TSEM

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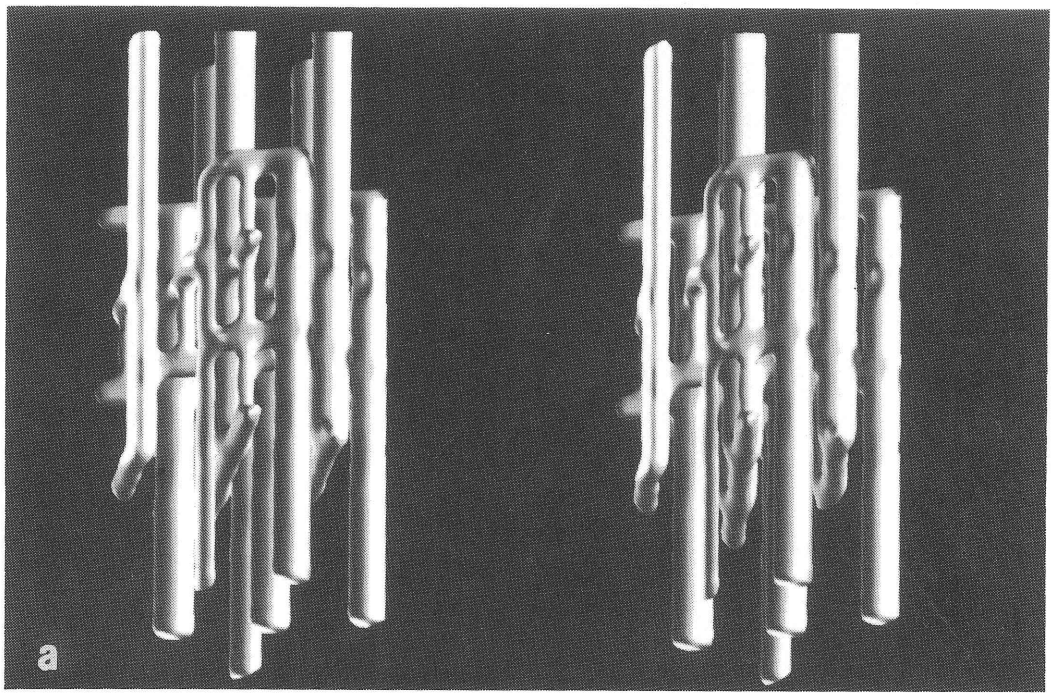

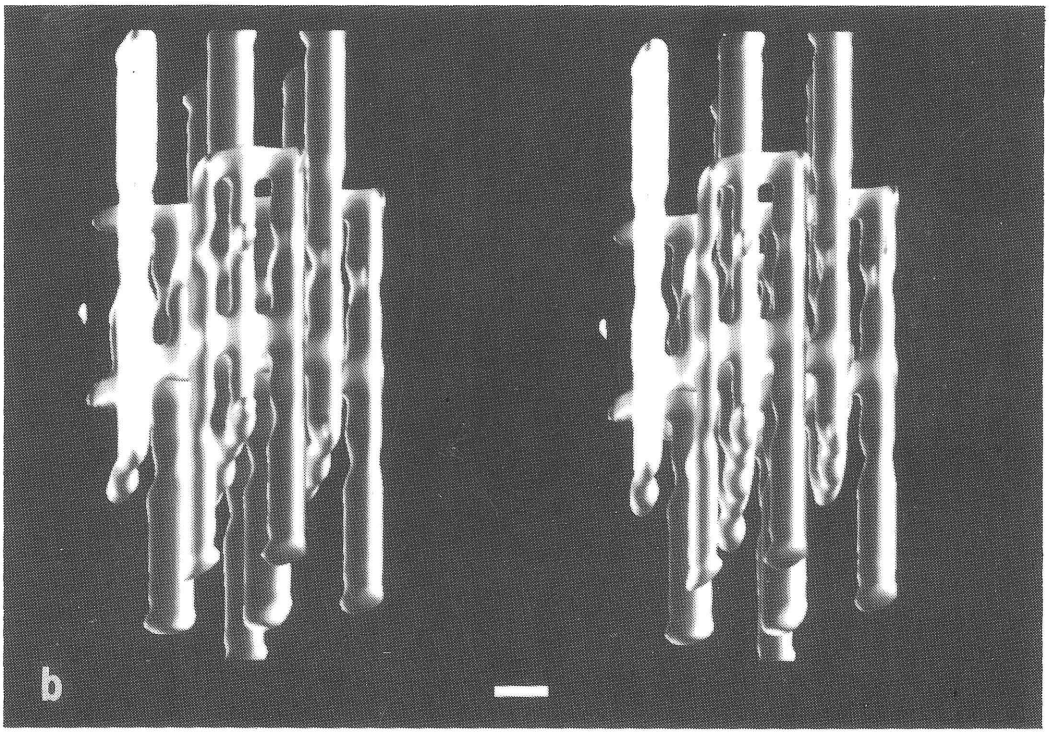

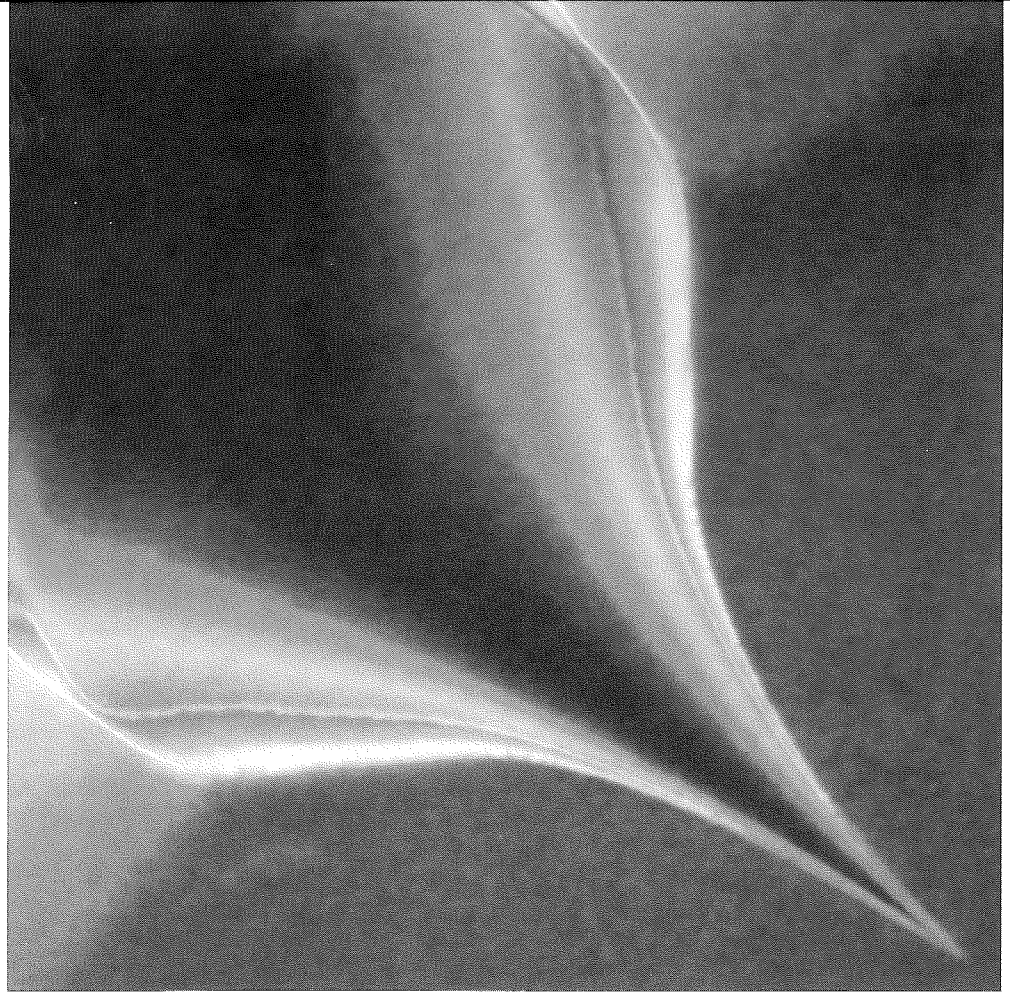

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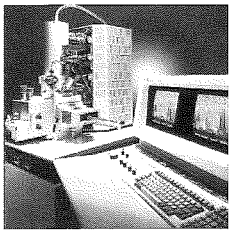

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Contents

TEXAS JOURNAL OF MICROSCOPY
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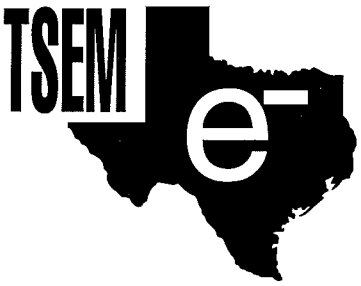
ISSN 0196-5662



David C. Garrett, Editor
Department of Biological Sciences, University of North Texas, Denton, TX 76203

Official Journal of the Texas Society for Electron Microscopy
"TSEM - Embracing all forms of microscopy."

ON THE COVER

Stereo shaded solid representations of a model of the Z band and the nearby I band in unstimulated skeletal muscle. The I band thin filaments enter the Z band from the top and bottom of the figures. Inside the Z band, the extensions of these filaments are interconnected by an array of smaller diameter Z band cross-connecting filaments. The structure shown is similar to structures observed in electron tomographic reconstructions of this Z band. a) A stereo shaded solid rendering of a portion of the model. b) For comparison, a similar rendering of a tomographic reconstruction of the model, produced by weighted back-projection from model projections spanning rotation angles from -60 to +60 degrees. The reconstruction exhibits more noise than the original, but reveals the same connectivity in spite of the missing projection data from -90 to -60 and +60 to +90 degrees. Bar = 10 nm.

Cover courtesy of John P. Schroeter and Margaret A. Goldstein, Baylor College of Medicine, Dept. of Medicine, Houston, TX 77030.

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ADVERTISER'S INDEX

President's Message

It hardly seems possible that it has been a year since I assumed the office of President of TSEM. The past year has been filled with many changes and exciting new challenges. Whether we have met and conquered these challenges will be judged by how successfully TSEM prepares for the 21st century.

