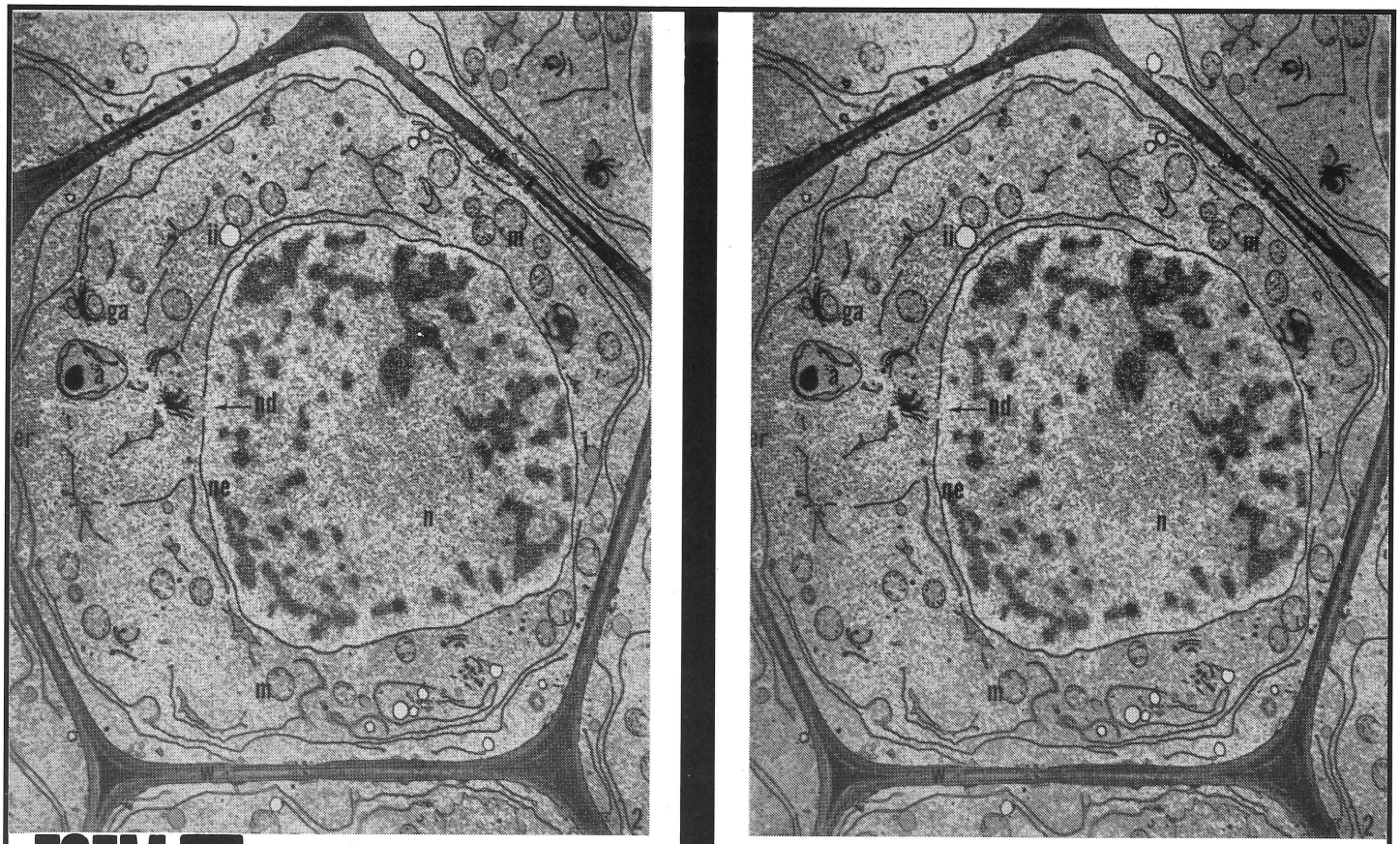
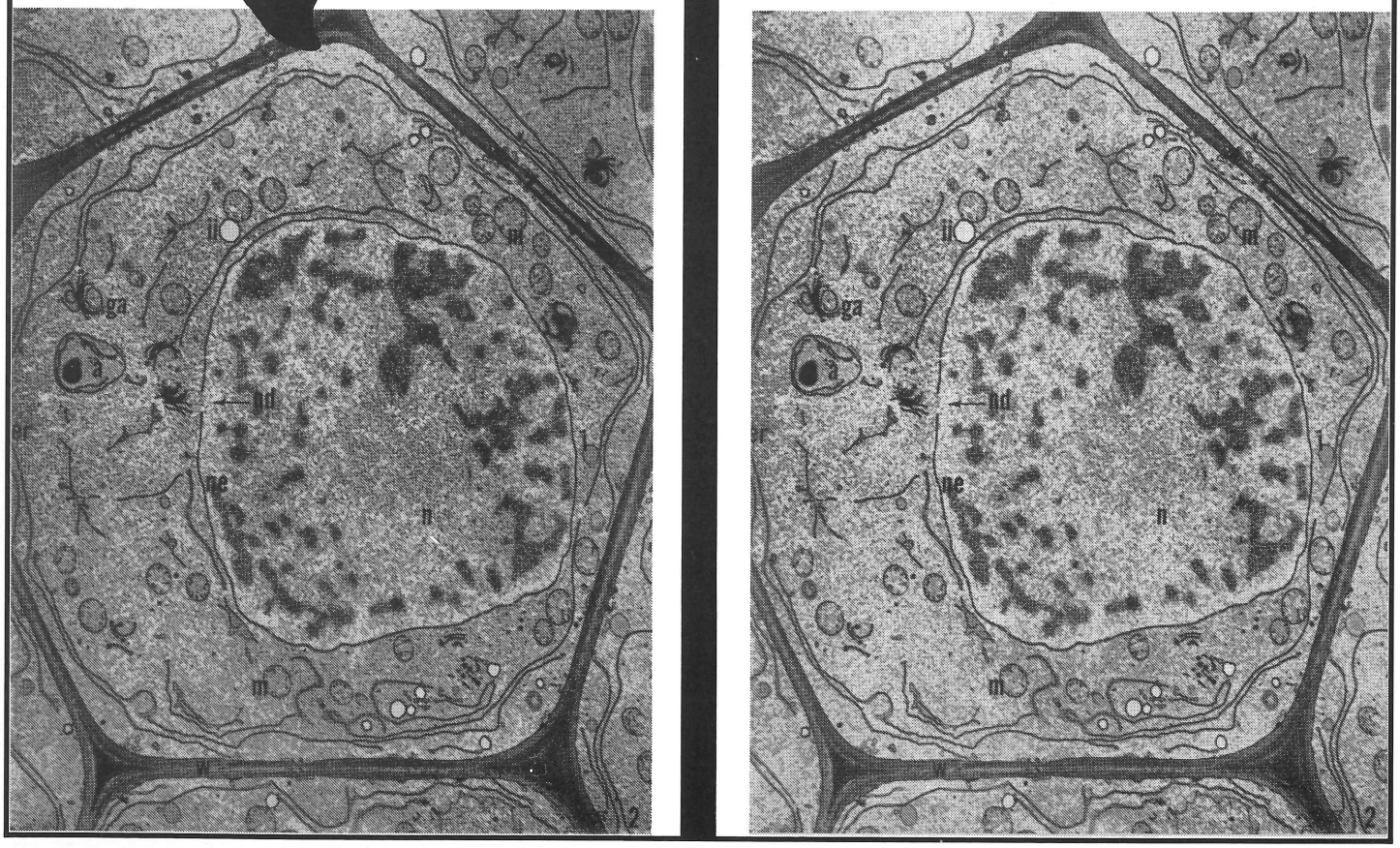


TSEM e- Journal

Texas Society for Electron Microscopy

Volume 22, Number 2, 1991 • ISSN 0196-5662



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TEXAS SOCIETY FOR ELECTRON MICROSCOPY
JOURNAL
VOLUME 22, NUMBER 2, 1991
ISSN 0196-5662

Louis H. Bragg, Editor
Department of Biology, The University of Texas at Arlington, Arlington, TX 76019

Texas Society for Electron Microscopy

"For the purpose of dissemination of research with the electron microscope."

ON THE COVER

Meristematic root cap cell fixed in 2.0% aqueous $KMnO_4$. From Whaley, Mollenhauer and Leech. 1960. The Ultrastructure of the Meristematic Cell. Am. J. Bot. 47:401-450.

Photo — Howard J. Arnott, Dept. of Biology, The University of Texas at Arlington, Arlington, TX 76019.

See Cover Comment on Page 9.

President's Message

As I write this, it is hard to believe that the Fall, 1991 meeting is just a few weeks away. The Spring, 1991 joint meeting with the Oklahoma Society is a pleasant memory. All participants have agreed that our confocal scanning workshop was a resounding success and that our symposia speakers were effective and very informative.

I'd like to remind you that the society's dues have increased slightly (from $10 to $15) to offset the loss of revenue imposed by IRS regulations for non-profit organizations. Those of you planning to attend the October 24-26 meeting can bring your dues up-to-date at that time if you haven't already done so.

Beginning with this meeting, we are offering *advance registration* to participants at a substantial savings in an effort to obtain a more accurate estimate of attendance. By this time most of you have learned that Dr. Hilton Mollenhauer, former President and perennial supporter of TSEM and one of the true pioneers and innovators in electron microscopy, is retiring this year. For this reason our fall meeting in Clear Lake is dedicated to Dr. Mollenhauer; a symposium will be conducted in his honor entitled "The Maize Root Tip, Impact on Modern Cell Biology", which will be presented by an international group of Hilton's friends, organized by Dr. D.J. Morre' of Purdue University.

A group tour of NASA's Johnson Aerospace Center will be held Friday afternoon. Mannie Steglich and Dr. Hal Hawkins have been working for several months to ensure a smooth, successful meeting. Participation by Materials' Science contributors is so strong that they will have their own platform session on Saturday morning.

Plans for our spring meeting are already well under way, thanks to Paula Williamson (local arrangements) and to Hal Hawkins. Tentatively we will meet in San Marcos at the Aquarena Springs Resort and Conference Center, probably March 26-28, 1992. The center features a sky-lift and glass-bottom boats within the park. In addition, we may have the opportunity to tour the new Science Building at Southwest Texas State University in which the Biology Department has state-of-the-art facilities. Details will be provided later.

I hope many of you will support your society by attending the Fall meeting and I look forward to seeing you at the Nassau Bay Hilton.

Sincerely,

Don A. Hay

Don A. Hay, President

ANSWER TO "WHAT IS IT"

Cross-section of the termite protozoan *(Reticulitermes flavipes).*

Photo — Howard J. Arnott, Dept. of Biology, The University of Texas at Arlington, Arlington, TX 76019.
(Magnification approximately 3,000X; 60kV)

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Certificate of Deposit No. 177576	$3,241.48	
Certificate of Deposit No. 0014483028	1,500.00	
Certificate of Deposit No. 0014483036	1,500.00	
Checking Account No. 0160041996	2,951.93	$ 9,193,41
RECEIPTS:		
Dues	$4,312.00	
Spring 1991 Meeting Registration	1,595.00	
Spring 1991 Workshop	185.00	
Spring 1991 Exhibitors Fees	1,370.00	
Journal Advertisements	2,500.00	
Checking Account Interest	48.26	
Donations and Grants	3,617.08	
Miscellaneous	1,007.75	$14,635.09
EXPENSES:		
Journal, Postage, and Stationary	$3,253.67	
Mailouts	845.00	
Spring 1991 Meeting	6,458.13	
Student Competition Spring 1991 Meeting	150.00	
Fall 1991 Meeting Deposit	500.00	
Treasurers Expenses	31.18	
Checking Account Service Charge	27.35	$11,265.33
ASSETS AS OF SEPTEMBER 1, 1991:		
Certificate of Deposit No. 113515	$3,392.76	
Checking Account No. 440059412	6,321.69	$ 9,714.45

