosenchymatous hyphae. It was attached to the leaf by a multicellular stalk, the columella. Numerous conidiogenous cells were associated with the under surface of the scutellum. The spore producing cells were phialidic, with a prominent collarette present at their apex.

A SCANNING ELECTRON MICROSCOPE STUDY OF THE STORAGE ROOTS OF *NYMPHAEA*. J. VAN DE VEIRE AND H. J. ARNOTT. The Department of Biology and Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019.

We have examined the storage roots of *Nymphaea helvola* and *N. mexicana* using light and scanning electron microscopy. The storage roots vary from two to 6 mm in diameter and two to 5 cm in length. They have a small stele and a thin epidermal layer. The major volume of the storage roots is made up of the cortex which is formed of mostly isodiametric parenchyma cells. These cells are approximately 100 μ m in diameter. In opposition to many other tissues of *Nymphaea* (leaves, stems, peduncles, flower parts) the parenchyma of the storage roots is very compact. There is a small air space system but it is not robust like that found in stems and leaves. The cortical parenchyma cells are notable because of the massive number of starch grains present in each cell. The starch grains completely fill each cell, leaving only a very small volume of the cell for other organelles. The starch grains are most often shaped like a short bullet (like a cylinder with one flat and one rounded end). The starch grains stain a very deep blue with IKI and show a Maltese cross under crossed polarizers. Some starch grains appear to be twins. Growing between the starch packed parenchyma cells there are many elongate crystalliferous sclereids. The sclereids are unbranched, sometimes bent, measure 10-40 μ m in diameter and are hundreds of μ m in length. The sclereids have a multilayered wall which in some places shows clearly defined pit cavities. The sclereids often have crystals of calcium oxalate partly embedded in their wall surface; it can be shown that most of the crystals are prismatic twins. Their bright birefringence indicates that they are probably crystals of calcium oxalate monohydrate (whewellite).

A STUDY OF d-LIMONENE AS A DEHYDRATION AND TRANSITION AGENT FOR LIGHT AND ELECTRON MICROSCOPY. M. A. DAVIS and H. J. ARNOTT, Dept. of Biology and Center for Electron Microscopy, The University of Texas at Arlington, Arlington Texas 76019.

Current dehydration techniques for light microscopy (LM) and scanning electron microscopy (SEM) often require the use of xylene which is expensive, hazardous and requires special precautions for its disposal and use. In this study we assess the use of d-Limonene, a volatile oil which is a natural extract of citrus fruits, in tissue fixation. d-Limonene has been characterized as noncarcinogenic and can be used in food products; it is relatively inexpensive. We hypothesize d-Limonene is a suitable alternative to xylenes in dehydration and other transitions (i.e., embedment in paraffin, etc.). The hypothesis indicates that d-Limonene will maintain morphological and dimensional characteristics of specimens after its use as well or better than xylene. Experiments were performed using 10 % formalin fixation, dehydration in ETOH, and transfer to either xylene or d-Limonene for LM investigation. SEM specimens were dehydrated in ETOH and transferred to d-Limonene for CO₂ critical point drying. Human red blood cells (RBCs) were chosen in our preparations due to their fragility and chemiosmotic sensitivity. Morphology of RBCs were characterized in plasma (*in vivo*). The results were compared to RBCs processed in xylene or in d-limonene after dehydration in ethanol. Effects on the RBCs were determined using LM, SEM, and digital image analysis.

A LIGHT AND ELECTRON MICROSCOPIC STUDY OF STOMATAL MORPHOLOGY IN *CITRUS SINENSIS* FRUITS. M.R. JOHNSON and H.J. ARNOTT. Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington. Arlington, Texas, 76019.

The guard cells of the stomata of the fruits of *Citrus sinensis* often appear to be a single, undifferentiated structure as opposed to the two differentiated guard cells commonly found in other plants and *Citrus* leaves. On commercial fruit, stomata are distributed irregularly on the epidermis. Examination of the stomata shows they are usually open and are covered with a thick layer of wax, which sometimes fills the stomatal pore, even plugging it. Specimens were prepared for scanning electron microscopy by treating the peel for brief periods with acetone and xylene and other lipid solvents to remove the thick, waxy cuticle from the epidermis layer. The samples were then fixed with 3% glutaraldehyde, dehydrated with increasing concentrations of ethanol, and sputter-coated with gold and palladium for EM viewing. LM sample were prepared by fixation with 3% glutaraldehyde, dehydrated with increasing concentrations of ethanol, embedded in paraffin, sectioned paradermally, and stained to enable the viewing of stomata apparatus. We are currently exploring two possibilities which may explain the apparent single structure seen by SEM. Namely, that guard cells are either masked by a sheath (possibly subsidiary cells), or that the "normal" guard cells of these fruits is located below the external pores which are visible on the surface of the peel.

CHARACTERIZATION OF SEED COAT OF FOUR LEGUMINOUS GENERA WITH SCANNING ELECTRON MICROSCOPIC INVESTIGATION. Nabarun Ghosh¹, Don W. Smith¹ and A. Chatterjee². Dept. of Biological Sciences, ¹University of North Texas, Denton, TX 76201. ²CAS, Dept. of Botany, University of Calcutta, India.