We were very fortunate to have Dr. Margaret Ann Goldstein, President, Microscopical Society of America (1996) at our Clear Lake meeting in the Fall. Dr. Goldstein, who is also a founding member and Past President of TSEM, performed double duty with not only a scientific presentation, but also a inspirational talk on MSA activities. Dr. Goldstein's enthusiasm for microscopy was evident as she presented the mission statement for MSA: "to promote and to communicate advances in microscopy". She emphasized that microscopy is going through a myriad of changes and that microscopists everywhere need to respond to these challenges. These are very exciting times in microscopy and we all need to be prepared to move forward.

We were also very fortunate to have Dr. David S. McKay give an invited lecture on his startling discovery of "Possible Past Life on Mars". Dr. McKay was kind enough to work the meeting into his schedule that, since the article appeared in the scientific literature, to say the least had been hectic. This is a topic that as you can imagine, quickly captured the general public's imagination and resulted in a flurry of speaking engagements for Dr. McKay. His lecture presented the finding of his research group at NASA in a concise manner and raised the level of interest in space exploration back towards what it was when we raced to the moon. We will have to wait until the new Mars probes provide more exciting data for Dr. McKay to complete his studies.

In addition to our invited speakers, the TSEM members lived up to their normal high standards and presented some excellent research. Reflective of our Society, the presentations were on a variety of topics with presenters ranging from undergraduate and graduate students through to one of our more senior scientists.

However, unlike the session audiences, the "Fun Committee" was frustrated in Clear Lake. Plans had been made to have the Social as well as the Friday banquet on the beautiful shore line. Plans had to be changed when the Texas skies opened and we experienced a "few light showers" on Thursday and Friday. Fortunately, the sun did come out on Saturday in time for everyone to have reasonable weather for their drive home. The Fun Committee has vowed to try again at the Spring meeting in Fort Worth.

The Spring meeting was originally planned for the first weekend in April. Discussions at Council indicated that this would solve our perennial problem of dealing with Spring break and still have the meeting in the early spring. What we didn't know was that the new Texas NASCAR track's inaugural race was that weekend with the result that 50,000 hotel rooms were booked in the Metroplex. While the race fans amongst us might have wanted to combine the two events, the possibility of finding a place to hold the meeting was impossible. Accordingly, the meeting was pushed back to April 17-19. This later date, it was reasoned would give everyone additional time to get their abstracts ready! I know that Robert Spears has put together an exciting scientific program in addition to a workshop being presented on "Computer Image Analysis Applications in Microscopy". I am very excited by this workshop for two reasons. First, I think this is a very timely topic and one with which those of us that still remember manual pumping controls on microscopes may still need some guidance. Second, and more important, this workshop is being presented by two of our student members. Student members of today will be the leaders of our State and National Societies in the next century. Their willingness to get involved and help TSEM gives me confidence that our Society is going to survive. In the interim, all of us must respond to the new challenges and keep our Society vital so they have a thriving TSEM to lead.

I guess one of the perks of being President is that it gives you the opportunity to reflect somewhat while others work very hard to make a meeting happen and keep the Society steaming forward. One does not really appreciate how hard other members of the Executive Council work when you are concentrating on one particular facet of a meeting. All of the members of the Executive Council work diligently for the members of this Society. When meeting time comes, however, I am most aware of how well the Program Chair, Secretary and Editor work together. The Program Chair acting as liaison with the vendors, speakers and hotel as well as designing a program to satisfy the needs of our wide interests. The Secretary who spends untold hours compiling our ever changing membership list not to mention being responsible for the numerous communications with everyone of us. The Editor goes hat in hand to our advertisers to insure that we can continue to publish our journal as well as making those numerous phone calls to get that extra abstract for our meeting program. Where would we be if the Treasurer didn't put in those long hours collecting dues, paying bills and keeping a steady hand on the assets of the Society. The Council also imposes on individuals by appointing them to positions. Your Student Representative holds a vital position as students are the bridge to our future. We all know how important our vendors are to the success of the meeting. The Vendor Representative fills a position where they have to go to their Competitors and reach consensus to help our Society. Then there are the Elect positions. Here are individuals working hard to learn the job that they will soon have on their shoulders, but still manage to contribute to immediate Society success by their suggestions. Last but not least, there is the position of Past President who's wise counsel is always a source of direction and comfort as we struggle with the issues of directing the Society.

All of the positions that I mentioned have outstanding individuals filling those demanding jobs. I could have listed them by name while expressing my thanks for all of their hard work and dedication. I certainly am thankful to the exceptional group of individuals that have served on the Council during my term as President. However, I am also grateful to those individuals that have served in the past and will serve in the future. The Members of TSEM owe all of these individuals a debt of gratitude. I hope that you will take a moment the next time you see one of them and thank them for all of their hard work.

The members of TSEM face many new and exciting challenges in the near future. I am confident that the Society has the expertise to conquer these challenges. It has been a privilege to serve this Society as President and I would like to thank the membership of TSEM for giving me the opportunity.

Sincerely,
Mitchell D. McCartney President, 1996-97

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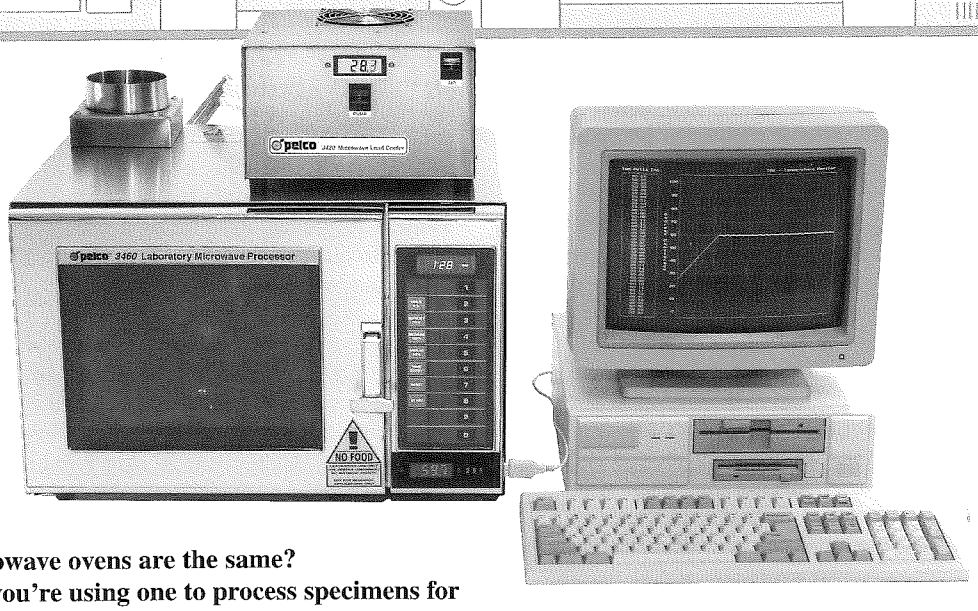

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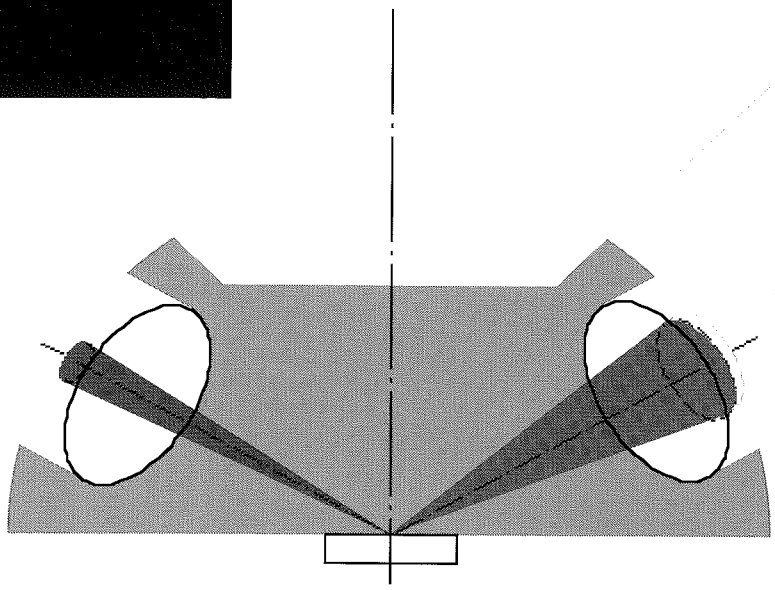

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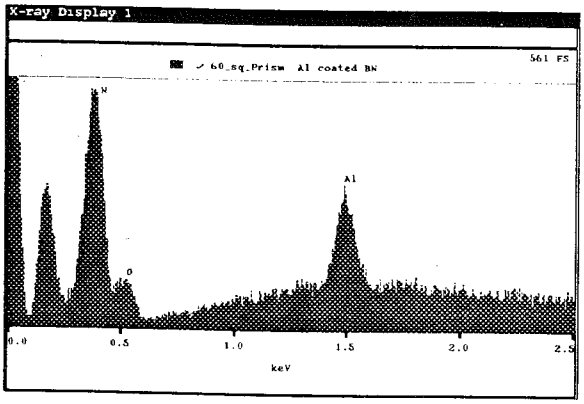

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Abstracts

MATERIALS SCIENCES
PLATFORM PRESENTATION—SPRING 1997

Nondestructive Evaluation of Cold-Rolled Cartridge Brass Using a Portable X-Ray Diffractometer. P. E. Diehl*, P. C. Schlesselman#, D. W. Mueller^x^, R. F. Pinizzotto*, J. de Alba° and L. E. Murr° *Materials Science Department, University of North Texas, Denton, Texas 76203-0308 # Engineering Technology Department, University of North Texas, Denton, Texas 76203-3198 ^x^ Department of Physics, University of North Texas, Denton, Texas 76203-5308 ° Department of Metallurgical and Materials Engineering, University of Texas-El Paso 79968.

A portable x-ray diffractometer which uses a CCD detector has been used to evaluate cold-rolled 70-30 cartridge brass. The method is based on the measurement of the x-ray diffraction line width, which depends on the microstructure of the material. The full-width-at-half-maximum for the (002) reflection increased linearly as the engineering strain increased from 0 to 80%. Optical microscopy and hardness measurements were also used to evaluate the microstructural changes of the brass at different stages of deformation. The results demonstrate that the x-ray diffraction instrument, which directly images the x-ray diffraction line via photon counting, can accurately and non-destructively evaluate this deformation process.