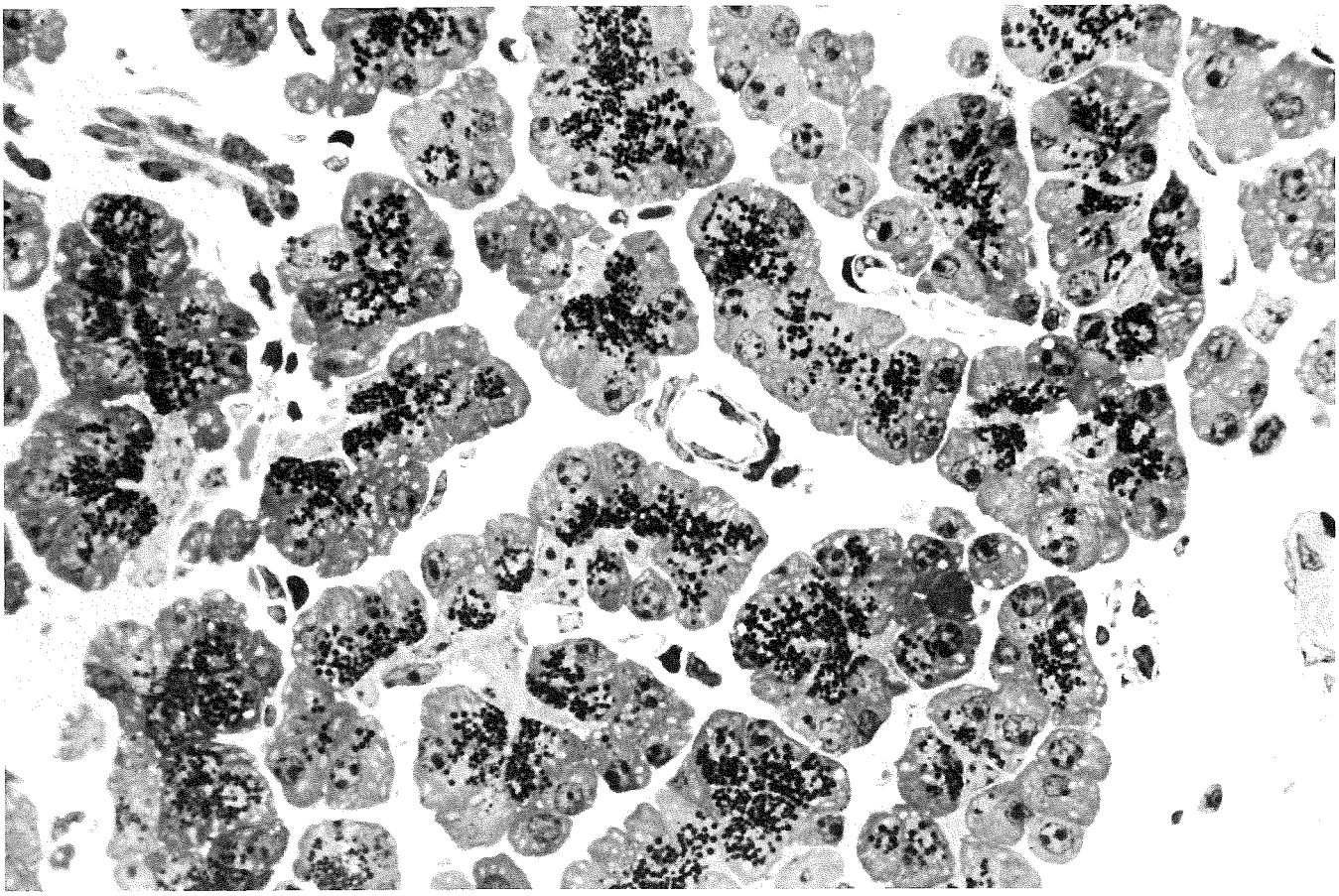

Photo courtesy of Maryann Fletcher, East Carolina University.

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COMMENTS ON COVER PHOTO

In the summer of 1960, as a Visiting Assistant Professor, I was teaching Botany at the University of California (Berkeley) when the now classical paper by Whaley, Mollenhauer and Leech was published. That summer and before, my major professor, Dr. Adriance S. Foster, at the time a world leader in plant anatomy, and I had conducted many discussions about published work on plants using electron microscopy. Often we laughed heartily and suggested to each other that the only use for most of them was as "wall paper" patterns. We could not see anything of value in that kind of work. The papers did not provide any new information about plant cells and the observations could generally not be validated by light microscopy.

For me, however, all that changed with the publication of the Whaley, Mollenhauer and Leech (1960) paper. As I discussed the paper with Foster we could see at last the value of electron microscopy in the study of plants. Their Figures (2 [see cover], 16, 19) could easily be related to light microscopy and hence provide a validation of electron microscopy. It was this paper, and others that followed that led me to study plant ultrastructure at the University of Texas at Austin in the Cell Research Institute. After arriving at Texas it didn't take long to find out who did the electron microscopy; clearly Hilton Mollenhauer was the "main man". On the eve of Dr. Mollenhauer's retirement I hasten to thank him, both personally, and also generically for all those who have been stimulated by his scientific and electron microscopic skills.

H.J. Arnott
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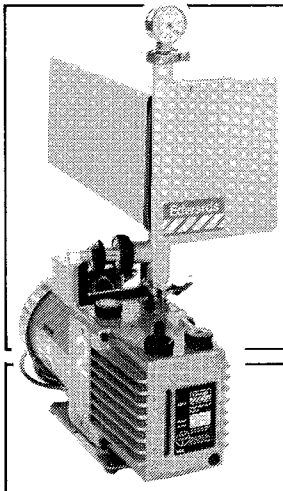

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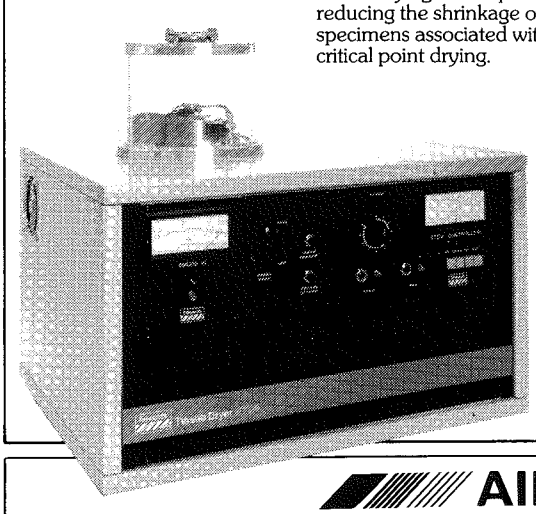

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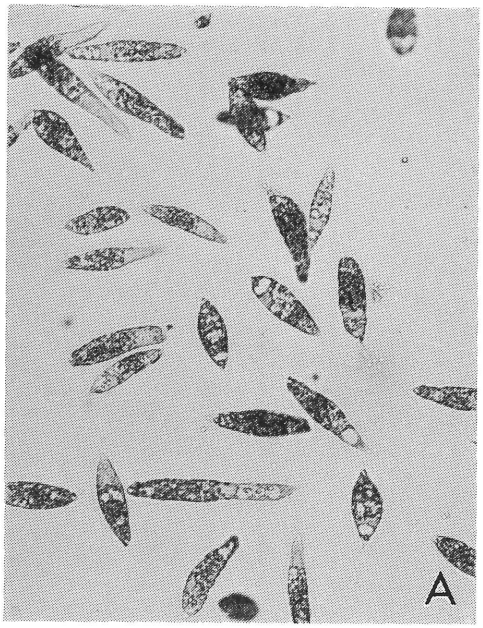
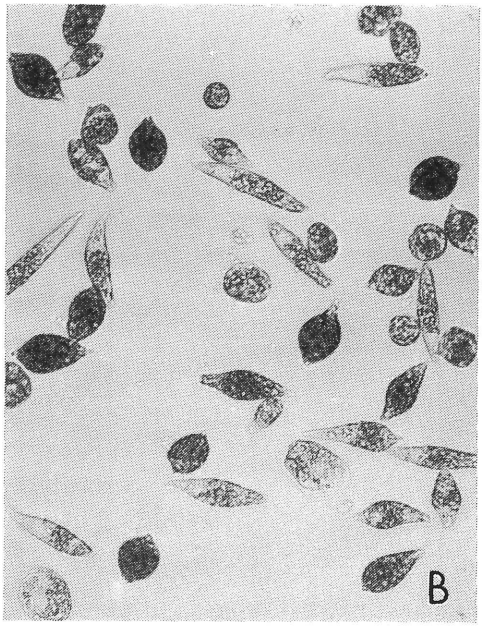
Questions & Answers

By Hilton H. Mollenhauer

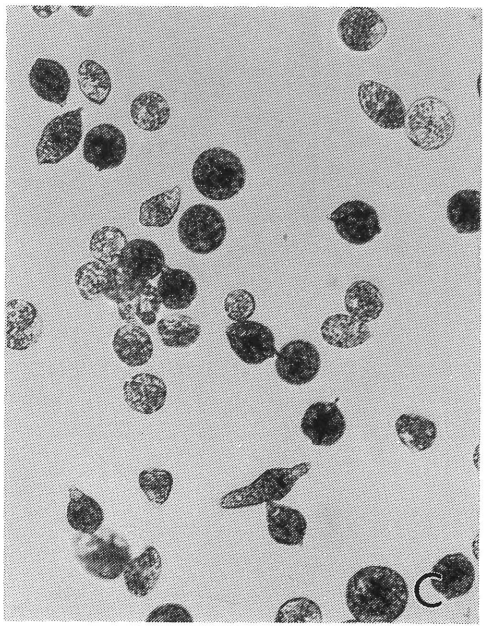
Q *These three light micrographs are of* Euglena *fixed in glutaraldehyde for electron microscopy. Why do the shapes differ and which is the most-nearly correct? All fixation conditions were the same except for one variable. Circle the appropriate variable: 1) osmolarity of the fixative. 2) temperature of the fixative. 3) concentration of glutaraldehyde. 4) pH.*





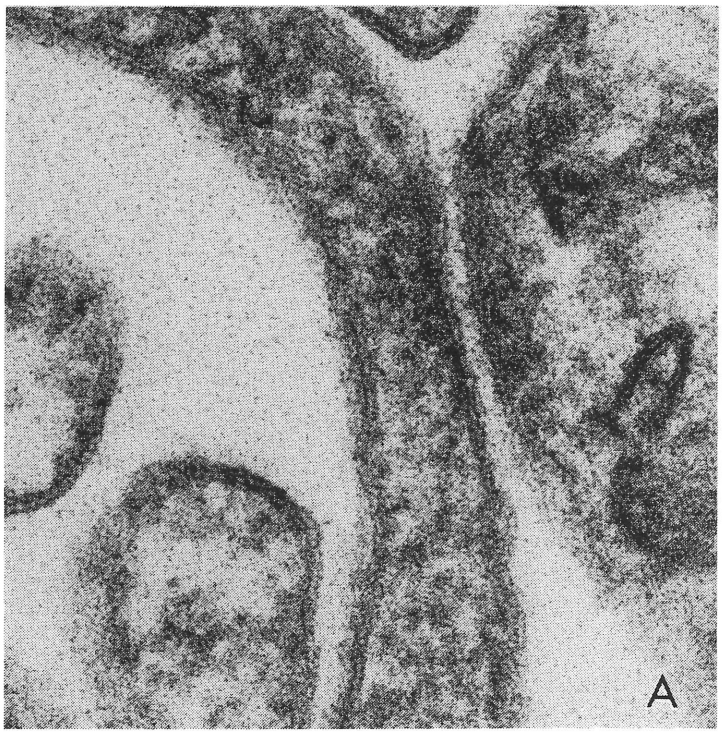
A The correct answer is item 2) temperature, and the most-nearly correct shape of *Euglena* is that shown in Fig. A. The fixation temperatures varied as follows: Fig. A (35°C); Fig. B (24°C); Fig. C (4°C). This illustrates two well-known facts; namely, that chemical fixation is slow and that cellular changes may occur before tissues can be stabilized. Do internal changes in cell ultrastructure correlate with cell-shape aberrations? Probably yes, although a spatial redistribution of microfilaments and microtubules is not easily recognized by cursory examination of electron micrographs. However, elevated temperatures may alter the way in which glutaraldehyde interacts with tissue elements and this may be noticeable in some tissues.

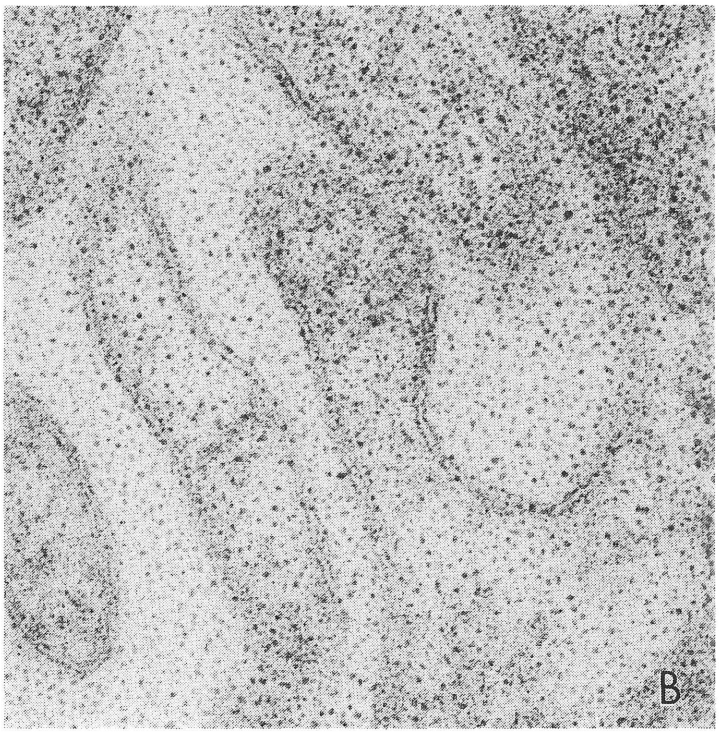
Do these data imply that all fixations should be at elevated temperatures to preserve cell shape and internal architecture? No, but they do suggest that temperature may be a useful variable when considering a fixation protocol. Cell shape is usually not a major consideration since most cells are constrained within a tissue mass and do not change much even if fixation is less than optimum. Internal architecture is usually more important than cell shape but, in most instances, it will differ only marginally within a fixation temperature range of 24-35°C. However, fixation at ice-bath temperature is not recommended since it often leads to significant ultrastructural changes, particularly in cytoskeletal elements of the cell.



