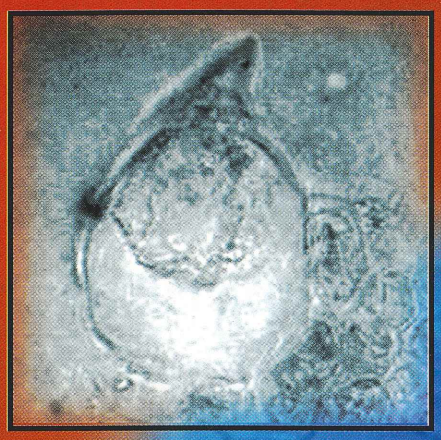
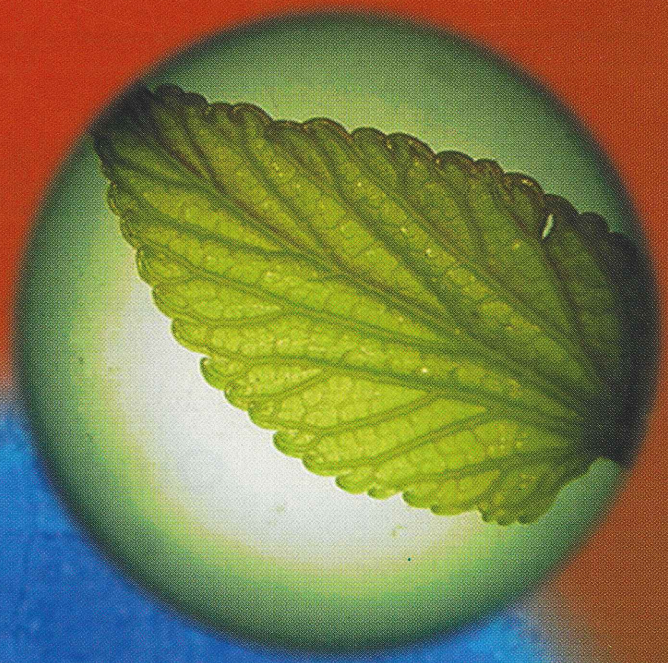
TSM e⁻

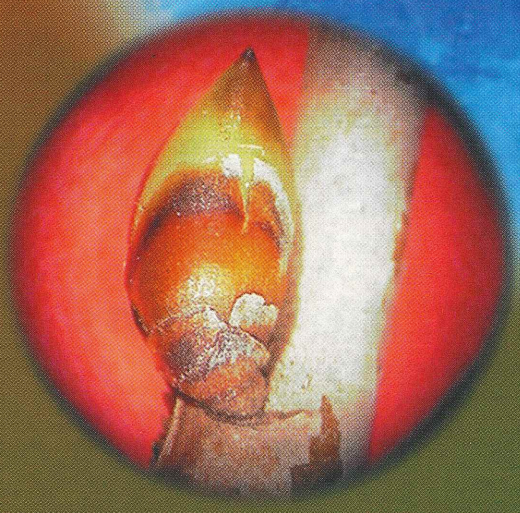
Texas Journal of Microscopy

Volume 32,
Number 1, 2001
ISSN 0196-5662

Visit our web site at:
www.microscopy.cjb.net



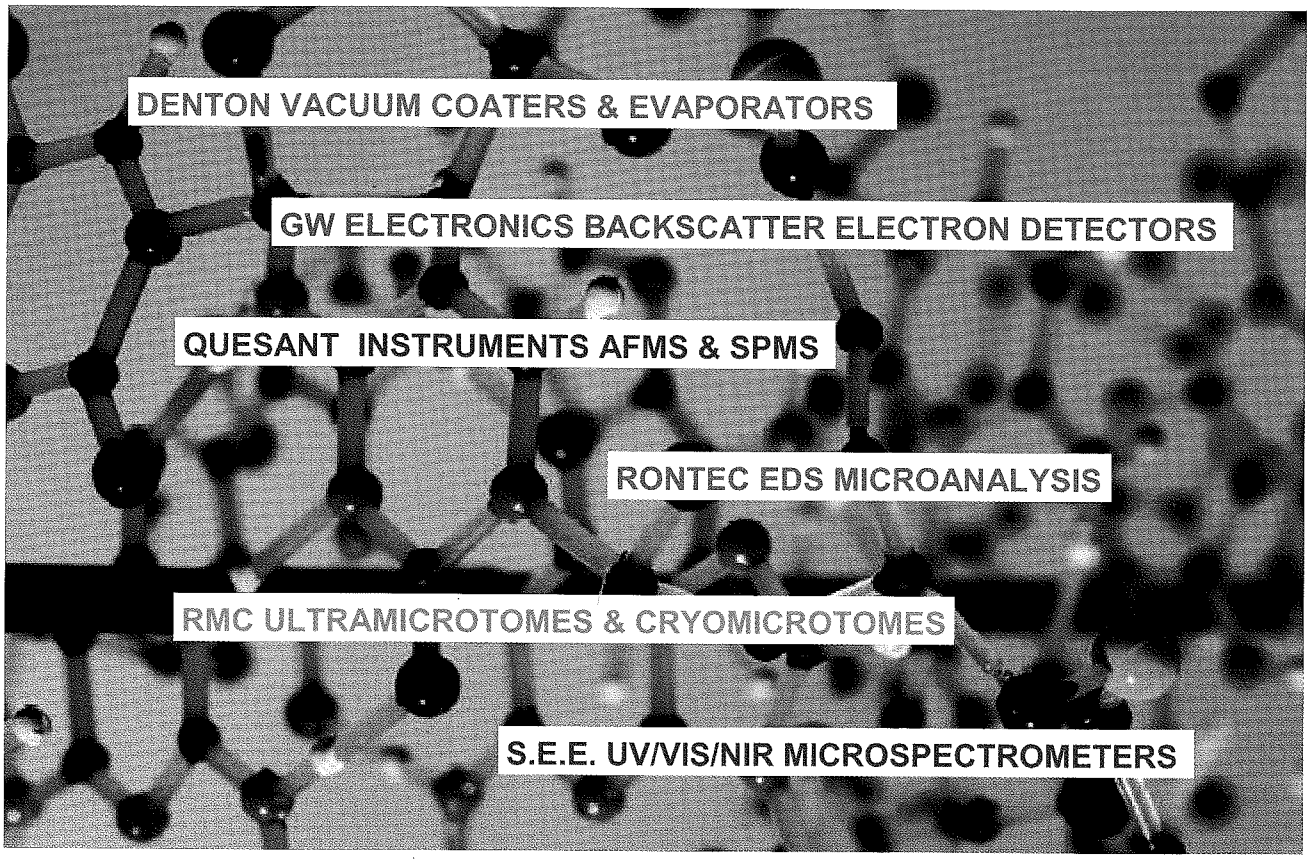




Crystals and Idioblasts in Mulberry Leaves

ATOMIC SPECTROSCOPY INSTRUMENTS

For
Quality Instrumentation
With
Innovation



Consider All the Options
Call 512 352 5340
Or Email grbird@thegateway.net

ATOMIC SPECTROSCOPY INSTRUMENTS, 3451 County Road 409, Taylor TX 76574

Contents

TEXAS JOURNAL OF MICROSCOPY
VOLUME 32, NUMBER 1, 2001
ISSN 0196-5662

Camelia G.-A. Maier, Editor
Department of Biology, Texas Woman's University, Denton, TX 76204

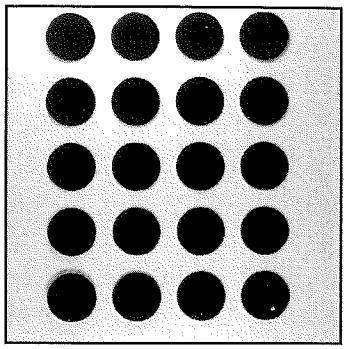
Official Journal of the Texas Society for Microscopy
"TSM - Embracing all forms of microscopy"
www.microscopy.cjb.net

ON THE COVER

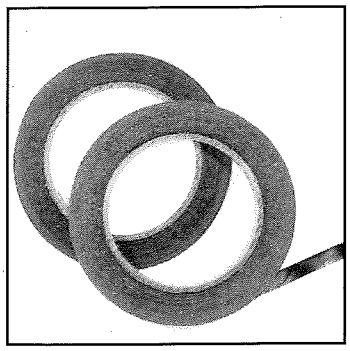
The four images represent calcium deposition and idioblasts at different stages in the mulberry leaf development. From downpage left, counterclockwise: mulberry bud in early March, beginning to open; prismatic crystals in the leaf primordia of an unopen bud; very young leaf in an open bud by middle of March; and a sharp-pointed cystolith in the upper epidermis of young leaves containing a globular mass of calcium carbonate. Pictures were taken with a digital camera directly through the ocular pieces of standard laboratory microscopes. Image size for all pictures was 640x480 JPEG. The picture of the prismatic crystals was taken with the 'NEGART' picture effect mode of the digital camera. Diedre L. Shepard, David C. Garrett, Catalina I. Pislariu, and Camelia G.-A. Maier, Department of Biology, Texas Woman's University, Denton, TX 76204 (DLS, CIP, and CGAM) and Department of Biological Sciences, University of North Texas, Denton, TX 76203 (DCG).

Microscopy Supplies ....."Taylored" to fit your application and your budget!

Carbon Paint is in an isopropanol base. Easy to apply with excellent conductivity.

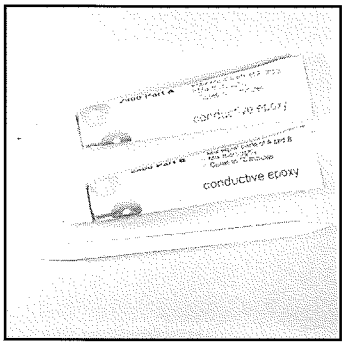


Carbon Conductive Tabs are a double-sided 30µ polycarbonate foil covered on both sides by a 30µ conductive glue.

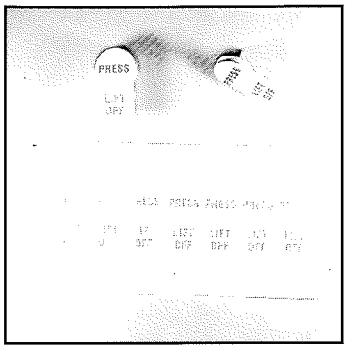


Carbon Tape is double-sided and highly conductive and easy to use for SEM and EDS applications.

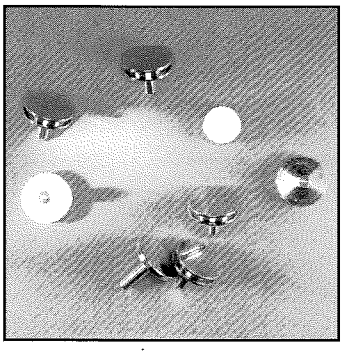
Conductive Silver Paint is easy to apply and provides excellent conductivity. 15 grams.



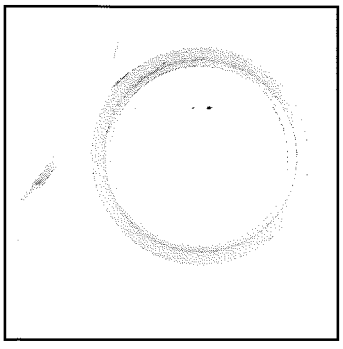
Use this 2-part **Conductive Silver Epoxy** for adhering microscope samples and solderless connections.



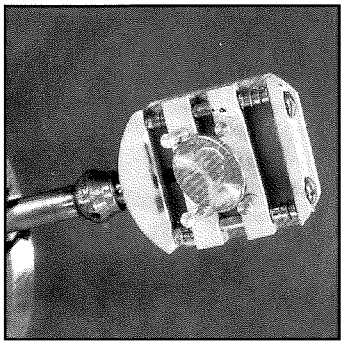
Our **Lift-N-Press Adhesive Tabs** are a convenient, clean, and economical method of mounting samples.



Quality aluminum specimen mounts for most EM's.



3M Copper Tape is a thin copper foil with a conductive adhesive, 1/4" x 18 yards.



Universal SEM Sample Holders allow the mounting of any specimen shape from 2mm to 29mm in size. 5 models available.

M.E. Taylor Engineering, Inc.

21604 Gentry Lane • Brookeville, MD 20833

Phone: 1-301-774-6246 • FAX: 1-301-774-6711 • e-mail: Metengr@aol.com

VISA, MASTERCARD AND AMERICAN EXPRESS ACCEPTED!

1/98

President's Message

A special welcome from all of us at TSM's Houston meeting to invited speaker, Dr. Scott Russell of the University of Oklahoma. He is both a distinguished microscopist and plant anatomist.

He does pretty well with the computer, too. His "Botanical Link of the Day" is amazing for its uncovering and display of interesting and educational botanical web sites the world over. The Botanical Society of America is a serious benefactor of Dr. Russell's computer talents. He is the recipient and organizer of abstracts that come in for the annual meetings. On the occasion of the XVI International Congress of Botany (August of 2000), everyone waited until the last minute to send their abstracts, and the 4000 plus abstracts that arrived in one day flooded his system. He does not have many gray hairs, but some of them came from that event. Scott is a member of the Oklahoma Society for Microscopy. Several of our members have had the pleasure of working with him both in the lab and in our local societies, OSM and TSM. He is an interesting person and an interesting speaker. We look forward to his presentation on Friday, April 6, 2001.

Our meetings all run smoothly, at least that is the way it looks to observers and participants. But when an event looks effortless you can bank on the fact that it is the result of considerable hard work. The program chair, Pam Neill, has put in countless hours to schedule papers, plan for and order foods, negotiate with hotels, and such. Our secretary, Sandra Westmoreland, also has hours and hours invested in getting information out on time and in organizing (and then staffing) the registration and sign-in desk at this year's meetings. Both have been absolutely delightful to work with. The society is fortunate to have them.

The transition in editors from David Garrett to Camelia Maier went smoothly as well. Camelia has put together the last two editions of the journal, and has added some new features. Among those, the 'Education' section for abstracts and 'Meeting Memories'. Please, respond to her invitations for material and manuscript submissions. They will show up in an even better and more interesting journal.

We are also in transition to the new web site, and the new web master is Becky Holford. Please, visit our web site at www.microscopy.cjb.net and give your input at the meeting and/or directly to Becky.

Jim Long has been the keeper of the money, what little we have to keep. The good news is, our income is up, the bad news is, so are expenses. We will hear the details at the business meeting.

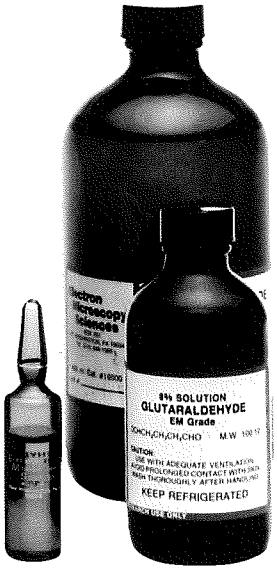
This meeting is an especially good one to come early and stay late, because of the scheduled workshops. We appreciate the work of Steve Zeigler and Al Coritz, who set up the workshops for Thursday, April 5 and Saturday, April 7, 2001. All 17 presentations this time are for the platform. Last time we had a significant increase in posters, but none this time.