Seeds exhibit a complex and high morphological and micro-morphological diversity, providing valuable taxonomic information. Their overall shape, color, size, and in particular, their ultra structural characters can be of high systematic significance. The present investigation on the seed micro-morphology of 26 species of the 4 Leguminous genera revealed interesting clues for characterization and proper identification. Seeds of 8 species of *Acacia*, 7 species of *Albizia*, 8 species of *Cassia* and 3 species of *Dalbergia* were used for stereo-microscopic and Scanning Electron Microscopic investigation. The surface debris was removed from the seeds and they were mounted on stubs with adhesives and coated with 100-150 Å thick gold coating. Silver paint was applied at the base of the seeds to prevent charging during scanning. The gold-coated seeds were scanned with a constant tilt angle 45° under the SEM. The observed surface features of these leguminous seeds can be grouped into meaningful categories according to four traits: 1. cellular pattern, 2. primary sculpture of a seed surface, 3. relief of the outer cell walls caused by cuticular striations, 4. epicuticular secretions, i.e., mainly waxes and related substances. Seeds of 5 species of *Cassia* and 2 of *Albizia* are very similar in their apparent morphology and can be very difficult to differentiate with naked eye. SEM shows strikingly different features that make separating species very easy.

THE DISTRIBUTION OF CALCIUM OXALATE CRYSTALS IN VEIN ROLL GALLS OF *QUERCUS SHUMARDII*. G. M. HUSE and H. J. ARNOTT, Dept. of Biology and Center for Electron Microscopy, The University of Texas at Arlington, Arlington Texas 76019.

The vein roll gall on the leaf of *Q. shumardii* (a red oak) is caused by a species of the gall midge, *Microdiplosis* sp. We have examined these galls using light microscopy (LM) and scanning electron microscopy (SEM). Materials for LM studies included clearings, hand and paraffin sections; for SEM we used glutaraldehyde/osmium fixed specimens which were then cryofractured. Like many plants the leaves of *Q. shumardii* contain crystalline deposits of calcium oxalate distributed throughout the lamina. The adaxial surface of galled leaves show areas along each side of the midvein and some lateral veins which are folded upward to form ridges up to 4 mm in height. The two folds extend upward and may touch. Each ridge forms an enclosed area in which the midge larva develops. Associated with the ridge is the formation of considerable amount of abnormal tissues, these tissues produce copious amounts of tannins. The distribution of tannins coincides with the aberrant leaf tissues. In clearings the tannins remain in the gall areas long after the remaining tissues are decolorized. In this paper we wish to examine the massive amounts of calcium oxalate crystals that are deposited in the gall tissues. Large druse crystals are abundant in the folded portion of the gall near the midvein, or lateral vein, about which the gall is formed. These crystals are highly birefringent and are probably whewellite (calcium oxalate monohydrate). In some cases files of cells can be found in which each cell contains a large druse which occupies most of the cell volume. A few cells containing prismatic crystals or crystal sand were also seen.

IMAGING MUSCLE: THE TECHNOLOGY, THE ART, THE SCIENCE. M. A. GOLDSTEIN AND J. P. SCHROETER, Department of Medicine, Baylor College of Medicine, Houston, TX 77030.

Power spectra of electron micrographs show that the Z band lattice in mammalian muscle has at least two structural states related to the contractile state of the muscle. Cross-sectional projections of relaxed muscle show the small square pattern, while projections of fixed contracted muscle show a pattern termed the basket weave. Differing three-dimensional models constructed to visualize the transition were consistent with the cross-sectional data; therefore, we produced three-dimensional reconstructions of Z band lattices from electron tomography of tilted specimens. The relaxed lattice shows features not imaged in micrographs of cross or longitudinal sections of muscle. Generating and analyzing these data lead to challenges in display of these "micro" structures not found in our "macro" world. Issues of perception and vision are of demonstrated importance in the display process.

ANTI-DUCTIN ANTIBODIES LABEL INTERCELLULAR MEMBRANES IN THE MIDGUT OF AN INSECT AS REVEALED BY CONFOCAL MICROSCOPY. P. V. PIETRANTONIO* and SARJEET S. GILL* (*Dept. of Entomology, Texas A & M University, College Station, TX 77843-2475; *Dept. of Entomology, University of California Riverside, Riverside, CA 92521)

The name ductin has been given to a family of highly conserved channel proteins of 16-17kDa that perform at least two distinct functions: proton transport in the ubiquitous vacuolar proton ATPase (V-ATPase), and acetylcholine transport in the electric ray electric organ. The presence and role of ductins in gap junctions in metazoan animals is currently under debate. We previously cloned a 16kDa protein from a tobacco budworm (*Heliothis virescens*, Lep. Noctuidae) larval midgut and Malpighian tubules cDNA library, which is highly homologous to the published sequences of other ductins (Pietrantonio and Gill, 1993). We also reported its immunolocalization with a specific affinity-purified anti-peptide antibody (AP-L2) in midgut and Malpighian tubule of feeding larvae and concluded that the cloned proteolipid encodes the Vacuolar ATPase proton-transporting subunit c from the V₀ sector (Pietrantonio and Gill, 1995).