NOTE: Only the first few minutes of fixation are critical. After the initial stabilization, fixation temperature can be lowered if so desired. In any event, it is probably best to lower the temperature to 4°C before moving the tissues into osmium tetroxide (which should also be at 4°C). This has two advantages; 1) the buffer rinse between glutaraldehyde and osmium tetroxide can usually be eliminated, and 2) this will slow the interaction between glutaraldehyde and osmium teroxide and help prevent the formation of pepper.

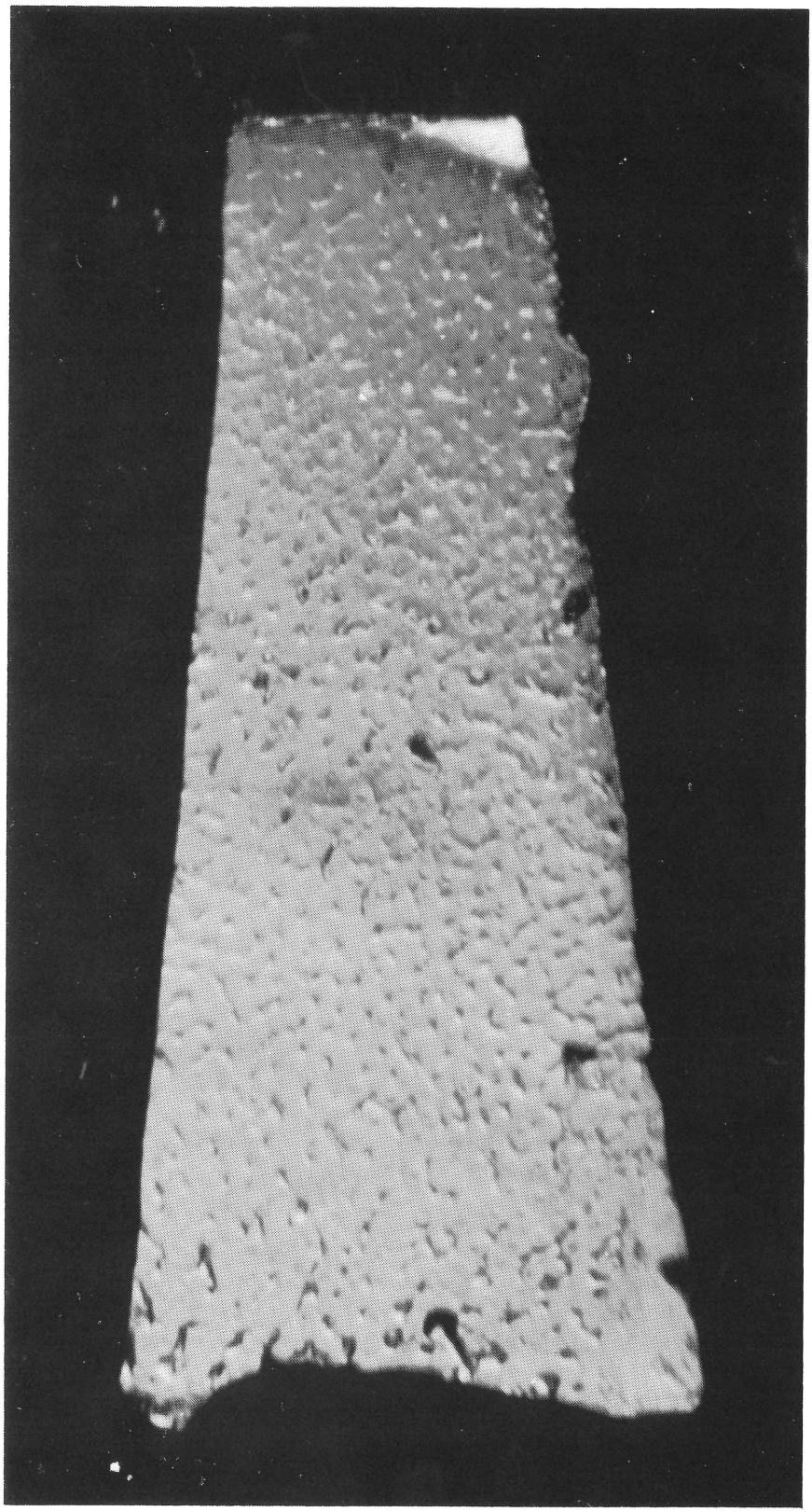
Q *The liver tissue in these two mircrographs were processed in the same manner, and the differences in appearance occurred after sectioning. What caused the pepper and reduced image contrast in Fig. B? Circle the correct answer. 1) Old lead citrate post-stain. 2) Reaction between uranyl acetate and lead citrate during post-staining. 3) Time. 4) Improper washing after staining. 5) Contaminant from microscope as might occur from poor vacuum. 6) Beam damage.*





A The correct answer is item 3) Time. The section in [B] differed from that in [A] only in that was stored 1 month before it was post-stained and examined. The section clearly deteriorated during storage, although what happened to it at the molecular level is not known. However, epoxy resins act very much like viscous fluids, and it is thought that unbound constituents within the polymer may have leached out of the section to form the pepper. Even whole cells have been shown to move within a block of polymerized resin. Although this example is extreme, the effect is probably universal and suggests that sections may have a finite life.

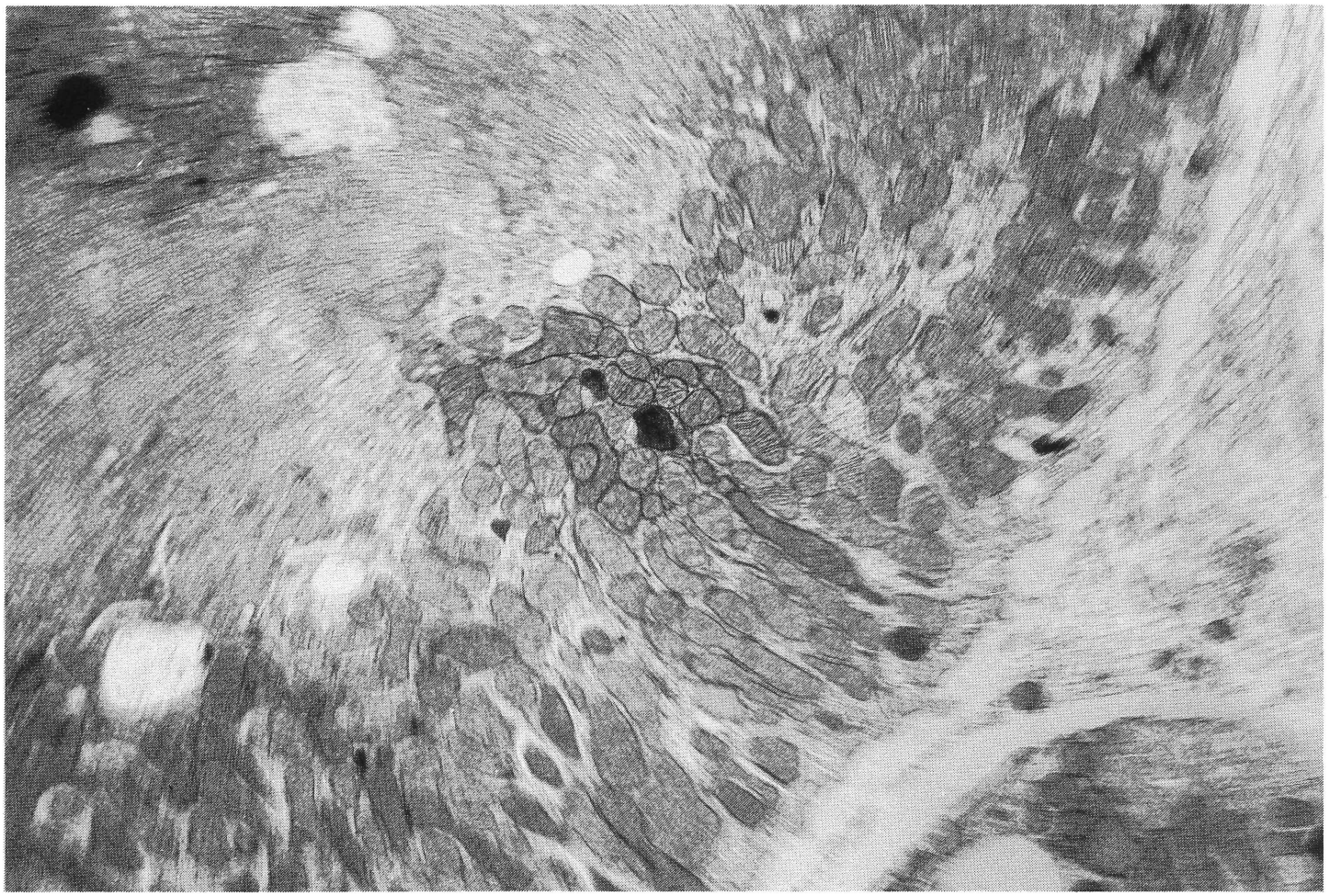
Q *This macro-photograph is of the face of a block of tissue that had been sectioned one month earlier. Why is the face not smooth as it always is immediately after sectioning?*



A The bumps on the surface of the block face are *Euglena* that moved during storage of the block. This is an extreme example in that such gross movement is not usually encountered. Nonetheless, movement almost surely occurs in all blocks of tissues, although at such a slow rate that it is seldom recognized. Tissues in sections probably move at a much greater rate since the tissues have less support from surrounding resin.

Epoxy resins commonly used for electron microscopy are seldom fully polymerized and only rarely maximally crosslinked, so that they act as viscous fluids. Tissues floating within them move, albeit very slowly. The stability of a resin formulation can be determined roughly by following the rate at which the surface of a sectioned block deforms. Visible deformation normally varies from several months to several years depending on resin formulation and quality of tissue impregnation.

Q *Why is this micrograph blurred around the edges but not in the center of the field?*



A The image rotated because the current through one of the magnifying lenses changed during exposure of the negative. The linear movement of the image increases as the distance from the center of rotation so that the image is relatively sharp only at the center of rotation. This is one of the reasons for aligning a microscope so that the center of image rotation coincides with the center of the viewing screen.

All magnetic lenses rotate the image as electrons move through them. The amount of rotation is dependent of lens strength and electron speed. *NOTE: Election speed is proportional to accelerating voltage.* Thus, anytime a lens currents or high voltage changes, the image rotates about an axis through the center of the lens. Image rotation is not normally noticeable in the modern microscope, even when changing magnification or focusing the image. This is because the inherent rotation of one lens is offset by an opposite rotation of another lens. Zero image rotation throughout the magnification range is a difficult design criterion, which should be recognized and appreciated by the user.

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In order to be considered for certification, the applicant must be a member in good standing in EMSA and submit either transcripts of college credit or training and evidence of transmission electron microscopy courses. Two letters of reference are required, with one letter coming from an EMSA member. A $50.00 fee must accompany the application.