Make plans now for the Fall 2001 TSM meeting in El Paso. It should be a good trip and a good meeting. I urge everyone, again, to commit yourself to preparing and giving a paper at the meetings. Every meeting. The lynchpin of our society is the presentation of our work. It is from that all else evolves. We are reasonably healthy at the moment, but if our society ever fails, it will be primarily from lack of participation in presenting our work. Low attendance is a result of lowered participation in presenting work, so the most productive single thing we can do for the society is to make sure we have lots of papers. It is a serious responsibility of all of us. This will translate into larger attendance, which translates into more income, more vendors, more papers, and a more vital society.

Don W. Smith
TSM President, 2000-2001

The Chemicals You Want
The Quality and Value You Need

At Electron Microscopy Sciences, We've Built a Business on it!



GLUTARALDEHYDE

For over 20 years we have been manufacturing the highest purity Glutaraldehyde available on the market; free from polymers and other contaminants. Prior to filling each lot is tested and assayed to assure consistent purity. Only if the Glutaraldehyde passes our rigorous quality control tests will we ship it to you.
Our EM grade is available in 8%, 10%, 25%, 50%, and 70% in 2ml, 5ml, 10ml ampoules as well as 100ml bottles. Our Biological grade is available in 25%, and 50% in 450ml and 1 gallon containers.

OSMIUM TETROXIDE

Crystalline (99.95%) and Solution
Each glass ampoule is pre-scored, pre-cleaned, and heat sealed in a plastic bag - guaranteeing you a contaminant-free solution.
Our solution is available in standard concentrations of 2%. and 4%, in 2ml, 5ml, and 10ml ampoules.
Our crystalline is available in 6gm, 5gm, 4gm, 2gm, 1gm, 1/2gm, 1/4gm 1/10gm ampoules.
Quantity discounts available - please call for special pricing.

Here at Electron Microscopy Sciences we have perfected the manufacturing and filling of the highest quality chemicals meeting all of your microscopy needs. In addition to the chemicals that are listed in our catalog we accept all special orders. If you have special size requirements, concentrations or purity specifications,
Electron Microscopy Sciences is the source.

For a copy of our newest catalog of supplies, accessories, chemicals, and equipment covering the entire field of Microscopy call or write us today. For the best results in your valued research, look for the name that is leading the way in the highest quality chemicals meeting all of your microscopy needs.

321 Morris Road • Box 251 • Fort Washington, PA 19034
Toll-free: 1-800-523-5874 • (215) 646-1566 • Fax: (215) 646-8931 Telex: 510-661-3280

Electron Microscopy Sciences

Treasurer's Report

TREASURER'S 2000 YEAR END REPORT
For period beginning January 1st, 2000 and ending December 31st, 2000

ASSETS AS OF JANUARY 1st, 2000:

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

Income:

Dues	$2,830.00
Spring Meeting 2000, San Antonio (Joint with Scanning)	
Meeting Registration	$0.00
Workshop	$0.00
Donations/Grants	$0.00
Fall Meeting 2000, Dallas	
Meeting Registration	$1,595.00
Donations/Grants	$350.00
Journal Advertisement Revenue	
30:2	$750.00
31:1	$1,750.00
31:2	$750.00
Checking Account Interest	$11.85
Interest on Certificate of Deposit No. 1882289323	$268.73
Misc. (Close out of J. Beaird Secretary Account)	$172.50
Total Income	**$8,478.08**

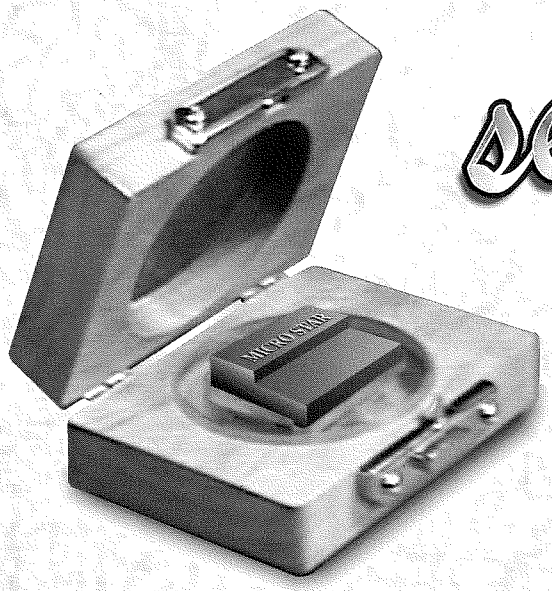
Expense:

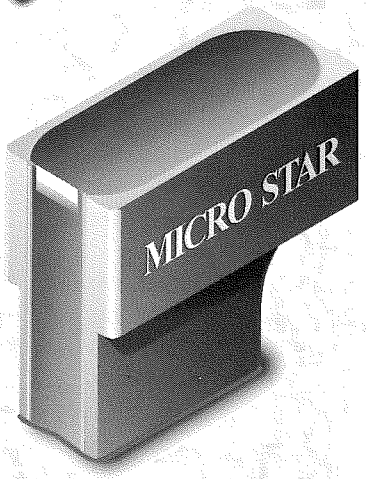
Journal Printing	
31:1	$1,899.63
31:2	$1,324.74
Student Travel	$575.50
Student Award	$0.00
Secretary's Account / Mailing & Office Expense	$1,400.00
Spring Meeting 1999 Expenses	$0.00
Fall Meeting 1999 Expenses	$4,472.32
Checking Account Fees	$32.24
Insurance Bond	$144.59
Postage	$13.10
PO Box Rental (Austin)	$64.00
Past President's Plaque	$67.66
Total Expense:	**$9,993.78**

ASSETS AS OF DECEMBER 31st, 2000

Checking Account No. 005772227833 (Bank of America)	$2,215.70
Certificate of Deposit No. 1882289323	$4,079.37
TOTAL	**$6,295.07**

Quality second to none.





Micro Star diamond knives superb quality is backed by an unprecedented 1 year guarantee. Micro Star, the leader in diamond knife technology is the choice of thousands of scientists around the world. See the reasons:

DIAMOND KNIFE BRAND	KNIFE TYPES	BOAT STYLES	ULTRAMICROTOMY KNIFE SIZES (mm)	KNIFE SIZES ABOVE 6mm	RESHARPENING FOR ALL BRANDS	SAFE NO TOUCH CLEAN SYSTEM	ONE YEAR GUARANTEE	3mm RESHARPENING	3mm NEW KNIFE PRICE
"D1"	6	4	1.5 to 4	NONE	NO	NO	NO	$ 1,650	$ 2,600
"D2"	6	5	1 to 5.5	NONE	NO	NO	NO	$ 1,700	$ 2,550
"D3"	1	1	2 & 3 only	NONE	NO	NO	NO	$ 1,750	$ 2,500
MICRO STAR	7	8	1 to 6	7 to 10	YES	YES	YES	$1,090	$1,990

Why pay more?

MICRO STAR DIAMOND KNIVES

800 533 2509 Fax 409 294 9861 e-mail: mistar@msn.com

Complete price list, specifications, dimensions and manual at our Web site: http://www.microstartech.com/

Treasurer's Report

TEXAS SOCIETY FOR MICROSCOPY TREASURER'S REPORT
For period ending March 15th, 2001

ASSETS AS OF JANUARY 1st, 2001:
Checking Account No. 005772227833 ..... $2,215.70
Certificate of Deposit No. 1882289323 ..... $4,079.37

TOTAL $6,295.07

Income:
Dues $244.61
Checking Account Interest $0.83

Total Income $245.44

Expense:
Secretary's Account / Mailing & Office Expense $250.00
Checking Account Fees $24.11

Total Expense: $274.11

ASSETS AS OF MARCH 15th, 2001:
Checking Account No. 005772227833 ..... $2,187.03
Certificate of Deposit No. 1882289323 ..... $4,079.37

TOTAL $6,266.40

Call For Papers

Manuscripts are needed for the next edition of the Texas Journal of Microscopy. Please send your work as short communications, full articles or review articles in biological sciences, material sciences or education to:

Camelia G.-A. Maier
TSM Journal Editor
Department of Biology, TWU
Denton, Texas 76204-5799
(940) 898-2358
cmaier@twu.edu

Manuscript deadline is July 15, 2001

MICRO STAR

ULTRAMICROTOMY

Diamond knives for all applications: cryo and ultramicrotomy, histology, and material sciences. From 1 to 12mm. Quality backed by one year guarantee. Resharpen and exchange all brands.

Cryo Ultramicrotome integrated in a single portable instrument. Designed for TEM and SPM sample preparation. Microprocessor controlled cryogenic system. Includes Dewar and complete set of attachments. Sections 25nm to 5µ, cryo temperatures to -130°C. Fully automatic or manual operation. High precision and stability at a fraction of the cost of other systems.

Request information, manuals and complete price list, or see them at the web.

800 533 2509
FAX 936 294 9861
MICROSTARTECH.COM

MICRO STAR
TECHNOLOGIES

STEREOLOGICAL PARAMETERS OF HEPATOCYTE ORGANELLES OF *PEROMYSCUS MANICULATUS* INHALING ETHANOL VAPORS

J. T. Ellzey, J. P. Drake, P. W. Boentges and L. L. Dader

Analytical Cytology Core Facility, Department of Biological Sciences,
The University of Texas at El Paso, El Paso, TX 79968-0519

ABSTRACT

In order to determine the possible toxic effects of ethanol on deer mouse (*Peromyscus maniculatus*) hepatocyte organelles, stereological parameters were obtained for the mitochondria, peroxisomes, and smooth endoplasmic reticulum of hepatocytes from four animal groups [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 observed significant changes, including a 10% increase in the volume density of mitochondria in the ADH-positive ethanol-treated deer mice, a 2.2% increase in the volume density of the smooth endoplasmic reticulum of the ADH-positive and ADH-negative deer mice, a 0.4% increase in the peroxisomal volume density for the ADH-positive, and a 0.6% increase in the ADH-negative ethanol-treated deer mice hepatocytes. These changes in stereological parameters are believed to be due to the toxic effects of ethanol when it is metabolized to acetaldehyde. This is the first stereological study of the effects of ethanol on deer mouse hepatocytes. *P. maniculatus* provides an excellent model for quantitatively comparing the effects of ethanol on cells that contain cytosolic alcohol dehydrogenase with cells lacking this major enzyme of ethanol metabolism.

INTRODUCTION

Ethanol has adverse effects on a variety of cells and tissues (1). As the primary site of ethanol degradation, the liver is vulnerable to injury from the toxic effects of ethanol metabolism (2, 3). Lindros (4) classified ethanol as an idiosyncratic hepatotoxin due to unpredictable effects, such as a lack of a clear dose dependency and the variability of toxicity within hosts. The deleterious effects of ethanol metabolism within the hepatocytes include the inhibition of numerous enzymatic pathways and stimulation of other pathways, the combination of which may result in alcoholic liver disease characterized by a progression from steatosis to hepatitis, fibrosis and cirrhosis (5).

The three primary enzymes that metabolize ethanol in the hepatocytes are alcohol dehydrogenase (AD) found in the cytoplasm, cytochrome P4502E1 located in the membranes of the smooth endoplasmic reticulum, and catalase located in the peroxisomes (5). The microsomal ethanol oxidizing system (MEOS) is induced in the presence of intoxicating levels of ethanol (5). In 1997, Lieber emphasized the correlation between the hypertrophy of the smooth endoplasmic reticulum and the induction of cytochrome P4502E1(6). He also suggested that acetaldehyde adducts, produced after ethanol is converted to acetaldehyde, alter the functions and morphology of mitochondria. More recently, Bailey *et al.* (1999) have demonstrated that ethanol stimulates the production of reactive oxygen species at mitochondrial complexes I and III (7).

Correspondence: Dr. Joanne T. Ellzey, Director, Analytical Cytology Core Facility, Biological Sciences, the University of Texas at El Paso, El Paso, TX 79968-0519. (915)-747-6880. E-mail jellzey@utep.edu.

KEYWORDS: Alcohol; hepatocytes; mitochondria; morphometry; *Peromyscus maniculatus*; peroxisomes; smooth endoplasmic reticulum; stereology; ultrastructure.

Bradford *et al.* (8) and Ito and Lieber (9) have utilized *P. maniculatus* to study ethanol metabolism in deer mice possessing cytosolic alcohol dehydrogenase (ADH^+) compared to deer mice lacking alcohol dehydrogenase (ADH^-). Bradford *et al.* (8) concluded that catalase is the primary enzyme for alcohol metabolism in the ADH^- deer mouse. Ito and Lieber (9) concluded from their experiments that cytochrome P4502E1 is the primary enzyme metabolizing ethanol in the ADH^- deer mouse.

Qualitative ultrastructural effects of ethanol metabolism on hepatocyte organelles have been reported by Lane and Lieber (10), Bruguera *et al.*, (11), Arai *et al.*, (12), and van de Wiel *et al.* (13). Weibel (14) suggested that morphometric analyses are needed to validate qualitative observations and to document changes not observed by qualitative evaluation.

Our null hypothesis was that no changes in the parameters of the mitochondria, peroxisomes or smooth endoplasmic reticulum would occur in the presence of intoxicating levels of blood ethanol in deer mice for two weeks. Our alternate hypothesis was that the parameters of these organelles would increase due to the induction of the enzymes that metabolize ethanol and the toxic effects of acetaldehyde. We postulated that changes in stereological parameters of hepatocyte organelles occur prior to histological observations of steatosis in the presence of intoxicating levels of blood ethanol in deer mice. Our expectations were that stereological parameters would increase in mitochondria if they were affected by an increase in acetaldehyde produced from ethanol metabolism and that an induction of catalase and cytochrome P4502E1 would result in increases in the stereological parameters of the peroxisomes and the smooth endoplasmic reticulum respectively. We rejected the null hypothesis when we found an increase in mitochondrial volume density, peroxisomal volume density and smooth endoplasmic reticulum volume density in the ADH^+ ethanol-treated deer mice. Weibel (14) observed a substantial increase in the volume density of the smooth endoplasmic reticulum in phenobarbital-fed rats compared to controls.

MATERIALS AND METHODS

Experimental Animals and Conditions

Thirty-two adult male pathogen-free deer mice were obtained from the Peromyscus Genetic Stock Center, the University of South Carolina. Sixteen of these mice were ADH^+ and sixteen were ADH^-. The ADH^+ mice produced cytosolic alcohol dehydrogenase in hepatocytes. The ADH^- mice were lacking cytosolic alcohol dehydrogenase in hepatocytes. Prior to the experiment, the deer mice were housed in cages in groups of eight for a minimum period of at least one week to allow for acclimation to a 12-hr photoperiod. The temperature was maintained between 23-26°C and a 12hr/ light/dark photoperiod was maintained with the lights automatically turned on between 0800 hr and 2000 hr. Purina lab chow and water were provided *ad libitum.* All of the deer mice were well-fed male adults (14.0-20.6 g).

