TSM

Texas Journal of Microscopy

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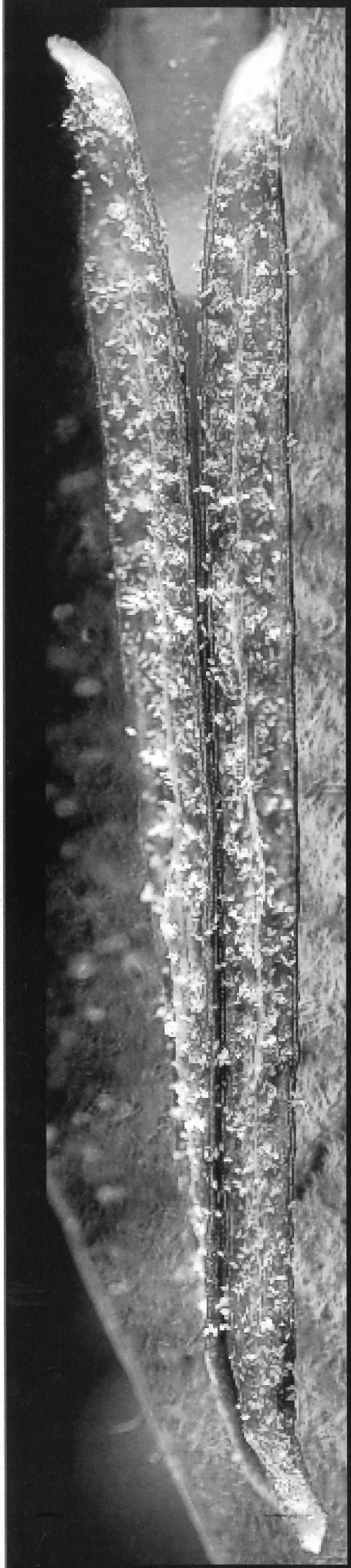

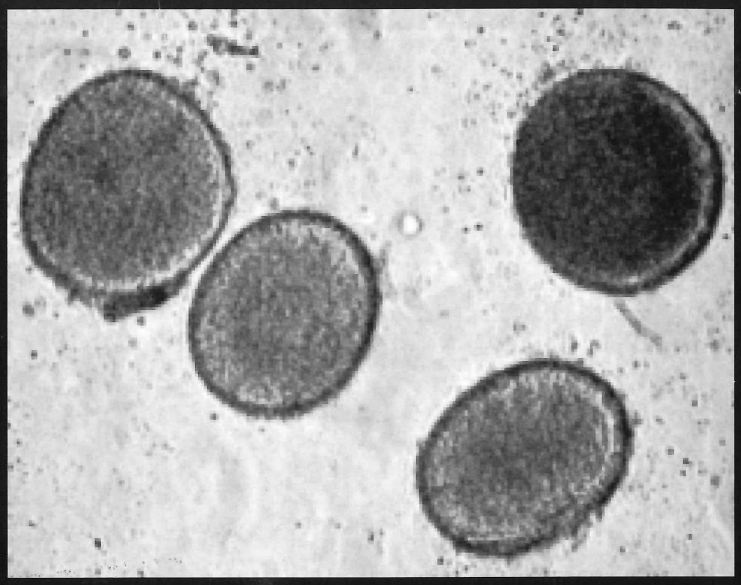

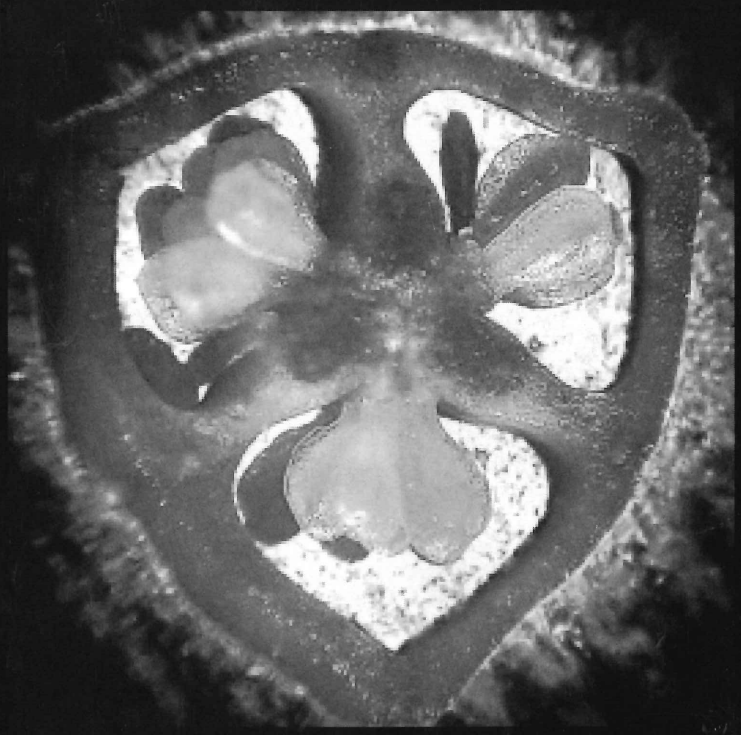

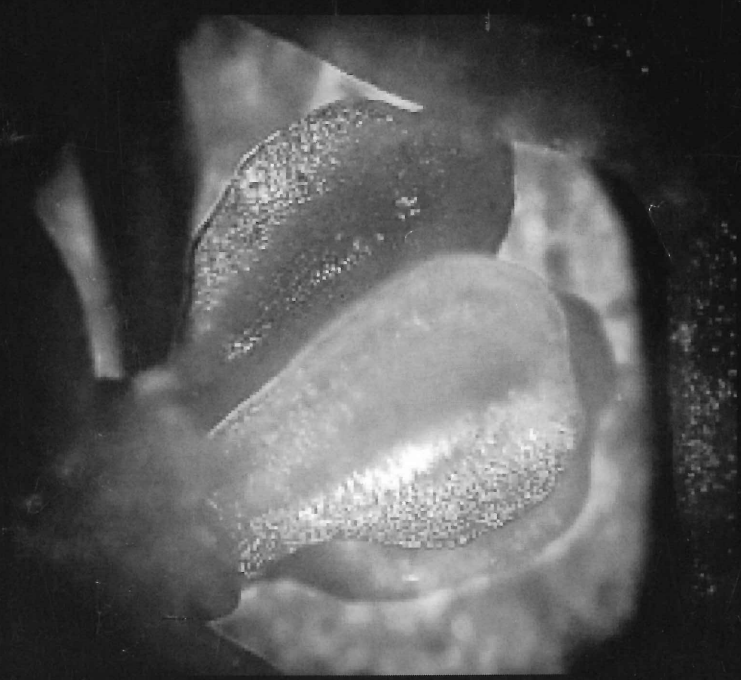

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TEXAS JOURNAL OF MICROSCOPY
VOLUME 31, NUMBER 2, 2000
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Camelia G.-A. Maier
Department of Biology, Texas Woman's University, Denton, TX 76204

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ON THE COVER

The four images represent *Gladiolus* sexual organs, from left clockwise: whole dehiscent anther, pollen grains, transverse section through the gynoecium, and a close-up of ovules. They are part of a series of innovative presentations designed for teaching botany laboratories to biology-major students at Texas Woman's University. Pictures were taken with a digital camera directly through the ocular piece of standard laboratory microscopes. Image size for all pictures was 640x480 JPEG. Pictures were adjusted for brightness and contrast with Corel Photo-Paint before being assembled for presentations with either Power Point or PixAround softwares. The three successive pictures of the *Gladiolus* anther were assembled with the PixAround software, which allows visualization of the whole specimen in slow motion on screen, with the possibility of zooming on the structures of interests. Catalin C. Lungu and Camelia G.-A. Maier, Texas Woman's University, Department of Biology, Denton, TX, 76204.

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in New York City

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ADVERTISER'S INDEX

Meeting Memories

Past Presidents at Spring Meeting 1998, Lake Texoma, Oklahoma. From Left: Bruce MacKay, Howard Arnott, Ann Rushing and Mitchell McCartney.

Lake Texoma

President's Message

Welcome to Dallas and to the facilities of Texas Instruments (TI). We expect to gain useful knowledge from the papers presented as well as from the Thursday seminars at the TI facility. Everyone should make it a point to say thanks to Kevin Cronyn, Midwestern Regional Sales Manager for Hitachi, for his work in setting up the Thursday seminars and for inviting a rather larger range of participants than we usually attract. Texas Instruments' Forest Lane Meeting Center is an excellent setting for the all-day seminars, so we are also indebted to TI for extending that courtesy.

We also welcome guest speakers Dr. Russ Pinizzotto, a member and frequent presenter at TSM while he was on the faculty at the University of North Texas, and Dr. Charles Mims, former president of TSEM as we were named then. Charles was at Stephen F. Austin at that time. He took his first ultrastructure course under Howard Arnott, and finished his Ph.D. at UT Austin.

We have for several years had 12-16 platform presentations and few or no posters. The total number of submissions this time is about the same, with about half offered as posters. Posters provide direct interaction with practically all interested members and they are becoming the preferred way of presenting our research. Actually, the number of papers is a little low for a society such as ours, and I hope several of us will increase the frequency with which we present papers and/or posters and will recruit presenters, especially among students.

Our meetings are excellent places for students to present their first conference paper. It is low pressure and non-threatening. At least as non-threatening as you can find for students presenting for the first time. At the same time the quality of papers has been rather good, rather competitive with regional and national meetings. We represent a good example of what a scientific society should be and do. Ours is a forum for presenting our work in progress for soliciting criticism and new insights into our work. This was once the only reason to have scientific conferences, but in modern times prestige, size, and scope of a conference are more important to our personnel evaluations, so some researchers do not present at state or regional meetings. We are all the poorer for it.