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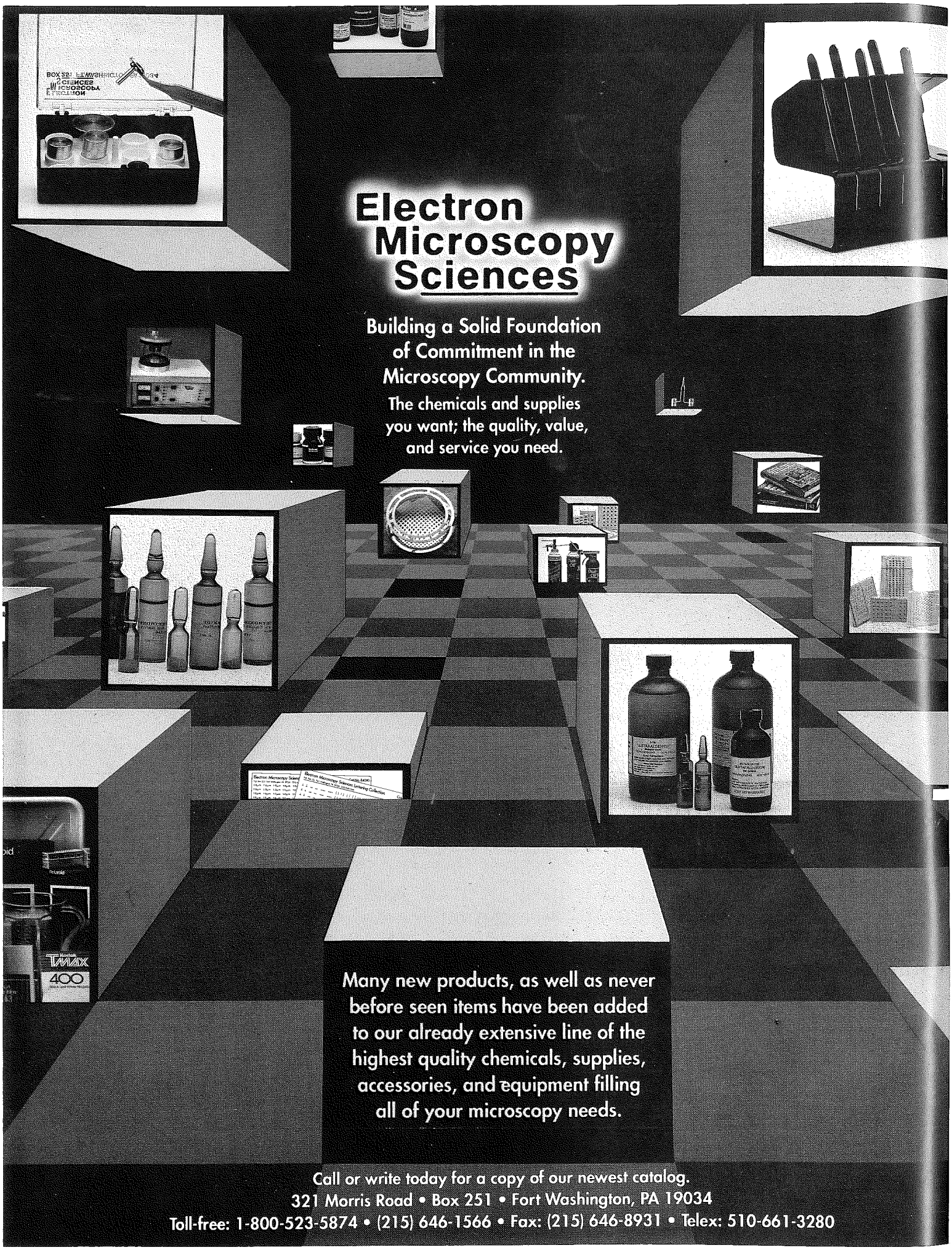
For more information contact: EMSA Certification Program, Southern Illinois University, Carbondale, IL 62901 (Telephone: 618-453-3730) and request either the general informational brochure or the application packet.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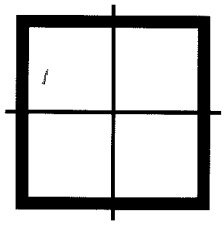

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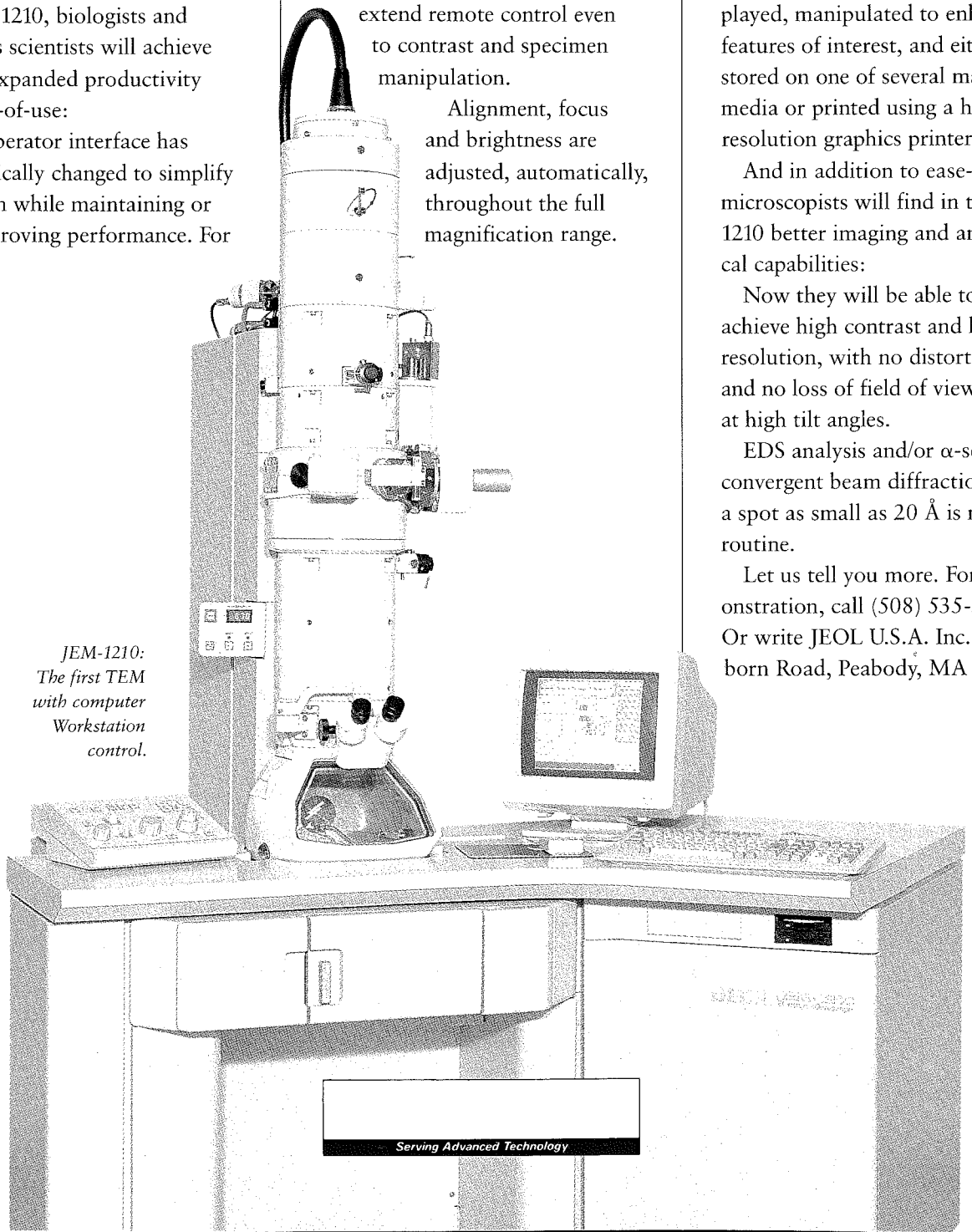

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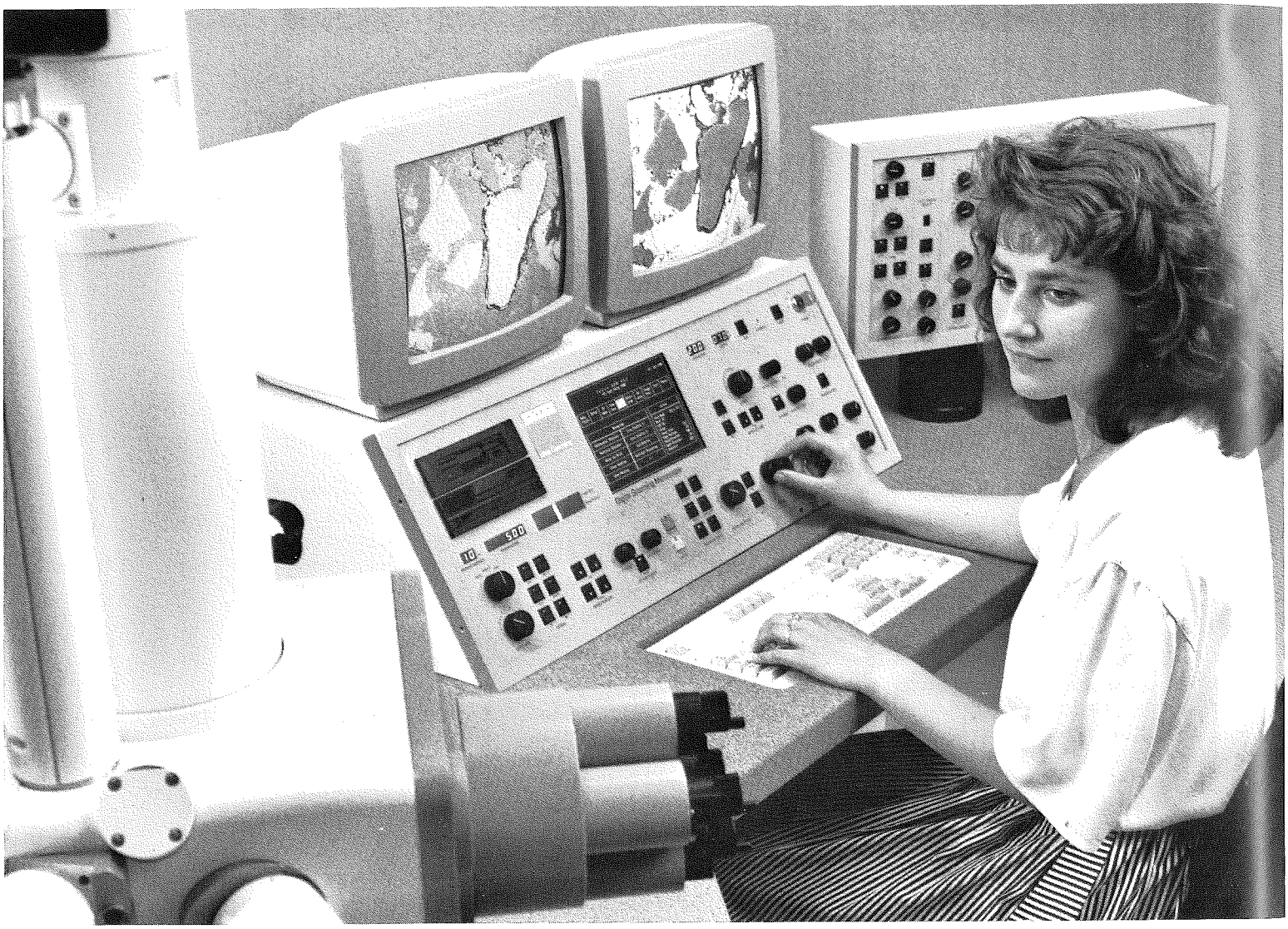

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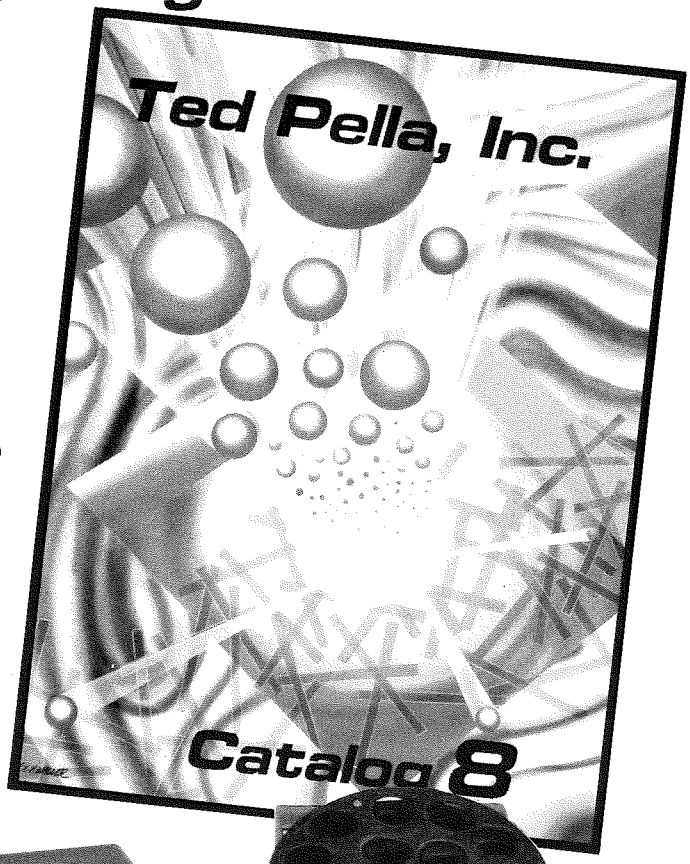

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Abstracts

BIOLOGICAL SCIENCES

PLATFORM PRESENTATION — FALL 1991

EVALUATION OF SEM PROCESSING TECHNIQUES OF CULTURED HUMAN LENS EPITHELIAL CELLS GROWN ON MICROCARRIER BEADS AND FLAT SUBSTRATES. D. Cantu-Crouch, W. E. Howe and M. D. McCartney, Alcon Laboratories, Inc., Fort Worth, TX 76134

In order to develop an in vitro model for cataract formation and treatment, human lens epithelial cells have been successfully grown in culture. In an effort to characterize the ultrastructure of these cells prior to model testing, it became apparent that alternate scanning electron microscope processing methods were needed. The present study has examined cultured human lens epithelial cells processed by critical point drying, Peldri II and tert-butyl alcohol on both flat and bead substrates. Critical point drying produced specimens that had numerous breaks and a series of microcracks on the cell surfaces. Peldri II and tert-butyl processing eliminated these filopodial and lamellipodial breaks as well as eliminating the microcracks on these large, flattened cells. Cells grown on Cytodex 3 beads appeared rounded with a convoluted membrane when they were processed by all three methods. Cytodex 3 beads were subsequently shown to shrink 60% during dehydration. Cells grown on polystyrene beads, which do not shrink, showed a morphology similar to the cells observed on on flat substrates. These results indicate that Peldri II and tert-butyl alcohol present an attractive alternative to standard SEM processing methods. In addition, interpretation of cultured cell morphology must consider substrate shrinkage as a possible contributor to artifactual changes.