Experimental Groups

The deer mice were divided into four groups of eight mice each. The experimental groups were as follows: ADH^+ deer mice exposed to ethanol, ADH^+ deer mice exposed to air, ADH^- deer mice exposed to ethanol, and ADH^- deer mice exposed to air. Each treatment was performed in an inhalation chamber described below.

The deer mice were housed in stainless steel 1/4 in hardware cloth cages built by Jonathan Drake. The cages measured 6 x 4 x 5 in^3 and one mouse was placed in each cage. Four of the wire-mesh cages were placed into each of two identical 20 x 15 x 10 in^3 ethanol inhalation chambers. The inhalation chambers were made of 1/4 in Plexiglas and modeled after chambers built by Terdal and Crabbe (15). The cages were raised to a height of 3 cm off of the floor of the inhalation chamber to ensure adequate circulation of air. Water and food were available *ad libitum* for each mouse. Each deer mouse and the remaining food were weighed each day.

In the experimental group of mice, ethanol was introduced into the chamber from a 1000 cc Erlenmeyer flask filled with 1 pint of 100% ethanol connected to the building air supply. The air was filtered with a Colman AL-431 filter to remove any contaminants before it passed into the ethanol inhalation chamber. The rate of airflow was monitored by a flowmeter to insure that air entered into the chamber at a rate of 10 L per min. After twenty-four hours, the air flow rates entering the flask were adjusted to provide a blood ethanol concentration in the deer mice of between 1.25 and 1.75 mg/ml for two weeks. In the control mice, the same type of chambers were used except that the Erlenmeyer flask was filled with one pint of distilled water, rather than 100% ethanol.

Breath Ethanol Sampling

The procedure of Bradford *et al.* (8) was used to determine blood ethanol concentrations (BEC) from breath ethanol sampling. Breath ethanol concentrations were also determined daily by gas chromatography (16, 17). The deer mice were exposed to sufficient ethanol to achieve an average blood ethanol level of between 1.49 to 1.54 mg/ml over a two-week period. Because three mice died during the experiment the number of animals used in the analyses was seven in each of the four groups. On the fifteenth day the mice were sacrificed between 9:00 - 10:30 a.m.

Electron Microscopy

Each deer mouse was perfused through the heart with 0.9% saline, followed by 0.25% glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.2). The liver was removed, weighed and minced into 1mm^3 pieces in 2% glutaraldehyde in sodium cacodylate buffer (pH 7.2). Vials were placed in a rotary agitator at room temperature for 1 hr. The glutaraldehyde was removed and the tissue was rinsed 3 times for 10 min each in 0.1M sodium cacodylate buffer (pH 7.2). After the third rinse, the tissue was rinsed with a 0.2M 2-amino-2-methyl-1,3 propandiol buffer, pH 9.4 (300mOs) once for 10 min.

The tissue was incubated in a 3,3'-diaminobenzidine (DAB) medium, pH 9.4 for two consecutive 30-min periods on a rotary shaker in a 37°C incubator. After two rinses in propandiol buffer and two rinses in sodium cacodylate buffer, the buffer was replaced with 1% aqueous osmium tetroxide and incubated for 1 hour at room temperature in the dark. The tissue was then rinsed 3 times with double-distilled water.

The tissue was stained *en bloc* in the dark at 4°C overnight with 2% uranyl acetate diluted to 1% with 0.1 M sodium cacodylate buffer (pH 7.2). The uranyl acetate was removed, the tissue was rinsed 3 times with distilled water and dehydrated through an ethanol series (75%, 95%, and 100%) for 5 min each. The ethanol was replaced by 100% double-distilled acetone in two 15-minute dehydration periods. The samples were embedded in PolyBed 812/BDMA plastic (Polysciences, Inc., Warrington, PA.) in BEEM capsules that were polymerized at 60°C for two days.

A total of ten BEEM capsules were prepared for each deer mouse. For each of the ten BEEM capsules, five were selected at random to ensure an unbiased data collection. Each of the five BEEM capsules for each deer mouse was sectioned with a Sorvall MT-1 ultramicrotome using a 3 mm Microstar diamond knife in order to obtain metallic-silver sections. The sections were collected onto tabbed 200 mesh Pelco copper grids. Two grids per BEEM capsule were obtained and stored in grid boxes, one grid box per mouse. The specimen grids were post-stained with 2% aqueous uranyl acetate and Reynold's lead citrate using the Kai Chien method (personal communication).

Morphometry

A Zeiss EM-10A transmission electron microscope operating at 60 kV was used to view the specimens at 6,300X. Before each photography session, the microscope was calibrated with a carbon grating replica with 2234 lines per mm (Electron Microscopy Sciences, Ft. Washington, PA) and a configuration file on the OPTIMAS 5.1 software was created with the calibration measurements. A Pulnix 1001 video camera was utilized to project the image of the liver tissue from the electron microscope to a PC 486 computer through the OPTIMAS 5.1 image analysis software and a Raptor driver. The image was magnified to a final magnification of 50,400X during this process and saved as a tiff file.

Three images were acquired for each BEEM capsule. One image was taken from the upper left corner of the first section found due north from the center of the grid. This was repeated again due south, and then west for a total of three micrographs for each capsule (18). This resulted in 15 images per mouse for a total of 105 images for each group of mice. With four groups of mice the grand total of images analyzed was 420 for this project.

Images were also recorded on Kodak S0-163 film with the Zeiss EM-10A transmission electron microscope operating at 60 kV. Custom printing techniques were used to prepare electron micrographs for qualitative analysis.

The nine stereological parameters measured were mitochondrial volume density (MVD), peroxisomal volume density (PERO), number of mitochondria (MC), number of peroxisomes (PC), mitochondrial surface density (MSD), peroxisomal surface density (PSD), mitochondrial numerical density (MND), peroxisomal numerical density (PDN) and smooth endoplasmic reticulum volume density (SERP). Stereological parameters were obtained for the mitochondria, peroxisomes and smooth endoplasmic reticulum of hepatocytes from four deer mouse groups (ADH-positive control (n=7), ADH-negative control (n=7), ADH-positive ethanol-treated (n=7), and ADH-negative ethanol-treated (n=7). The raw data from 420 images were analyzed. These results were consistent with the results based upon averaging 15 micrographs per mouse using a two-way multivariate analysis (MANOVA), followed by a two-way univariate analysis (ANOVA).

The volume density, surface density, and numerical density of the mitochondria and peroxisomes were obtained from the stored images according to Borunda *et al* (18). The volume density of the smooth endoplasmic reticulum was obtained by point counting on micrographs after photographing the tissues from the four groups of mice on the Zeiss EM-10 TEM at X 6,300.

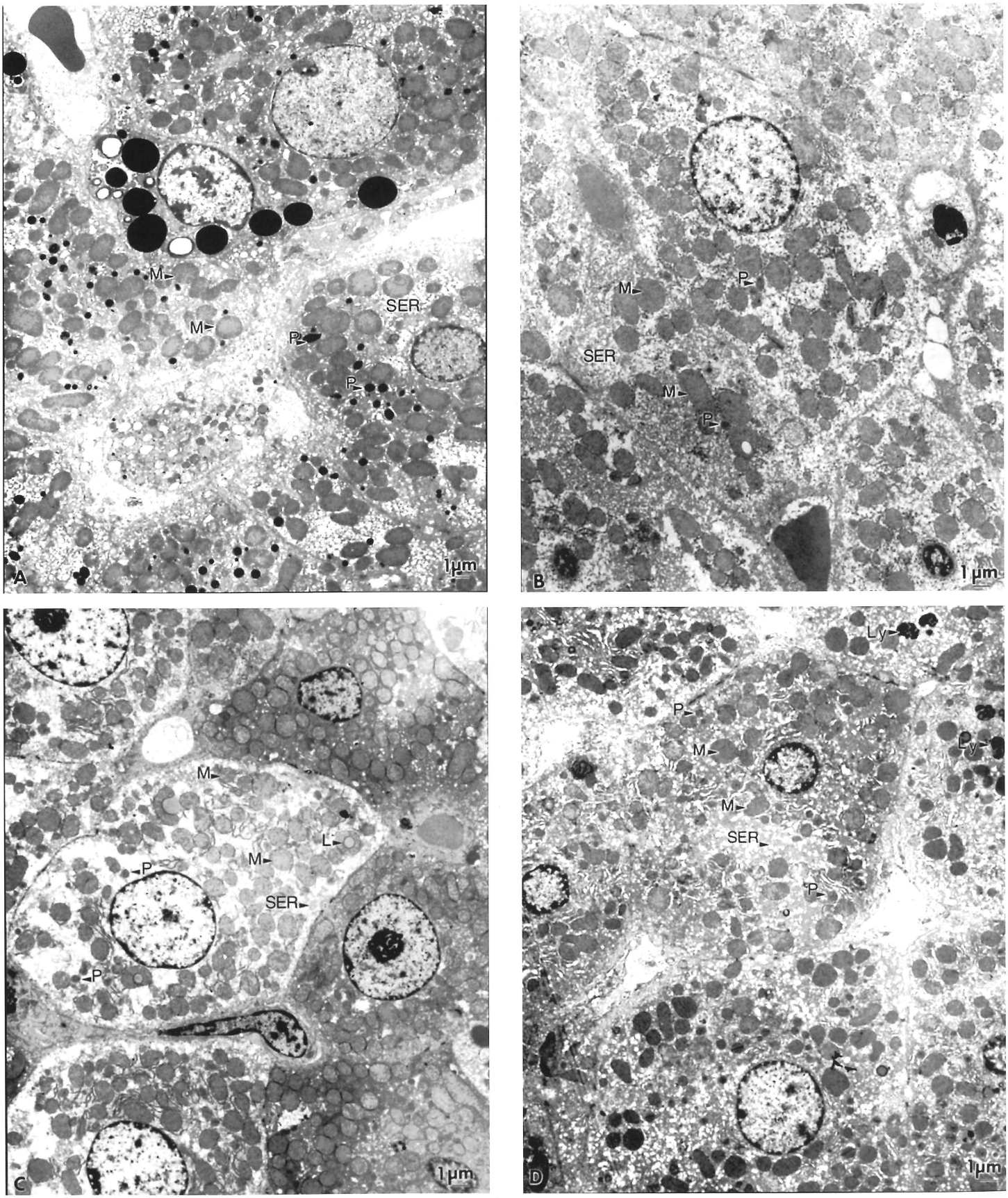


FIGURE 1. Transmission electron micrographs of *P. maniculatus* hepatocytes (X6,000). **A** ADH^+ control; **B** ADH^+ ethanol-treated; **C** ADH^- control and **D** ADH^- ethanol-treated. (L = lipid; Ly = lysosomes; M = mitochondria; P = peroxisomes; SER = smooth endoplasmic reticulum.)

RESULTS AND DISCUSSION

Qualitative transmission electron microscopy of *P. maniculatus* hepatocytes is presented at a low magnification (X6,000) for the four deer mouse groups in Fig. 1. The ADH^+ control hepatocytes contain spherical nuclei, lysosomes, numerous mitochondria, and less abundant peroxisomes, rough and smooth endoplasmic reticulum (Fig. 1A). In the ADH^+ experimental hepatocytes, the nuclei are of similar size to those found in the controls. The mitochondria and peroxisomes appear larger and the smooth endoplasmic reticulum is more abundant (Fig. 1B). The ADH^- control hepatocytes have similar nuclei (Fig. 1C) to those of the previous two micrographs (Fig. 1A and 1B), large mitochondria associated with rough endoplasmic reticulum and lipids, and small peroxisomes. The ADH^- ethanol-treated hepatocytes (Fig. 1D) have nuclei that appear similar to the those in the first three micrographs; lysosomes and mitochondria were also similar in size and appearance to the ADH^- control deer mice; larger peroxisomes and more abundant smooth endoplasmic reticulum than the ADH^- control deer mice hepatocytes.

With the two-way MANOVA to compare mitochondrial and peroxisomal parameters, we observed that the genetic by treatment interaction (p-value = .0203) is significant. Then, using the univariate two-way ANOVA's, we see that the genetic by treatment interaction is significant for mitochondria (p-value = .0071) and not significant for peroxisomes (p =.3927). There was a 10% increase from the control in the volume density of mitochondria in the ADH^+ ethanol-treated deer mice, with a small decrease from the control in the volume density of mitochondria in the ethanol-treated ADH^- deer mice (Fig. 2A).

There was a significantly higher mean hepatocyte peroxisomal volume density for the ethanol-treated mice (both ADH^+ and ADH^-) than the control mice (Fig. 2A). The smooth endoplasmic reticulum (SER) appears to be induced in both the ADH^+ and the ADH^- ethanol-treated mice (Fig. 2B). The smooth endoplasmic reticulum volume density (SERP) had a significantly higher mean for the ADH-negative mice than the ADH-positive mice (Fig. 2B).

For the variables mitochondrial count, mitochondrial volume density, mitochondrial surface density, peroxisomal count, and peroxisomal numerical density there was a significant interaction genetic to treatment (Table 1). The ethanol-treated mice always resulted in a larger mean than the controls for the ADH-positive deer mice. The ethanol-treated ADH-negative mice always demonstrated a smaller mean than the controls for the organelles of the liver (Table 1).

A previous study in our laboratory (18) showed that there were no qualitative ultrastructural differences and no significant differences between the hepatocyte peroxisomal volume density of ADH^+ and ADH^- deer mice that inhaled air rather than ethanol.

Weibel (14) demonstrated that morphometric analyses provide an excellent method for the validation of morphological observations of cells and organelles in determining the toxicological effects of drugs. Very few morphometric investigations have been performed on mouse hepatocyte organelles (18, 19, 20).

The hypertrophy of the smooth endoplasmic reticulum in both the ADH^+ and ADH^- deer mice is consistent with Ito and Lieber's results of an induction of cytochrome P4502E1 in the presence of chronic alcoholism (9). The significant increase in the volume density of mitochondria in the ADH^+ ethanol-treated deer mice could be explained by a response to the increased production of acetaldehyde where all three enzymes, alcohol dehydrogenase, cytochrome P4502E1 and catalase are functional in the metabolism of ethanol. In contrast, we found a significantly smaller mean for the volume density of mitochondria from the ADH^- ethanol-treated deer mice. This could be due to a lack of comparable acetaldehyde production in the ADH^- deer mice that lack cytosolic alcohol dehydrogenase. A small, but significant increase in the volume density of peroxisomes (p = .0175) may be correlated with an increase in catalase in both the ADH^+ and ADH^- ethanol-treated deer mice. Bradford *et al.*, (8) reported substantial increases in catalase in ethanol-treated deer mice. The genetic by treatment interaction was not significant for peroxisomal volume density (p-value = .3927) in our research.