We had a pretty fair turnout of members and presenters for the spring meeting in San Antonio. Meeting with SCANNING 2000 had several advantages, and the San Antonio location is always attractive. Some of us took advantage of the setting to see some to the sites, quite including the Alamo. When I have students from foreign countries, I never miss the opportunity to give them that insight into Texas History that can only be gotten by a visit to the most significant of Texas shrines.

For several years the cost of meetings has exceeded the income, not by much, but we have operated at a deficit. Due to reserves accumulated over the years we have been able to survive this continuing decline in net worth, but we must make necessary changes. I propose that we increase the meeting registration to a rate more like other state and regional conferences we attend, while leaving it at a nominal rate for students. For most of us, our departments and companies will reimburse an increased amount just as they now reimburse our almost nominal lower figure. We can avoid causing a hardship on students if we leave their registration fee at the current low rate.

Enjoy Dallas and the meetings. Probably several will want to go the West End on Friday night. Try to get back in time for the start of the Saturday morning session.

Don W. Smith
TSM President, 2000-2001

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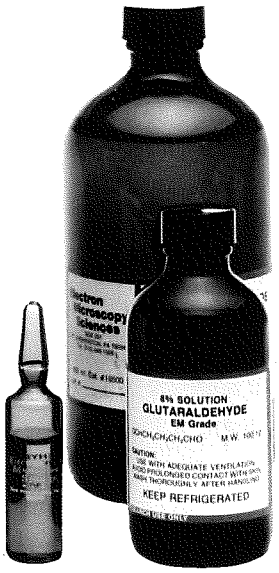

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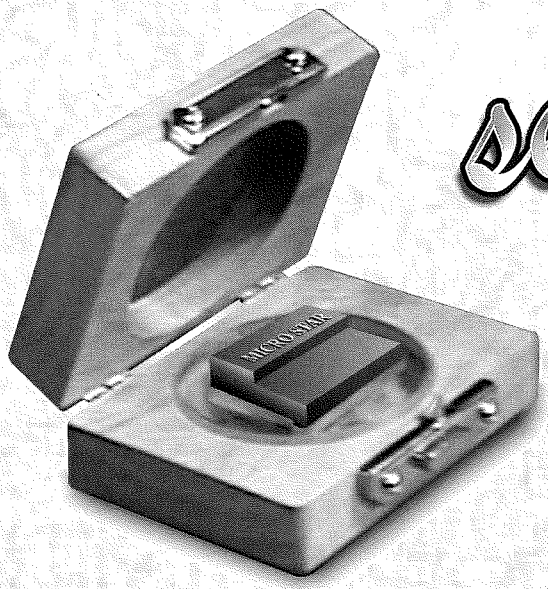

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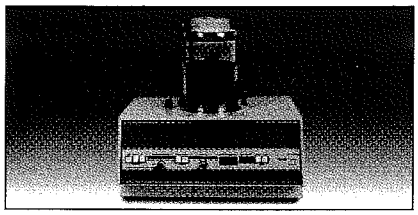

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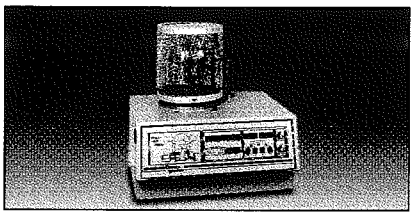
Desk II TSC System offers manual or automatic operation and includes a mechanical pump, turbomolecular pump for ultra-high 10^{-6} vacuum, and starter target for Pt coating. Ready to operate in minutes, the system provides ultra-thin, fine-grained, continuous films and sputters Au/AuPd, Cr, and Pt materials.



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Bench Top Turbo System is a compact, turbo-pumped high vacuum 10^{-6} torr evaporator for carbon or metal evaporation and general TEM/SEM sample prep. A large 10" diameter x 12" high Pyrex bell jar and stainless steel base plate with eight available feed-throughs enhance flexibility of the system by permitting installation of multiple evaporation accessories, specimen holders, and substrate handling fixtures.

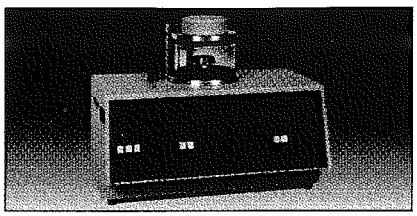

Table Top Cold Sputter/Etch System

Desk II System offers manual or automatic operation and includes a mechanical pump and starter target for Au/Au Pd coating and routine preparation of SEM specimens. It is available in three models to accommodate wafers up to 8.0" diameter, provides a uniform, conductive, fine-grained 100Å coating in less than 5 minutes from pump down through venting and utilizes an etch mode to clean nondelicate, contaminated specimens prior to coating.

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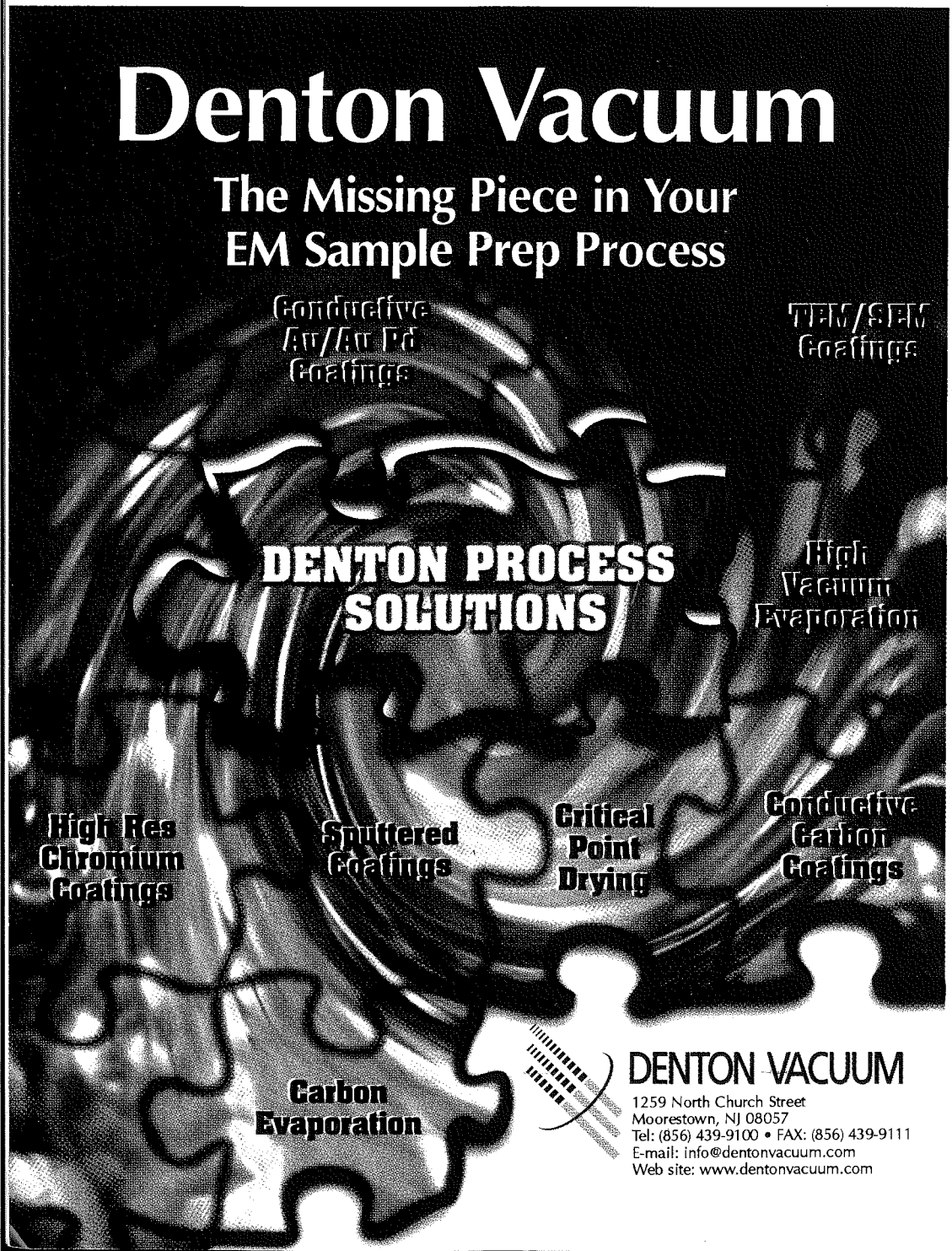

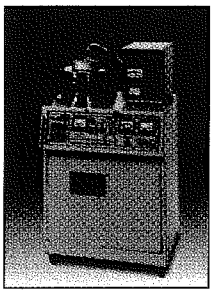

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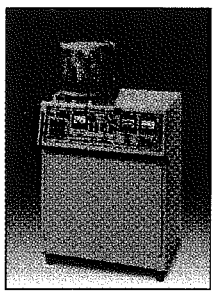

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Treasurer's Report

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Checking Account No. 005772227833	$3,731.40
Certificate of Deposit No. 1882289323	$4,079.37
TOTAL	**$7,810.77**

INCOME:

Dues	$1,779.00
Spring Meeting 2000, San Antonio Meeting Registration	$0.00
Journal Advertisement Revenue	
30:2	$750.00
31:1	$1,750.00
Checking Account Interest	$4.72
Interest on Certificate of Deposit No. 1882289323	$214.75
Misc. (Close out of J. Beaird Secretary Account)	$172.50
Total Income	**$4,670.97**

EXPENSES:

Journal Printing 31:1	$1,899.63
Secretary's Account/ Mailing & Office Expense	$1,400.00
Student Travel	$495.00
Insurance Bond	$144.59
Past President's Plaque	$67.66
PO Box Rental	$64.00
Checking Account Fees	$1.68
Spring Meeting 1999 Expenses (Joint Meeting with Scanning)	$0.00
Total Expenses	**$4,072.56**