EXFOLIATION AND RELATED MICROSTRUCTURES IN 2024 Al BODY SKINS ON AGING AIRCRAFT. M. POSADA, L.E. MURR, C-S. NIOU, R.M. ARROWOOD, D. LITTLE, D. ROBERSON, Dept. Metallurgical and Materials Engineering, University of Texas at El Paso, El Paso, TX 79968

Exfoliation, a directional attack along elongated grain boundaries in rolled 2024 aluminum sheet and plate, has been examined in some detail for KC-135 aging aircraft body skin samples using optical metallography, SEM, and TEM. A series of SEM micrographs reveal the severity of the exfoliated region on the skin panel. A detailed analysis of grain boundary and matrix precipitates was performed as well as detailed analysis of elemental depletion profiles across grain boundaries. These observations suggest that anodic sites along the grain boundaries play a far less significant role in the propagation of exfoliation than the hard corrosion products that are forming which provide wedging stresses as they grow within the elongated grain boundaries. The grain boundaries seem to exhibit unique and unusual structural and/or energetic features which could play a significant role in the directionality of the corrosion characterized by exfoliation. This feature is being explored by measuring the misorientations determined for the elongated grain boundaries having identical (110) grain surface orientations in neighbor grains identified by TEM and selected-area electron diffraction techniques. This represents a new approach to a very old and unsatisfactorily resolved problem in related 2XXX plate. Research supported by the AFSOR-FAST program of the University of Texas at El Paso (Grant no. F49620-95-1-0518).

MICROSTRUCTURAL PHENOMENA ASSOCIATED WITH IMPACT CRATERS IN 1100 ALUMINUM TARGETS. Alicia Ayala, Dept. Metallurgical and Materials Science Engineering, University of Texas at El Paso, El Paso, TX 79968.

The cratering process has been a topic of interest for researchers since man first set foot on the Moon. The importance of this process is that it allows for the understanding of the long-term response of structural materials existing in low Earth orbit (LEO) and more general extended space environments. In this study, normal incidence impact craters in 1100 aluminum targets formed by spherical soda-lime glass projectiles at velocities ranging from 0.571 km/s to 6.1 km/s were examined along crater cross-sections using transmission electron microscopy. Analysis of microscopy data revealed that crater microstructure was mainly characterized by heavily deformed grains. Post-impact grains appeared to be elongated with respect to original grains and contained and increased dislocation density. This was observed along the crater walls as well as in the base of the crater. Accompanying diffraction patterns show that some dynamic recrystallization may have occurred as a result of the high velocity impact. Vickers microhardness tests indicated that changes in microhardness values extended 3 mm below the crater base. Vickers microhardness values (VHN) at a distance of 0.05 mm below the crater axis increased from a base target hardness of approximately 45 VHN to a hardness range of 60-70 VHN and then gradually decreased to the base hardness.

Alicia Ayala, (915) 747-5718.

MICROSTRUCTURAL OBSERVATIONS AND COMPUTER SIMULATIONS OF HYPERVELOCITY IMPACT CRATERS IN OFHC COPPER TARGETS. S.A. QUINONES, J.M. RIVAS AND L.E. MURR, Department of Metallurgical and Materials Engineering, University of Texas at El Paso, El Paso, Texas 79968

A series of impact craters in OFHC copper (Cu) targets were characterized based on optical metallography, transmission electron microscopy (TEM) and Vickers microhardness. The impact craters were produced by 3.17 mm 1100 aluminum (Al) spheres that impacted the target material at impact velocities between 1 and 7 km/s. Optical metallography of each of the crater cross-sections was performed along the impact axis and demonstrated an evolution of microstructures. Four distinct zones were observed which included (1) dynamic recrystallization, (2) a highly deformed region, (3) a region containing microbands within deformed grains, and (4) a region containing microbands within undeformed grains. The presence of dynamic recrystallized grains and microbands was confirmed based on TEM studies. The extent of each of these zones varied with impact velocity, and the total affected area extended further into the target material with increase in impact velocity. Measurement of the Vickers hardness number (VHN) along the impact axis resulted in trends which support the microstructural evidence. The hardness values were converted to residual yield stress values by the relationship σ_{ys}=H/3.0. The computer simulation program Autodyn-2.0 was used to simulate the series of impact craters into OFHC copper. Crater geometry comparisons were made of experimental versus simulated craters. Yield strength contour maps of simulated crater cross-sections were produced using Autodyn-2.0 and were compared to experimentally derived yield stress contour maps. The results of a computer simulation for an impact produced at velocities exceeding those attainable in the laboratory (u_o > 7 km/s) was also compared to the results at lower velocities (u_o < 7 km/s).

GOETHITE PARTICLE MORPHOLOGY IN SOILS AND SEDIMENTS: EXAMPLES FOR INSTRUCTION. J.B. DIXON, Soil and Crop Sciences Dept., Texas A&M University, College Station, TX 77843-2474, USA.

Goethite is common in soil clays and some sediments. Also, it is an important adsorbent of phosphorus in soils. It is an excellent choice for demonstrating lattice imaging and electron diffraction with a transmission electron microscope. It is rather stable in the electron beam, occurs in small particles thin enough to image lattice fringes, and the crystals often take a favorable orientation for imaging the fringes. Also, it is more interesting than many other minerals because it frequently forms twins and the exterior surfaces are often complex. Twinned goethite was observed in a student's sample from a water well. A twin was found in a soil clay that was so small that it was not evident that it was a twin until the electron diffraction pattern was examined. Clay from a Vertisol from Oklahoma contains twins and single crystals with sharp ends and only evident at high magnification (ca. 300,000x). In teaching students about clays with a transmission electron microscope goethite provides several special opportunities to demonstrate instrumental versatility and to explore relationships among the minerals present and the character and history of the sample. (Reproduced from Program of 11th Int. Clay Conf. Ottawa, Canada, 1997).

MATERIALS SCIENCES
POSTER PRESENTATION—SPRING 1997

TRIOCTAHEDRAL SMECTITE IN MG-CARBONATE SPELEOTHEMS. V.J. POLYAK AND N. GÜVEN, Dept. Geosciences, Texas Tech University, Lubbock, TX 79409-1053.

A magnesian trioctahedral smectite is the major constituent of insoluble residue from Mg-carbonate speleothems after removal of carbonate by the sodium acetate method. The smectite was identified by x-ray diffraction, electron microscopy, and energy dispersive spectroscopy. It is intimately associated with dolomite crusts and huntite moonmilks in Carlsbad Cavern and other dolostone caves of the Guadalupe Mountains of New Mexico. Smectite crystals are fibrous and make up decimicron-sized filamentous masses that envelope crystals of dolomite, huntite, and magnesite; individual fibers are submicron-sized. The trioctahedral smectite is authigenic and penecontemporaneous with the Mg-carbonate minerals. In water films, progressive evaporation and carbon dioxide loss result in the sequential crystallization of Mg-calcite, aragonite, dolomite, huntite, hydromagnesite, and magnesite. Not all of these minerals will be found in each setting, however, this sequence of carbonate precipitation will remove all Ca and greatly enrich Mg in the cave water. Silica is normally available in the alkaline, high pH thin film microsetting where Mg-carbonate minerals form, and consequently, trioctahedral smectite crystalllizes. The Mg-silicates are also associated with opal, quartz, and uranyl vanadates.

BIOLOGICAL SCIENCES
PLATFORM PRESENTATION—SPRING 1997

CRYSTAL TRICHOME DEVELOPMENT ON THE ADAXIAL LEAF SURFACE OF *NELUMBO NUCIFERA*. C. SCHWARTZ , J. VAN DE VEIRE AND H. J. ARNOTT. The Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019.

The sacred lotus, *Nelumbo nucifera,* is a common waterplant in the family Nelumboaceae. Because of its cellular microstructure, water beads and rolls off the adaxial (but not the abaxial) surface of the leaves. Light and SEM observations show that the upper epidermis is formed by a lawn of cells. Each of these lawn forming cells has a short apical cone-like extension which averages 15.6 mμ in height; technically each cell is termed a trichome. Similar epiderminal cells are not found on any other part of the plant. Scattered within this trichome lawn one finds a series of multicellular crystal-bearing trichomes. Each crystal-bearing trichome is several times the height and many times the volume of those constituting the lawn. The crystal-bearing trichomes appear to be formed by a "stack" of 10 to 50 cells. The terminal cell of each of these large trichomes contains a "star-shaped" druse-like crystal of calcium oxalate. The "star-shaped druse" is a multiple interpenetrant twin which is twinned in a single plane; they are almost identical to those found in the leaves of ochra, *Hibiscus esculentus*. In apposition to the lawn trichomes the large ones develop in an asynchronous manner. Because of this, different stages in the development of the large trichomes can easily be studied by selecting small leaves of the correct size. These leaves were fixed by glutaraldyde and osmium tetroxide, dehydrated, freeze fractured, dried using the critical point method, mounted on stubs, and studied in the SEM. We have noted that when water is applied to the upper epidermis, air is "captured" (trapped) around the crystal-bearing trichomes. Air trapping seems to be an important part of the mechanism through which water beads on the adaxial surface. The exact structure of the crystal bearing-trichomes seems to be important in air trapping. Therefore, their development and final structure is of considerable interest.

THE USE OF EMBEDMENT-FREE SECTIONS TO EXAMINE THE CYTOSKELETON OF TRABECULAR MESHWORK CELLS IN INTACT HUMAN TRABECULAR MESHWORK TISSUE. K. WILSON, M. ENGLER, D. LANE, A. F. CLARK and M. D. McCARTNEY, Alcon Laboratories, Inc., Fort Worth TX, 76134

The development of ocular hypertension in glaucoma has been shown to be a result of decreased aqueous outflow through the trabecular meshwork (TM). Previous studies on cultured human glaucomatous TM cells have shown a variety of differences from normal TM cells including the accumulation of unusual polygonal cytoskeletal structures, called cross-linked actin networks (CLANS). In order to study the possible role of CLANs in ocular hypertension, a method of embedment-free sections was developed to determine if CLANs were present in trabecular meshwork cells in situ. Normal and glaucomatous human anterior segments were fixed with 2% glutaraldehyde, embedded in diethylene glycol distearate (DGD), sectioned, the DGD removed, immunogold antibody labelled, critical point dried and examined using TEM. Similar to previous studies of in vitro TM cells, glaucomatous TM cells appeared to be larger with larger nuclei. Embedment-free sections of intact normal and glaucomatous TM cells showed actin labeled microfilament bundles (stress fibers), approximately 70-165 nm in diameter, that ran along the longitudinal axis of the cells. Microtubules were also evident and labelled with immunogold tubulin antibody. Normal and glaucomatous embedment-free TM cells also showed areas with abnormal cross bundling of the microfilaments. These preliminary data suggest that these abnormal bundling may represent the CLANs observed in cultured TM cells. Future studies utilizing these techniques will further investigate these cytoskeletal structures.