In summary, we have shown that when *P. maniculatus* inhales sufficient ethanol vapor within a two-week period to produce intoxicating levels of blood ethanol, significant changes occur in the stereological parameters of the mitochondria, peroxisomes and the smooth endoplasmic reticulum. In mitochondrial volume density there are significant differences in the responses of the ADH^+ compared to the ADH^- deer mice expressed as the genetic by treatment interaction. The ADH^+ ethanol-treated deer mice had a 10% increase in the volume density of mitochondria (Fig 2A). There is a slight increase in the peroxisomal volume density for both the ethanol-treated ADH^+ and ADH^- deer mice (Fig. 2A). The smooth endoplasmic reticulum volume density is induced in parallel for both the ADH^+ and the ADH^- deer mice (Fig. 2B). We also concluded that *P. maniculatus* provides an excellent model system for quantitative comparisons between the organelles of deer mice that have cytosolic alcohol dehydrogenase in hepatocytes and those that lack this enzyme.

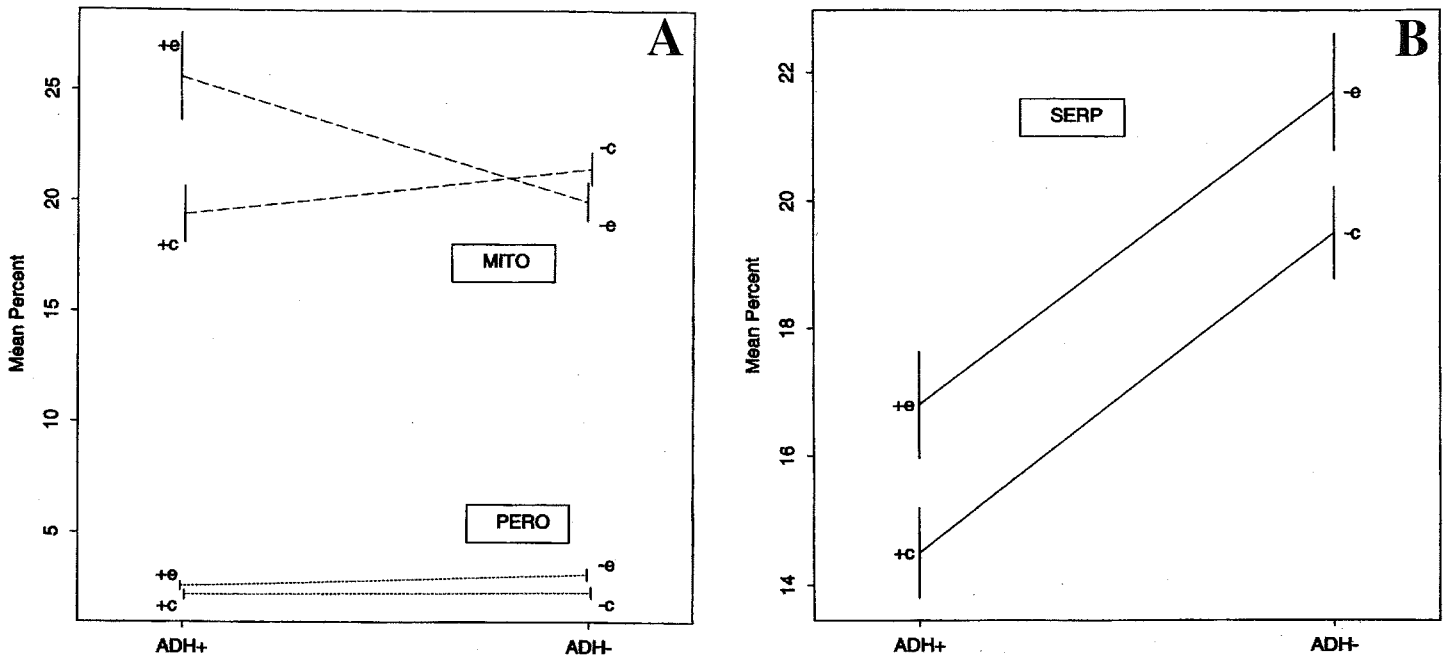


FIGURE 2. Mean percentage of the volume density for **(A)** the mitochondria (MITO), peroxisomes (PERO), and **(B)** smooth endoplasmic reticulum (SERP) of the control and experimental groups of *P. maniculatus*. Vertical bars represent the standard errors of the means. Note that the vertical scale of 2B is expanded over 2A.

	ADH^+control	ADH^+ ethanol	ADH^- control	ADH^- ethanol
MC(n)	11.7±1.33	14.4±2.65	13.9±1.95	12.6±1.47
MITO(V_V%)	19.36±3.39	25.6±5.21	21.5±1.92	20.5±3.03
MSD (μ^2/μ^3)	1.15±0.143	1.48±0.296	1.35±0.154	1.20±0.132
PC (n)	6.21±1.50	8.14±1.76	7.75±2.08	6.65±1.22
PDN (n/μ^3)	0.452±0.115	0.619±0.203	0.611±0.170	0.421±0.0847
SERP (V_V%)	14.5±3.78	16.7±4.54	19.5±3.78	21.7±4.91

TABLE 1. Mean values of stereological parameters for mitochondria, peroxisomes and smooth endoplasmic reticulum of deer mice hepatocytes.

ACKNOWLEDGMENTS

Portions of this research were undertaken for a Master of Science degree in Biological Sciences, the University of Texas at El Paso by Jonathan Drake. The data collected by Paul Boentges were supported by an NSF-MIE grant # S990708 . The research of Joanne T. Ellzey is supported by NIH-RCMI grant # 5G12RR08124. Statistical consultations were provided by Drs. Joan Staniswalis and Julia Bader, the Statistical Consulting Laboratory supported by NIH-RCMI grant # 5G12RR08124. The contents of this paper are solely the responsibility of the authors and do not necessarily represent the official views of NIH.

REFERENCES

(1) G. M. Pastino, E. J. Flynn, and L. G. Sultatos. Genetic polymorphisms in ethanol metabolism: issues and goals for physiologically based pharmacokinetic modeling. Drug Chem. Toxicol. 23 (1) (2000) 179-201.

(2) C.S. Lieber. Clinical biochemistry of alcohol and its metabolic and hepatic effects. J. Ann. Diabetol. (1992) 183-210.

(3) C. S. Lieber and C. S. Abittan. Pharmacology and metabolism of alcohol, including its metabolic effects and interactions with other drugs. Clin. Dermatol. 17(4) (1999) 365-379.

(4) K. O. Lindros. Alcoholic liver disease: pathological aspects. J. of Hepatology 87 (1996) 7-15.

(5) C. S. Lieber. Hepatic and other medical disorders of alcoholism: from pathogenesis to treatment. J. Stud. Alcohol 59 (1998) 9-25.

(6) C. S. Lieber. Cytochrome P-4502E1: its physiological and pathological role. Physiol. Rev. 77 (1997) 517-544.

(7) S. M. Bailey, E. C. Pietsch, and C. C. Cunningham. Ethanol stimulates the production of reactive oxygen species at mitochondrial complexes I and III. Free Radic. Biol. Med. 27 (7-8) (1999) 891-900.

(8) B. U. Bradford, C. B. Seed, J. A. Handler, D. T. Forman, and R. G. Thurman. Evidence that catalase is a major pathway of ethanol oxidation *in vivo*: dose-response studies in deer mice using methanol as a selective substrate. Archiv. of Biochem. and Biophys. 303 (1993) 172-176.

(9) D. Ito and C. S. Lieber. Ethanol metabolism in deer mice: role of extrahepatic alcohol dehydrogenase. Alcohol Clin. Exp. Res. 17 (1993) 919-925.

(10) B. P. Lane and C. S. Lieber. Ultrastructural alteration in human hepatocytes following ingestion of ethanol with adequate diets. Am. J. Pathol. 49 (1966) 593-603.

(11) M. Bruguera, A. Bertran, J. A. Bombi, and J. Rodes. Giant mitochondria in hepatocytes: a diagnostic hint for alcoholic liver disease. Gastroenterology 73 (1977) 1383-1387.

(12) M. Arai, E. R. Gordon, and C. S. Lieber. Decreased cytochrome oxidase activity in hepatic mitochondria after chronic ethanol consumption and the possible role of decreased cytochrome aa content and changes in phospholipids. Biochem. Biophys. Acta 797 (1984) 320-327.

(13) J. A. G. Van de Wiel, C. M. P. Duijf, J. C. L. M. Pertijs, J. H. J. Copius Peereboom-Stegeman, and R. P. Bos. Growth and liver morphology after long-term ethanol consumption of rats. Lab Animals 24 (1990) 265-272.

(14) E. Wiebel. Stereological Methods. Vol. 1. Practical Methods for Biological Morphometry. (Academic Press, New York, 1979) 2.

(15) E. S. Terdal and J. C. Crabbe. Indexing withdrawal in mice: matching genotypes for exposure in studies using ethanol vapor inhalation. Alcohol Clin. Exp. Res. 18 (1994) 542-547.

(16) G. P. Redmond and G. Cohen. Sex difference in acetaldehyde exhalation following ethanol administration in C57BL mice. Nature 236 (1972) 117-119.

(17) L. A. Pohorecky and J. Brick. A new method for the determination of blood ethanol levels in rodents. Pharm. Biochem. Behav. 16 (1982) 696.

(18) D. Borunda,. J. T. Ellzey, and T. L. Stevens. Ultrastructure of *Peromyscus maniculatus* hepatocytes. Texas J. of Microscopy 26 (1995) 50-53.

(19) J. Meijer and B. A. Afzelius. Effects of clofibrate treatment and starvation on peroxisomes, mitochondria and lipid droplets in mouse hepatocytes: a morphometric study. J. Ultrast. and Mol. Struc. Res. 102 (1989) 87-94.

(20) D. De Craemer, J. Vamecq, F. Roels, L. Vallee, M. Pauwels, and C. Van den Branden. Peroxisomes in liver, heart and kidney of mice fed a commercial fish oil preparation: original data and review on peroxisomal changes induced by high-fat diets. J. of Lipid Res. 35 (1994) 1241-1250.

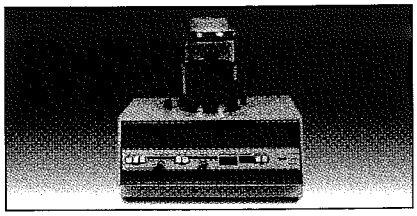
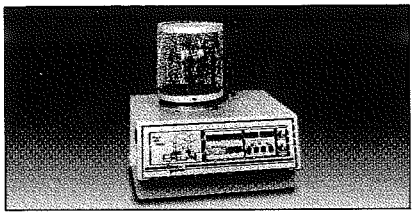


Table Top Turbomolecular Sputter/Etch System

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.



Bench Top Turbo System

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.

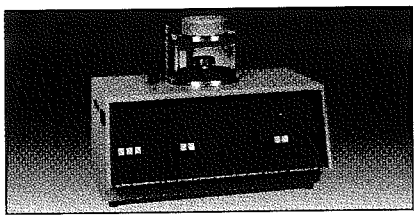
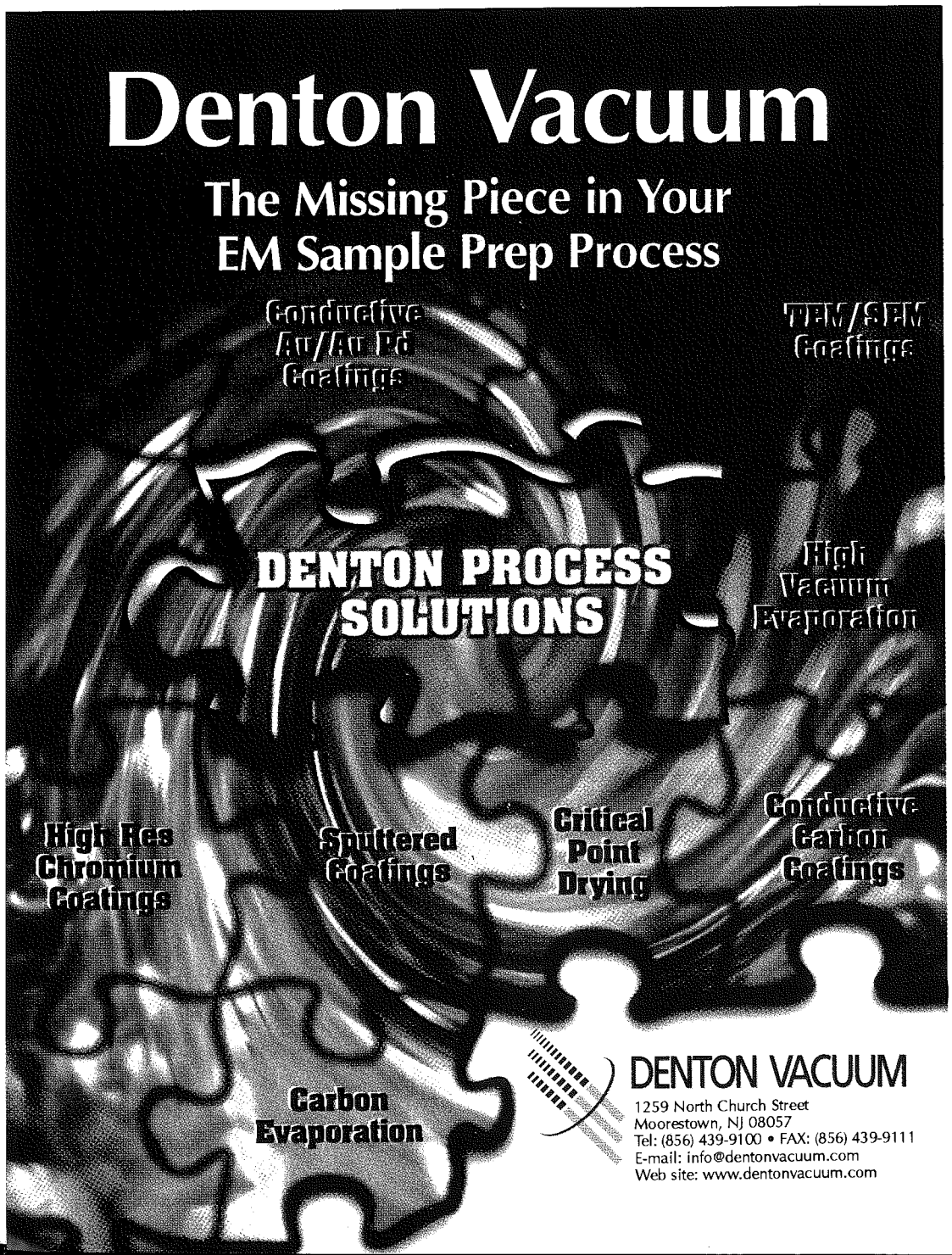


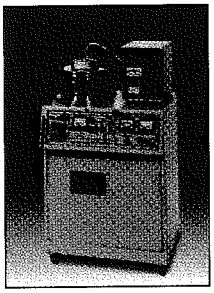
Table Top Cold Sputter/Etch System

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.