We now present the immunolocalization of this protein in the midgut during the L₄-L₅ larval molt and early post ecdysis into the fifth instar in *H. virescens*. The results indicate that the 16kDa protein spatial expression is developmentally regulated. Labeling by AP-L2 varies during the molt in the midgut goblet cell apical plasma membrane and the goblet cell apical valve. Epifluorescence and confocal microscopy revealed strong anti-ductin labeling in intercellular areas during the molt, and most dramatically during early post ecdysis into the fifth instar. The characteristic labeling pattern observed in intercellular areas is consistent with the claimed involvement of ductins in gap junctions. V-ATPase regulation during the molt was also investigated by simultaneous immunohistochemistry with an anti-B subunit antiserum, which was used as a tracer for the V-ATPase V₁ sector.

BIOLOGICAL SCIENCES

POSTER PRESENTATION—SPRING 1996

STRUCTURAL CHARACTERIZATION OF PERIPHERAL NERVE CELLS AND NERVE - MUSCLE JUNCTIONS OF THE OVIDUCT OF THE STABLE FLY, *STOMOXYS CALCITRANS* (DIPTERA: MUSCIDAE). B.J. COOK AND N.W. PRYOR, USDA-ARS-FAPRL, College Station, TX 77845

Ultrastructure of both peripheral nerve cells and neuromuscular junctions associated with the oviduct of the stable fly, *Stomoxys calcitrans* (L.) was described. Twelve or more multipolar peripheral neurons were found along major branch nerves that enter the ovipositor. Several were suspended in the haemocoel and others were in close proximity to the surface of the oviduct. Some peripheral neurons contained an abundance of neurosecretory granules that ranged in size from 32 to 180 nm in diameter. No glial elements enveloped the perikarya of such cells. Neurosecretory axons were usually found in the boundary region of large nerves just beneath the stroma. Peripheral nerve cells in close apposition to oviduct muscles were generally non-neurosecretory and were ensheathed in a glial perineurium. Peripheral neurons were surrounded occasionally by an extensive network of extracellular spaces in the glial perineurium. Other smaller neurons were found within large nerve trunks.

Nerve - muscle junctions contained large clusters of synaptic vesicles and occasionally included small groups of neurosecretory granules. Many nerve terminals on the surface of the oviduct contained a complex postsynaptic folding of sarcolemma. Active transmission sites were indicated by increased densities along the neurollemma. Some neurohemal release sites were also evident. This ultrastructural evidence demonstrates that neurosecretory processes are involved in the function of some peripheral nerve cells in the stable fly.

THE THREE DIMENSIONAL STRUCTURES OF Z BANDS IN UNSTIMULATED AND RIGOR SKELETAL MUSCLES. J. P. SCHROETER, R. J. EDWARDS, AND M. A. GOLDSTEIN, Department of Medicine, Baylor College of Medicine, Houston, TX 77030.

We have previously observed a dimensional and structural transition in the Z band lattice associated with the change from the inactivated small square (ss) lattice form seen in unstimulated skeletal muscle to the activated basket weave (bw) form seen in the rigor muscle. We have further investigated this transition by three dimensional reconstruction of the Z band lattices using electron microscope tomography on longitudinal thin sections of rat soleus muscle. An increase in both the longitudinal and cross-sectional dimensions of the rigor Z lattice was observed. Shaded solid renderings of parts of the Z band reconstructions reveal substantial re-arrangement of the cross-connecting Z-filaments in the ss to bw transition. The density due to the connecting structures was observed to decrease. This complex re-arrangement of the Z band lattice is consistent with a redistribution of Z band forces concomitant with changes in A band cross bridge binding. (Supported by NIH grant HL17376.)

ENHANCEMENT OF IMMUNOHISTOCHEMICAL REACTIVITY USING STRECK TISSUE FIXATIVE. ANN S. BURKE, ROBERT A. COX, HAL K. HAWKINS, Shriners Burns Institute, Dept. Electron Microscopy, UTMB, Dept. Pathology, Galveston, TX 77550

Immunohistochemical staining using horseradish peroxidase-DAB was improved for identification of structural antigens with polyclonal and monoclonal antibodies against laminin, collagens, Type IV and VII and TNF- α , when Streck Tissue Fixative (S.T.F.), a non-crosslinking fixative, was used for fixation instead of buffered formalin. S.T.F. contains Diazolidinyl urea, 2-Bromo-2-nitropropane-1,3-diol, Zinc Sulfate, and Sodium Citrate.

Tissue samples included human, pig and rat skin, that were fixed overnight in formalin or S.T.F. at room temperature, then processed for paraffin embedding. Five micron sections were dried overnight at 60 °C, deparaffinized and rehydrated. Duplicate slides were predigested with 0.1 % pepsin in 0.5M acetic acid or 0.0025% protease (Sigma) in Tris-buffered saline (TBS), pH 7.6, depending on the protocol. Vector ABC kits, PK 4001 and PK6102 were used for detection with DAB kit, SK-4100.