THE EFFECTS OF HYPOXIC HYPOXIA AND CARBON MONOXIDE ON SMALL-GRANULE CELLS WITHIN TRACHEAL EPITHELIUM. M.V. CHOKSHI AND A.D. PEARSALL, Dept. of Anatomy, Baylor College of Dentistry, Dallas, TX 75246

Scattered throughout the epithelial lining of the respiratory system is a group of cells, Small-Granule Cells (SGCs), with features that are similar to Amine Precursor Uptake and Decarboxylation cells. In the intrapulmonary airway, these SGCs occur either singly or in organized clusters, whereas, in the extrapulmonary airways, they occur singly. Numerous studies have demonstrated that clustered SGCs are sensitive to airway pO_2; however, the functional significance of the solitary occurring cells remain conjectural. Accordingly, the purpose of this investigation was to characterize the cellular response of tracheal SGC by utilizing ultrastructural morphometric techniques. Fifteen New Zealand white rabbits (28 days old) were exposed to one of the following conditions for six hours: control (20.6% O_2), hypoxic hypoxia (13% O_2), or tissue hypoxia (750 ppm CO). Following the induction of anesthesia, the animals were exsanguinated by vascular perfusion; subsequently, the trachea was removed *en block*, and tissue samples were prepared for routine TEM analysis. Morphometric analysis demonstrated a decrease in the volume percent of the infranuclear cytoplasm occupied by the dense-core vesicles in both hypoxic hypoxia (8.5%) and tissue hypoxia (5.3%), as compared to the control (12.7%). By comparison, the volume percent of the entire cytoplasm occupied by mitochondria and rough endoplasmic reticulum remained unchanged. This data supports the premise that the tracheal SGCs are sensitive to tissue hypoxia, irrespective of the airway pO_2. Financial support has come from NIH Grant #HL-42615.

COMPARISON OF SCANNING ELECTRON MICROSCOPY PROCESSING TECHNIQUES ON SOLVENT SENSITIVE INTRAOCULAR LENS MATERIALS. J. Drab, D. Cantu-Crouch and M.D. McCartney, Alcon Laboratories, Inc., Fort Worth, TX 76134

Scanning electron microscopy (SEM) examination of intraocular lenses (IOL) is important for the evaluation of new designs, coatings and surface features. The problem arises when previously implanted IOLs must be processed to preserve adherent biological material and yet not effect these sensitive polymers. The present study compares the appearance of three types of lens materials, poly-methyl methacrylate (PMMA), molded acrylic soft lens (MASL), and IOGEL after each has been processed by critical point drying (CPD), Peldri II and tert-butyl alcohol. Lenses not subjected to any processing were the standard for the appearance of their optic surface, optic edge, positioning holes, haptic junctions and haptic arms. PMMA lenses showed slight distortion of the positioning holes with CPD and Peldri II while tert-butyl provided good preservation. MASL lenses were adversely affected by CPD and tert-butyl while Peldri II did not distort the surfaces. In contrast, IOGEL lenses were adversely affected by all three processing methods. PMMA haptics were deeply grooved by CPD and Peldri II but were similar to controls with tert-butyl. MASL haptics were severely distorted when processed by all three methods. In conclusion, acceptable preservation was achieved with tert-butyl for PMMA optics and haptics while Peldri II maintained the surface features of MASL lenses.

A STUDY OF THE CRYSTAL PACKETS OF *VITIS* USING X-RAY ANALYSIS AND BACKSCATTERED ELECTRON IMAGING. H. J. ARNOTT, L. E. LOPEZ, AND M. A. WEBB. Department of Biology, University of Texas at Arlington, Arlington, TX 76019-0489 and Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907.

Using techniques described elsewhere we have been able to isolate the crystal packets of several species of *Vitis* (*V. mustangensis*, *V. labrusca*, and *V. vulpina*). The crystal packets contain several hundred crystals of calcium oxalate and a matrix in which the crystals are embedded. These two constituents represent the major contents of the vacuole in these large idioblastic cells of the grape leaf. The crystal packets are extremely hydrophilic and the addition of water to isolated packets causes their immediate breakdown. Thin sections of the packets show that the matrix is composed of a filamentous reticulum. X-ray analysis of the isolated packets shows that the elements Ca and K are differentially distributed; calcium is of course found in the space occupied by the calcium oxalate crystals, whereas both calcium and potassium are associated with the region of the packet occupied by the matrix. The matrix has been shown to consist of a PAS positive material which is, at least in part, composed of the following sugars: arabinose, mannose, galactose, glucose, and glucuronic acid. In addition a mixture of proteins has been found in the crystal packets. We are currently investigating the distribution of other (trace) elements within the crystal packet.

THE MANY FACES OF AMYLOID IN DIAGNOSTIC NEUROPATHOLOGY. S.C. BAUSERMAN, M.D., Department of Pathology, Scott & White Clinic and Texas A&M University College of Medicine, Temple, TX 76508.

Amyloid deposition in the central and peripheral nervous system presents in a wide variety of often puzzling clinical scenarios. Its neuromuscular manifestations are also poorly characterized in the literature. We present representative case material including special stains and TEM studies of the affected tissues. Two cases of neuromuscular disease presented with a chief complaint of jaw claudication, a symptom clinicians often associate with a diagnosis of Temporal Arteritis. Amyloid Neuropathy is a well-known entity with protean manifestations frequently including the autonomic nervous system. A relatively recent awareness of amyloid deposition in intracranial blood vessels and in vascular malformations of the brain has brought our attention to this as a cause of non-traumatic intracranial hemorrhage (strokes) especially in the elderly and in the population of Alzheimer's Disease patients where it has been called Congophilic Angiopathy. The presence of amyloid in the mature neuritic plaques of Alzheimer Disease patients may have pathogenetic significance for research into this pervasive disease. Representative cases from our diagnostic material are presented to illustrate the morphologic spectrum of these diseases. Possible pathogenetic mechanisms for Amyloid deposition in these various Neuropathologic settings are discussed.

TARGETING OF ERYTHROCYTES TO T-LYMPHOCYTES USING SPECIFIC MONOCLONAL ANTIBODY. LAURA CHIARANTINI[1,2], ROBERT DROLESKEY[2], MAURO MAGNANI[2], HEINZ KIRCH[1] AND JOHN R. DELOACH[1], [1] USDA/ARS Food Animal Protection Research Laboratory, Route 5, Box 810, College Station, Texas 77845, [2] Istituto di Chimica Biologica "G. Fornaini" Universita' degli Studi di Urbino, Via Saffi, Urbino 61029, Italy.

Carrier erythrocytes are cells which had been reversibly hemolyzed to encapsulate drugs, toxins or biological response molecules for delivery purposes. In order to guide carrier erythrocytes to specific cells of the immune system we explored a method for targeting erythrocytes to T-lymphocytes. The targeting system was tested by coupling monoclonal antibody (Mab) to the surface of mouse erythrocytes and subsequent exposure to T-cells *in vitro* and *in vivo*. Erythrocytes coupled to T-cell surface specific Mab (anti-mouse Thy1.2) formed rosettes with a mouse derived clone of cytotoxic T-cells *in vitro*. Examination of rosettes by scanning and transmission electron microscopy showed that these T-cells had an average of 4-5 erythrocytes attached to them but there was no evidence of membrane fusion. If a nonspecific Mab (control mouse ascites fluid) was used rosette formation did not occur. Erythrocytes coupled to specific Mab injected into mice IV retained their ability to recognize T-cells since rosettes were found in circulating blood. Thus, carrier erythrocytes coupled to Mab offer the potential for delivering molecules directly to target sites.

NUCLEAR POLYHEDROSIS VIRUS IN THE HEPATOPANCREAS OF THE SPIDER CRAB, *LIBINIA EMARGINATA*. T.Z. MC NEILL, Dept of Biology, Trinity University, San Antonio, TX 78212. E.F. COUCH, Dept of Biology, Texas Christian University, Fort Worth, TX 76129.

A nuclear polyhedrosis virus was found in the nucleus and cytoplasm of hepatopancreas cells but not in the green gland, ovary or mandibular organ of *Libinia emarginata* from the Wood's Hole, Massachusetts area. The viruses were encapsulated in a protein crystal inclusion body within the neucleoplasm. The viral particles were also found in the cytoplasm but, at this location they were not included in a crystalline matrix as seen in the nucleus. These nuclear crystals, and the individual virus particles contained within them had a morphology like that of other arthropod polyhedrosis viruses. Similar viruses (*Baculo virus pinaei*) have been found in crustacea, including pink shrimp (Couch, 1974). This viral group is also commonly found in insecta (Gouranton, 1972). The nucleocapsid itself is somewhat rod-shaped but has a slight bend making it closer to kidney-shaped. The capsid is 35 nm in diameter and 85 nm in length. The crystalline inclusion body inside the nucleoplasm is trapezoidal and measured 5 um in length along the major axis and 3 um in length along the minor axis. The lattice spacing was 7.5 nm between protein subunits. The protein subunits were 16 nm in diameter.

LEAF STRUCTURE OF *ABRONIA MACROCARPA* GALLOWAY (NYCTAGINACEAE). GENA K. HAMILTON AND PAULA S. WILLIAMSON, Dept of Biology, Southwest Texas State University, San Marcos, TX 78666.

Abronia macrocarpa, large-fruited sand verbena, is an endangered East Texas endemic restricted in distribution to Leon, Freestone, and Robertson counties. The plant occurs in sandy soils in the Post Oak Savanna. The plant is an herbaceous perennial with a deep taproot. The species is characterized by large, papery, thin-walled anthocarps (fused sepals surrounding the fruit). The leaves are ovate with entire margins. The epidermis consists of irregular shaped epidermal cells, anomocytic stomata, and multicellular glandular trichomes. Sand typically adheres to the trichomes. The sand covering the leaf may reflect light or reduce herbivory. Stomata are distributed on both epidermal surfaces. Large substomatal chambers are present in both the palisade and spongy layers. The mesophyll consists of one palisade layer and three spongy parenchyma layers. Venation is eucamptodromous. The vascular bundles are numerous, but small and lack a well developed bundle sheath. Football-shaped idioblasts, containing bundles of raphide crystals, are distributed throughout the mesophyll.

SEM TECHNIQUES FOR IDENTIFICATION OF POLLEN ADHERING TO CORN EARWORM MOTHS. M.W. PENDLETON AND P.D. LINGREN, Texas A&M Electron Microscope Center, College Station, TX 77843 and USDA-ARS, Crop Insect Pests Management Research Unit, College Station TX 77840.

The migration patterns of the corn earworm, Helicoverpa zea (Boddie), moth, a significant pest of grain crops, can be determined through the identification of pollen grains which become attached to moths. Following the collection of moths from Oklahoma and Texas, a comparison was made between dissecting light microscopy and scanning electron microscopy to assess which procedure or combination of procedures might best determine whether pollen was present on a moth sample and/or determine which pollen taxa was present on a particular moth. Of the almost 400 moths studied, 301 were screened using a dissecting microscope and those moths which exhibited material which could be pollen (111 moths) were then examined using scanning electron microscopy (SEM). The remaining 98 moths were examined using only SEM. A comparison of the time spent for various procedures associated with the preparation and analysis of pollen on moths suggest that the dissecting scope is inadequate for accurate determination of the presence or identification of the pollen types present on the moths used in our study. Observation of moths only by SEM is more accurate and reliable than initial screening using dissecting light microscopes for pollen recognition and identification. Alternative mounting media used for attachment of insects to stubs is also discussed.

OBSERVATIONS OF CALCIUM CRYSTALS IN OXALIS DILLENII DURING PLANT GROWTH. K.G. GHANEM AND L.H. BRAGG, Department of Biology, The University of Texas at Arlington, Arlington, TX 76019.

In earlier studies of mature Oxalis dillenii seed coats and leaves we observed concentrations of calcium oxalate crystals, but less amounts to none were present in the roots and stems of these plants. Other researchers have speculated that the exposed crystals in the seed coats may alter the soil's pH, thus providing a more favorable environment for these plants. SEM observations were made of the seed coat crystals before and after sprouting of the plants to detect possible changes in crystal sizes and numbers. Cotyledons and embryos within dormant seeds as well as young seedlings with photosynthetic cotyledons and the root-shoot axis were examined using light microscopy to detect crystal occurrence. All vegetative parts from sprouting through maturation were examined for the presence of crystals. Some reduction in crystal size occurred on the seed coat. Crystals were heavily concentrated in the mesophyll of the cotyledons and foliage leaves but were not associated with the vascular tissue of these structures. Crystals were absent at the apex of the cotyledons, the apical meristems of the shoot and root, and the root hairs.

~~SAFETY IN THE EM LABORATORY: STAYING HEALTHY AFTER~~ "MANY" YEARS. J.A. MASCORRO, Department of Anatomy, Tulane Medical School, New Orleans, LA 70112.