Denton Vacuum

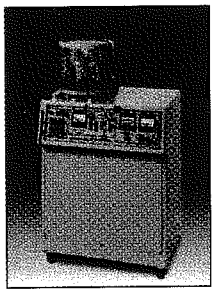
The Missing Piece in Your EM Sample Prep Process





Hi-Res 100 Chromium Coating System

Hi-Res 100 Chromium Coating System provides fast pumping and cycle times with excellent cleanliness for high resolution FESEM sample prep. A patented Anode Grid® and low deposition rate allow controlled ultra-thin 10Å high purity Cr films on substrates to 8.0″ diameter. High vacuum 10^{-7} torr and high water vapor pumping speed prevent sample and film contamination while a quartz crystal monitor and shutter provide automatic deposition for thickness repeatability.



DV-502A High Vacuum Evaporator

DV-502A System is a general purpose, high vacuum evaporator for the preparation of TEM Support films and conductive carbon coatings for X-ray microanalysis. Diffusion, turbo or cryo pumped, the system utilizes state-of-the-art electronics and an advanced mechanical vacuum design to rapidly and repeatedly cycle from atmosphere to high vacuum. The DV-502A is ideally suited for a wide range of EM and R&D lab applications, and can also be used for various other applications in the compact disc, microelectronic, and semiconductor industries.

DENTON VACUUM
1259 North Church Street
Moorestown, NJ 08057
Tel: (856) 439-9100 • FAX: (856) 439-9111
E-mail: info@dentonvacuum.com
Web site: www.dentonvacuum.com

Abstracts

BIOLOGICAL SCIENCES
PLATFORM PRESENTATION—SPRING 2001

CALCIUM DEPOSITION AND IDIOBLAST DEVELOPMENT IN BUDS OF DIOECIOUS MULBERRY. DIEDRE L. SHEPARD, DAVID C. GARRETT, CATALINA I. PISLARIU, AND CAMELIA G.-A. MAIER, Department of Biology, Texas Woman's University, Denton, TX 76204 (DLS, CIP, and CGAM) and Department of Biological Sciences, University of North Texas, Denton, TX 76203 (DCG)

Our interest in sexual dimorphism of dioecious plants, as part of ongoing phytoestrogen research, prompted us to study calcium deposition and idioblast development in mulberry. Samples of spring buds were taken from male and female mulberry trees in various locations in Denton County, Texas, one week apart. Early in March, male buds had a mean length of 22 mm and contained 6-7 leaf primordia. Female buds averaged 7.5 mm in length with 3-5 leaf primordia. After a week, male buds started opening exposing the inflorescences. Entire bud leaves or portions of them were observed under light microscopy and crystals were counted at three locations: base, middle, and tip of the leaf. Leaves of both male and female buds showed a trend of very few prismatic crystals near the petiole with an increasing number of crystals towards the tip, illustrating developmental differences. The oldest or outside primordia and leaves contained more crystals than primordia and leaves inside the buds. Cells of inflorescence bracts did not contain crystals. In general, female bud leaves contained more prismatic crystals than the corresponding male bud leaves. No idioblasts were observed in leaves from closed buds. Both hooked or unhooked idioblasts were observed in the upper epidermis of very young leaves from open male and female buds. Elemental analysis of idioblast content indicated that they contain calcium depositions, visualized as central, globular masses. Further work will focus on the chemical structure of the crystals and the development of the idioblasts in dioecious mulberry.

MEASUREMENT OF HETEROCHROMATIN LEVELS IN MELOIDOGYNE (NEMATODA) INDUCED GIANT CELL NUCLEI IN PISUM SATIVUM. DAVID T. SILVEY, JOSEPHINE TAYLOR, AND ROBERT J. WIGGERS, Dept. Biology, Stephen F. Austin State University, Nacogdoches, TX 75962.

Members of the *Meloidogyne* genus of root-knot nematodes induce large multi-nucleate giant cells with elevated DNA contents in host plants. Previous research has shown that the DNA is non-systematically amplified and examination of giant cell DNA for evidence of specific gene amplification has proven negative. Giant cell nuclei at 1, 2, 3, and 4 weeks post inoculation (PI) in *Pisum sativum* were fixed and sectioned for transmission electron microscopy. Resulting images were analyzed to determine the distribution of heterochromatin in giant cell nuclei as compared to control root tip nuclei. Statistical analysis indicated that at 1, 2, and 3 weeks PI, heterochromatin was significantly under-represented in giant cell nuclei while at 4 weeks PI, returned to levels not significantly different from root tip nuclei.

ESTIMATION OF EGGSHELL PORE SIZE USING THE METHOD OF WATER VAPOR GAS CONDUCTANCE. SANDRA L. WESTMORELAND AND HOWARD J. ARNOTT. The Department of Biology and The Center for Electron Microscopy, University of Texas at Arlington, Arlington, Texas 76019.

Gas exchange, which occurs through the eggshell pore system, is crucial to the optimum growth of the avian embryo. Eggshell "porosity" must be carefully balanced to accommodate the embryo's needs. "Much of the rapid progress over the last decade in understanding the principles governing gaseous diffusion [in avian eggs] is attributable to the simple technique suggested by Ar, et.al. in 1974" (Carey, 1983). Ar quantified shell porosity by studying the loss of water from eggs stored under conditions of known humidity. Ar stated that water vapor gas conductance (G_{H2O}) through pores is a function of both the "total functional pore area" (A_p) and shell thickness (L). Using Ficke's first law of diffusion, Ar derived a formula for calculating functional pore area using G_{H2O} values. The method used in this study for determining eggshell porosity as measured by water vapor gas conductance is that described by Arad and Marder (1982). Nonfertile eggs were collected from six different caged White Leghorn hens of the same breeding line. The eggs were placed in desiccators above dry KOH pellets at room temperature. The desiccators were vented to room air through a short, $CaSO_4$-filled column, to ensure pressure equilibration. The eggs were weighed daily for ten days to determine water loss. Simultaneous weighing of additional small $CaSO_4$-filled columns, placed in the desiccators, ensured zero humidity. Water vapor conductance (G_{H2O}) and functional pore area (A_p) were calculated according to the method of Ar, et.al. (1974). These values were compared with the predicted G_{H2O} values and predicted A_p values based on initial egg weight. In a future study functional pore area data will be compared to the values obtained for pore areas using image analysis.

POLLEN COLLECTION BY EUROPEAN AND AFRICANIZED HONEY BEES. R. RUIZ, A. KUANG, W. RUBINK, G. JONES, Dept. of Biology, The UT-Pan American, Edinburg, TX 78539 and USDA/ARS, Subtropical Agricultural Research Center, Beneficial Insects Research Center, Weslaco, TX 78569

With the advent of the Africanized honey bee, ways to differentiate it from the European honey bee have been greatly sought in attempts to better understand the role of Africanized bees in pollination. It has been largely theorized that while the European honey bee specializes in high nectar content flowers, the Africanized honey bee is a generalist and exploits any flower sources available. Pollen and nectar collecting strategies for both honey bee races have been explored in attempts to exploit their pollinating capabilities. This project examines pollen collected by honey bees from feral and managed European and Africanized colonies. Two relatively isolated sites (USDA/APHIS (Animal and Plant Health Inspection Service) at Moore Air Base in Mission, TX and Rob and Bessie Welder Wildlife Foundation north of Sinton, TX) with twelve paired-colonies are used for obtaining samples. Through an acetolysis procedure (a series acid washings), the collected pollen from the individual foragers is fixed and observed with light and scanning electron microscopes for identifying pollens.

AN SEM STUDY OF BRISTLECONE PINE LITTER FROM 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 major grove of bristlecone pines, *Pinus longaeva*, can be found on the eastern flank of Wheeler Peak in The Great Basin National Park, Nevada. Part of the grove is located on a rocky lateral moraine which extends upward and Southeast forming a ridge. Near the top of the ridge the remains of WPN 114 (Currey, 1965), also called Prometheus, can be found between other living and dead bristlecone pines. WPN 114, is (was) the world's oldest known living tree (4800+ year's old). The tree was cut down in 1964 in order to obtain accurate data concerning its growth rate and age in an effort to establish a local climate chronology (Cohen, 1998). At present the stump and parts of the stem and roots can be found in a rocky area of the ridge at about 10,700 feet elevation. We visited the site and with permission of the park officials collected litter samples from areas of the grove including a collection less than one meter from the Prometheus stump (Fig 1).

The litter we collected on the ridge has components similar to that found in many coniferous forest. It consisted of needle leaves, needle fascicles, bud scales, small branches, cones and other plant parts (Fig. 2). In some areas the litter was completely unconsolidated, the component plant parts lying directly on course soil which has accumulated between the ubiquitous rocks making up the major substrate of the ridge. In other sites we found some consolidated litter which formed matts one to 2 cm in thickness. The matts were not extensive in size and also reside on course soil and are overlain by unconsolidated plant materials. Within the matts, plant parts could be identified but the most common element was various kinds of fungal hyphae. Bristlecone leaves can be easily identified in both matts and in the unconsolidated litter. Within matts they were often completely enshrined in a web of fungal hyphae which were also found penetrating into the interior of the leaves. Many of these fungal hyphae produced surface crystals which are believed to be calcium oxalate. The crystals varied from typical euhedral shapes to druse and even raphide-like shapes. Considerable variation occurs in the distribution and number of crystals found on the hyphae, some areas being completely covered by crystals.

Examination of small bristlecone stems found in the litter was interesting for several reasons. Like the main stem of many living and most dead bristlecone trees the bark and the vascular cambium are missing from most or all of the stems (Fig 2). However, even when the surface was eroded, the internal tissue structure appears to be very well preserved (Figs. 3-6). Fungi can be seen on the external surface but they were not found among the internal stem tissues as was true in leaves (Figs 3-6). Transverse sections of the branches showed some small radial cracks but most of the wood remains much as it would be in life. The pith consisting of large thick walled parenchyma cells also remains stable (Fig 3). Stems as small as three millimeters in diameter had several annual rings in which the early and late wood could easily be identified (Fig 4). Radial/longitudinal sections of the stems revealed tracheids with circular bordered pits characteristic of coniferous wood (Fig. 5). The pits were even seen at the very blunt ends of some tracheids (Fig 6).

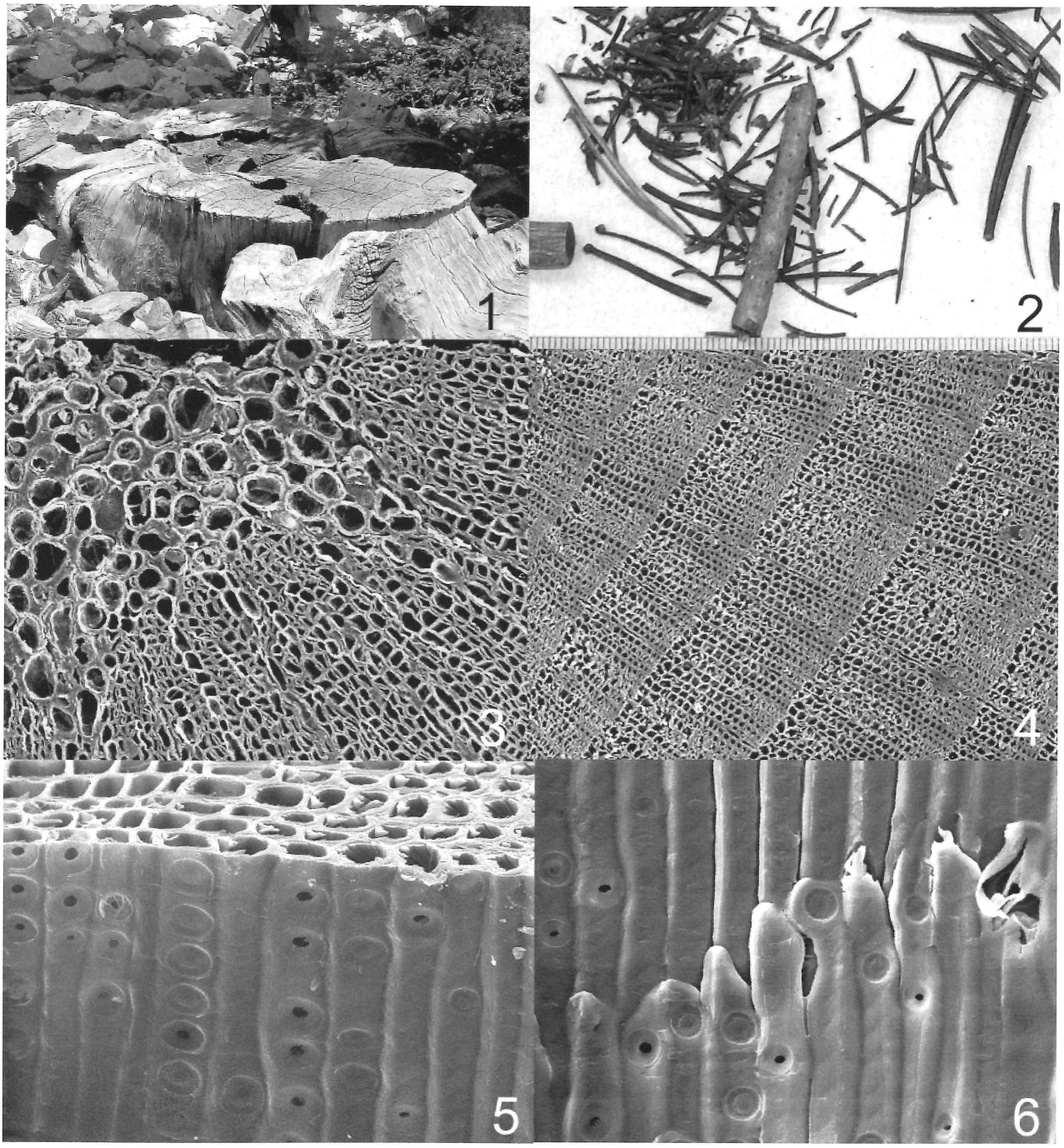


Figure 1. Litter collection site, showing the stump of WPN 114 (Prometheus) growing at approximately 10,700 feet in the Great Basin National Park, NV. Figure 2. Unconsolidated litter gathered a approximately 50 meters from the sight shown in Fig. 1. and consisting mostly of leaves, small branches, male cones, etc. Millimeter grid shown along bottom of figure. Figs. 3-6 Stem of *Pinus longaeva*. Figure 3. SEM micrograph showing a section of a small branch with a portion of the star-shaped pith and primary xylem. The pith cells (upper left) are large and thick walled. Magnification 60 X. Figure 4. SEM micrograph showing the secondary xylem of a small branch with six evident growth rings. Each annual ring shows both early and late wood in a well defined radial series which are usually continuous from one growth ring to the next. Magnification 47 X. Figure 5. SEM micrograph showing the intersection of a transverse and radial section of the early wood formed by thick walled tracheids. Tracheids possess numerous circular bordered pits as seen in the radial portion of the micrograph. Magnification 210 X. Figure 6. SEM micrograph showing a radial section of the early wood. Note the ends and overlay of several tracheids, one has a circular border pit near its termination. Magnification 225 X.