ASSETS AS OF SEPTEMBER 31, 2000

Checking Account No.005772227833	$4,329.81
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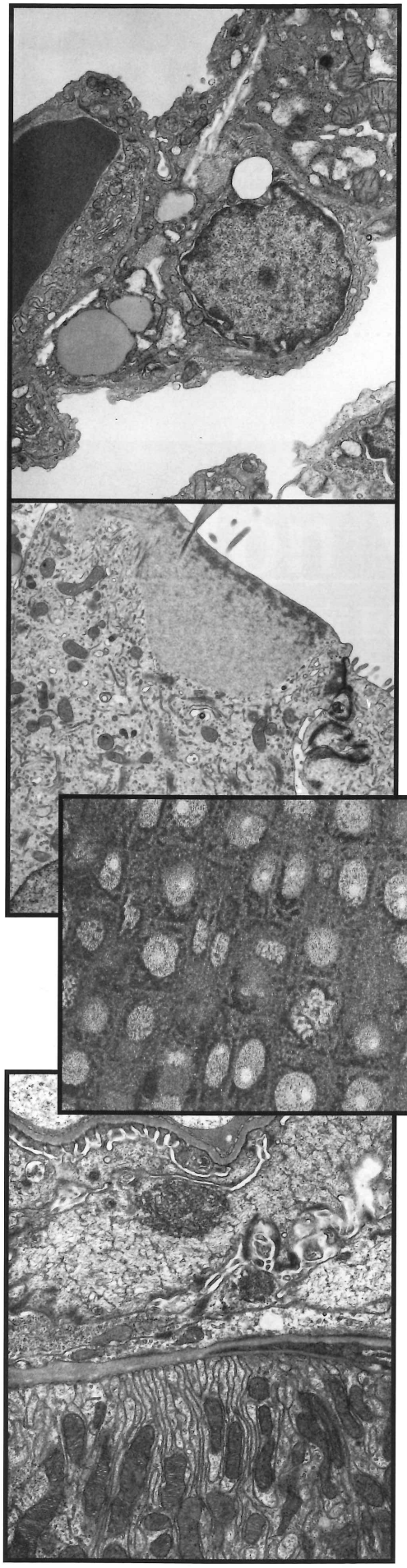
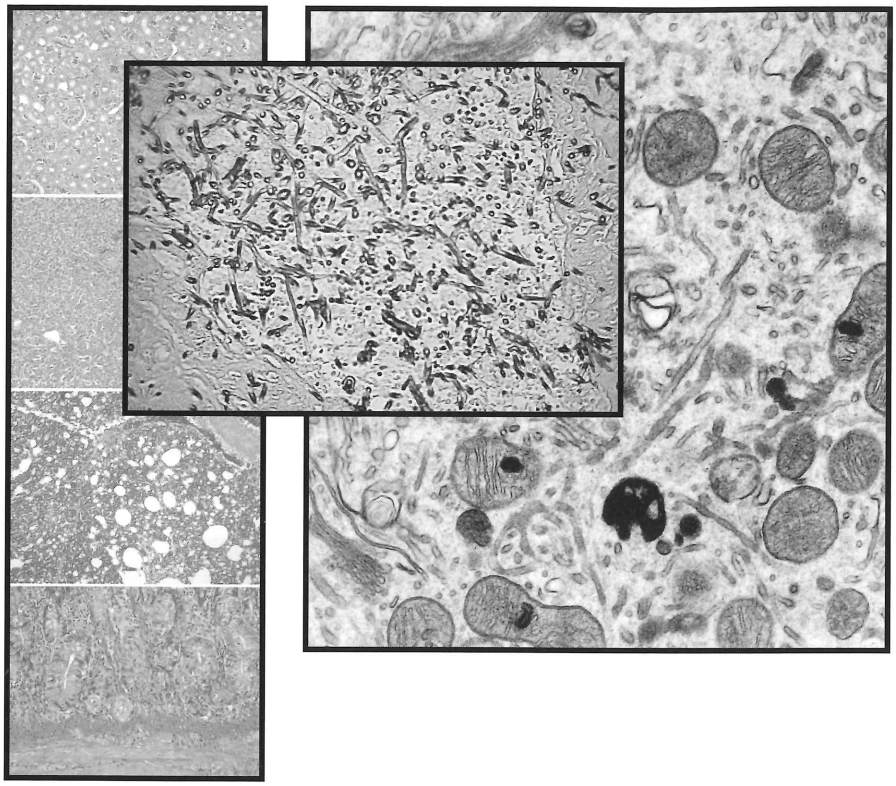
Manuscript deadline is January 31, 2001





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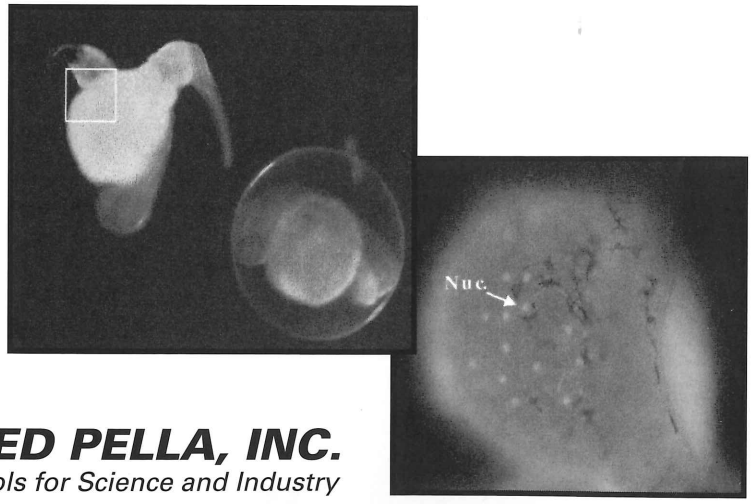

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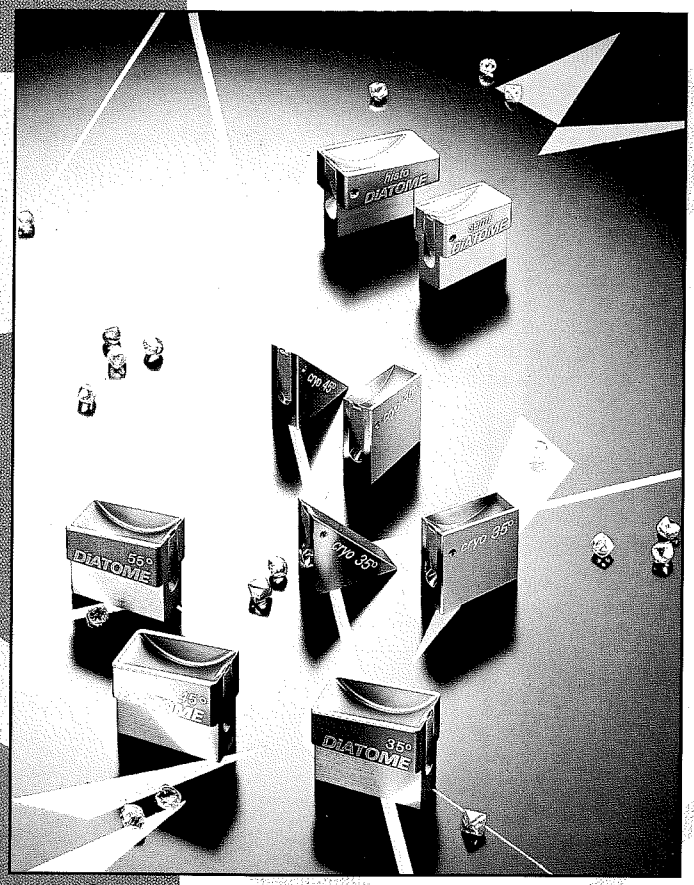

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GUIDELINES: Manuscripts written in English will be considered for publication in the form of original articles, historical and current reviews, case reports and descriptions of new and innovative techniques. It is understood that the submitted papers will not have been previously published. Accepted manuscripts become property of the TEXAS JOURNAL OF MICROSCOPY and may not be published elsewhere without written consent of the Editor. The author should retain one complete copy of the manuscript. The JOURNAL is not responsible for manuscripts lost in the mail.

PAGE PROOFS/REPRINTS: The editor will be responsible for proof-reading the type-set article. Reprints may be ordered from the printer.

MANUSCRIPT PREPARATION: Manuscripts should conform with the following guidelines:

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TITLE PAGE: Include:

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SECTIONS: The text of each original article and technical report should be divided into four major sections entitled INTRODUCTION; METHODS AND MATERIALS; RESULTS; AND DISCUSSION.

Historical and current reviews and case reports do not need to be divided into the aforementioned sections.

ABSTRACT: Summarize the article in no more than 150 words. This takes place of a final summary paragraph.

REFERENCES to other work should be consecutively numbered in the text using parentheses and listed at the end, as in the following examples:

(1) A. Glauert, Practical Methods in Electron Microscopy. Vol. 2 (North-Holland. Amsterdam, 1974) 82-88.
(2) P.S. Baur, Jr., G.F. Barratt, G.M. Brown and D.H. Parks. Ultrastructural Evidence for the Presence of "Fibroclasts" and myofibroclasts" in Wound Healing Tissues. J. of Trauma. 19 (1979) 774-756.
(3) D. Gabor. Information Theory in Electron Microscopy, in: Quantitative Electron Microscopy. Eds. G.F. Bahr and E. Zeitler (Williams and Wilkins, Baltimore, 1956) 63-68.

(NOTE: Authors are responsible for the accuracy of references.)