ALTERED STOMATAL MORPHOLOGY OF GLOSSY LEEK PLANTS OBTAINED *IN VITRO*. C.G.-A. MAIER AND D. POST-BEITTENMILLER, The Samuel R. Noble Foundation, Plant Biology Division, Ardmore, OK 73402

Among 187 regenerated leek plants from tissue culture and transferred to the greenhouse, 3 glossy mutants were found and designated S1, S2 and S3. Two plants, S2 and S3, had a different stomatal morphology, mainly extra adjacent stomata, compared to wild type plants in addition to the glossy phenotype. After 4 months from transferring to soil, plant S1 had 50% and plant S2 36% total wax load on their epidermis compared to the control, based on GC analysis. The wax load on plant S2, however, may be an overestimation due to the difficulties of estimating surface area of its non-smooth surface. Extra cells and abnormal subsidiary cells that which were larger, smaller or displaced from their normal position, contributed to the wavy surface of plant S2 leaves. While S1 showed crystalline wax structures under SEM, the S2 plant had no wax crystals (on the mid-leaf section examined). The S1 plant presented normal stomata while S2 and S3 plants had double, triple and occasionally quadruple stomatal complexes, as well as abnormal (with 1-3 guard cells, asymmetric guard cells, unopened pore, twice larger) and clustered stomata, which represented approximately 34% of total stomata. GC and SEM analyses were performed on newly developed leaves of these glossy plants after 6 months in the greenhouse. Although the amount of total wax on plants S2 did not change, wax crystals were present on its epidermis at this stage in the development. The stomatal morphology with double, triple, abnormal and grouped stomata was maintained representing 31% of total stomata for plant S2 and 21% for plant S3. Variation of the percentage of abnormal, double, and triple stomatal complexes was observed among the base, middle section and tip of the lamina. More abnormalities were found at the base of the lamina, on both adaxial and abaxial sides. Only two other stomatal mutants were previously described in the literature: the *too many mouths* (*tmm*) and *four lips* (*flp*) in Arabidopsis, a dicot (Yang and Sack, Plant Cell 7/1995: 2227-2239) and double, triple and grouped stomata in the *cer-g* mutant of barley, a monocot (Zeiger and Stebbins, Amer. J. Bot. 59/1972: 143-148). In leek, as well as in barley, the primary effect of this mutation appears to be on stomatal production rather than on wax biosynthesis.

EVALUATION OF THE TEMPORAL RELATIONSHIP BETWEEN HAUSTORIUM FORMATION AND EPIDERMAL CELL DEATH CAUSED BY BLACKSPOT DISEASE ON ROSE PLANTS. ERIC P. WARTCHOW* AND JOSEPHINE TAYLOR, Department of Biology, Stephen F. Austin State University, Nacogdoches, TX 75962.

The fungus *Diplocarpon rosae* is the causal organism of blackspot disease of rose. As part of its infection cycle, epidermal cells are penetrated by the fungal hyphae and intracellular feeding structures called haustoria are formed. Following haustorium formation, epidermal cell death may occur. Since some rose varieties are known to display resistance to blackspot disease we inoculated resistant (*Rosa roxburghii* and *Rosa wichuraiana*) and susceptible (*Rosa hybrida* cv. 'garden party') roses and used light microscopy, scanning electron microscopy, and transmission electron microscopy techniques to investigate the possible modes by which resistant roses achieve their resistance. We then attempted to establish if the timeliness of haustorium formation is related to epidermal cell death, and if cell death is related to host resistance.

SELECTED PHARMACEUTICAL DOSAGE FORMS AND CHARACTERISTICS OF THEIR SURFACE MORPHOLOGY USING SCANNING ELECTRON MICROSCOPY. A. STACEY AND H.J. ARNOTT. The Department of Biology and The Center for Electron Microscopy, University of Texas at Arlington, Arlington, Texas 76019.

Considering the proprietary nature of pharmaceutical agents, very few studies have been published with respect to scanning electron microscopy (SEM). In this study, four different pharmaceutical delivery systems were examined using SEM. The four delivery systems investigated were immediate-release, controlled-release, sublingual and transdermal. Micrographs were taken of individual specimens and details of their surface morphologies were observed. The most interesting observations were the variations in the shape, size, and surface characteristics of micropellets in the immediate and controlled-release samples. Variations in morphologies may be due to manufacturing processes or the presence of additional ingredients in the specimens.

A COMPARISON OF MAMMILLARY CONES OF INCUBATED AND NON-INCUBATED AVIAN EGGS. S.L. WESTMORELAND AND H.J. ARNOTT. The Department of Biology and The Center for Electron Microscopy, University of Texas at Arlington, Arlington, Texas 76019.

The avian eggshell has a stable structure consisting of an external organic cuticle, a calcite layer, with a columnar portion and an internal mammillary cone portion, and two interior proteinaceous membranes. This was confirmed by the scanning electron microscope examination of eggshell samples of twenty-one bird species, each representative of a different avian order. An exception to this pattern was observed in the eggshell sample of Oxyura jamaicensis (Ruddy Duck) which lacked the two shell membranes, revealing the mammillary cone layer. Mechanical and chemical methods were used in attempts to remove the shell membranes from eggshells of Gallus domesticus (domestic chicken). Scanning electron micrographs showed the solid crystalline mammillary cones of Gallus domesticus differed from those of Oxyura jamaicensis which had central voids in each cone. Decalcification of the eggshell during embryogenesis is reported to begin in the center of the mammillary cones. Examination of this eggshell layer can be used to determine if a shell is from an egg that had been incubated, according to Burley and Vadehra, 1989 (The Avian Egg, John Wiley and Sons). Using the mammillary cones as an indicator, it is theorized that the Gallus domesticus egg was non-incubated and that of Oxyura jamaicensis was incubated.

SCANNING ELECTRON MICROSCOPIC STUDIES ON THE SEED ANATOMY OF TWELVE LEGUMINOUS SPECIES
Nabarun Ghosh[1], A. Chatterjee[2] and Don W. Smith[1]
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The application of seed data is increasingly included and discussed in revisions and circumscription of angiosperm families and orders. In practice, most data have been provided by SEM examination of the epidermal surfaces. Sometimes data from seed surface only are not enough for proper identification of the species and taxonomic interpretation. In the present investigation we cut sections of three different species of four leguminous genera to study the seed anatomy under the dissecting microscope and the SEM. Under the dissecting scope the color of the different layers of tissue could be seen, and this is helpful. But detailed cellular patterns were revealed only under SEM. It was possible to observe the cellular organization of the epidermis, endosperm and internal structural details of the seeds with SEM. We found distinctive anatomical features in the cross sections of seeds of diffeerent species of the same genus. Main distinguishing characters observed were uniseriate or multiseriate epidermis, epidermal projections, number of rows and nature of columns of the hypodermal layer, especially the distinctive cell shapes in the endosperm which may have diagnostic value.Three different species of *Dalbergia* and two species of *Albizia* are difficult to distinguish externally even with seed coat study under SEM, but this study with cross sections provided enough characteristic features to isolate one from the other. Our study revealed notable anatomical features of the epidermal and endospermic tissues of the seeds that will help in further application of seed data in different fields.

BIOLOGICAL SCIENCES
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ACETYLCHOLINE BROMIDE CELL MEMBRANE RESPONSE IN *TETRAHYMENA SP.* J.G. COKE IV, Dept. Biology, Sam Houston State University, Huntsville , Texas 7734

Cholinergic chemosensory response in *Tetrahymena sp.* is well documented (Tsang et al., *J. Protozool.* **30**. (A), 1983; Koppelhus et al., *Biol. Bull.* **187**. 1994), however explicit plasma membrane response to acetylcholine in *Tetrahymena* remains uninvestigated. Acetylcholine bromide (Mallinckrodt Chemical Works #1850) was elected to study potential cell aberrations due to it's cholinergic properties and undetermined lethal dose toxicity (Lenga, R.E. *The Sigma-Aldrich Library of Chemical Safety Data Edition* 2, Vol. 1. Sigma-Aldrich, Milwaukee Wisc. 1988; *Merck* **11**. Rahway, N.J. 1989). Three 125 mL Erlenmeyer flasks containing 50 mL of 1% proteose peptone medium were inoculated with 0.5 mL log-phase *Tetrahymena* to produce axenic cultures. One culture served as control, remaining cultures were treated to produce 2 ppt and 5 ppt concentrations of acetylcholine bromide. A 30% decline in cell harvest was noted in the 5 ppt culture as compared to control culture harvest after 48 hours. TEM examination revealed cell morphology alterations in treatment cultures when evaluated by ANOVA morphometric analysis.

ACTINOMYCIN D EFFECTS ON NUCLEOLAR ORGANIZER REGIONS IN *TETRAHYMENA*. M.L. ATTEBERY, Dept. of Biological Sciences, Sam Houston University, Huntsville, TX 77341

Actinomycin D has been shown to scatter the proteins of the nucleolar organizer regions which can be differentially stained with silver nitrate (Yokoyama, *et al.*, Experimental Cell Research. 202:77, 1992). Treatment with Actinomycin D on *Tetrahymena* was shown to cause a block of all postzygotic development (Ward and Herrick, Developmental Biology. 173:174, 1996). *Tetrahymena sp.* were cultured in 50 mL of 1% proteose peptone in 125 mL Erlenmeyer flask, treated with 10^{-1} μg/mL Actinomycin D for 3h and 6h, fixed with 1.6% glutaraldehyde in 0.1M phosphate buffer for 1h at 4°C and post fixed with 1% osmium in 0.1M phosphate buffer for 1h at room temperature. Cells were dehydrated, infiltrated and embedded in Epon 812, gold/silver sections were cut and viewed using Hitachi HS-8 TEM, cells were dehydrated with HMDS at the 100% ETOH dehydration and sputter coated using Pelco Sputter Coater 9000 and viewed using a Jeol J5M-35 SEM. All instruments were made available at Micro Star Technologies, Huntsville, TX. Differences were noted in treatment levels and culture. Photographic morphometric analysis on TEM micrographs showed Nucleolar differences which were analyzed using ANOVA of silver-stained nucleolar organizer centers. Actinomycin D significantly altered nucleolar configurations in *Tetrahymena*.