ANALYSIS ON DEPOSIT RESERVES OF WHEAT AND MUSTARD SEEDS DEVELOPED UNDER MICROGRAVITY ENVIRONMENT.
R. I. RUIZ AND A. KUANG, Dept. of Biology, The University of Texas- Pan American, Edinburg, TX 78539

Deposit reserves of *Brassica rapa* and *Triticum aestivum* seeds produced under microgravity environment on MIR space station have been studied. Mature and dried seeds harvested from MIR space station were fixed in 2.5% glutaraldehyde and 2% formaldehyde after being softened in distilled water. After dehydration, seeds were embedded in LR White resin and then sectioned. Sections were stained with periodic acid-Schiff's reagent and Aniline Blue Black for carbohydrate and protein, then observed and photographed with microscopes. Starch grains and protein bodies were counted and measured in the size and density. Data were analyzed by analysis of variance using SPSS software. Results show significant differences in protein bodies; however, no statistical differences in starch are observed between space seeds and the ground seeds. Supported by NASA grant NAG2-1020 and the UT-Pan American graduate program.

A LIGHT AND SCANNING ELECTRON MICROSCOPE STUDY OF BRISTLECONE (*PINUS LONGAEVA*) WOOD FROM SHEEP MOUNTAIN, INYO COUNTY, CALIFORNIA. HOWARD J. ARNOTT, The Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019.

The White Mountains of California have some of the most interesting stands of bristlecone pine (*Pinus longaeva*) in existence. Research workers have been studying this area for some time and have documented the presence of many trees over 4000 years of age. Likewise they have also demonstrated that bristlecone wood is stable for millennia after it dies and even when it has fallen. This wood has importance in developing the bristlecone chronology. In the summer of 2000 a number of individuals from the Laboratory of Tree-Ring Research collected materials in parts of the White Mountains. Through the generosity of Chris Baisan and Rex Adams I was fortunate to receive a slab of wood from the Sheep Mountain collections of 2000. It was designated SHP2000-CHB 05 and was about 10" x15" x1"in dimensions. It contained 334 annual rings which had been cross-dated from 432 B.C. to 766 B.C. Of considerable interest was the presence of a frost ring at 570 B.C. After careful documentation several pieces were cut from the main slab, sectioned, and viewed in the light microscope or sputter coated for viewing in the scanning microscope. The first sections examined were from B.C. 670 to 665. The most notable aspect of these rings was the thickness of the cell walls in both the early and late wood. Secondly, was the substantial development of horizontal resin ducts. The resin ducts were usually filled with resin. Vertical resin ducts were also filled, for the most part, with resin. In some areas of the wood the tracheids were also filled with resin, sometimes it was individual tracheids and in other places many contiguous tracheids were filled with resin. The resin contained in the lumen of tracheids often seem to have "substructure" consisting of clear areas (probably bubbles before sectioning) within the solid resin. Other areas of the Sheep Mountain wood also showed extremely thick cell walls which added to the dense characteristics and resinous character of this wood.

MICROSCOPY OF SEVERAL POPULATIONS OF *HYPOTHYCE MIXTA* HOWDEN FOUND IN TEXAS (COLEOPTERA: SCARABAEIDAE). KIMBERLY OSBORNE AND WILLIAM W. GIBSON, Department of Biology, Stephen F. Austin State University, Nacogdoches, TX 75961

Hypothyce mixta is a relict scarab found in isolated areas in Texas. *Hypothyce* is found in deep sandy areas with sparse vegetation. Adults are found between June and August primarily between the hours of 4:00 and 8:00 PM. The type specimen was collected in Anderson County, Texas. The allotype was collected in Nacogdoches County, Texas. Until recently very few populations of *Hypothyce* have been collected but now with many specimens from various geographically isolated populations there is a need for morphologic comparison of these populations to determine whether they are species, subspecies or only local variations. In this study, external morphology of several populations of *Hypothyce* was viewed using a dissecting microscope and a scanning electron microscope. Specimens were first observed under the dissecting scope for gross anatomical and color differences. After observation one or two specimens from each location were disarticulated and dehydrated using a graded alcohol series dehydration. Specimens were then critical point dried, sputter coated, and viewed with a Hitachi S405-A scanning electron microscope. Morphologic differences were quantified and will be used in further taxonomic work with this (these) species.

CHANGES IN FLORAL ANATOMY OF *NELUMBO NUCIFERA*, THE SACRED LOTUS, DURING THERMOREGULATION. C.L. SCHWARTZ AND H.J. ARNOTT, The Center for Electron Microscopy at the University of Texas at Arlington, Arlington, TX 76019.

Lotus, *Nelumbo nucifera* , is a water plant found throughout the southeastern United States. They reside in slow-moving waters and are found locally at The Fort Worth Nature Center. Lotus flowers are large, about 20cm in diameter when fully opened, and extend above water level on a long, thin peduncle. The flowers have 2-4 sepals, 10-20 petals, and 50-100 stamens that are arranged around a large, central receptacle. The conical receptacle or carpophore contains anywhere from 10-20 carpels, depending on subspecies. The stamens of lotus have three parts; a short filament, an anther about twice as long as the filament, and a staminal appendage that is densely packed with starch filled cells. Lotus is one of six species of plants, which are known to have thermoregulatory flowers (Patino, 2000). Lotus flowers can maintain a temperature of 30-36°C during a 2-4 day period when ambient temperature fluctuates from 10-45°C (Seymour & Schultze-Motel, 1998). The receptacle generates 54% of the heat during thermoregulation, while the petals and stamens generate about 23% each (Seymour & Schultz-Motel, 1998). The exact mechanism by which lotus generates heat is unknown. It is believed through calorimetric experiments that the energy comes from the breakdown of carbohydrates (Seymour & Schultze-Motel, 1998). There are three main stages of thermoregulation. Prior to thermoregulation, the flowers undergo normal floral development. The first stage of thermoregulation consists of "day 1" flowers. Day 1 flowers are cup-shaped and only open enough to allow insects to enter and pollinate the receptive stigmas. At this stage, the staminal appendages block any insects from reaching the pollen-filled anther sacs. At night, the flower closes and enters the second stage or "day 2" flowers. Day 2 flowers open to reveal the anther sacs that are releasing pollen. However, the stigmas are no longer receptive to pollen. This minimizes self-fertilization. At night, the flower closes (more loosely than the previous night), and enters the third stage or "day 3" flowers, during which, thermoregulation ceases. During day 3, the stamens dehisce, and along with the petals, fall off, leaving only the central receptacle. The receptacle enlarges and eventually sclerotizes, protecting the developing seeds embedded within.

A PRELIMINARY SCANNING ELECTRON MICROSCOPIC STUDY OF THE WOOD OF WPN 114 (PROMETHEUS), A 4800-YEAR OLD TREE OF *PINUS LONGAEVA*. HOWARD J. ARNOTT, The Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019.

Large stands of bristlecone pines (*Pinus longaeva*) are well known from various parts of the West, especially California and Nevada. A well-developed grove occurs just east of Wheeler Peak, Nevada. Perhaps the most famous tree in this grove is Prometheus (WPN 114) which until 1964 lived on the upper part of a rocky moraine just below Wheeler Peak at 10,700 feet. Late in the summer of 1964 this tree was cut down in order to obtain accurate information about its age and growth rate. Much of the tree remains at the site in Great Basin National Park, however, portions of it including well-documented slabs and partial sections have been dispersed. Through the much appreciated aid of Rex Adams and Tom Harlan and other members of the Laboratory for Tree-Ring Research, Univ. of Arizona, I was able to acquire some well documented and properly dated samples of Prometheus wood. In this study I will report on wood, in which the annual rings were produced between BC 1701 and BC 1677. In other words this wood is between 3677 and 3701 years of age, the annual rings having developed when Prometheus was between 1149 and 1173 years of age. After carefully recording the nature of the sample, sections of the wood were made, sputter coated the specimens and studied them in the SEM. In general the annual rings were similar in thickness with only modest variation. Six of the oldest annual rings had an average thickness of 506 μm, or approximately 0.5 mm. Other annual rings in this sequence seem to be similar in thickness, however, one very thin ring measured only about 50 μm, or about one tenth of the usual thickness and consisting of only three to four cells in total. The annual rings were also unusual in that there were very few latewood cells. In general only two to four cells preceded the cessation of growth in the fall where as 10-15 earlywood cells were found in many rings. Resin ducts are common but not closely spaced. Two or three layers of cells surrounding the resin ducts are often filled which what appears to be resin, however, the resin ducts themselves are usually empty.

MATERIALS SCIENCES
PLATFORM PRESENTATION—SPRING 2001

THE EFFECT OF SOLULTIONIZING TIME ON THE AGING BEHAVIOR OF 7075 ALUMINUM ALLOY. E. V. ESQUIVEL, Metallurgical and Materials Engineering Department, The University of Texas at El Paso, El Paso, TX 79968-0520

The aircraft and automotive industry have found extensive applications for age- hardenable aluminum alloys due to their high strength-to-weight ratios. Strengths for such alloys can be further enhanced if proper heat treatments are performed. The solutionizing effect on the aging behavior of 6061 and 2014 aluminum alloys has been previously studied at length. In both cases, it was reported that an increase in solutionizing time at an isothermal solution heat treatment caused a decrease in the time required to achieve peak hardness during aging. This same trend has been observed in the 7075 aluminum alloy system.

Keeping with the previous experimental techniques, 7075 aluminum samples were solutionized, quenched in ice water, aged and re-quenched in ice water. Optical microscopy was used to compare the grain size obtained at different solutionizing times while transmission electron microscopy was used to study the microstructure obtained after solutionizing and aging. Aging plots were obtained with the help of a Vickers microhardness tester. It was observed, as expected, that there was a decrease in the time required to achieve peak hardness with an increase in solutionizing time.

Research supported by the General Services Administration.

MICROSTRUCTURAL ANALYSIS OF THE IMPACT CRATERING PHENOMENA OF IRON AND STAINLESS STEEL TARGETS, E. TRILLO, A. A. BUJANDA, N.E. MARTINEZ, AND L. E. MURR, Metallurgical and Materials Engineering Department, The University of Texas at El Paso, TX, 79968

The frontier known as space is a region of vast possibilities. As we explore this region, we unknowingly leave behind remnants of our presence in the form of debris. In low earth orbit (LEO), there is at present billions of man-made space particles that may compromise any craft in orbit. Previous studies have shown that at velocities lower than hypervelocity (< 5 km/s) there exists a regime where the depth of penetration may be greater than at higher velocities. This study analyzes the effects of Fe and stainless steel penetrators impacting Fe and stainless steel targets over a range of velocities (0.45 – 3.85 km/s). Macrophotography, optical, and transmission electron microscopy reveal the unique crater formations and band-like structures that are present in both target materials. This is in contrast to the base materials that exhibit equiaxed grain structures and relatively low dislocation densities. SEM micrographs reveal the brittle-like nature of the penetrator as it breaks up inside the crater. Depth (p) to diameter (D_c) ratios were calculated and compared to other material systems at different impact velocities. This research was supported by NASA MURED Grand NAG-9-1171and NASA Grant NAG-9-100.

ATOMIC SCALE STRUCTURE-PROPERTY RELATIONSHIPS OF INTERFACES IN MULTICOMPONENT OXIDE THIN FILMS. S. STEMMER, Department of Mechanical Engineering and Materials Science, Rice University, Houston, TX 77005-1892, J.-P. MARIA AND A. I. KINGON, Department of Materials Science and Engineering, North Carolina State University, Raleigh, NC 27695, S. K. STREIFFER, Argonne National Lab, Materials Science Divison, and N.D. BROWNING, Physics Department, University of Illinois at Chicago

Interfaces in multicomponent oxide films for electronic applications play an active role in determining the performance of a device. Determining the two-dimensional atom column arrangement around the interface is often not sufficient to relate the interface structure to the electronic properties of the interface. Vacancies, segregation, and bonding changes at the interface are determining the electronic properties of the interface. It has been shown that a combination of electron energy-loss spectroscopy (EELS) and Z-contrast imaging at atomic resolution can provide this information. For these techniques a microscope capable of producing a probe size of atomic dimensions is required. We show that an instrument originally designed for conventional transmission electron microscopy has sufficient stability to perform these experiments with a spatial resolution of < 0.2 nm. We investigate the accommodation of excess titanium at grain boundaries of $(Ba,Sr)TiO_3$ thin films for memory applications. The observed microstructures and sub-nanometer scale bonding changes at interfaces and grain interiors are correlated with the dielectric and electric response of the films. We present investigation of novel gate dielectric stacks to reveal interdiffusion and reactions on a sub-nanometer scale that have a profound influence on the dielectric properties of these novel oxides.

NANOSTRUCTURED SEMICONDUCTOR MATERIALS FOR PHOTOELECTROCHEMICAL APPLICATIONS. C.L. SCHWARTZ[1], N.R. TACCONI[2], K. RAJESHWAR[2], H. J. ARNOTT[1], [1]Center for Electron Microscopy, [2]Department of Chemistry and Biochemistry, The University of Texas at Arlington, Arlington, TX 76019.

Nanostructured semiconductor materials are especially of interest in electrochemical devices such as solar cells, photoelectrochromic windows and displays, and nanosensors. This communication reports the preparation and SEM characterization of semiconductor nanostructures electrodeposited into two contrasting template structures – one based on ordered arrays of polystyrene spheres and the other based on alumina self-organized cylindrical holes. Polystyrene templates (PST) with two-dimensional periodical arrays are used to prepare cadmium selenide nanocrystals using a completely new strategy involving three steps: (1) PST are electrophoretically deposited on gold or ITO surfaces, (2) selenium dots are electrochemically grown in the PST void lattices, and (3) the Se dots are cathodically stripped as Se^{2-} in a Se(IV)-free electrolyte medium dosed with the requisite amount of Cd^{2+} ions. This new approach brings about high purity CdSe nanocrystals, and avoids Se admixed with CdSe. Alumina templates, with self-organized cylindrical holes, are used for the preparation of semiconductor nanorods with different lengths and diameters. Specifically, CuSCN semiconductor nanorods are fabricated atop of electrochemically deposited gold nanowires following a two step procedure: (1) electrodeposition of the required length of copper nanorods, and (2) copper electrooxidation in a SCN^- solution. Finally, co-functional template-assisted architectures of metal-semiconductor composites wherein the metal component is chosen to enhance the adsorption cross-section of a targeted substrate candidate will be also described. Such strategies provide an avenue for improving quantum yields in photocatalytic reactions.