Laminin (E-Y Labs) and Type IV collagen (Biogenex), polyclonal antibodies, showed intense staining of the basal lamina and dermal cells. Type VII collagen (Sigma), monoclonal antibody, intensely stained the basal lamina. TNF- α , (Boehringer Mannheim), monoclonal antibody, stained the basal lamina and epithelial cells.

Previously, formalin-fixed samples showed absent or light staining product. Using the S.T.F. fixative, antibody solutions could be diluted 10-fold and gave more uniform results than formalin-fixed samples. This could significantly save on antibody purchases. Reactivity with various antibodies on S.T.F.-fixed tissues was increased for all antibodies tested. We routinely compare the presence of these antigens in normal and proliferating epidermal tissues. Since we can qualitatively compare these tissues, we are now working on quantitating the staining intensity.

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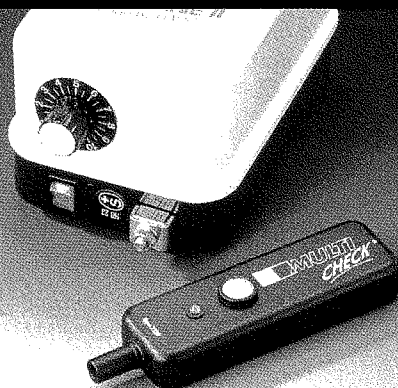
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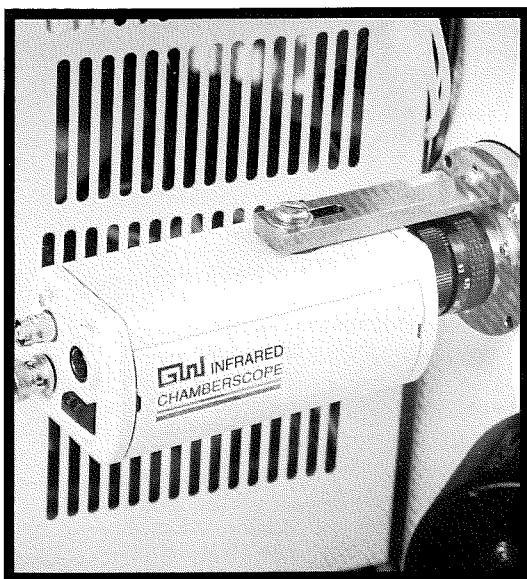
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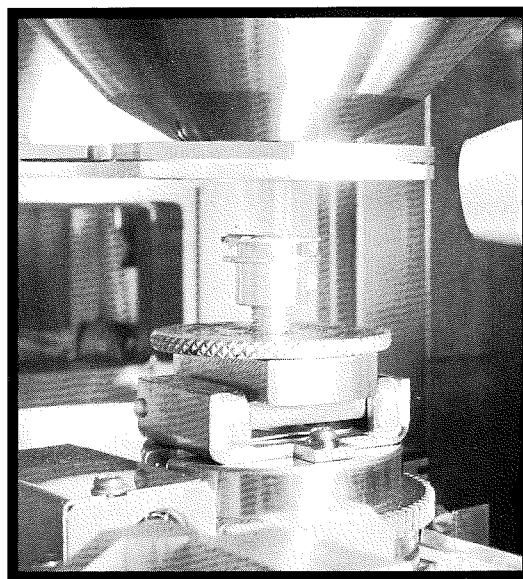
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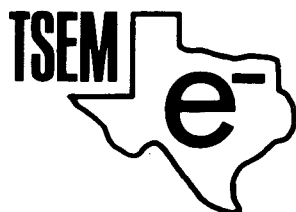
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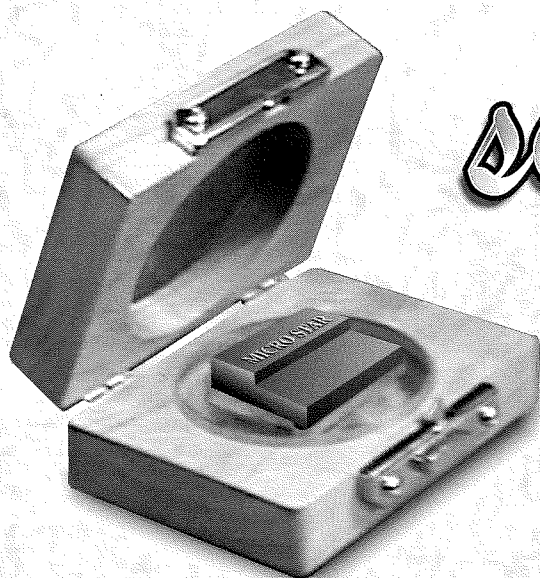