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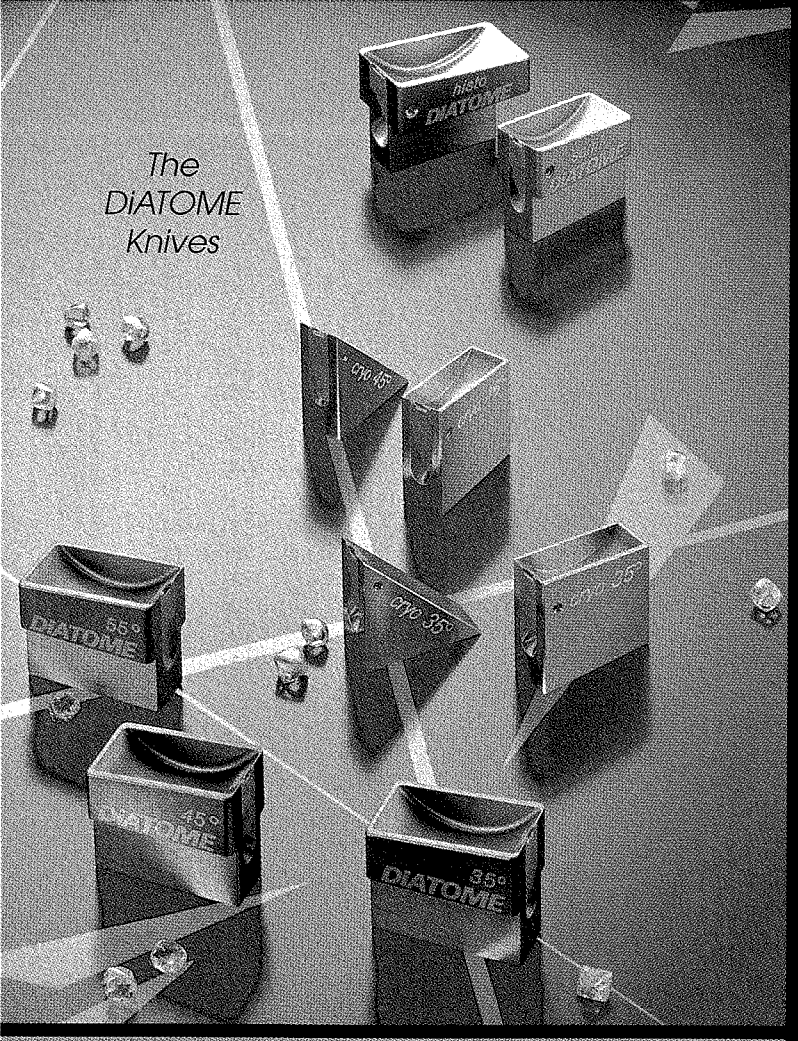

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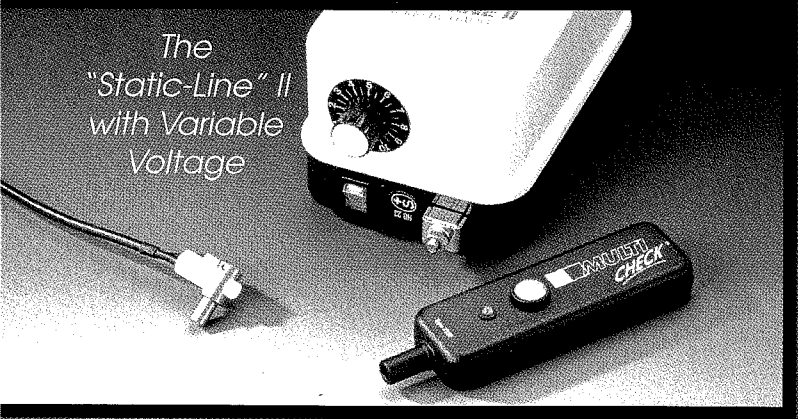

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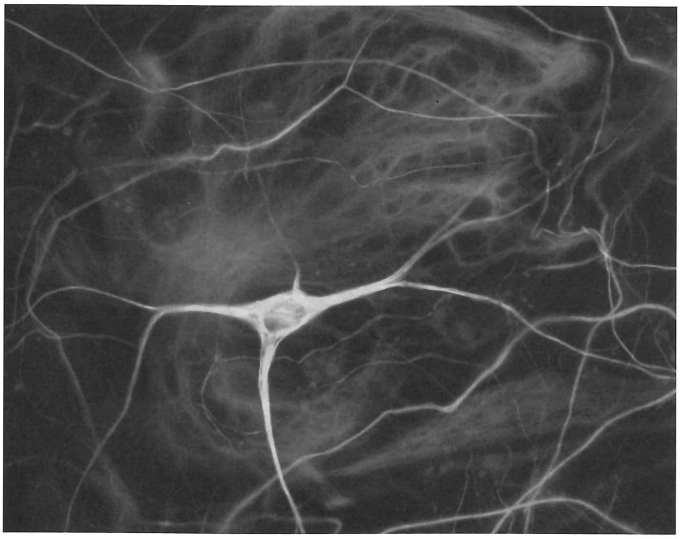
ANSWER TO "WHAT IS IT"

from Texas Journal of Microscopy 31:1



The picture on the back cover of Volume 31, Number 1, shows a dissociated spinal cord network, 35 days *in vitro*. The neurons were stained against neurofilament with monoclonal anti-68kD-neurofilament, FITC-conjugated (Sigma); glia cells (astrocytes) were stained against glial fibrillary acidic protein (GFAP) with monoclonal anti-GFAP, Cy-3-conjugated (Sigma); the cell nuclei were stained with the DNA dye Hoechst 33258 (Molecular Probes). The fluorescence pictures were taken at 3 different filter wavelength with a cooled CCD camera and recomposed in Photoshop.

Alexandra Gramowski, University of North Texas, Center for Network Neuroscience, P.O. Box 5218, Denton, TX 76203.

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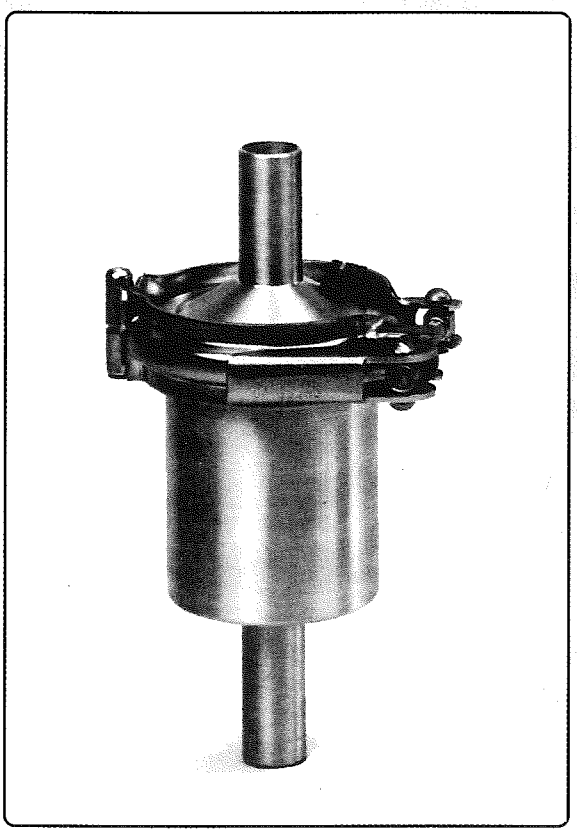

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Abstracts

BIOLOGICAL SCIENCES
PLATFORM PRESENTATION—FALL 2000

CRYSTALIFEROUS FUNGI ASSOCIATED WITH BRISTLECONE (*PINUS LONGAEVA*) LITTER FROM THE GREAT BASIN NATIONAL PARK. HOWARD J. ARNOTT AND CATHERINE J. ARNOTT-THORNTON. The Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019.

A large grove of bristlecone pines, *Pinus longaeva*, exists on the eastern flank of Wheeler Peak in The Great Basin National Park, Nevada. The grove is located on a rocky moraine and in its upper part the remains of the Prometheus Tree (WPN-114) can be observed among many living and dead bristlecone pines. Prometheus, the world's oldest known living tree (4800+ years old), was cut down in 1964. Currently the stump and parts of the stem can be found in a rocky area of the moraine at about 10700 feet elevation. With permission we visited the site and collected litter samples from among the upper part of the grove including a collection about 1 m from the Prometheus stump. These samples were returned to the laboratory for analysis by light and electron microscopy. For the most part the samples consisted of unconsolidated leaves, small branches and cones lying directly on the surface of the soil. In a few cases, including the Prometheus site mentioned above, just below the surface the litter was consolidated into a grey-brown matt from 1 to 12 cm in thickness. A series of white fungal rhizomorphs which run through the matt are 1-2 mm in diameter and several centimeters in length. Rhizomorphs and leaves extracted from the litter samples were attached to stubs, sputter coated with gold/palladium and examined in the SEM. The rhizomorphs consisted of hyphal strands, 1 to 2 μm in diameter, surrounding small pieces of litter. Often these hyphae have crystals associated with their surface. Among the rhizomophs four types of crystaliferous hyphae were seen. In the first, numerous very elongated crystals extend from a hypha simulating a a bottle brush. These needle-like crystals are 0.5μm in diameter and up to 22μm in length; they appear similar to calcium oxalate of previous studies, but in these as well the next three, positive identification awaits x-ray diffraction study. In a second class, numerous crystals 2 to 3μm in size completely encrust the hypha, they appear to be calcium oxalate dihydrate. In a third class, a multitude of small calcium oxalate-like crystals about 0.1μm are found forming a tightly encrusting layer. In a fourth calcium oxalate-like class, "regular-spaced" triangular crystals extend at various angles from a hypha. In addition to these, large hyphae of 7 -10 μm diameter are found, these may be smooth or have numerous small vesicles attached to their surface.