INVESTIGATION OF MICROTEXTURES IN VOLCANIC ROCKS. M. HORN, Dept. of Geology, UT Arlington, Arlington, TX 76019

The crystallization kinetics of lava is a fundamental aspect of both petrology, the study of rocks, and volcanology. Central to the issue are the processes and controls of crystal growth from an aluminosilicate melt at atmospheric thermodynamic conditions. In this setting, rock crystallization kinetics is most strongly influenced by the chemical composition of the magma, the abundance of gaseous compounds, especially water, and the degree of undercooling. The effects of these factors are displayed as microtextural features in the final rock. The examination of the shapes, sizes, and orientations of mineral crystals and gas bubbles are essential to understanding the crystallization process. This study sets out to further interpret volcanic microtextural features based upon SEM and thin section (transmitted light) imagery.

CEMENTUM ANALYSIS USING TRANSMITTED POLARIZED MICROSCOPY TO DETERMINE ARCHAEOLOGICAL SEASONALITY. JOHN H. TAYLOR, 2201 Delcrest Drive, Austin, Texas 78704

Cementum analysis is a recent technique which assesses archaeological seasonality, essential in reconstructing economic prehistory. A tooth root tissue, cementum is comprised of increments which when examined using transmitted polarized microscopy reveal seasonality much as tree-rings reveal age. This study analyzes a sample of faunal specimens excavated at Konispol Cave in Albania, significant because its rare complete cultural sequence across the Mesolithic-Neolithic transition spans the Neolithic diffusion of agriculture from the Near East across the Balkan Peninsula into Europe. Traditional faunal analyses suggest an early spring site occupation, perhaps as a lambing den. Cementum analysis in this study suggests a seasonal site occupation centering on late summer and early fall. The combination of results from traditional seasonality attributions and those of cementum analysis represent a bimodal model for annual site occupation. The results suggest that transhumance, the practice of moving herds to richer highland summer resources and returning them to lowland shelter for the winter, may be an early feature of Balkan economic prehistory.

EDUCATION

PLATFORM PRESENTATION—FALL 2000

USING THE INTERNET TO ORGANIZE TEACHING MATERIALS. SANDRA L. WESTMORELAND AND HOWARD J. ARNOTT, The Department of Biology and The Center for Electron Microscopy, University of Texas at Arlington, Arlington, Texas 76019.

A website on the Internet can be used to organize and present teaching materials. This allows instructors to make available to their students items such as syllabi, worksheets, instructions, tutorials, and notes in a paperless manner. These materials can be added to or changed to keep them current and may become a body of materials to be used by other instructors teaching the same course. This enables instructors to provide consistency among sections of the same course and continuity from semester to semester while maintaining flexibility. On our Human Anatomy I Lab website we have five major categories of materials: Schedule/ Important Information, Student Teaching Information, Omission Sheets, Supplemental Worksheets, and Password-protected presentations. The Schedule contains the syllabus for the course with reading and workbook assignments and dates of scheduled exams. The Student Teaching Information includes the duties for undergraduate teaching assistants and a self-evaluation form. Omission Sheets inform the students specifically what parts of the laboratory manual to read and which parts may be omitted. In the Supplemental Worksheets section we provide items that are used as study aids. For example, a table is provided for students to make notes and drawings as they study the histology slides. Password-protected presentations include power point shows that can be used to present lecture concepts and are also available for students to review at will. We have found that the tutorial format is especially helpful for studying histology. Using a light microscope and a digital spot cameral attachment, micrographs were made of the histology slides that our students must identify. These images were placed in a tutorial providing, with successive mouse clicks, first the image, then a clue to its identity, and finally the tissue's name. The password protection feature enables the instructor to permit only his students to view certain materials rather than allowing universal access. The Internet website is a flexible tool that could be widely utilized by instructors to improve the presentation of instructional materials.

CORPORATE MEMBERS

Atomic Spectroscopy Instruments,
Graham R. Bird.
3451 County Rd. 409,
Taylor, TX 76574.
(512) 352-5340.

Barry Scientific, Inc., Margrit Barry.
P.O. Box 173, Fiskdale. MA 01518.
(800) 348-2257. FAX (508) 347-8280.

Denton Vacuum, Inc., John Crow.
Electron Microscopy & Research Systems.
1259 N. Church Street, Moorestown NJ 08057.
(856) 439-9100. FAX (856) 439-9111.

EDAX Inc., Curtis Gold.
1413 Hunter's Ridge Cr., Denton, TX 76205.
(940) 484-6656. FAX (940) 484-6756.

Emitech, John Fitzpatrick.
3845 FM 1960 West, Suite 345,
Houston TX 77068.
(713) 893-2067.

Electron Microscopy Sciences/Diatome,
Richard Rebert/Stacie Kirsch.
321 Morris Road, P.O. Box 251,
Fort Washington, PA 19034.
(800) 523-5874. FAX (215) 646-8931.
sgkcck@aol.com

FEI Company,
Jo L. Long. 1410 Gemini, Houston, TX 77058.
(281) 480-4015. FAX (281) 480-2708.
jo_long@pei.philips.com

Hamamatsu Photonic Systems,
Butch Moomaw.
360 Foothill Rd., Bridgewater, NJ 08807.

JEOL (U.S.A.), Inc., Bill Sousa.
11 Dearborn Rd., Peabody, MA 01960.

JEOL (U.S.A.), Inc., Richard Lois.
256 Green Cove Drive, Montgomery, TX 77356.
(409) 449-4141. FAX (409) 597-6200.
lois@jeol.com

LEICA, Inc., Michael Boykin.
310 9th Street NE, Atlanta, GA 30309.
(800) 248-0665. FAX (404) 577-9044.

Micro Star Technologies, Inc.,
CathyRyan.
511 FM 3179, Huntsville, TX 77340.
(936) 291-6891. FAX (936) 294-9861.
mistar@msn.com

NSA Hitachi,
3109 Skyway Circle North, Irving, TX 75038.

Oxford Instruments, Inc., Joyce Bosher.
130A Baker Ave. Extension, Concord, MA 01742-2204.

Oxford Instruments, Inc., Mike Crowley.
3536 Flora Vista Loop, Round Rock, TX 78681.

Physical Electronics, Mike Craig.
8753 Yates Drive, Suite 200,
Westminster, CO 80030.

Princeton Gamma-Tech. Inc., Bob Green.
458 Sherman Way, Decatur GA 30033.

RMC Inc., David A. Roberts/Steve Miller.
3450 S. Broadmont, Suite 100, Tucson, AZ 85713.
(520) 903-9366. FAX (520) 903-0132.
Steve.Miller@RMC-Scientific.com

Rontec USA, Jessica Wheeler.
20 Main Street, Acton, MA 01720.
(978) 266-2900. FAX (978) 929-9313.
wheeler@rontecusa.com

SCANNING/FAMS, Inc., Mary K. Sullivan.
P.O. Box 832, Mahwah, NJ 07430.
(201) 818-1010. FAX (201) 818-0086.
scanning@fams.org

Scientific Instrumentation Services,
Alexander E. Greene.
PMB-499, 1807 West Slaughter Lane, No. 200,
Austin, TX 78748-6200.
(512) 282-5507. FAX (512) 280-0720.
ablue@io.com

Vital Image Technology, Steve Rapp.
33811 Hanawalt Rd., Agua Dulce, CA 91350.

EDITORIAL POLICY

LETTERS TO THE EDITOR

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

MICROGRAPHS AND COVER PHOTOS

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

EMPLOYMENT OPPORTUNITIES

The JOB OPPORTUNITIES section will be comprised of a "Jobs Available" and a "Jobs Wanted" sub-section. Anonymity of individuals listing in the Jobs Wanted or Jobs Available sub-sections may be maintained by correspondence routed through the Editor's office.

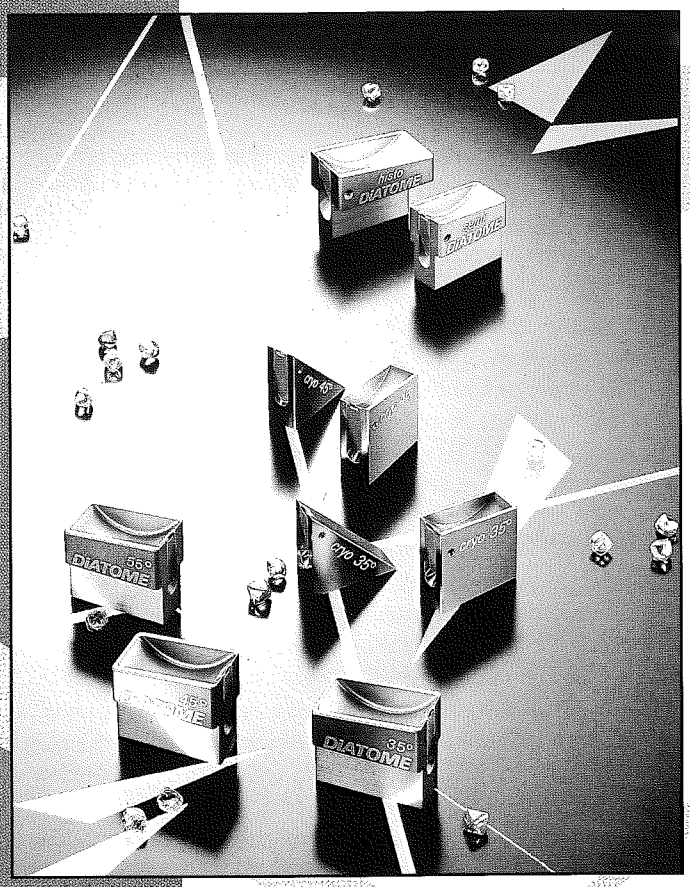
TECHNICAL SECTION

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

PUBLICATION PRIVILEGES

The right to publish Abstracts in the TEXAS JOURNAL OF MICROSCOPY is restricted to TSM members or to those whose membership is pending. A membership application form can usually be found in each issue of the TEXAS JOURNAL OF MICROSCOPY. Membership dues are as follows: student $2.00; regular members $15.00; Corporate members $75.00. Research articles are accepted from both members and non-members. Individuals who belong to TSM by virtue of a corporate membership are invited to participate in Journal submissions as are our regular or student members. However, papers of a commercial nature, either stated or implied, will not be accepted for publication as a Research Report or Techniques Paper. Such papers may be acceptable as advertising copy.

ADVERTISER'S INDEX



For The Performance You Expect:

The DiATOME resharpening service.

When Diatome resharpens a Diatome Knife, we restore it to its original condition. **That is our Guarantee!** Your resharpened Diatome Knife will have the same length, the identical cutting edge and carry the same guarantee of quality as the day it first left our factory.

Only Diatome can make this claim!

No other company can successfully resharpen a Diatome Diamond Knife. We have found that when other companies try to resharpen our knives, the original parameters of our knives are either altered or totally lost (the diamond cutting edge is shorter or in some cases our diamond has been removed and replaced with a diamond of inferior quality and shorter service life). Hence, returning to you an inferior knife that does not perform as the original.

The Diatome Diamond Knife is also guaranteed for an **unlimited** number of resharpenings.

Each Diatome Diamond Knife, whether new or resharpened, is subjected to extensive testing for its ability to cut accurately without scoring or compression. Only if its performance passes our tests will we ship it to you.

This too is guaranteed!

Diatome is committed to customer satisfaction. Therefore, in the unlikely event that you experience any difficulties, or for any reason you are unhappy with the performance of your knife, please contact us immediately. You can be sure that any problem with your knife will be corrected.

We guarantee it!

We stand by our commitment to quality and customer satisfaction.

For Quality
For Accuracy
For Satisfaction
Forever

DiATOME U.S.

Call or write for our complete set of literature today.

P.O. Box 125, Fort Washington, PA 19034
(215) 646-1478 • (800) 523-5874

Information for Authors

GENERAL INFORMATION

PURPOSE: The goal of the TSM Journal is to inform members of the society and the Journal's readers of significant advances in microscopy, research, education, and technology. Original articles on any aspect of microscopy are invited for publication. Guidelines for submission of articles are given below. The views expressed in the articles, editorials and letters represent the opinions of the author(s) and do not reflect the official policy of the institution with which the author is affiliated or the Texas Society for Microscopy. Acceptance by this Journal of advertisements for products or services does not imply endorsement. Manuscripts and related correspondence should be addressed to David C. Garrett, Editor, TEXAS JOURNAL OF MICROSCOPY, Department of Biological Sciences, University of North Texas, Denton, Texas 76203-5218.

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:

FORMAT: Submit an original and two copies of the entire manuscript, typed, double-spaced, on 8 ½ x 11 white paper, leaving ample margins. Number each page and identify the article by placing, at the top left of the page, a shortened form of the title, followed by the last name of the first author.

TITLE PAGE: Include:

a. Full title of the article
b. Initials and last names of all authors
c. Current positions of each author (department, institu tion, city)
d. Full name, telephone number and address of the author to whom reprint requests are to be sent.

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.)

TABLES:

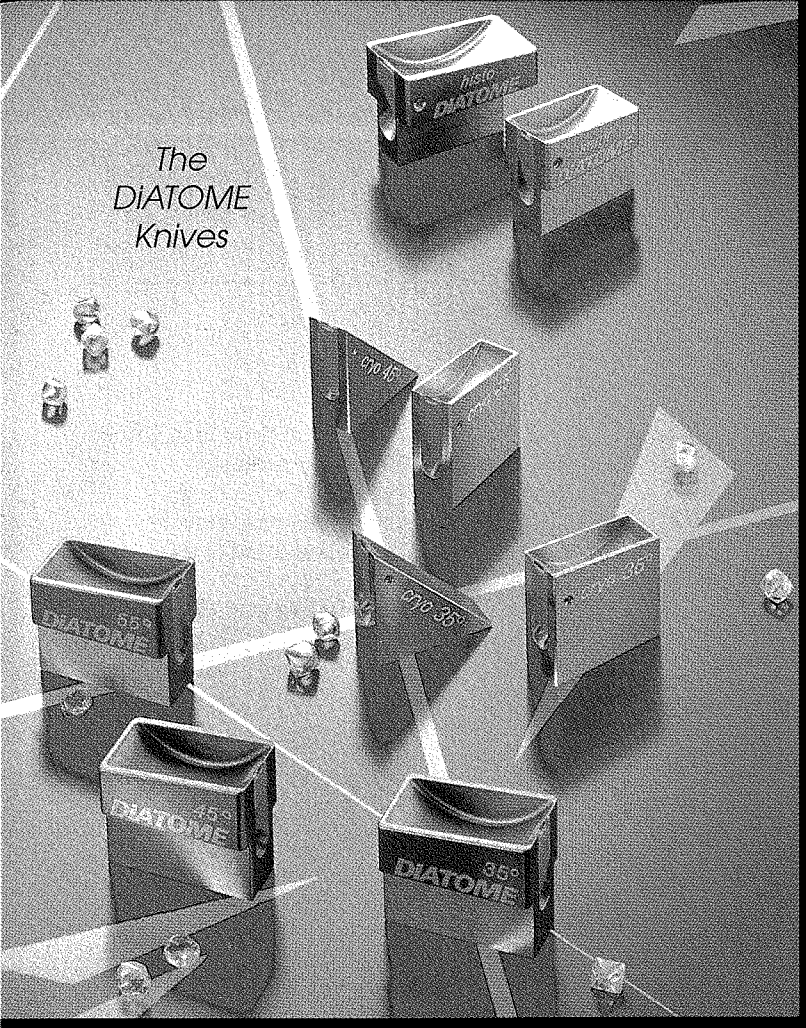
a. Type double-spaced each table on a separate sheet.
b. Number in order in which they are referred to in the text.