CHEMOKINETIC AND ULTRASTRUCTURE EFFECTS OF HUMAN CHORIONIC GONADOTROPIN RECEPTOR INDUCTION IN *TETRAHYMENA*. S.A. BRADFORD, Department of Biological Sciences, Sam Houston State University, Huntsville, Texas, 77341.

The effect of hormones and their precursors on the response of *Tetrahymena* is well documented using radiography (Christopher, G. and Sundermann, C., *Exp. Cell Res.* **201**, 1992; Csaba G. and G. Nemeth, *J. Biochem. Physiol.* **65**B, 1979), however selective receptor induction response to human chorionic gonadotropin using antibody localization of the receptors remains undetermined. Ultrastructural localization studies are lacking on binding sites for human chorionic gonadotropin ,(human pregnancy urine; Sigma CG-5 Lot 65H0755) this hormone was selected. Four 125 ml Erlenmeyer Flasks containing 50 ml of 1% proteose peptone media were inoculated with 3 ml of log phase *Tetrahymena* to produce axenic cultures. After 48 hours of incubation, one culture served as a control, three remaining cultures were treated individually with 3 μl/50 ml, 6 μl/50ml, and 12 μl/50 ml concentration of human chorionic gonadotropin for one hour at 20 C. Receptor induction was performed by centrifugation and resuspension in 1 % proteose peptone media at 28 C for 24 hours. Cultures were fixed in phosphate buffered saline (PBS) 4% formalin for five minutes and incubated for one hour with 0.2 μ l/ml HCG in PBS at 37 C. Cultures were washed in 0.01M phosphate buffer (pH 7.4) and post-fixed in PBS 0.5 % OsO_4 for 30 minutes. Cells were dehydrated to 100 % ETOH, and portions were dried on aluminum stubs with HMDS, and gold sputter coated using a Pelco Sputter Coater 9000 in preparation for viewing in JOEL-JSM 35 SEM. Cells were infiltrated with 1:1 acetone and EPON 812 and embedded in EPON 812 and DMP 30 for TEM. Sections were cut with a Microstar Technologies diamond knife. Immunolabeling was performed using a primary antibody to HCG and a secondary antibody to bovine with a peroxidase label. The sections were counterstained with uranyl acetate and viewed using a Hatachi HS-8 TEM. Morphometric analyses using ANOVA showed differences in treatment effects and culture morphology differences.

CREATION OF STEREOSCOPIC IMAGES USING PHOTOEDITING COMPUTER SOFTWARE. Mike Davis and H.J. Arnott, Department of Biology and Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019.

The creation of stereo pairs, a very common and useful practice in scanning electron microscopy, involves photographing a specimen at two different perspectives and viewing the two images simultaneously to produce a three-dimensional image. This composite image is often helpful in discerning three-dimensional structures of objects as well as the relative positions of objects in three-dimensional space. Oftentimes, stereo pairs are not photographed due to time constraints, difficulty in acquisition, or other reasons and important 3-dimensional information is not examined. We report a novel method of creating stereoscopic images from a single image utilizing photoediting computer software. Original SEM photographs are digitized by a scanner and opened in a photoeditor. The images are tilted in the Z plane approximately 8 degrees utilizing a method by which to retain focus and detail and are printed for viewing with the original image using a stereoscope. Other examples of stereoscopic enhancements of images using photoediting software are also addressed.

ULTRASTRUCTURAL CHANGES DUE TO NICOTINE TREATMENT ON *TETRAHYMENA SP.* J.R. DOYEN, Dept. Biological Sciences, Sam Houston State University, Huntsville, TX 77341

Nicotine is a potent stimulant of the hypothalamopituitary-adrenal axis, resulting in rapid secretion of ACTH in a dose dependent manner (Matta *et al.* Endocrinology 1993). Individual cell response was characterized by membrane transport of secretory product, but there was no individual cell localization or measurement. Single celled organisms would provide a model cell evaluation. However, there is a paucity of information of nicotine effects involving single celled organisms. *Tetrahymena sp.* were cultured in 50 mL of 1% proteose peptone in 125 mL flasks for 24 hours at 21°C. Treatment doses of 2.5 µL and 5.0 µL of nicotine (98%) were administered every 12 hours for 48 hours with one culture maintained as a control. Examination was made on cells fixed with phosphate buffered 3% glutaraldehyde and post fixed with 2% osmium tetroxide. Dehydration was accomplished through an increasing concentration ethyl alcohol series. Cells were stained with 1% uranyl acetate in 100% EtOH. SEM-cells were dehydrated with HMDS at 100% EtOH and gold sputter coated. Cells were viewed using a Joel J5M-35 SEM. Cells were infiltrated with 1:1 acetone and epon 812, then embedded in epon 812. Silver to gold thin sections were cut using an MT2B and MicroStar Technologies diamond knife. Sections were viewed using a Hitachi HS-8 TEM. *Tetrahymena* population volumes were determined and nicotine treated cells were 55% less than control by volume. Alterations in surface morphologies were noted in scanning micrographs. Morphological analysis of TEM micrographs showed structural differences which were analyzed using ANOVA.

EPICUTICULAR WAX STUDIES ON *IN VITRO* REGENERATED LEEK (*ALLIUM PORRUM* L.). C.G.-A. MAIER and D. POST-BEITTENMILLER, The Samuel R. Noble Foundation, Plant Biology Division, Ardmore, OK 73402

An efficient and reproducible leek regeneration system has been developed for the purpose of epicuticular wax (EW) studies and the application of transformation techniques. EW studies were accomplished by GC and SEM on developmental stages *in vitro*, from callus as stage 0 to rooted shoots as stage 4. The GC profile was the same for all stages in tissue culture as well as for seedlings and commercially available plants. However, the amount of C31 ketone, the dominant wax compound on leek epidermis used as a marker for total wax, increased with later *in vitro* stages and was significantly higher on shoots regenerated in dry environmental conditions than on those from a relatively high humidity environment. More variation in the amount of C31 ketone, based on the standard error, was observed for shoots grown in environmental conditions close to 100% humidity than for those grown in a drier environment. C31 ketone is produced even in humidity conditions close to 100% and especially on shoots that already developed a small bulb and adventitious roots. Plants obtained from shoots in stages 3 and 4 kept in high humidity conditions had 36% less C31 ketone on new leaves after one month in the greenhouse when compared to the regenerants obtained from dry *in vitro*-culture conditions. A correlation between the chemical composition of wax by GC and its crystalline structure on leaf epidermis by SEM was found. In general, at low levels of C31 ketone (up to 1µg/cm^2) no wax crystals were present on the surface of the epidermis at all stages. EW was deposited as a film on the leaf surface. At levels above 1µg/cm^2 tubular, parallel and branched crystals were present on the epidermis of shoots (mostly stages 3 and 4) and of regenerated plants obtained under a variety of humidity conditions. In earlier stages wax crystals were observed around the stomates only. In conclusion, the stages of leek tissue culture and regenerated plants in the greenhouse represent a suitable model system for EW biosynthesis studies since they are comparable to stages *in vivo*.

TESTOSTERONE PROPIONATE RECEPTOR INDUCTION RESPONSE IN *TETRAHYMENA*. ACSA M. ZAVALA, Department of Biological Sciences, Sam Houston State University, Texas 77341.

The selective responsiveness of *Tetrahymena* to hormones and their precursors is well documented using radioactive labeling techniques (Csaba. G. and G. Nemeth, *J. Biochem. Physiol.* **65**. (B), 1979; Christopher, G. and C. Sundermann, *Exp. Cell Res.* **201**, 1992), however exclusive receptor induction response to testosterone in Tetrahymena has not been studied. The androgenic hormone testosterone propionate ($C_{22}H_{32}O_3$, MW 344.48) (Ward's Natural Science Establishment, Inc., #38W5230) was selected to study hormone receptor induction since documentation of this steroid's transport system in *Tetrahymena* was not seen in the literature. Four Erlenmeyer flasks (125 mL) containing 50 mL of 1% proteose peptone media were inoculated with 3 mL each of log phase *Tetrahymena* to produce axenic cultures. Following 48 hours of incubation, one untreated culture served as the control, and three experimental cultures were treated with 3 µl/ml, 6 µg/ml, and 12 µg/ml of testosterone respectively for one hour at 20 C. They were centrifuged and resuspended in proteose-peptone media, and incubated at 28 C to permit time for receptor induction. Cells were centrifuged in phosphate buffered saline (PBS) 4 % formalin for five minutes, incubated with 0.2 µl/ml of testosterone in PBS for one hour at 37 C. The cultures were washed in 0.01 M phosphate buffer and post-fixed in PBS 0.5 % OsO_4 for 30 minutes. Cells were dehydrated to 100 % ETOH, samples were dried on aluminum stubs (using HMDS) and gold sputter coated using a Pelco Sputter Coater 9000 in preparation for viewing in a JOEL-JSM 35 SEM. Remaining samples were infiltrated with 1:1 acetone and EPON 812 and embedded in EPON 812 and DMP-30 for TEM. Sections were cut using a Microstar Technologies diamond knife. Immunolabeling consisted of a primary antibody to testosterone and a secondary antibody to bovine with a peroxidase label. Thin sections were counterstained with uranyl acetate and viewed using a Hatachi HS-8 TEM. Morphometric analyses of receptor sites using ANOVA showed differences in treated cultures.

RADIOACTIVE *IN SITU* HYBRIDIZATION IN MOLECULAR BIOLOGY. S.C. Williams and M. Davis, Molecular Pathology Core Laboratory, Department of Internal Medicine, The University of Texas at Southwestern Medical Center, Dallas, TX 75235.