PHOTOGRAPHY OF LOTUS. C.L. SCHWARTZ AND H.J. ARNOTT. The Dept. of Biology and The Center for Electron Microscopy at The University of Texas at Arlington, Arlington, TX 76019.

Lotus, *Nelumbo nucifera*, is one of six plants, which is known to have thermoregulatory flowers[1]. How and why lotus flowers regulate their temperature is under investigation via light and electron microscopy. Using microscopy, we have found heavily laden starch containing cells. Starch is the assumed fuel source for thermorgeulation in lotus. An understanding of thermoregulation starts with understanding the basic biology and ecology of these plants. Although microscopy is a very useful tool for biologists, the integration of photography at the "macro" scale can enhance the value of either light or electron microscopy. Sometimes, it's a good idea to step away from the microscope and look at the subject as a whole. The photographic tools used in this study of lotus include a SLR Nikon N70 camera, several lenses and filters, a tripod, and several types of film. The first film used was Kodak Gold (ISO 100) to show general morphology of the flowers, a little about their ecology, and experimental set-up. Two other films used were Kodak B/W Infrared and Kodak Color Infrared. B/W infrared film records a subject that emits, reflects, or transmits infrared radiation. The goal of using this B/W infrared film was to see if the infrared radiation given off during thermoregulation would show an image on the film. Photographs were taken at both day and night to see the effect of the sun's infrared radiation. Color infrared film was also used to try to capture the infrared radiation released during thermoregulation. Although images from both types of film were artistically beautiful and quite compelling, neither film was able to capture the infrared radiation from the flower.

[1]Patino, S., Grace, J., and Banziger, H. (2000). *Oceologica* **124:**149-155.

THE FLOWERS OF THE "MOTHER TREE" OF THE CALIFORNIA PEPPER TREE, *SCHINUS MOLLE*. HOWARD J. ARNOTT. The Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019.

Schinus molle L., a native of Peru, has been cultivated in California since around 1830. A specimen of *Schinus molle* growing on the grounds of the Mission San Luis Rey in Oceanside, California has been designated by some as the "First-Tree" and by others as the "Mother-Tree." The use of either term implies that this specimen is the tree from which all other California Pepper Trees are derived and that it was the first to be grown in California. Examination of historical photographs, especially those of C. E. Watkins, demonstrates that the "Mother-Tree" was present on the grounds of Mission San Luis Rey as a large tree before 1880. Historical anecdotes give two stories about this tree. The first, indicated that in 1830 seeds were given to Father Peyri by a sailor from Peru; one of seven that germinated survived. A second anecdote, given by Engelhardt[1] says "a sailor from Peru, in 1830 brought a sprig of a pepper tree and planted it in the Mission garden. This was the first of its kind in California." Father Peyri left California in 1834 and subsequently Mission San Luis Rey was abandoned. By good fortune the San Luis Rey Pepper Tree survived the almost half century period of desertion and lives today. Currently it is well maintained and is easily be seen by hundreds of visitors each year. The tree has a crown of about 15m in diameter with a trunk is over 2.5m in diameter, the latter which shows the knobby burls typical of older specimens. I visited the "Mother-Tree" and with permission collected flowers from it. The buds and flowers were cream colored with five sepals, five petals, three carpels and ten stamens characteristic of the species. The flowers of the "Mother-Tree" appeared to be the typical male flowers of *S. molle*. Subsequent examination by light and electron microscopy confirmed that the flowers were male with well developed stamens and aborted ovaries. The flowers of *S. molle* are functionally unisexual and found on separate trees. Hence it is clear that the "Mother-Tree" or "First-Tree" is male.

[1] Z. Engelhardt. 1921. "San Luis Rey Mission." J. H. Barry Co.

SOURCES OF VARIATION IN PORE DENSITY IN EGGSHELLS OF WHITE LEGHORN CHICKENS. Sandra L. Westmoreland. The Department of Biology and The Center for Electron Microscopy, University of Texas at Arlington, Arlington, Texas 76019.

The study of the pores of avian eggshell is of interest as they perform the important function of permitting gas exchange through the shell during incubation. Attention has been given to pore distribution by various researchers. The distribution of pores within an eggshell has long been said to be non-uniform (Tyler, 1955). Rahn, et.al. (1977) and Packard, et.al. (1977) demonstrated that the pore area of the eggshell changed when a bird was moved to a different altitude, possibly preventing dehydration. Carey (1983) noted that the way in which the shell gland creates a pore or how it makes the appropriate number of pores per egg is not understood.

Investigations of pore density require that the sources of variation of this density be understood. Sources of variation in pore density in eggs of birds of the same breed may include the variation between different birds, the variation between eggs of a given bird, and the variation in different regions of the same egg. The purpose of this study is to measure the distribution of pore density in eggshell of White Leghorn chickens and determine the sources of variation of this density. Six sequentially laid eggs from each of six White Leghorn chickens were obtained from the Poultry Science Department of Texas A&M University. Samples from each of the thirty-six eggs were taken in three egg regions: the blunt (airspace) region, the equator region, and the pointed end-region. These samples were imaged, digitized, and analyzed using image analysis software. The pore density data was analyzed using an ANOVA to determine the sources of variation. The results from this study may aid researchers in interpreting the implications of pore density in the avian eggshell.

TRANSMISSION ELECTRON MICROSCOPY ON THE SUPERIOR CERVICAL GANGLIA OF THE MICE LACKING THE α3 NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNIT

NABARUN GHOSH[1], LAURA Y. MACKEY[2] DAWNA ARMSTRONG[3], JAMES P. BARRISH[3] and MARIELLA DE BIASI[1]

[1]Division of Neuroscience, [2]Department of Molecular Physiology and Biophysics, Baylor College of Medicine and [3]Department of Pathology, Texas Children Hospital, Houston, TX 77030.

Autonomic ganglia are the places where the neural information is processed and integrated before reaching the target organ. We previously showed that the absence of α3 containing nicotinic acetylcholine receptor (nAChR) subunit produces multiorgan autonomic dysfunction (Xu *et al.* 1999) and compensatory mechanism involved in heterozygous α3 animals (Ghosh *et al.* 2000). Light microscopy of the sympathetic superior cervical ganglion (SCG) showed architectural and cellular changes in the α3 -/- ganglia. Quantitative analysis showed that the number of neurons and glial cells were higher in the α3 -/- animals.

The aim of our investigation was to determine whether the absence of α3 causes ultrastructural changes in α3 +/- and α3 -/- mice compared to the wild type. SCGs were excised from anesthetized animals and fixed in 2.5% buffered Glutaraldehyde, captured in agar beads for tissue processing with LYNX Automatic Tissue Processor, and postfixed with osmium tetroxide followed by partial dehydration. After complete dehydration the samples were infiltrated with resin, embedded in flat embedding mold with complete resin. After tissues were oriented under a dissecting scope, embedding molds were placed in 100°C oven to cure overnight.

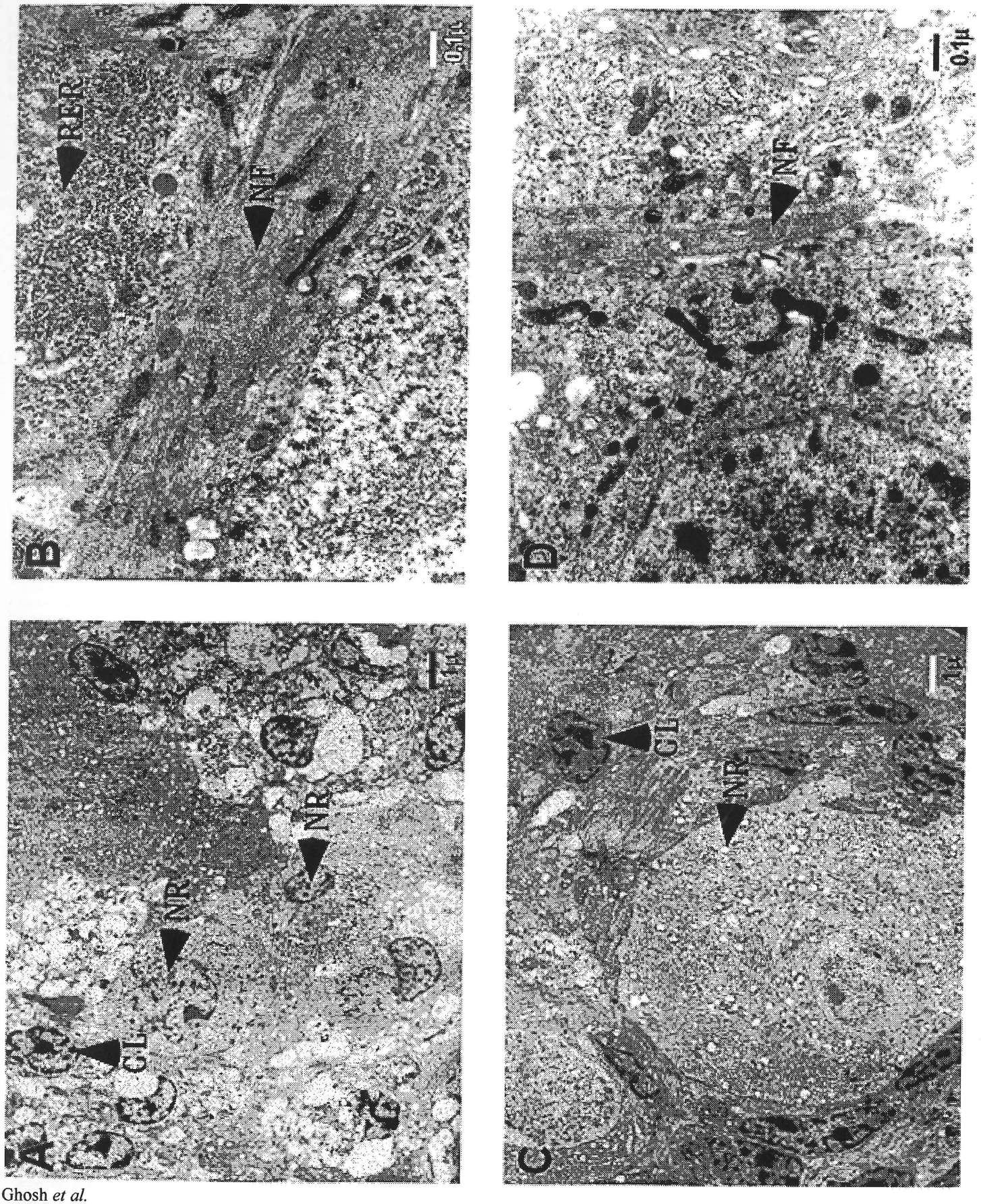
Semithin sections (0.50μm) were cut on a Leica Reichert Ultracut. The sections were placed on a glass slide, stained with 1% Toluidine Blue and observed under light microscope. Ultrathin sections (70-80 nm) were cut and captured on grids from floating turf. Only the ultrathin sections were accepted for viewing with TEM because of the limited ability of the electron beam to penetrate an embedded sample.