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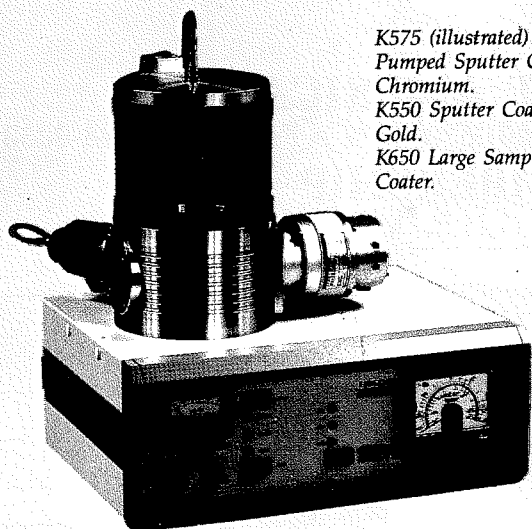
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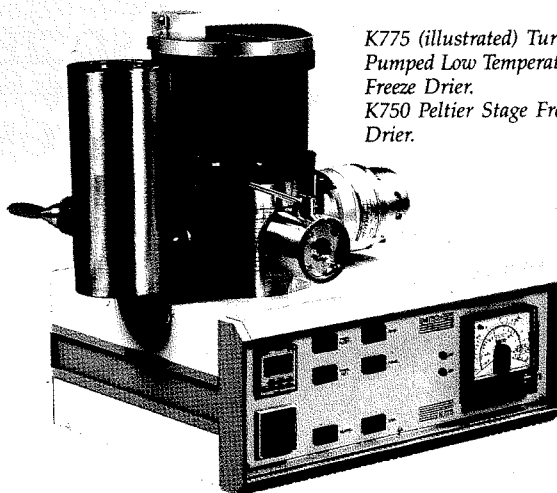
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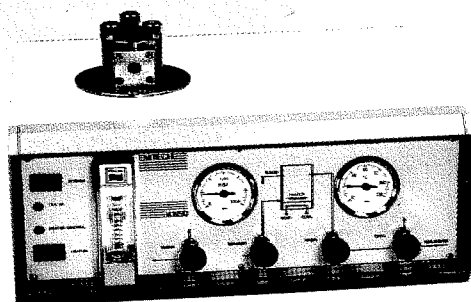
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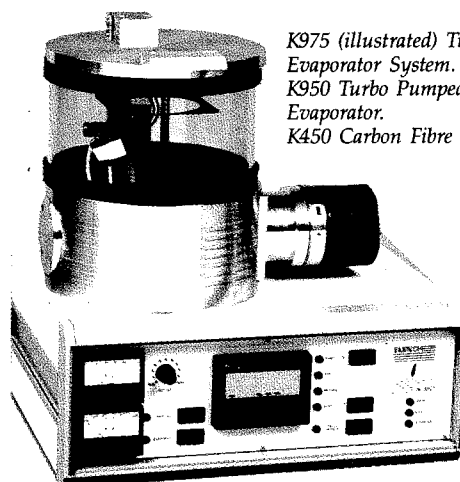
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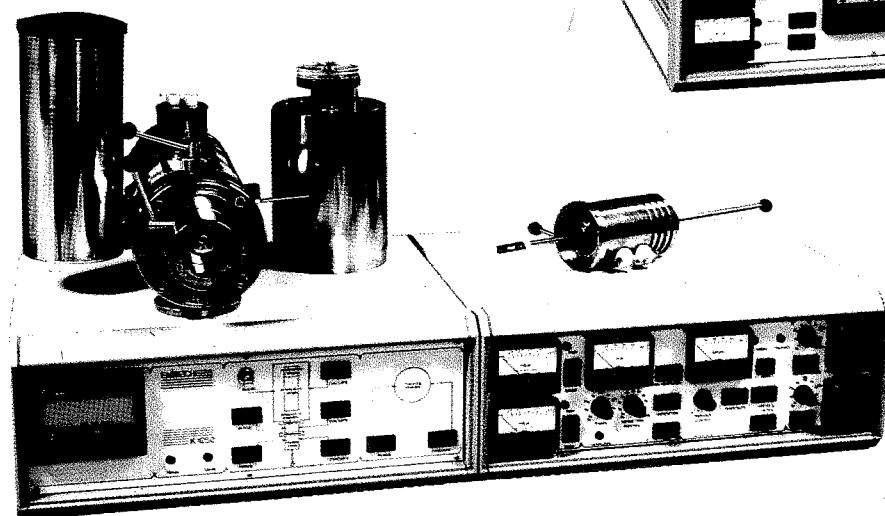
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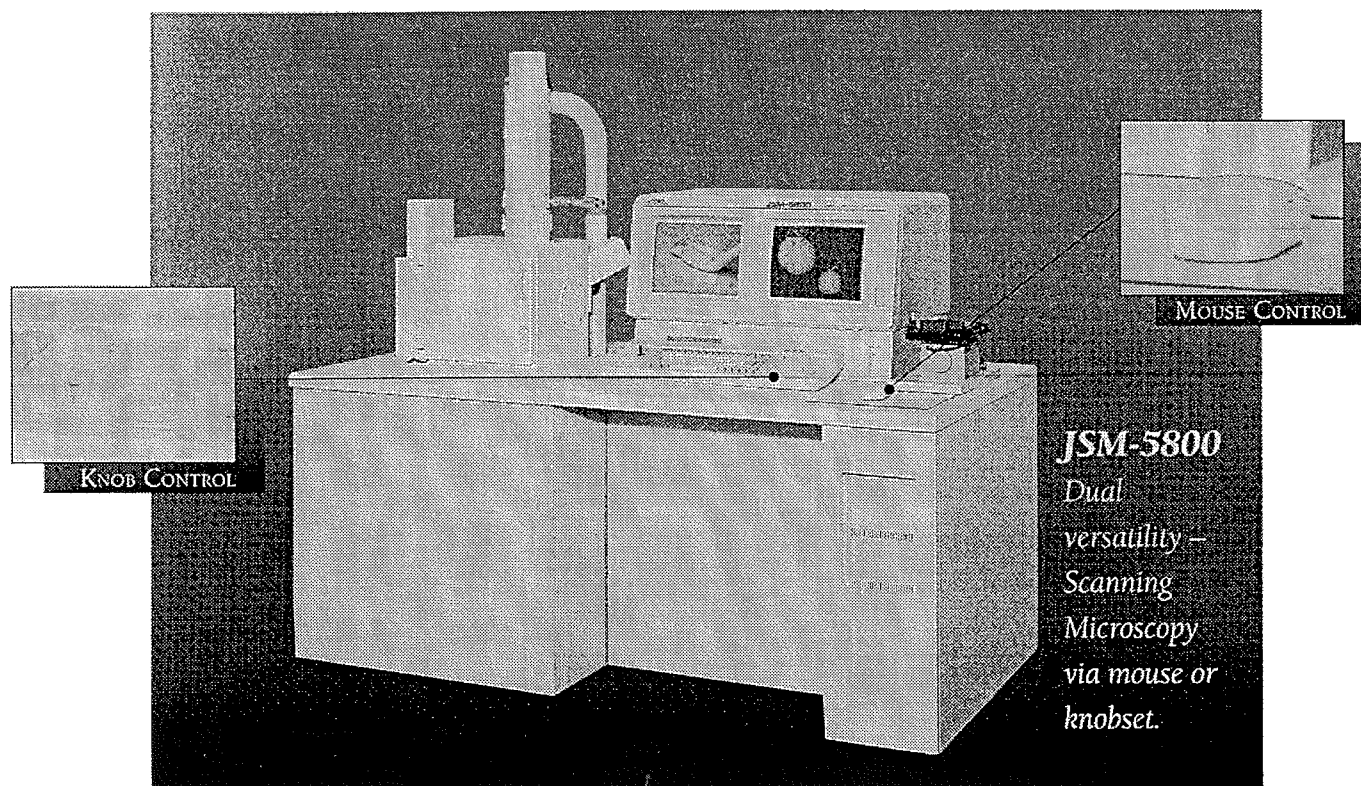
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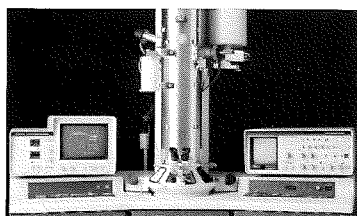