Radioactive *in situ* hybridization (ISH), now a common practice in molecular biology, is ever becoming a more powerful tool in scientific research with highly specific and visually stunning results. It involves the use of probes, typically RNA, tagged with radioactive sulfur (^{35}S) or other isotopes. The probe is an antisense sequence of RNA that is literally spread across thin tissue sections that contain single-stranded mRNA of specific molecules of interest. The probe interacts with single stranded mRNA in the tissue to form a double-stranded hybridized RNA. The probe is highly specific to its compliment of RNA found in the tissue itself, and radioactively tags it. Hybridized tissue sections on slides are then dipped in photographic emulsion, and the radioactive probes expose the emulsion, forming silver grains. After developing slides with conventional photographic chemicals, tissue sections may be viewed under darkfield microscopy. The results are very effective, highly specific, and visually appealing. We discuss the steps involved in ISH and through examples demonstrate the effectiveness and specificity of probes encountered in our laboratory.

A COMPARISION OF THE RAYS OF *ASTREUS* AND *GEASTRUM* (EARTHSTARS). J. M. ULSES AND H. J. ARNOTT. The Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019.

The process of the exoperidium (rays) opening and closing is cyclic and repeatable in *Astreus* and some species of *Geastrum*. However, the exoperidium of other species of *Geastrum* remain open. These differences provide an opportunity to study the rays of both genera with the hope of better understanding the hygroscopic ray movement. The bending of the exoperidium depends on its structure and the presence or absence of water. Examination of ray structure may allow an understanding of the hygroscopic mechanism that causes the rays to open and close. In this study we confirmed that there are four layers in the rays of *Astreus* whereas in species of *Geastrum* there may be fewer. Evidence for this was found by using light microscopy to examine thin sections of the rays which were made using a freezing microtome. In *A. hygrometricus* there is a thin mycelial layer on the adaxial surface that becomes thin and cracked upon drying. Below that there is a thin pseudoparenchymous layer, a thick fibrous layer and finally another mycelial layer that wears off with age. In *Geastrum corollinum*, there are thick pseudoparenchymous and fibrous layers covered by a thin mycelial layer. In *Geastrum asper*, the rays are constructed in a different manner than other hygroscopic species. A thick fibrous layer exists on both surfaces separated by a layer of loose parenchyma-like cells. Two nonhygroscopic species were also studied, *Geastrum saccatum* and *Geastrum minimum*. They had a single dense pseudoparenchymous layer and a very thin fibrous layer.

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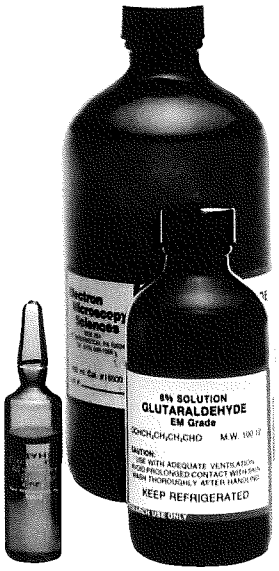

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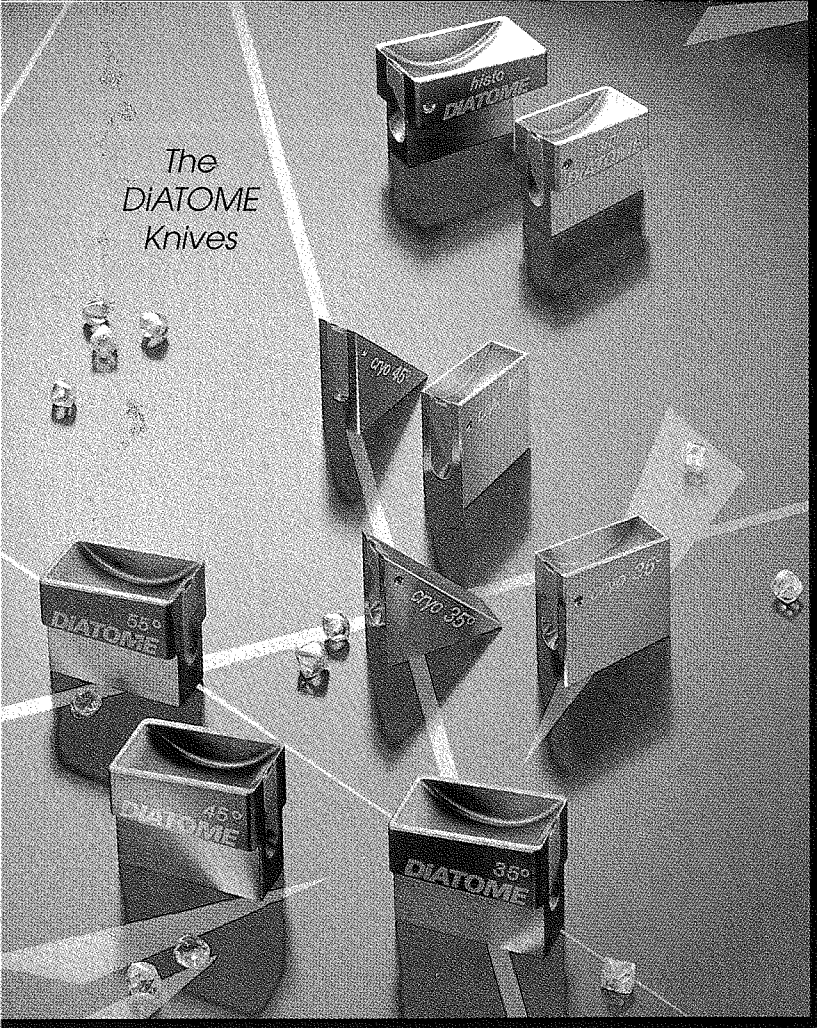

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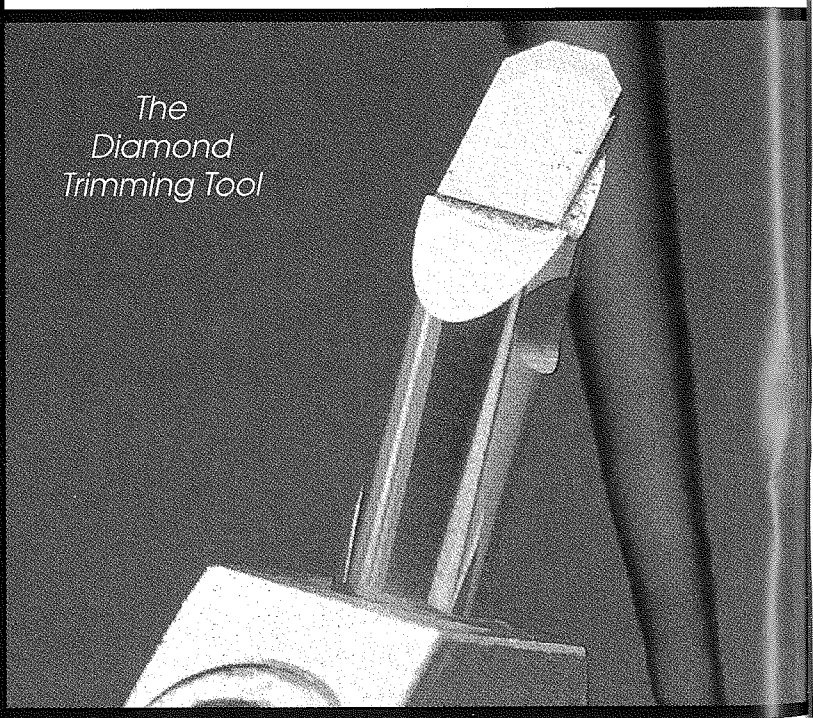

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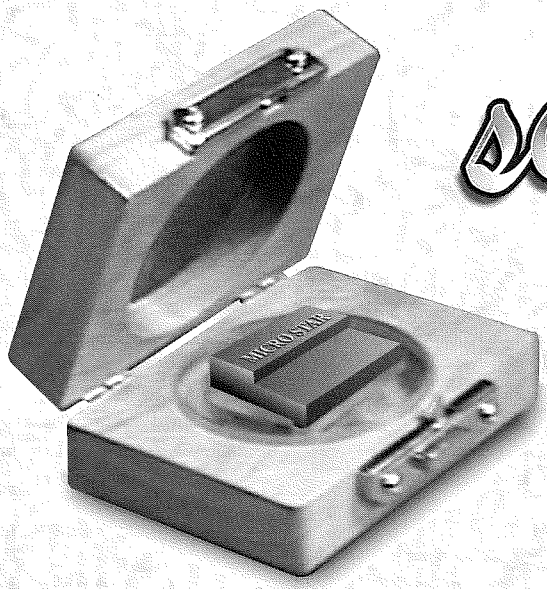