The 60-70 nm ultrathin sections on grids were subsequently stained with 2% Uranyl-Acetate and Lead Citrate. The sections were observed with a JEOL JEM100C Transmission Electron Microscope. Observation with low magnification revealed the darkly stained glial cells and lightly stained neurons. The nuclei and the nucleoli were visible as dark electron dense structures. High magnification examination showed rough endoplasmic reticulum (ER), mitochondria and Golgi bodies. Collagen fibers, intermediate filaments and vesicles were visible at high magnification as well. We also observed axons and dendrites in cross section. Studies conducted in α3 +/+ and +/- animals revealed no significant ultrastructural differences. Studies are being conducted to determine the ultrastructural features of SCGs of α3 -/- mice.

Reference:

Xu, W. Gelber, S. Orr-Urtreger, Avi, Armstrong, D. Lewis, R, Ou, C. Patrick, J. Role, L. De Biasi, M. and A. L. Beaudet, (1999). Megacystis, mydriasis, and ion channel defect in mice lacking the α3 neuronal nicotinic acetylcholine receptor. *Proceedings of the National Academy of Sciences USA.* 96:5746-51.

Ghosh, N. Salas, R. Mackey, L. Y. Yu, W. Broide, R. S. and M. De Biasi (2000). Expression of neuronal nicotinic acetylcholine receptor α3 and α3 subunits in mouse and compensatory mechanism in heterozygous animals. *The Journal of Scanning Microscopies.* Vol. 22,3: 191-192.



Ghosh *et al.*

LEGEND TO THE FIGURES:

A and B are the Transmission Electron Micrographs of the Superior Cervical Ganglia (SCG) of wild type (+/+) mice. C and D are the Transmission Electron Micrographs of the SCGs of heterozygous (+/-) mice. GL = Glial cells, NF = Neurofilament, NR = Neuron, RER = Rough Endoplasmic Reticulum.

IDENTIFICATION OF MITES (ACARI) FROM LIZARD STOMACH CONTENTS USING SCANNING ELECTRON MICROSCOPY. M. M. GERSON, Dept. Biology, University of Texas, Arlington, TX 76019-0498

Three types of mites (Acari) from zebratail lizard (*Callisaurus draconoides*) stomach contents were examined and identified using scanning electron microscopy (SEM). Careful examination of the mouthparts, genital orifice, and anal opening revealed that the mites belong to two separate taxa. The more sclerotized, bristly mites were identified as members of the Order Mesostigmata, and both types present in lizard stomach contents have the appearance of lizard or arthropod parasites. The less chitinous, slender-legged mite was identified as a member of the Order Prostigmata; this type of mite appears to be free-living. Despite the partially digested state of many of the specimens, the use of SEM allowed observation of sufficient detail to classify these small arthropods.

CYCLOPOIDA OR HARPACTICOIDA? DETERMINATION OF TAXONOMY OF FRESHWATER COPEPODS USING SEM IMAGES. M.H. DOWNING. Dept. Biology, University of Texas at Arlington, Arlington, TX 76019.

An unknown species of freshwater planktonic Copepoda was collected from two Tarrant County, Texas lakes over a four-month period beginning in November 1999. Identification by traditional methods of dissection and light microscopy was difficult to achieve because of the species' small .45 mm body length and cylindrical body shape. SEM images were used to examine the appendages required to follow taxonomy keys for copepods. The new specimens were identified as genus Canthocamptus of the suborder of Harpacticoida.

MEMBRANE INTERACTIONS WITHIN ERYTHROCYTES PARASITIZED BY *BABESIA* ISOLATED FROM WILD RUMINANTS. R.E. DROLESKEY, P.J. HOLMAN AND G.G. WAGNER. Dept. of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843.

Isolates of *Babesia* from white-tailed deer (*Odocoileus virginianus*), bighorn sheep (*Ovis canadensis nelsoni*), caribou (*Rangifer tarandus caribou*), and two isolates from elk (*Cervus elaphus*) were examined by both transmission and scanning electron microscopy. Parasites were examined from in vitro culture in homologous and heterologous erythrocytes. A distinguishing characteristic displayed within erythrocytes parasitized by these isolates was the appearance of membranous structures in close proximity to the parasite as well as the erythrocyte membrane. Some of the vesicular structures resembled components of a documented *Plasmodium* mediated protein trafficking system. Feeding structures that resembled the coiled organelle of *Babesia microti* were identified in cultures of *B. odocoilei*. Trophozoites from all isolates frequently contained host cell based cytoplasmic structures. These observations may indicate possible ways in which these parasites incorporate nutrients from both the surrounding medium and the parasitized erythrocyte

BIOLOGICAL SCIENCES
POSTER PRESENTATION—FALL 2000

EVOLUTIONARY RELATIONSHIPS BETWEEN RAMALINA CELASTRI AND RAMALINA WILLEI AS DETERMINED BY MERISTEM INITIALS AND BRANCHING PATTERNS. Valerie L. Jackson and Ann E. Rushing, Department of Biology, Bethel College, Minneapolis, MN 55112 and Department of Biology, Baylor University, Waco, TX 76798.

Ramalina is a genus of fruticose lichens with erect, three-dimensional growth of flattened thallus branches. This research characterizes the meristem initials, bundles of fungal hyphae that give rise to new thallus growth, and branching patterns of two Ramalina species, R. celastri and R. willei. Lichen samples of both species were obtained through field collections in central Texas. Observations with the scanning electron microscope were used to describe the meristem bundles and the resulting branching patterns. In R. celastri, a broadly ovoid meristem gives rise to a broad, flattened thallus. Terminal bifurcations or divisions of the broad meristem establish the overall branching pattern in R. celastri. Divisions of the meristem may give rise either to two meristems of equal size or to two unequal meristems, the larger of which may divide again soon after. The meristem of R. willei is spherical and much smaller than the meristem of R. celastri and it gives rise to a narrow thallus. Terminal bifurcations and branching from the margins of the thallus are both found in R. willei. Both terminal and side branches result from similarly sized spherical meristems. Based on these observations, R. willei displays evolutionarily advanced features as compared to R. celastri. The small, spherical meristem and extensive branching are considered to be advanced features.

SCANNING ELECTRON MICROSCOPY OF THE DEGRADATION OF BONES. Cory S. Rice and Susan Wallace, Department of Sociology, Anthropology, Gerontology, and Forensic Science, Baylor University, Waco, TX 76798.

Forensic anthropology and osteological research collections are regularly challenged with the need to effectively and efficiently remove non-osseous tissue from bone while preserving the integrity of the bone. In this pilot study, we compared methods routinely used for processing bones and documented changes to the bone using scanning electron microscopy. Samples were taken from four vertebral columns of the domestic pig (Sus scrofa). All columns were cleaned using dermestid beetles (Dermestid maculata) at the following levels: Column A (control), complete skeletonization; B, early skeletonization; C, advanced decomposition; and D, early decomposition. Individual vertebrae from B, C, and D then were subjected to the following treatments: simmer, Biz®, bleach, ammonium hydroxide, 10% hydrogen peroxide, trypsin, papain, and pepsin, to determine the amount of time necessary to remove all remaining tissue. Cores taken from Column A (control) then were subjected to identical treatments and times determined by the individual vertebrae treatments described above. A diamond core 3/8" drill bit was used to make the core samples from the spinous process of lumbar vertebrae and from the transverse process of the thoracic vertebrae. Dried core samples from each treatment then were examined using the scanning electron micrscope and micrographs at 100X of top, middle and bottom portions of each core were taken for comparison. Bleach and simmering treatments were macroscopically and microscopically the most invasive with extensive pitting and fracture lines evident. The least invasive were papain and trypsin treatments. This pilot study will lead to further analysis of the effect of different chemicals, enzymes, and thermal conditions on the degradation of bone. This research will also provide important information for a future National Maceration Site where the cleansing and preservation of skeletal remains will take place.

POSTANTIBIOTIC EFFECT AND ULTRASTRUCTURAL CHANGES IN CORYNEBACTERIUM PSEUDOPIPHTHERITICUM EXPOSED TO VANCOMYCIN. Heather D. O'Dell, Sally W. Jackson, and Ann E. Rushing, Department of Biology, Baylor University, Waco, TX 76798.