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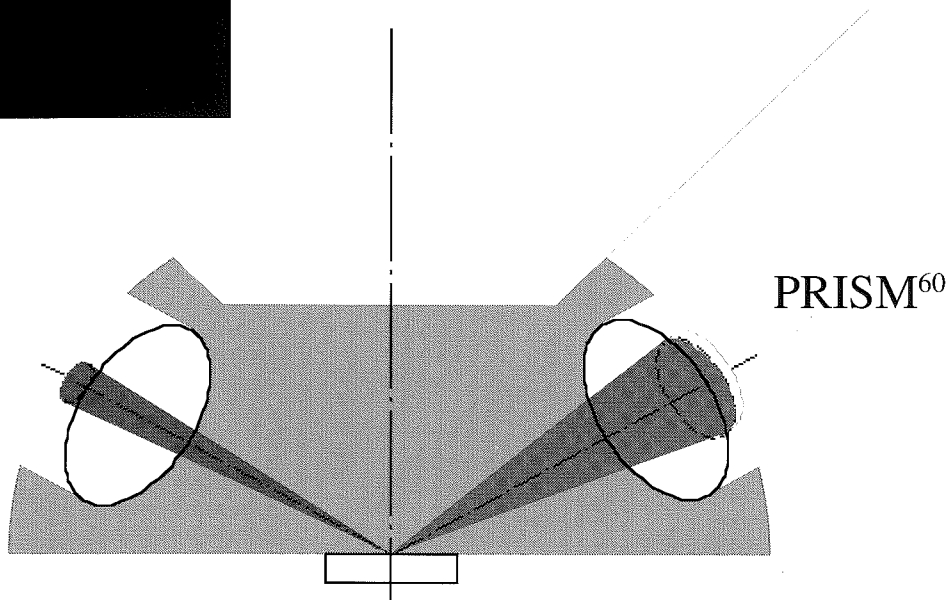
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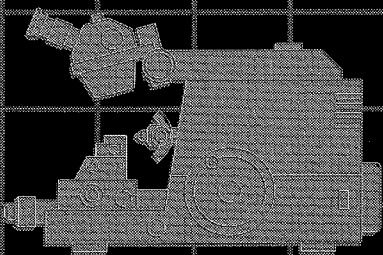
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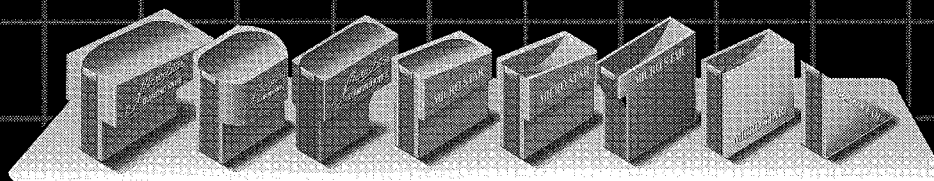
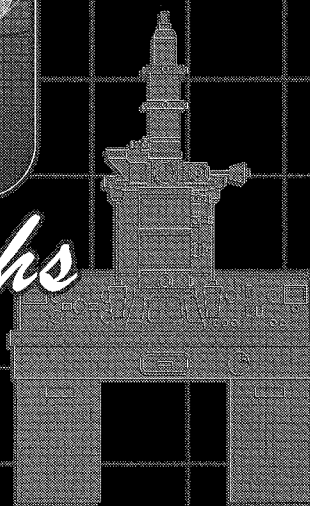
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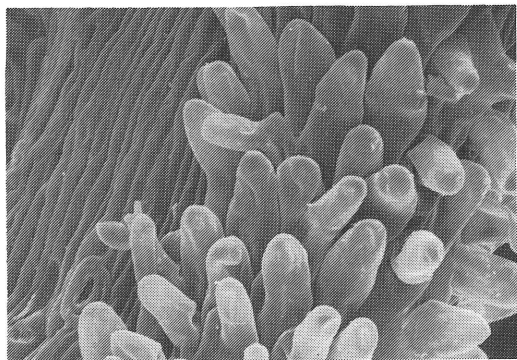
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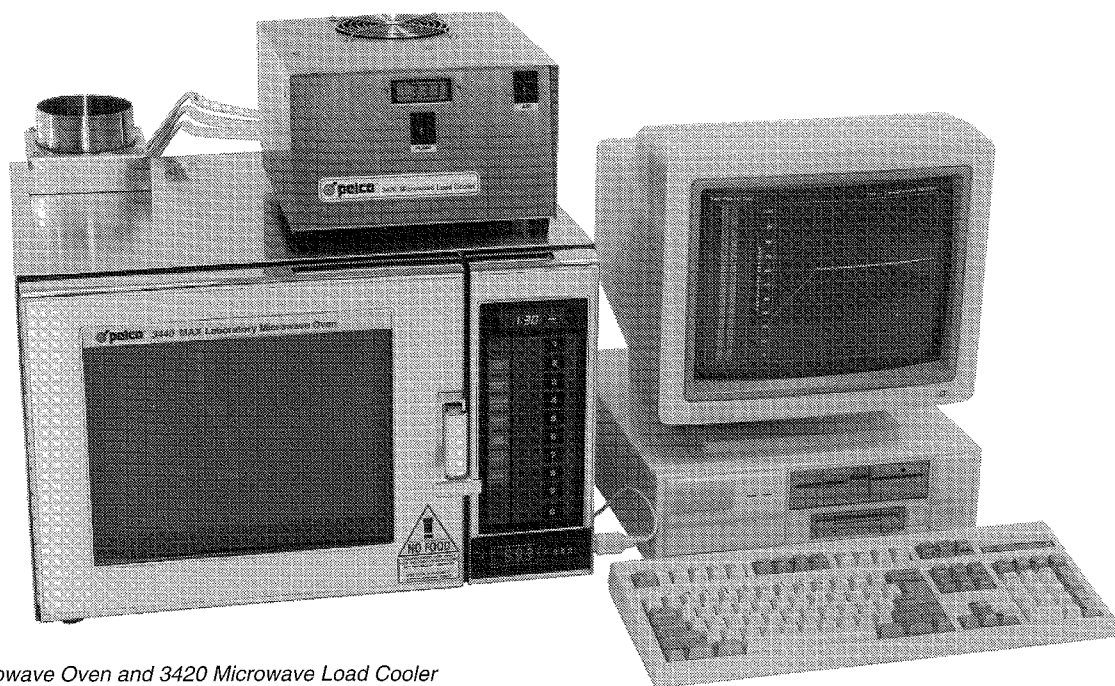
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The PELCO™ Prep-Eze™ makes the handling of specimens during microwave processing easy. A small plastic basket holds the specimens and fits snugly onto a plunger. Specimens can be transferred from vial to vial or dish to dish, using the plunger as a carrying tool. In the microwave application, a temperature probe fits through the plunger.