Caution and common sense are parameters that this microscopist has practiced faithfully since his introduction into the field of electron microscopy in 1962. In the absence of standardized procedures or rules to insure safety within the electron microscope laboratory, safeguards to a large measure become the responsibility of the individual microscopist, a factor that must be addressed very seriously in order to guard against occupational injury. Areas that most often concern the worker usually include the epoxy resins (especially the low viscosity dioxides), fixatives, heavy metal stains, and organic solvents. In addition, however, one must not take for granted that activities such as breaking glass knives are safe, for glass chips in the eye could be as devastating as osmium vapors in the lungs. This worker recalls quite well the early days of perfusion fixation of the central nervous system with osmium tetroxide, with nothing more than a room fan to keep the osmium vapors away. And also the practice of preparing embedding media without particular concern over skin contact with the individual ingredients. Dehydration and infiltration often were performed without the benefit of a hood area, thus coming into contact with the volatile vapors of propylene oxide. Since those years, a combination of experience, common sense, caution, avoidance of all laboratory vapors, and safeguards against skin contact with chemicals have combined to (presumably) keep this microscopist in a condition of good health.

THE THREE DIMENSIONAL DISTRIBUTION OF MANGANESE IN THE HYPHOPODIA (APPRESSORIA) OF *GAEUMANNOMYCES GRAMINIS* IN CULTURE. H. J. ARNOTT and L. E. LOPEZ, Department of Biology, The University of Texas at Arlington, Arlington, TX 76019-0498.

Take-all, *Gaeumannomyces graminis* var *graminis*, is a soil-borne fungus which attacks wheat and other small grains; a second variety, *G. graminis* var *tritici*, produces the black stem rot of rice. Both varities are widespread and are important disease agents in Texas. Abundant hyphopodia (appressoria; cells involved in the penetration and infection of the host) are formed *in vitro* when the Take-all organism, *Gaeumannomyces graminis* (Ggg 502.1L) is grown on 4% potato dextrose agar. When supplemental manganese is added to cultures ($MnSO_4$ supplied as 50 $\mu g\ g^{-1}$) a yellow brown precipitate forms in association with the hyphopodia and certain other lateral branches. We are using x-ray maps and backscattered electron imaging to study the location of the manganese deposits. When grown with added manganese a fibrous coating is formed on the hyphopodia and on certain lateral branches. It appears that this fibrous coat is associated with the manganese biomineralization. X-ray maps demonstrate that the major region of manganese deposit is in this fibrous layer. Using Topas, a solid modeling program, we are developing a 3-dimensional model from both light and electron microscopic studies. With the model we are attempting to define the exact region of deposit in an effort to develop a 3-dimensional understanding of the process of biomineralization.

THE ROLE OF ENDOCYTOSIS IN ENCAPSULATION USING HYPOTONIC DIALYSIS. Robert E. Droleskey[1], Kathleen Andrews[1], Laura Chiarantini[1,2] AND John R. DeLoach [1]UDSA/ARS, FOOD ANIMAL PROTECTION RESEARCH LABORATORY, ROUTE 5, BOX 810, COLLEGE STATION, TX 77845, AND [2]INSTITUTO di CHIMICA BIOLOGICA, UNIVERSITA degli STUDI, VIA SAFFI, 2, 61029 URBINO, ITALY.

When examined by transmission electron microscopy (TEM) carrier erythrocytes prepared by hypotonic dialysis contain a percentage of cells with what appear to be endocytic vesicles. To determine the role which endocytosis might play in the encapsulation process human and murine erythrocytes from specific stages in the process were fixed and examined using TEM. Additionally, cells from these stages were incubated with fluorescent probes (Lucifer Yellow-CH, Lucifer Yellow-Dextran and FITC-Dextran) in an attempt to rapidly quantify endocytosis. The uptake of fluorescent probes was monitored using a fluorescent microscope, fluorescent spectrofluorimeter, and a fluorescent activated cell sorter. Vesicles did not appear in sectioned erythrocytes until the annealed stage for human (15%), which was at maximum, while vesicles were present in 5% of resealed murine cells. Maximum endocytosis for the mouse (13%) was found to occur following annealing and an isotonic buffer wash, while cells washed in hypotonic buffer showed no increase in vesiculation from the 9% present after annealing. After a similar hypotonic wash for annealed human cells, the percentage of cells with vesicles dropped to 8%, while an isotonic wash resulted in a reduction to only 12%. The number of vesicles present in human carrier cells dropped to between 10 and 11%, while 8-10% of murine carrier cells contained vesicles Active endocytosis, as determined by the uptake of FITC-Dextran, was found to be occurring in only 1-2.25% of the human carrier cells.

ELECTRON MICROSCOPIC EXAMINATION OF LIPID DEPOSITS IN HUMAN AORTIC FATTY STREAKS. K.F. KLEMP AND J.R. GUYTON, Departments of Medicine and Cell Biology, Baylor College of Medicine, Houston, TX 77030

The earliest distinctive lesions in human atherosclerosis a fatty streaks, characterized by initial foam cell formation. Fibrous plaques, the clinically important lesions have fibromuscular proliferation, extracellular lipid deposits in a core region, and foam cells. We investigated the hypothesis that some or most fatty streaks might develop extracellular lipid deposits consistent with core region formation. Thirty-two unilateral fatty streaks from 24 autopsied aortas were selecte and control tissue blocks from the contralateral aortic walls were also examined. Tissues were processed for cytochemical E by osmium-thiocarbohydrazide-osmium (OTO) and osmium-tannic acid-paraphenylenediamine (TA-PDA) techniques. The OTO protoc lent itself well to morphometric analysis of the tissue while the TA-PDA procedure allowed superior ultrastructural analysis We found extracellular osmiophilic material consistent with lipid deposits to be increased ($p < .05$) in the deep intima of fatty streaks. Evidence of foam cell necrosis contributing to extracellular lipid deposition was not common. Neutral lipids in the form of droplets tended to be intimately associated wit elastic fibers while membranous lipid was often found enmeshed in collagen bundles. Some lipid deposition was so extensive as to resemble the lipid-rich core region of fibrous plaques. Cholesterol clefts, a hallmark of the fibrous plaque core region, were present in four (12.5%) of the specimens examined These observations lend support to the hypothesis that extracellular lipid deposition in the deep intima underlying aortic fatty streaks may be involved in the progression of these lesions to fibrous plaques.

TISSUE RESPONSE TO POROUS HYDROXYAPATITE IMPLANTS IN RAT ALVEOLAR BONE: AN ELECTRON MICROSCOPIC EXAMINATION. R. Spears, B. Schaulin and A.D. Pearsall, Dept. of Anatomy, Baylor College of Dentistry, Dallas, TX 75246

Numerous investigations have demonstrated that hydroxyapatite (HA) provides an effective replacement for autogenous bone in many surgical and reconstructive procedures. Such implants are often requisite for various dental procedures, including alveolar ridge reconstruction and augmentation. In addition to being biocompatible, the implant must be strong enough to withstand the rigors of mastication. At the present time, most studies of HA implants have examined the tissue-implant interface via light microscopy. Therefore, the purpose of this investigation was to characterize the tissue response of rat alveolar bone to Interpore-200 (IP), a porous HA implant, using scanning (SEM) and transmission electron microscopy (TEM). Eighteen adult male Sprague-Dawley rats were anesthetized and both mandibular second molars were extracted. The sockets were flushed with saline, filled with IP, and subsequently sutured. The animals were sacrificed at 14, 28, and 56 days by vascular perfusion and samples prepared for either SEM or TEM examination. At two weeks, few signs of inflammation were apparent. SEM demonstrated that the implants were surrounded by a fibrous envelope (FE) that blended with the implant surface. TEM analysis revealed the presence of an inner cellular layer (ICL) composed primarily of fibroblasts. At 28 days, SEM and EDXA analysis demonstrated that the FE was covered with osteoblasts. However, the FE showed no signs of mineralization. TEM investigation revealed that the ICL had become thinner and the cell types less diverse, comprised almost entirely of fibroblasts and a few foreign body giant cells. By day 56, bone was observed to contact the outer boundary of the FE, but remained separated from the implant. The results from this study demonstrated that IP was biocompatible, but a fibrous cellular layer was always observed at the implant-bone interface. (Supported by NIH Grant #DE07188.)

ULTRASTRUCTURAL ANALYSIS OF CHEETAH SPERM: A POTENTIAL METHOD FOR ASSESSING SPERM QUALITY FOR BREEDING PROGRAMS. *L.D. GRAY, R.S. SIMMONS, L.S. THEDFORD, D.B. HOLIDAY, Depts. of Cell Biology & Environmental Sciences, The Vivarium, and Epidemiology & Biomathematics, The University of Texas Health Center at Tyler, P.O. Box 2003, Tyler, TX, 75710.*

Breeding programs utilizing captive cheetahs (*Acinonyx jubatus*) are currently of great interest to zoological parks throughout the United States and the world due to the endangered status and genetic nondiversity of this animal. There is relatively little published scientific information concerning reproductive physiology and anatomy in this species. Only a handful of articles concerning cheetah sperm morphology were located by computer literature search. It is possible that qualitative and semiquantitative methods of assessing sperm ultrastructure will be beneficial in choosing the best breeders for these breeding programs. In the present investigation, semen was collected by electroejaculation from healthy cheetahs housed at the Caldwell Zoo in Tyler, TX. The semen samples from individual animals were pooled and an aliquot from each was fixed in 0.1M sodium cacodylate buffered 3% glutaraldehyde. The samples were postfixed in OsO_4, *en bloc* stained in uranyl acetate and dehydrated in ethanol. A sample was then removed and placed dropwise on glass coverslips. The samples were air dried on the coverslips and these were mounted on Al stubs and then coated with Au/Pd for SEM analysis. The remainder of the cells were embedded in Spurr's epoxy resin and processed for TEM study. Qualitative SEM indicated that sperm cells were over 30 µm long with dorsolaterally flattened heads. Anomalies noted by SEM included coiled tails, microcephaly and numerous cytoplasmic droplets. The sperm cells were usually in aggregates. TEM observations confirmed the presence of numerous abnormal forms including coiled tails, disorganized axonemes, vacuolated nuclei and large cytoplasmic droplets. For quantitative TEM studies, sections from several blocks were collected on coordinate marker grids. The squares to be sampled were predetermined and evenly distributed in order to insure adequate sampling of the entire block face. Sperm profiles were scored for having or lacking a specific defect and this was recorded for later statistical analysis. Defects likely to affect motility and acrosomal integrity were of principal interest. Specific percentages versus types of defects found in the cells will be reported in the presentation.

BIOLOGICAL SCIENCES

POSTER PRESENTATION — FALL 1991

DISTRIBUTION OF LAMININ IN BASAL LAMINA OF GROWING RAT AIRWAYS R.A. Cox, Q.Y. Zhu, A.S. Burke, M.J. Evans, Shriners Burns Institute, 610 Texas Avenue, Galveston, TX 77550

During growth of rat tracheas, several morphological changes occur in the epithelium. As the circumference of the trachea increases, the epithelial height increases. Accompanying this height increase, the epithelial distribution of basal cells changes with the number of basal cells increasing from 3.2/100 um to 9.6/100um. In addition, anchoring junction components of basal cells (desmosomes and hemidesmosomes) also increase. The changes in basal cells are related to attachment of columnar epithelial cells to the basal lamina. The purpose of the present study was to determine if the major adhesive protein in the BL (laminin) also changed as the trachea grew in circumference. To accomplish this, 30 and 90 day-old rat tracheas were studied. Using post-embedded immunocytochemistry, the distribution of laminin in the basal lamina was determined. Preliminary results suggest that the concentration of laminin in basal lamina does not change (19.1 and 19.8 gold particles/um of basal lamina, respectively). These data indicate the adhesive properties of laminin are maintained in the basal lamina as the airway grows in circumference. This suggests that increased attachment strength of the epithelium to the basal lamina is accomplished by increased junctional attachment (hemidesmosomes) and not increased concentrations of laminin in the BL. This interpretation does not exclude the possibility of increased numbers of laminin receptors on the basal cells.

This research was supported by Shriners Hospital for Crippled Children grants #15813 and #15853.

THREE-DIMENSIONAL STRUCTURES OF NATIVE AND CHYMOTRYPSIN-HUMAN α_2-MACROGLOBULIN COMPLEXES. S.J. Kolodziej,[1] J.P. Schroeter,[1] T. Wagenknecht,[2] J.P. Bretaudiere,[1] D.K. Strickland,[3] and J.K. Stoops.[1] [1]The University of Texas Health Science Center, Houston, TX 77030, [2]New York State Department of Health, Albany, NY 12201-0509, [3]American Red Cross Biomedical Research and Development, Rockville, MD 20855.