The postantibiotic effect (PAE) is a delay in the recovery of bacteria after short-term exposure to antimicrobial agents. The importance of PAE is in its potential to influence dosing schedule of antibiotics. Corynebacterium pseudodiphtheriticum, considered part of the normal flora of the skin and nasopharyngeal mucosa, recently has been designated as a respiratory pathogen. The focus of this study was to determine the PAE of C. pseudodiphtheriticum after exposure to vancomycin and to examine the ultrastructural changes in cells after exposure to and removal from the antibiotic. Using standard methods, four trials of PAE determinations for C. pseudodiphtheriticum after exposure to vancomycin resulted in a mean PAE duration of 281.69 minutes. For electron microscopy observation, cells were harvested by centrifugation and were fixed prior to antibiotic exposure, after 1 hour exposure to antibiotic and at the end of the PAE. Immediately following the 1 hour antibiotic exposure, cell wall degradation, multisegmenation, numerous ghost cells, and abnormally large cells were observed. However, many cells maintained normal morphology. At the end of the PAE, some cells had morphology similar to the contol cultures. However, there were many ghost cells, cells with multisegmentation, and cells with evidence of wall degradation. Due to the low yield of cells present in cultures at the end of the PAE, this study may not be a conclusive analysis of ultrastructural alterations induced by vancomycin. Nevertheless, results of this study provide evidence that C. pseudodiphtheriticum exhibits a positive PAE, and thus intermittent dosing could be used. Also, the presence of ultrastructural alterations at the end of the PAE may provide evidence that the rate of physiological repair is faster than the rate at which the cell structure is repaired.

STEREOLOGICAL PARAMETERS OF HEPATOCYTE ORGANELLES OF *PEROMYSCUS MANICULATUS* INHALING ETHANOL. J. T. ELLZEY, J.P. DRAKE, P. BOENTGES AND L. DADER, Biological Sciences, The University of Texas at El Paso, El Paso, TX 79968-0519.

In order to determine the possible toxic effects of ethanol on deer mouse hepatocyte organelles, stereological parameters were obtained for the mitochondria, peroxisomes, and smooth endoplasmic reticulum of hepatocytes from four groups of *Peromyscus maniculatus* ADH-positive control (n=7); ADH-negative control (n=7); ADH-positive ethanol-treated (n=7); and ADH-negative ethanol-treated (n=7). We postulated that in the presence of intoxicating levels of blood ethanol in deer mice for two weeks, morphometric measurements of hepatocyte organelles would demonstrate changes in stereological parameters that occur prior to the histological observations of steatosis. We measured nine parameters and observed significant changes including a 10% increase in the volume density of mitochondria in the ADH-positive ethanol-treated deer mice compared to the controls. The observed increase in the volume of the smooth endoplasmic reticulum of both the ADH-positive and ADH-negative deer mice is expected with an induction of cytochrome P4502E1 due to the ethanol treatment. A slight increase in the peroxisomal volume density for both the ADH-positive and the ADH-negative deer mice hepatocytes may be indicative of an induction of catalase in the ethanol-treated deer mice.

LOCALIZATION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) PROTEIN, RECEPTORS AND M-RNA IN NORMAL AND DISEASED CAROTID PLAQUE. ANN S. BURKE[1], A. HENDERSON[2], ROBERT A. COX[1], HAL K. HAWKINS[1,3], GLENN C. HUNTER[2], Dept. of Electron Microscopy, Shriners Hospital for Children, Galveston, TX 77550[1]. Depts. of Vascular Surgery[2] and Pathology[3], University of Texas Medical Branch, Galveston, TX 77555

Vascular endothelial growth factor (VEGF), an endothelial cell-specific mitogen that enhances vascular permeability and regulates endothelial differentiation and angiogenesis, has been implicated in wound healing, embryogenesis, atherosclerosis, restenosis and tumorigenesis.

In order to evaluate the distribution of VEGF in atherosclerotic plaque, we studied normal and diseased human carotid specimens using automated immunohistochemistry and *in situ* hybridization to identify the presence of VEGF, VEGF receptors (flt-1 and Flk-1) and VEGF m-RNA within the lesions. Cell specific localization was determined using antibodies against endothelial cells (Factor VIII-related antigen), macrophages (CD68) and smooth muscle cells (α-actin). The presence of VEGF m-RNA was confirmed by *in situ* hybridization using a 618bp cDNA probe.

VEGF protein was identified in normal and diseased carotid tissues, primarily in endothelial lining cells and microvessels in the plaque and adventitia, smooth muscle cells and macrophages. Expression of VEGF m-RNA was also identified in endothelial lining cells, microvessels and areas of intimal thickening in carotid plaque. VEGF receptors, flt-1 and Flk-1 were localized in endothelial and smooth muscle cells.

Although present in normal tissue, VEGF expression appears to be increased in carotid plaque. Reactive oxygen species and the relative anoxia in the vessel wall, associated with an increase in the plaque mass, may contribute to the up-regulation of VEGF gene expression in the atherosclerotic plaque. The contribution of other cytokine and growth factors, such as basic fibroblast growth factor, transforming growth factor-alpha and epidermal growth factor to the initiation and progression of atherosclerosis are presently being investigated.

ULTRASTRUCTURAL ANALYSIS OF CORYNEBACTERIUM PSEUDODIPHTHERITICUM AFTER EXPOSURE TO SUB-MICS OF VANCOMYCIN AND CIPROFLOXACIN. Hilary D. Clark, Sally W. Jackson, and Ann E. Rushing, Department of Biology, Baylor University, Waco, TX 76798.

Sub-minimal inhibitory concentrations (sub-MICs) of antibiotics have been reported to cause morphological and ultrastructural alterations in bacteria. Corynebacterium pseudodiphtheriticum, a normal member of the oropharyngeal flora that recently has been recognized as an emerging respiratory pathogen, was examined by transmission electron microscopy after exposure to sub-MICs of either vancomycin or ciprofloxacin. Cells were grown directly on membrane filters placed on brain heart infusion agar for 36 hours until mid-log phase, and then transferred to agar containing one-fourth the minimal inhibitory concentration (MIC) or either vancomycin or ciprofloxacin for 48 hours. Cells were fixed immediately after exposure to each antibiotic and after membranes had been placed onto drug-free agar for a 36 hour recovery period. Vancomycin- and ciprofloxacin-exposed cells both contained multilayered membranous invaginations that appeared to be continuous with the cytoplasmic membrane, associated with cross walls, or adjacent to the nuclear region. These lamellar membranes, which were not observed in untreated control cells, resembled those reported in other bacteria after sub-MIC exposure to selected antibiotics. Additional abnormalities such as multisegmentation, cell wall degradation, and a less dense nuclear region were also observed in vancomycin-treated cells. Ciprofloxacin-treated cells, on the other hand, appeared to have a considerably diffuse nuclear region. Cells from each treatment group examined after a recovery time of 36 hours contained abnormalities consistent with the above observations although with less frequency. Results of this study provide further evidence that antibiotics in concentrations well below the MIC can cause structural changes in bacterial cells.

EPICUTICULAR WAXES ON LEEK (*ALLIUM PORRUM* L.) ORGANS DURING DEVELOPMENT. **CAMELIA G.-A. MAIER*[1] AND DUSTY POST-BEITTENMILLER[2]**, [1]Texas Woman's University, Department of Biology, Denton, TX, and [2]Monsanto, St. Louis, MO.

Epicuticular waxes (EW) on aerial organs of leek plants at different stages of development were studied by GC-MS, high resolution SEM, confocal and fluorescence microscopy. All aerial organs presented EW by GC-MS but not all of them presented crystalline wax as shown by SEM. No crystalline structure was observed on organ segments not exposed to light and/or at a young stage in the development, such as stems inside the culm of leaves and overlapping leaf sheaths. Hentriacontan-16-one, odd-chain alkanes and even-chain aldehydes were the predominant classes of compounds detected in the leaf blade EW. The branched-rods and waffle-shaped patterns of EW crystals on leaf blade and ligule, presumably due to the abundance of hentriacontan-16-one, was replaced by mostly plates on stems and buds exposed to light, and thick truncated columns on inflorescence bracts. Weathering of crystalline structures was observed on stem surfaces as well as on the leaf blade. The composition of EW on other organs was different than that on the leaf blades. GC-MS analysis of EW on leek organs indicated changes in wax composition and load due to the organ developmental stage primarily, but also due to the environmental and microenvironmental factors such as light and humidity, especially inside culm. Confocal microscopy along with SEM gave useful insights into the leek EW microstructure. Recrystallization studies along with fluorescence microscopy on fresh samples established that plant EW fluoresces. The natural fluorescence of EW can be used as a method of screening for wax mutants in different species.

TRANSMITTED AND CONFOCAL MICROSCOPY ANALYSIS OF PALATE FUSION AND THE ROLE OF PI-3 KINASE. P. KANG* AND K.K.H. SVOBODA. Texas A&M University System, Baylor College of Dentistry, Dallas, TX 75266-0677

Cleft palate results from the failure of fusion between two palatal shelves. Previous studies demonstrated that epithelial-mesenchymal transformation is a key mechanism for the fusion of the rodent palate. During this phenotype transition, epithelial cells lose cell-cell adhesion, change cell shape, degrade basement membrane, and migrate into mesenchyme. As PI-3 kinase activity is involved in regulating cytoskeletal reorganization and cell migration, we investigated the role of PI-3 kinase in epithelial-mesenchymal transformation and basement membrane degradation during palatal fusion *in vitro*. Dissected palatal shelves were cultured in serum free media and treated with a specific PI-3 kinase inhibitor – LY294002 (0, 100ηM, 1μM, and 10μM). Tissues were harvested from 40 to 72 hours and processed for H&E staining and immunohistochemical analysis of a specific marker for basal lamina (laminin). The fate of midline epithelia was traced by carboxyfluorescence labeling and analyzed by confocal microscopy. In control and 100 ηM inhibitor treated cultures, basal lamina was absent in the midline and the mesenchyme achieved confluence after 72 hours. However, in the groups treated with 1 μM and 10 μM LY294002, medial edge epithelia remained in the midline and laminin staining was positive after 72 hours. In conclusion, our results demonstrated that PI-3 kinase activity is necessary for basement membrane degradation and epithelial-mesenchymal transformation during palatal fusion *in vitro*.