Microwave/EM Bulletin Board: 916-243-9456
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MICROSCOPY SOCIETY OF AMERICA CERTIFICATION BOARD EXAMINATIONS

ELECTRON MICROSCOPY TECHNOLOGIST

—(BIOLOGICAL SCIENCES)—

GENERAL ELGIBILITY REQUIREMENTS:

1. Membership in MSA.
2. ONE of the following conditions must be met:
 - 2 years (60 credits) college or equivalent, including science and TEM (1 year laboratory) courses; science courses to include one each of chemistry, physics and biology; math through trigonometry
 - 1 year (30 credits) college or equivalent, including one course each of chemistry and physics, and 1 year of recent full-time work experience (within the past 5 years) in a TEM laboratory
 - high school diploma and 2 years of recent full-time work experience in a TEM laboratory
 - 3 years of recent full-time work experience in a TEM laboratory
 - 6 years full-time TEM work experience within the past 8 years.

IMPORTANT DEADLINES:

Examinations are administered twice a year (two cycles per year).

Deadlines for receipt of applications are: October 1 and April 4.

FOR APPLICATIONS AND ADDITIONAL INFORMATION:

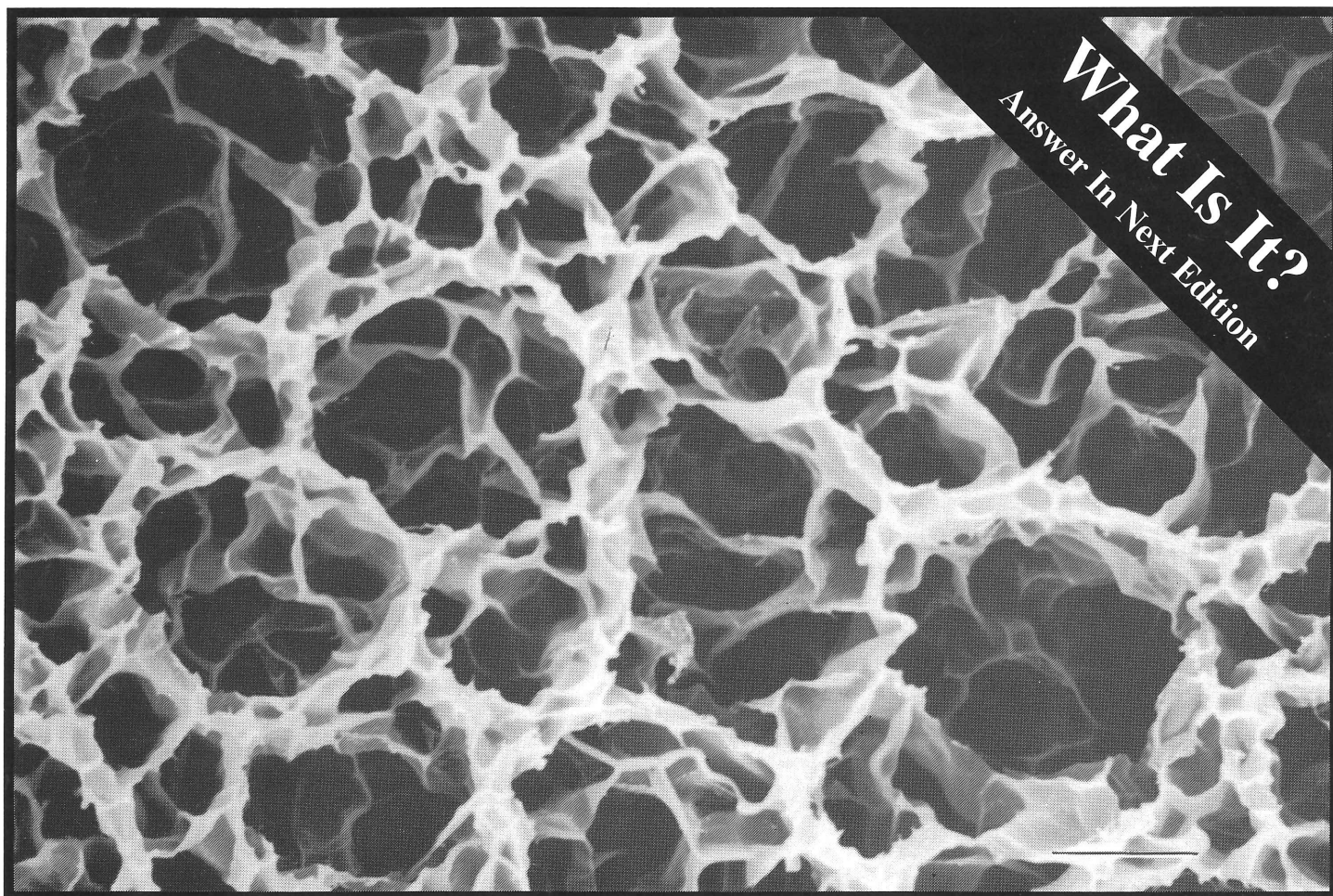
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Texas Society for Electron Microscopy

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