Human α_2-Macroglobulin (α_2-M) (M_r=720,000) consists of four identical subunits bound together to give a complex consisting of two protomers. α_2-M binds two moles of chymotrypsin per mole of α_2-M subsequent to the cleavage of its "bait" region by the protease resulting in a substantial change in its structure. As part of our effort to elucidate the mode of action of α_2-M, the three-dimensional structures of native and chymotrypsin-treated human α_2-M were determined by the single-exposure random conical tilt series method using negative stain electron microscopy to visualize the molecules. Two-dimensional projections of both structures showed close correspondence to the characteristic views seen in negative stain and cryo-electron microscopy indicating that the reconstructions are reliable. Furthermore, tilt experiments demonstrated the relatedness of the various projections and were in accord with those obtained from the three-dimensional structure. The arrangement of the two protomeric units in the native and proteolyzed molecules could be deduced, thus making it possible to determine the manner in which the structure rearranges on binding of the protease. We propose that the native molecule in the shape of a twisted oval loop opens at the contacts between the two protomeric units at opposite ends of the structure and that they become co-planar in the transition to the proteolyzed structure.

ABSENCE OF ATHEROSCLEROSIS IN THE 12 MONTH OLD HYPERLIPIDEMIC OBESE ZUCKER RAT, E.Wilson, R.Verani, K.L.Berens and L.M.Buja, Department of Pathology and Laboratory Medicine,University of Texas Medical School and The University of Houston.

The genetically obese Zucker rat (OZR) exhibits hypertriglyceridemia and hypercholesterolemia and has been proposed as an animal model of atherosclerosis. We examined the aortic arch and abdominal aorta of 5 OZR and 5 lean littermates(LZR)at 12 months of age, by light(LM),transmission(TEM), and scanning electron (SEM) microscopy. No significant differences were observed in serum glucose(192±106 vs 184±65 mg/dl), and systolic blood pressure (129±12 vs 126±4 mgHg) at 12 months of age between OZR and LZR. OZR manifested marked hyperlipidemia compared to LZR: serum triglycerides 2147±2100 vs 329±368 mg/dl ($p<0.05$) and serum cholesterol 255±165 vs 99±55 mg/dl ($p<0.10$). Histological examination showed similar findings in the OZR and LZR,e.g. minimal focal intimal thickening and rare subendothelial cells. SEM and TEM showed intact endothelial cells, and TEM showed smooth muscle cells, collagen and elastin in the areas of intimal thickening.Foam cells in the intima were not seen. We conclude that despite hyperlipidemia,the OZR at 12 months of age does not develop atherosclerosis. Minimal histological changes were observed in both LZR and OZR; however, these changes were interpreted as mild age-related pathology. It is suggested that, in the absence of other factors such as hypertension and hyperglycemia, the hyperlipidemia per se is not sufficient to initiate the process of atherosclerosis in the OZR.

MATERIALS SCIENCES

PLATFORM PRESENTATION — FALL 1991

AN ELECTRON MICROSCOPY STUDY OF COPPER SHAPED CHARGE COMPONENT MICROSTRUCTURES. ALAN GUREVITCH, L. E. MURR & W. W. FISHER. Department of Metallurgical and Materials Engineering, The University of Texas at El Paso, El Paso, TX 79968.

A recovered copper shaped charge jet fragment has been built-up by copper electrodeposition to allow it to be systematically sectioned and polished for detailed observations by optical and electron microscopy for the first time. The residual jet fragment microstructure was observed to have a recrystallized grain structure and dislocation substructures similar to those in the undeformed copper shaped charge liner cone. However the grain size in the recrystallized jet fragment was 15 μm compared to 45 μm for the liner. More significantly, however, SEM observations of the jet fragment exhibited a high density (7%) of voids and coalesced void tunnels elongated axially within the fragment geometry which resulted during jet elongation and breakup by diffusion and viscous growth at high strain and strain rate. This observation of large porosity in the shaped charge jet indicates the potential role that impurities may have in jet formation and stability to the extent that these impurities may influence void nucleation, growth, and coalescence. Supported by Zernow Technical Services Contract ZSC-90-002(4172).

ANALYTICAL TEM ANALYSIS OF PARTICULATES EXTRACTED FROM THE NASA-LDEF SATELLITE. J. M. RIVAS, B. MARQUEZ, A. H. ADVANI, & L. E. MURR, Department of Metallurgical and Materials Engineering, The University of Texas at El Paso, El Paso, TX 79912

A modified two-stage carbon replication technique has been developed to extract fine particles from the surface of an aluminum alloy exposed in space in the Long Duration Explosure Facility Satellite (LDEF) in low earth orbit (LEO) for 5.7 years. More than 100 individual particulates or particulate clusters have been examined in an analytical TEM to routinely document bright and dark-field images, the associated selected-area electron diffraction pattern, and the elemental composition by energy dispersive X-ray spectrometry (EDS). The particles have been identified to consist of debris from other surface features on the LDEF Satellite (including micro-meteoroid impact debris), atmospheric (earth)/stratospheric particles such as NaCl, reaction products unique to the space environment, and interplanetary dust particles (IDPs). A histogram of chemical element frequency has been developed and compared with IDPs and ice forming nuclei (IFN) in the upper atmosphere. Supported by NASA-Johnson Space Center Grant 9-481.

HIGH RESOLUTION TRANSMISSION ELECTRON MICROSCOPY STUDIES of $BaSrTiO_3$/Si INTERFACES. R.F. PINIZZOTTO, H. YANG, S.R. SUMMERFELT* and B.E. GNADE,* Center for Materials Characterization, University of North Texas, Denton, TX; and *Materials Science Laboratory, Texas Instruments Incorporated, Dallas, TX.

High resolution transmission electron microscopy was used to examine the microstructures of $BaSrTiO_3$ thin films deposited on silicon substrates. The samples were fabricated using pulsed laser ablation in ultra-high vacuum. The (100) Si substrates were HF cleaned, rinsed, mounted in the chamber and heated to 500°C before deposition. The BST was deposited using pulse rates of 20 Hz. The oxygen overpressure was varied during the deposition. TEM samples were prepared using standard cross-sectional preparation methods, concluding with ion milling to electron transparency. A JEOL 100CX STEM was used to determine general microstructural properties and a Hitachi H-9000 HRTEM was used for high resolution lattice imaging.

The BST is columnar throughout the film thickness with an average grain width less than 20 nm. The growth occurs more uniformly with oxygen overpressure. An additional phase is observed at the BST/Si interface, consistent with earlier XPS studies. This phase is of non-uniform thickness with triangular grains up to 20 nm tall. HREM demonstrates that in some regions, the BST grew epitaxially on the Si substrate. The grains contain a high density of microtwins, but no dislocations were observed. These results indicate that it may be possible to grow epitaxial ferroelectric materials on silicon substrates as part of future three-dimensional structures.

A TEM STUDY OF CARBIDE PRECIPITATION IN AUSTENITIC STAINLESS STEELS. A. H. ADVANI, DIXIE MATLOCK & L. E. MURR. Department of Metallurgical and Materials Engineering, and Institute for Manufacturing and Materials Management, The University of Texas at El Paso, El Paso, TX 79968.

There is no real evidence or systematic observations of where precipitates form in the grain boundaries of austenitic stainless steels or specific sites within the matrix. Using dark-field TEM, we have observed carbide precipitation to be highly correlated with the regions formed by intersecting micro-shear bands which are composed of heterogeneous bundles of stacking faults which include twin and epsilon martensite. These observations have been made in both 316 and 304 stainless steels. This site specific nucleation leads us to believe that grain boundary nuclei are also site specific, probably associated with grain boundary ledges. Since α'-martensite in 304 stainless steel has already been unambiguously identified to nucleate at micro-shear band intersections having specific crystallographic compositions, it may be that subtle alterations in these site specific nucleation phenomena can account for both α'-martensite and carbide formation. We illustrate these features in detail in this paper. some preliminary TEM of precipitation on coherent twin boundaries will also be included to illustrate the novelty of utilizing structure and energy invariant interfaces to study grain boundary sensitization and precipitation in austenitic stainless steels. Supported by Directorate of Stockpile Grant DN-0009: Institute for Manufacturing and Materials Management.

SEM STUDIES OF INTERMETALLIC INTERFACES IN COMPOSITE SOLDERS. Y.WU and R.F.PINIZZOTTO, Center for Materials Characterization, University of North Texas, P.O.Box 5308, Denton, TX 76203-5308.

A systematic study is presented on the effects of aging time and metallic particle additions on the formation of intermetallic compounds in Pb/Sn solders on Cu substrates. Scanning electron microscopy and x-ray energy dispersive spectroscopy revealed the presence of Cu_3Sn and Cu_6Sn_5 at the solder/substrate interface. Cu_6Sn_5 forms during the soldering operation and Cu_3Sn forms during aging. Both increase in thickness with the square root of aging time. A model for the formation and growth of the intermetallic compounds is presented which involves the Cu and Sn reacting to form Cu_6Sn_5 at the solder/Cu interface when Sn is liquid. During aging, Sn diffuses through the solder/Cu interface, including the Cu_6Sn_5 intermetallic. At low Sn concentrations, Cu and Sn react to form Cu_3Sn. At high Sn concentrations, Cu and Cu_3Sn react with Sn to form Cu_6Sn_5. XEDS was used to determine that Cu_6Sn_5 is the final product when Cu is present, either from the substrate, or from particle additions of pure Cu, Cu_6Sn_5 and Cu_3Sn. Ag_3Sn and $AuSn_4$ are the final products when Ag and Au particles are added to the solder. The Ag and Au additions also were found to reduce the thickness of Cu_3Sn formed at the solder/Cu substrate interface.

ULTRAMICROTOMY: A UNIQUE APPROACH TO PREPARE SOLDER FOR TRANSMISSION ELECTRON MICROSCOPY. L.A.FOSTER, Y.WU, A.R.WILSON and R.F.PINIZZOTTO, Center for Materials Characterization, University of North Texas, Denton, TX 76203.

Solder joints provide a crucial link in electronic packaging by interconnecting integrated circuits and devices, distributing electric power, and dissipating heat. TEM analysis allows one the correlate the microstructure of composite solders with thermal aging. To date, solder samples have been prepared for TEM analysis by mechanical thinning, electrochemical polishing and ion milling. Ultramicrotomy or thin-sectioning of samples with a diamond knife is a preparative technique used by life scientists and polymer chemists to prepare samples for transmission electron microscopy. This technique is being explored by material scientists as an additional method to prepare thin samples of metals and ceramics for TEM analysis. We have found that solders are easily prepared for TEM studies by embedding solder in epoxy resin and thin sectioning with an ultramicrotome and a diamond knife. Thin sectioning of solder allows observation of all phases, including intermetallics and interfaces. Growth of intermetallic regions during thermal aging may be used as an internal microstructural monitor. TEM analysis of solder may aid in elucidating mechanisms that control solderability.

TRANSMISSION ELECTRON MICROSCOPE OBSERVATIONS OF CRYSTAL LATTICE DEFECTS AND GRAIN BOUNDARY MICROSTRUCTURES IN HIGH-TEMPERATURE SUPERCONDUCTORS.
L. E. MURR, A. H. ADVANI, W-P. LI, C.-S NIOU & R. BIRUDAVOLU. Department of Metallurgical and Materials Engineering, The University of Texas at El Paso, El Paso, TX 79968.

It has been observed that the explosive (shock-wave) consolidation of superconducting powders: $YBa_2Cu_3O_7$ and $Bi_7Pb_3Sr_{10}Ca_{10}Cu_{15}O_x$, and the shock deformation of sintered samples of these powders, results in altered and degraded resistance-temperature (R-T) signatures. This shock-induced degradation appears to be related to the production of crystal defects which include dislocations, twins, and clusters of point defects which often exhibit local strain fields. Doping sintered Y-Ba-Cu-O and Bi-Pb-Sr-Ca-Cu-O with silver has a dramatic effect on the degradation of the R-T signature, but the mechanism remains a mystery since no significant segregation of the silver to grain boundaries or other microstructural features are observed. Lattice images of grain boundaries in fact illustrate very smooth transitions even for misorientations greater than 45°, and short-time, high-temperature anneals in Y-Ba-Cu-O have exhibited transport supercurrent densities to approach 10^3 A/cm^2. A search for unique and specific microstructures/defects to explain this behavior has not uncovered any apparent effects in Y-Ba-Cu-O. Supported by a Mr. and Mrs. MacIntosh Murchison Endowed Chair (LEM).

TRANSMISSION ELECTRON MICROSCOPY OF ROTOR STEELS, H.V. MALLELA, R.F. PINIZZOTTO, V. GOVINDARAJU, *D.W. BRASWELL AND *B.M. HOLMAN, Center for Materials Characterization, University of North Texas, Denton, TX 76203, *Texas Utilities Electric Company, Dallas, TX 75201.

Conventional and scanning transmission electron microscopy (STEM) with x-ray energy dispersive spectroscopy (XEDS) were used to study various steel samples from critical power plant components obtained from Texas Utilities Electric Company. Samples were prepared using chemical and jet thinning techniques. Selected area diffraction patterns were obtained at the grain boundaries and from the matrix to explore the carbide structure and to study the grain boundary morphology. The remaining creep life for Cr-Mo-V steels may be related to carbide types and their volume fractions. One possible failure mechanism is temper embrittlement; therefore, STEM was used to test for sulphur segregation on grain boundaries. Preliminary observations also show finely dispersed precipitates along the grain boundaries.