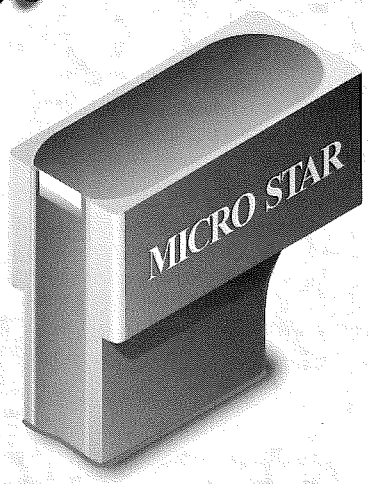

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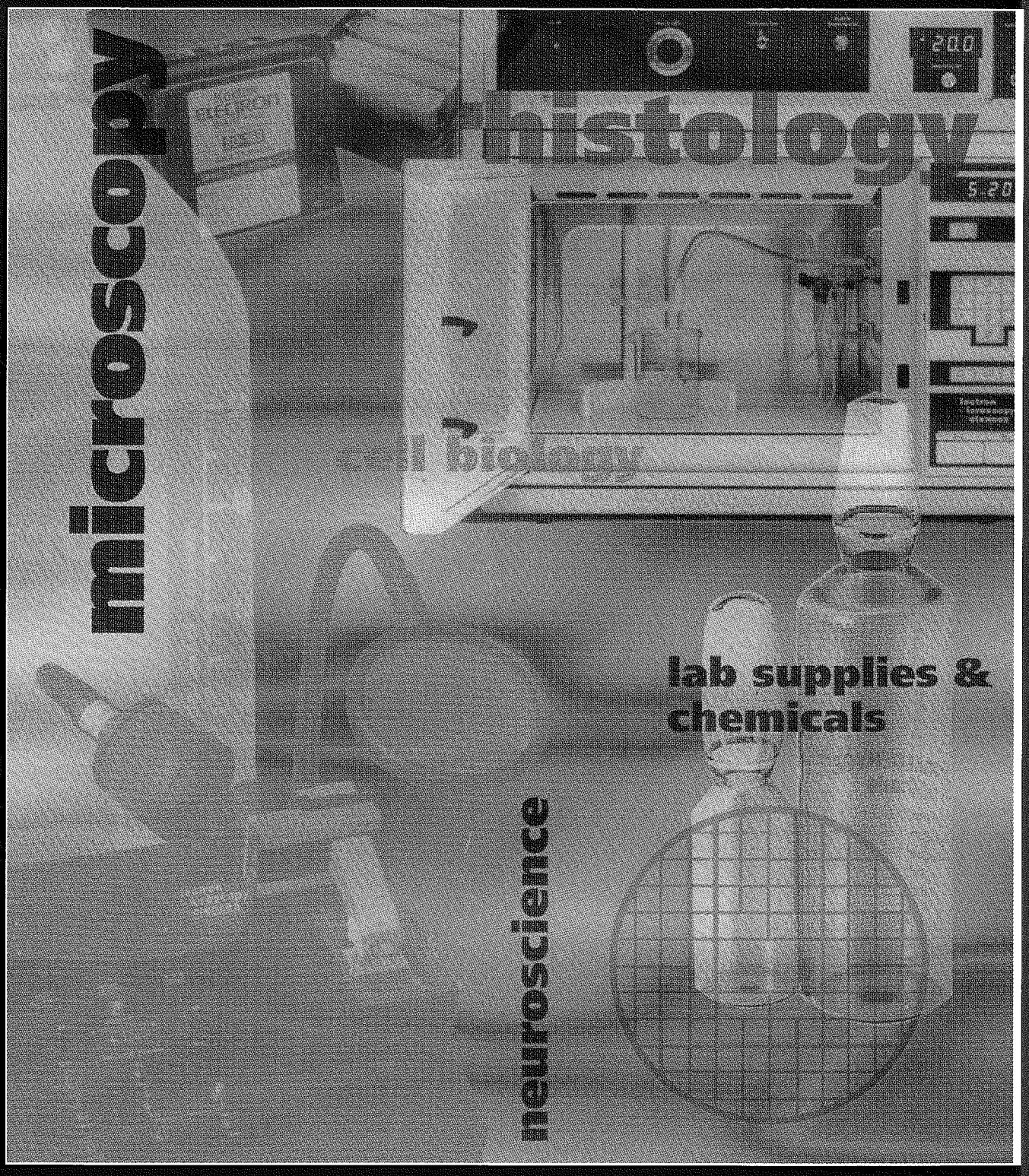

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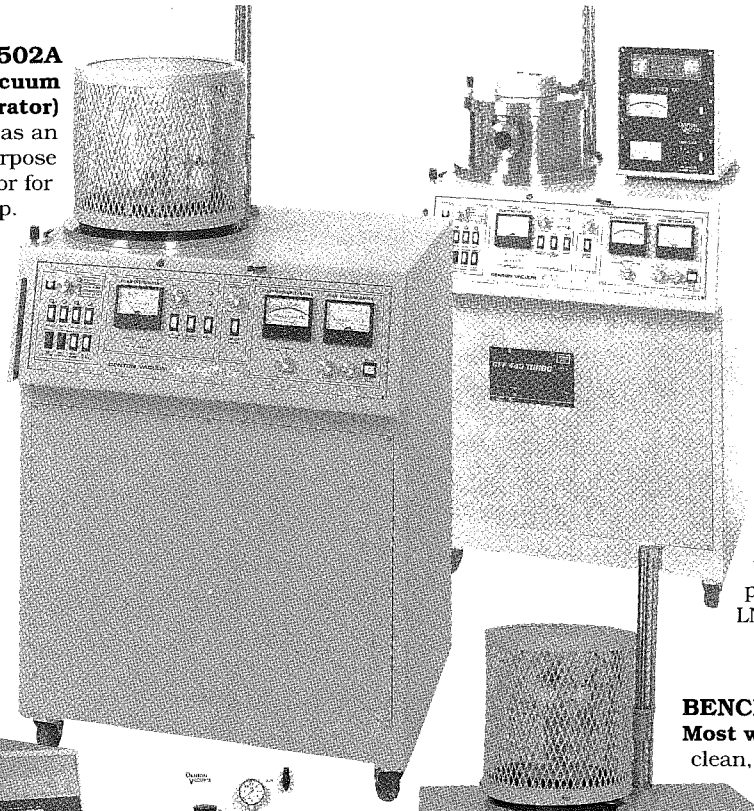

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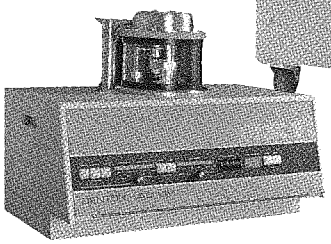

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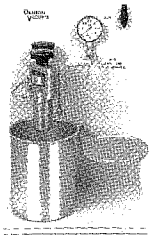

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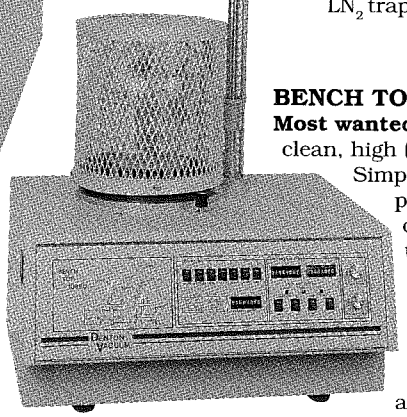

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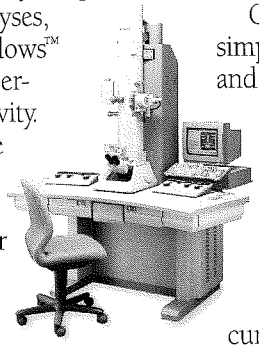

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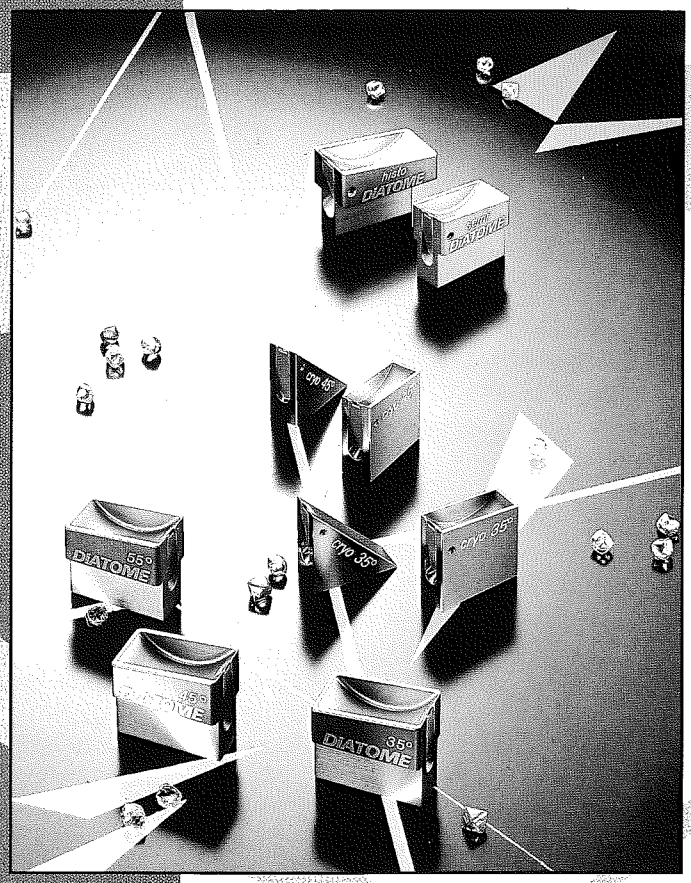

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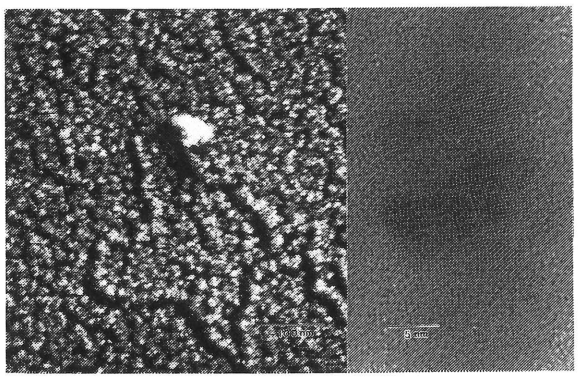

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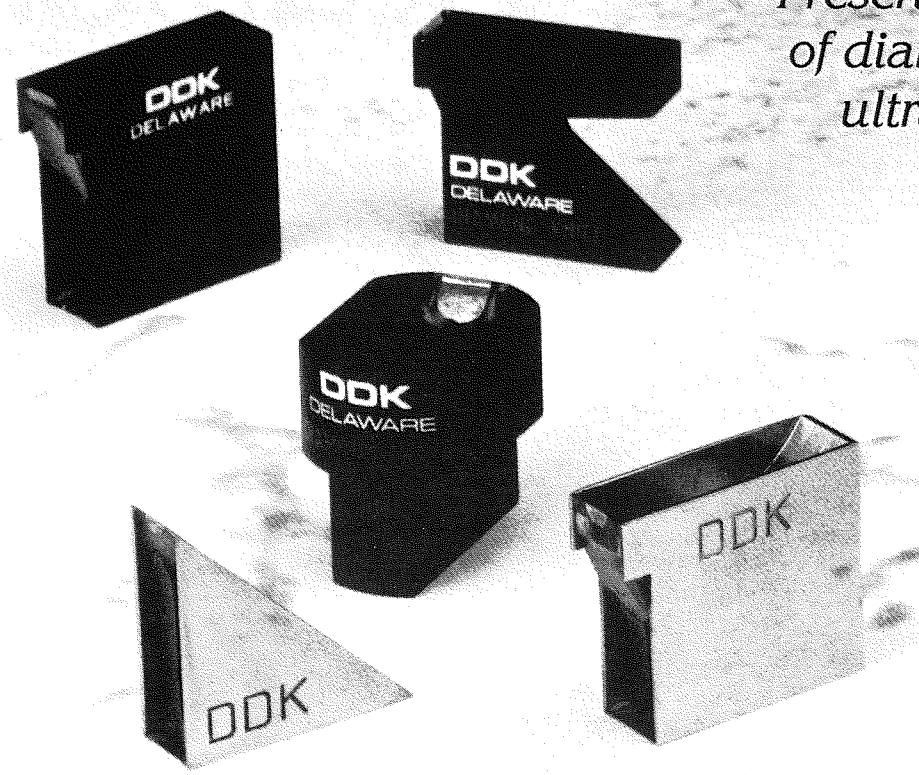