MATERIALS SCIENCES

PLATFORM PRESENTATION—FALL 2000

TEMPLATE-DIRECTED SYNTHESIS OF ORDERED ARRAYS OF SEMICONDUCTOR NANOSTRUCTURES. **C.L. SCHWARTZ[1], AND N.R. TACCONI[2].** [1]The Center For Electron Microscopy, [2]Dept. of Chemistry and Biochemistry at The University of Texas at Arlington, Arlington, TX 76019.

Nanostructured semiconductor materials are attractive for their technological uses in electronic and electrochemical devices such as nanoelectronic circuits, nanorobotics, solar cells, and chemical nanosensors. This communication reports the fabrication and SEM characterization of template assisted electrochemical and chemical deposition of semiconductor arrays using two contrasting template structures – one based on polystyrene spheres and the other based on porous alumina (alumite). Polystyrene sphere templates with two-dimensional (2D) periodical arrays are made from nanosized particles suspended in solution, and the void lattices are filled with precursor solutions for semiconductor deposition. Porous alumina templates, with self-organized cylindrical, uniformly sized holes ranging from 20 to 200 nm in diameter, are prepared by anodic oxidation of aluminum in acidic electrolyte, and are used for the fabrication of semiconductor one-dimensional (1D) nanowires. The two types of templates provide nanostructured semiconductor materials with structural ordering (in 1D and 2D arrays), and due to their nanometer sizes, their electrical and optical properties differ from those of the corresponding semiconductor bulk materials.

EDUCATION
PLATFORM PRESENTATION—FALL 2000

IN-HOUSE EDUCATIONAL AIDS FOR TEACHING BOTANY LABS. CATALIN C. LUNGU AND CAMELIA G.-A. MAIER, Texas Woman's University, Department of Biology, Denton, TX.

Educational aids under the form of Power Point and PixAround presentations were obtained in our plant biology teaching laboratories by using basic equipment and standard, laboratory-grade microscopes, a digital Mavica camera with tripod and a computer. Pictures were taking directly through the ocular lenses of either a Zeiss microscope or a Ken-A-Vision stereoscope. Image size for all pictures was 640x480 JPEG. Pictures were adjusted for brightness and contrast with Corel Photo-Paint before being assembled for presentations with either Power Point or PixAround softwares. Successive pictures of sections through monocot and dicot leaves, and of anthers and carpels were taken and assembled with PixAround software in order to visualize the whole specimens in slow motion. Cyclosis was visualized by using the 100x objective and recorded with the digital Mavica camera as short, 15 s movies of 320x240 MPEG each. The presentations are meant to accompany the lab manual in use at our university ('Plant Biology Lab Manual' by Don W. Smith and Camelia G.-A. Maier, ISBN 0-7872-1279-2) with the main goal of encouraging the study of botany among biology major students. Our innovative approach to accomplish in-house educational aids for teaching plant biology can be used by any college and high-school science laboratories, which lack videomicroscopy systems but possess basic equipment and a digital camera.

EDUCATION
POSTER PRESENTATION—FALL 2000

WEB GRAPHICS TO SUPPLEMENT LABORATORY CLASSROOM MATERIALS. P. GREGORY AND C.CORN, Department of Biology, Tyler Junior College, Tyler TX 75711

Access to laboratory materials (microscope slides, specimens, and models) outside the regularly scheduled laboratory class is limited due to high enrollment, long student commuting distances and student work schedules. Several methods are used to increase availability of materials: (1) review laboratory is available one afternoon a week, (2) models may be checked out on a limited basis, and (3) a narrated video was created. However, microscope slides and specimens cannot be checked out. To increase availability of the materials for review, we have begun posting images to the college web page. These postings include labeled and unlabeled images of histologic slides, specimens, and models. The students can access the files from the campus computers or their personal computers.

Histologic slides are scanned using a 35mm slide adapter with a desk scanner. Specimens and models are photographed using a digital still camera and the photos are imported into a graphics program. Labels can be added, highlighted, enlarged or colors changed as necessary. Images are indexed and students can "bookmark" the site for easy access. Information can be printed or copied to disk. Copyright laws do not apply because these are original images produced by our faculty. Several examples of material which is featured on the web site are included in the poster. Images include both light and electron microscopy, fresh and preserved specimens, models and diagrams. Web-delivered graphics can be updated easily and routinely, thus providing students supplemental access to laboratory material.

Current laboratory postings available to our students may be seen at: "http://*www.tyler.cc.tx.us/science/course/biology/a&p/anat.htm*".

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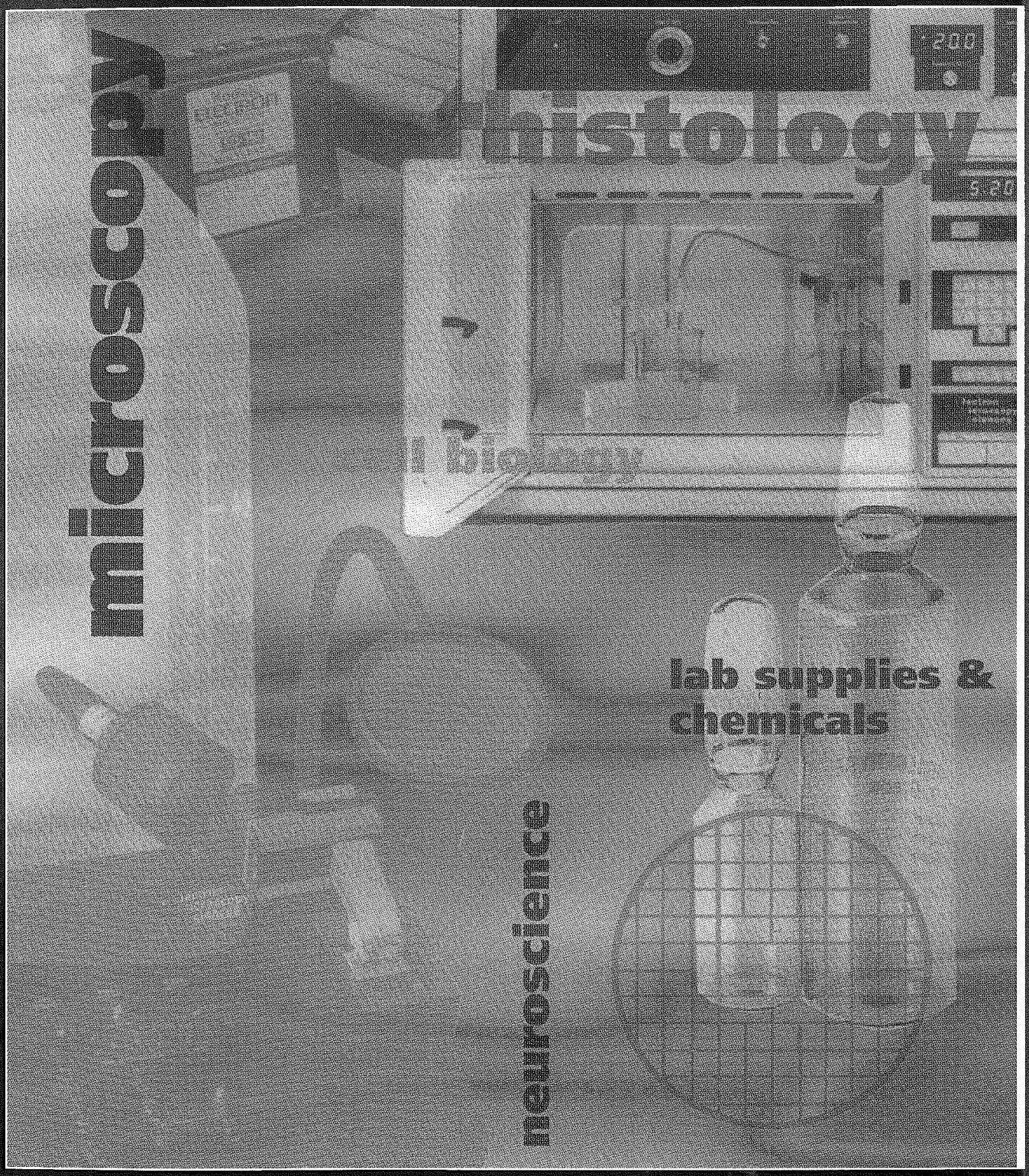

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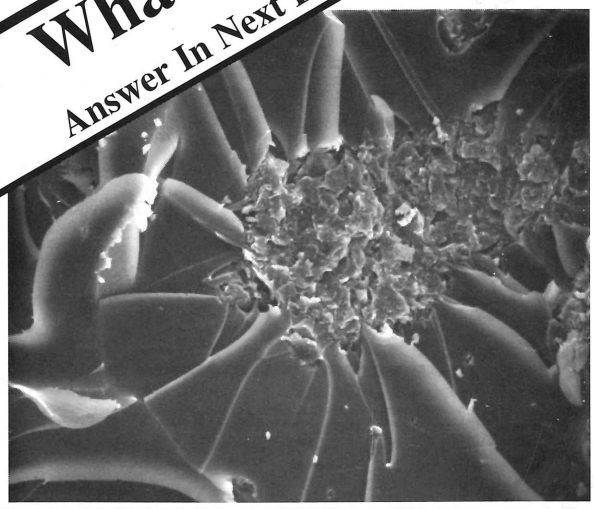

Micrograph on left by Prakaipetch Punchaipetch; Department of Material Sciences, University of North Texas, Denton, TX 76203. Micrograph on right by David Garrett; Department of Biological Sciences, University of North Texas, Denton, TX 76203.