STRUCTURAL STUDY OF $NISI_2/SIO_2/SI$ AND $NISI_2/SI$ MATERIALS BY TRANSMISSION ELECTRON MICROSCOPY. H.YANG and R.F.PINIZZOTTO, Center for Materials Characterization, University of North Texas, Denton, TX 76203-5308, L.LUO, Center for Materials Science, Los Alamos National Lab, Los Alamos, NM 87545 and F.NAMAVAR, Spire Corporation, Bedford, MA 01730.

Nickel disilicide thin films on SiO_2/Si substrates with (100) and (111) orientations were examined and compared to epitaxial $NiSi_2$ layers on single crystal silicon substrates. Samples were made by e-beam evaporation of high purity Ni onto conventional SIMOX samples and then annealed at 800°C for one hour. This converts Ni and top Si layer into $NiSi_2$. $NiSi_2$/Si samples were made using the same procedure. Samples were examined by conventional transmission electron microscopy (CTEM) and high resolution electron microscopy (HREM). Our results show that high quality, single crystal $NiSi_2$ thin layers were formed on both types of substrates. With the SiO_2/Si substrate, the $NiSi_2$ layer has a uniform thickness of 200 nm. Some twin boundaries were observed. With Si substrate, the silicide layer is disrupted at the silicide-Si interface by step-like silicon incursions from the substrate. The substrate orientation has little effect on the microstructure.

CORRELATION OF EELS CORE EXCITATIONS IN POLYMERS AND MOLECULAR ANALOGUES
E. Rightor*, A. Hitchcock**, S. Urquhart**, and A. Wen**
* Dow Chemical, B-1470B, Freeport, Texas 77541
** Institute for Materials Research, McMaster Univ., Hamilton, Ontario, Canada L8S 4M1

The near-edge region of core ionization edges can be used as a probe of the local bonding and structure of atoms excited by incident radiation, such as in electron energy loss spectroscopy (EELS). Efficient parallel EELS spectrometers in ATEMs now allow study of these transitions in radiation sensitive materials such as polymers. To study relationships between core edge transitions and structure of a polymer such as poly(ethylene terephthalate) [PET], we have compared polymer EELS spectra with molecular analogues and explored molecular orbital energies using extended Hückel theory calculations.

Comparison of spectra for the analogues, PET, and molecule-based simulations reveals strong similarities demonstrating the viability of using molecular spectra to aid studies of EELS transitions in macromolecules. This work also shows the ability of core excitations to distinguish functional groups by fingerprint analysis and integration of theoretical calculations to further clarify spectral interpretation.

This information is presented in good faith, but no warranty is given, nor is freedom from any patent inferred.

MATERIALS SCIENCES

POSTER PRESENTATION — FALL 1991

AN SEM STUDY OF MICROMETEOROID IMPACT CRATERS AND RELATED SURFACE PHENOMENA ON SELECTED LDEF SPACECRAFT MATERIALS. STELLA QUINONES, C.-S. NIOU & L. E. MURR. Department of Metallurgical and Materials Engineering. The University of Texas at El Paso, El Paso, TX 79912.

Micrometeoroid impact craters ranging from roughly 1 μm to 100 μm have been examined by scanning electron microscopy (SEM) and energy dispersive X-ray spectrometry (EDS). The crater base and ejecta rims often do not contain elements indicative of complex alloying between the impacting meteoroid particle and the aluminum alloy (6061-T6) surface impacted. This observation support the kinetic models for simulating hypervelocity particle impacts which assume the impacting particle is completely vaporized. However, detailed studies of micrometeroid sections must be conducted in order to unambiguously support this contention. The angle of impact can often be observed from the symmetry of the ejecta rim. The apparent impacts of other irregular fragments probably in the low-earth orbit have also been observed. These impact craters and related impact features on metal surfaces are a contrast to the erosion of plastic (polymer) surfaces exposed to high fluences of atomic oxygen (< 10^{21} atoms/cm^2) on the leading edge of the LDEF Satellite. This erosion is caused by polymer molecule fragmentation from the impacted polymer surface. This research is supported by a NASA-Johnson Space Center Grant NAG-9-481.

TRANSMISSION ELECTRON MICROSCOPY OF DEFORMED BERYLLIUM. C.-S. NIOU, A. H. ADVANI, L. E. MURR, L. VEGA & S. W. STAFFORD. Department of Metallurgical and Materials Engineering, The University of Texas at El Paso, El Paso, TX 79968.

Beryllium sheet and extrusions have found uses in secondary aircraft structures, and components of the NASA Space Shuttle Orbiters have been fabricated from hot-pressed Be. Although Be has a high stiffness-to-weight ratio, its use as a critical structural material is limited by its low ductility and toughness. Sheet Be is particularly susceptible to brittle fracture, and this brittle behavior has been attributed to slip limitations and twinning on $(10\bar{1}2)$. However twinning has never been crystallographically observed in the transmission electron microscope. In this study, we have made a detailed examination of microstructures in SR-200-E Be sheet (98 wt. % Be ~ 2% BeO as oxide inclusions, and impurities of Al, C, Fe) deformed in tension and torsion. No evidence of deformation twinning was found and dislocation densities increased only slowly (to a maximum of ~ 10^9 cm^{-2}) only a few microns from the fracture surfaces. Consequently the strains were extremely localized and characteristic of a truly brittle material. No dislocation pile ups were observed, and the truly unique microstructural features were associated with the grain boundaries which exhibited an unusually high density of ledge structures and associated large strain fields which were observed by strain-field diffraction contrast in the TEM. The nonhomogeneous, brittle fracture in Be therefore appears to be almost exclusively dominated by the grain boundaries and ductility exhibited by Be is probably due primarily to grain boundary sliding. Supported in part by NASA-Johnson Space Center (Contract 27400 TE) and by a Mr. and Mrs. MacIntosh Murchison Endowed Chair (L.E.M.).

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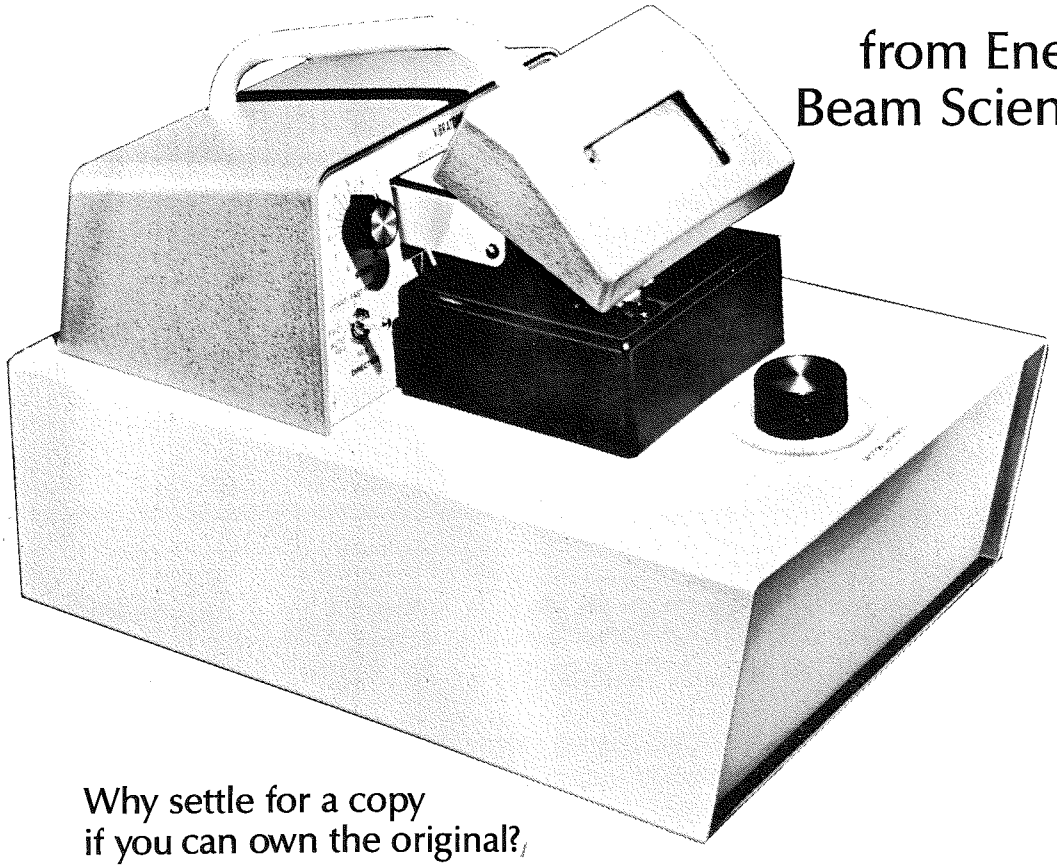

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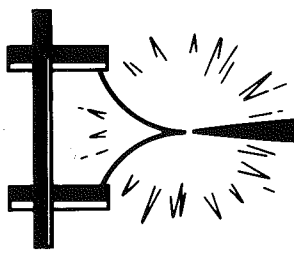

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SPI Supplies, Division of Structure Probe, Inc., Charles A. Garber. P.O. Box 656. West Chester, PA 19381. (800) 242-4774.

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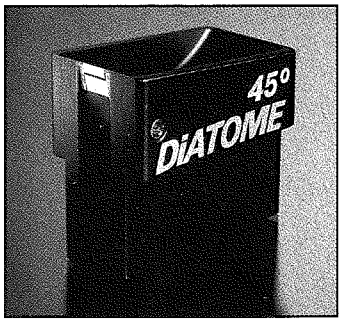

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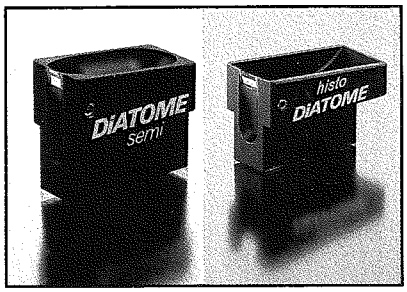

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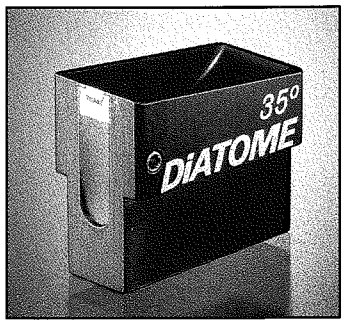

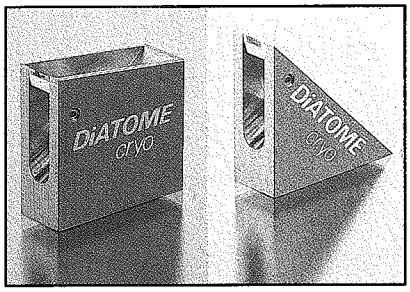

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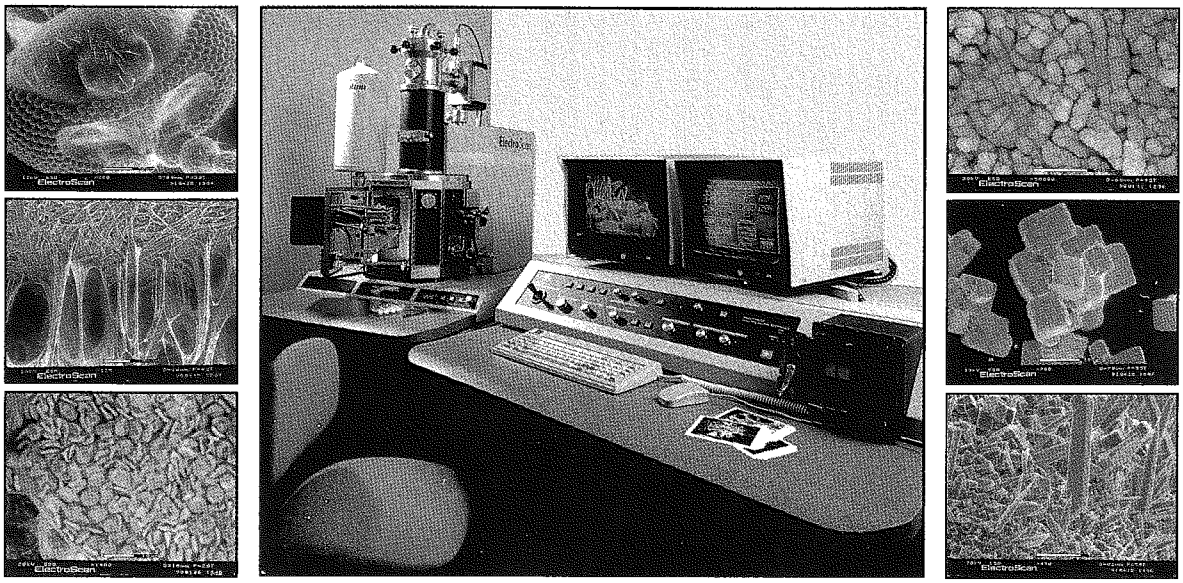

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