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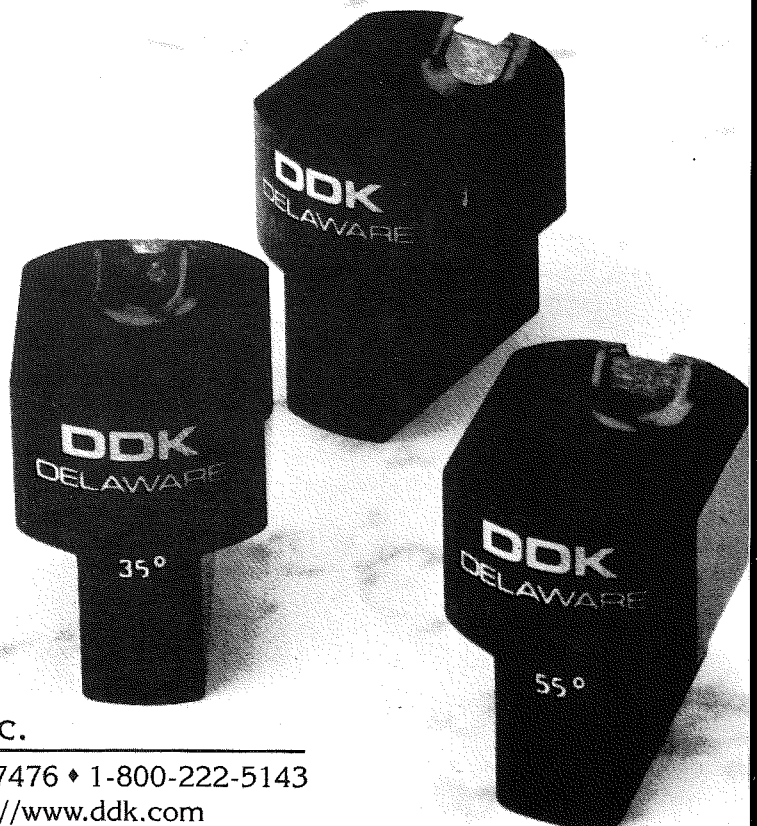

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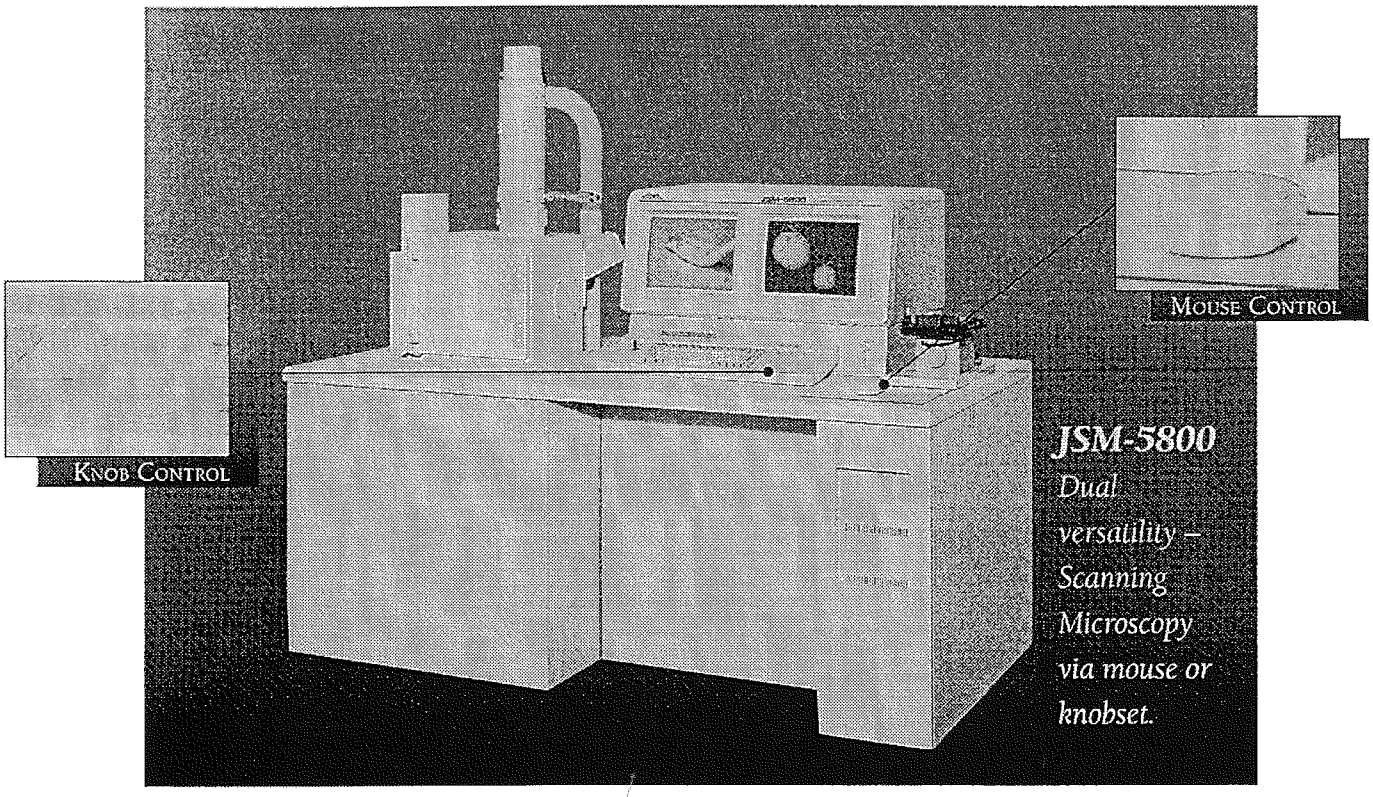

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GENERAL ELGIBILITY REQUIREMENTS:

1. Membership in MSA.

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   — 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

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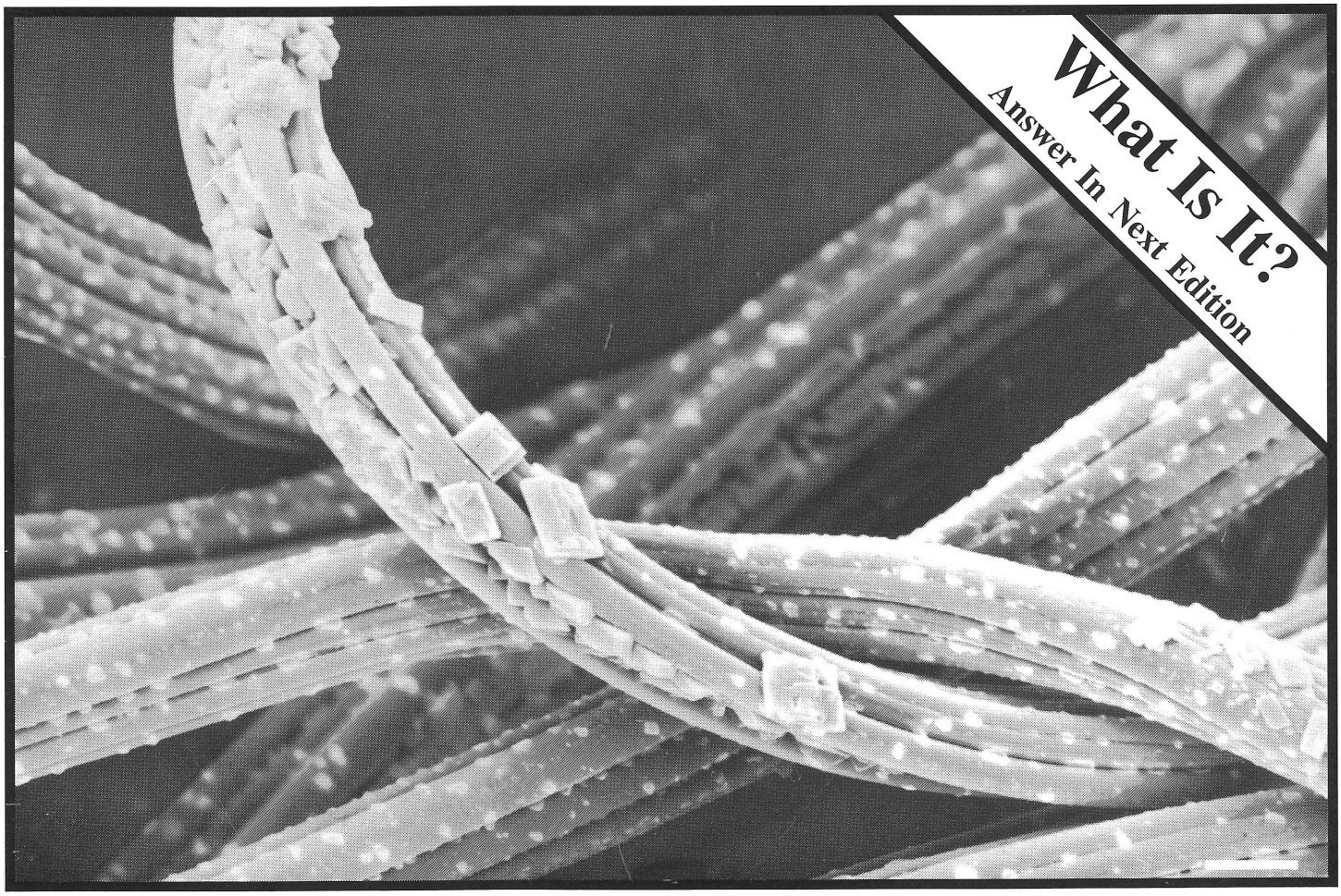

Micrograph by Howard J. Arnott, Department of Biology, University of Texas at Arlington. Arlington, TX 76019. Bar=10μm.