ILLUSTRATIONS:

A. Submit three complete sets of illustrations. Copy machine reproductions of photographs will not be accepted. Indicate which set is the original photograph or illustration.
B. Number the figures in the order in which they are referred to in the text.
C. For black and white illustrations, submit sharply focused, glossy prints, or line drawings, 1.5 times larger than they are to appear in print (¼ or ½ page). Scale should be drawn on the photograph itself, not below.
D. For color illustrations, if needed, submit positive 35-mm color transparencies (not prints) for the original (prints may be used for the two copies). Authors will bear the entire cost of color reproductions.
E. Identify all illustrations (author, title of paper, and number) by a gummed label on the back of each. Do not mount the illustrations, write on the back of them, clip them, or staple them.
F. Illustrations taken form other publications require reprint permission and must be submitted in the form described above.

NOMENCLATURE AND ABBREVIATIONS: Journal abbreviations used should be those listed by the "Index Medicus." Nomenclature abbreviations should be similarly standardized.

ACKNOWLEDGEMENTS should appear as a footnote which will appear at the top of the first page of the article.



The DIATOME Knives

DIATOME U.S.

diamond knives, accessories, and services.

custom knives and services

1 OUR resharpening service of DIATOME knives guarantees that your resharpened DIATOME knife will be restored to its high quality and original condition (same length and identical cutting edge). DIATOME knives are guaranteed for *unlimited* resharpenings.

2 OUR diamond knife exchange program* allows you to trade in most competitors knives and in return receive a new DIATOME knife (any size) at the resharpening cost. **limited time offer*

3 OUR standard boats are blue but black boats are now in stock and available upon request.

4 OUR custom diamond knives and tools are available for those applications where the standard knives are not applicable.

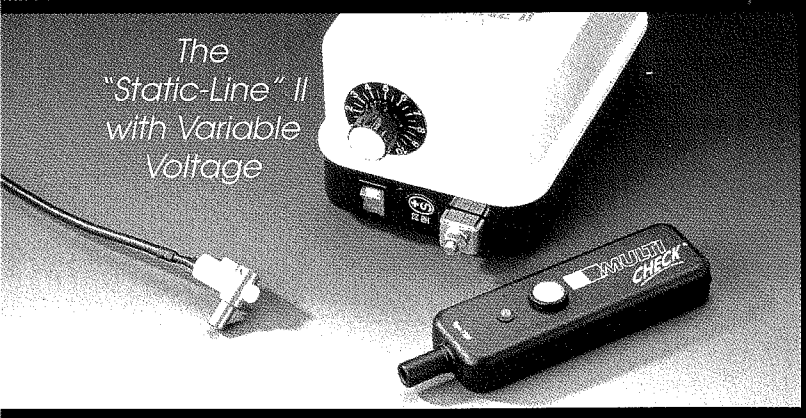
5 OUR free sample sectioning and evaluation program is available to customers either experiencing a problem sectioning a particular specimen or need advice on a knife for a specific application.

6 OUR guarantee on our new and resharpened knives ensures your complete satisfaction. If you are experiencing difficulties with any of our knives, please contact us.

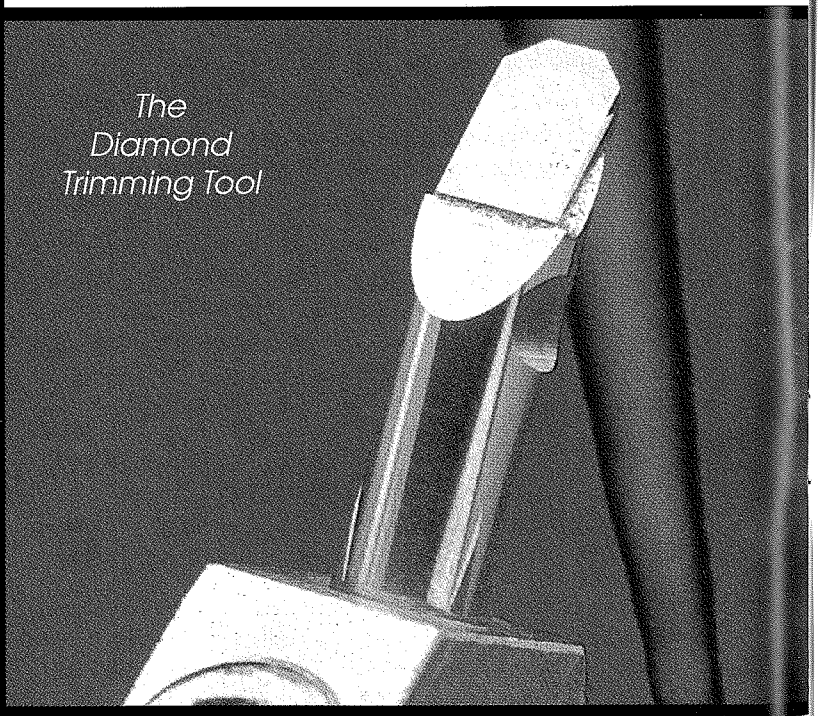
products

Whether your needs are Biological or Materials related, in E.M or L.M., at ambient or low temperatures, DIATOME has the answer. With three different knife angles (35°, 45°, 55°) and six different types of knives (ultra-thin, semi-thin, cryo-wet, cryo-dry, histo and histo-cryo) covering the entire microscopy spectrum.

Included in our line is our diamond trimming tool for ambient or cryo temperatures as well as our updated "Static-Line" II with variable voltage.



The "Static-Line" II with Variable Voltage



The Diamond Trimming Tool

delivery time

We now keep an extensive inventory of new knives for immediate delivery. However, if your knife is not in stock we can ship within 3 weeks.

Resharpened knives will be returned 4 weeks after receipt from customer.

For more information on any of our products or services please call or write us today.

DIATOME U.S.
321 Morris Road • P.O. Box 125 • Fort Washington, PA 19034
(215) 646-1478 • Fax (215) 646-8931

APPLICATION FOR MEMBERSHIP OR CHANGE OF ADDRESS TEXAS SOCIETY FOR MICROSCOPY, INC.

Date _____

Please type or print legibly. Fill out completely. The numbers in parentheses are the maximum number of characters and spaces the computer can accommodate for that blank. Though we will mail to your home address, we prefer to have your work address. Please note that membership is for Jan. - Dec. for each year.

Check One: ☐ I am applying for new membership in T.S.M.

☐ I am a member and wish to change my address.

☐ I am a STUDENT and wish to upgrade to REGULAR membership.

Are you a member of MSA? ☐ Yes ☐ No

Name (last name first) _____ (35)

Institution _____ (35)
(Please write out completely. We'll abbreviate it.)

Department _____ (35)
(Please write out completely. We'll abbreviate it.)

Street & Number / P.O. Box _____ (35)

City _____ (20) State _____ (2) Zip _____ (10)

Work Phone (_____) _____ (13) Extension _____ (4)

Electronic Mail (_____) _____ (40)

Home Phone (_____) _____ (13) FAX No. (_____) _____ (13)

Category of Membership (circle only one): **Regular** **Corporate** **Honorary** **Library**

Student: _____ Degree Program _____ Signature of faculty sponsor

Broad field of interest in which you utilize Microscopy (Circle only one):

Zoology	**Botany**	**Microbiology**	**Cell Biology**	**Biochemistry**
Medicine	**Vet. Medicine**	**Chemistry**	**Sales**	**Service/Repair**
Materials	**Petroleum**	**Semiconductor**	**Environment**	**Minerals**

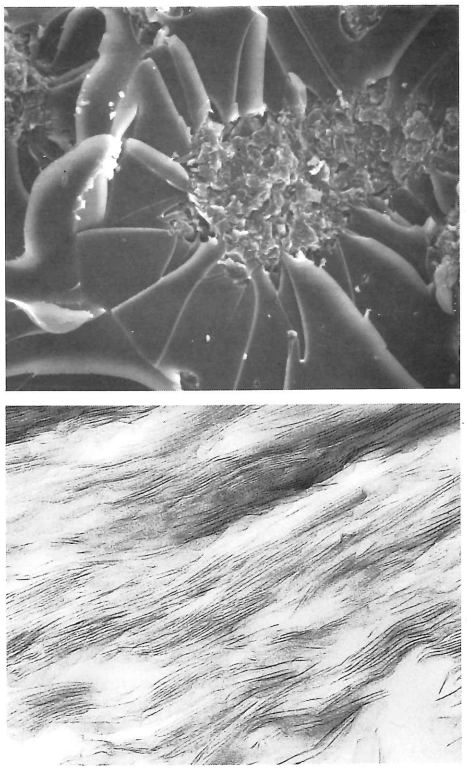
If you are a member changing your address, please attach an old mailing label to help us identify your previous record in the computer. Applicants for membership should include a check or money order for one year's dues with application (Regular: $15.00; Student: $2.00; Corporate: $75.00).

Applications for new membership, or for upgrading of membership category from STUDENT to REGULAR, will be presented to the Executive Council at their next meeting for their approval (majority vote). The applicants will then be presented by the council to the membership at the next general business meeting for their approval (majority vote). Applicants will be added to the membership rolls at that time.

Please Return To: Sandra Westmoreland
Texas Society for Microscopy
University of Texas at Arlington
P.O. Box 19498
Arlington, Texas 76019

ANSWER TO "WHAT IS IT"

from Texas Journal of Microscopy 31:2



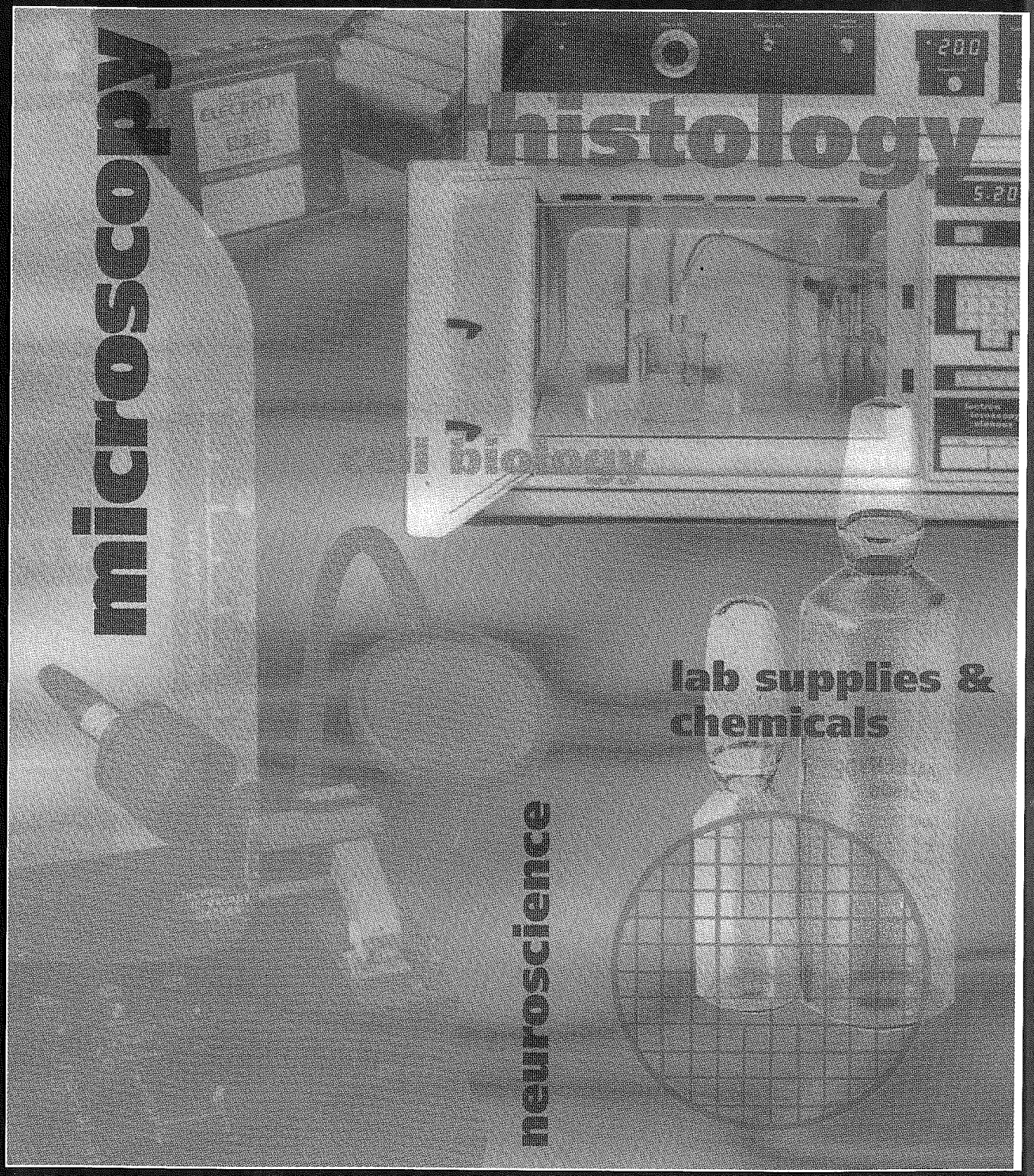
The pictures on the back cover of Volume 31, Number 2 represent micrographs of SEM (top) and TEM (bottom) analyses of epoxy + montmorillonite nanocomposites. Polymer nanocomposites are a recent development arising from the introduction of reinforcements having a nanometer size in at least one dimension. Current thinking on the dispersion of the montmorillonite platelets in polymers is based on TEM images as shown on the right indicating platelets dispersed throughout the polymer matrix. However, SEM images reveal that these dispersions are actually aggregates. Alkyl ammonium treated montmorillonite layers are spaced periodically with a 2-nm spacing. As the TEM indicates, the individual platelets swelled from 2 nm to a separation of 3-6 nm. Further, the interaction between the platelets and montmorillonite was large enough to disrupt the directional c stacking so individual platelets were well distributed. SEM images were taken from tensile specimen crossections and were coated with Au/Pd prior to observing under SEM. Sections were obtained with a diamond knife for TEM.

Meeting Memories

Camelia Maier, the journal editor, presenting a poster at the Fall Meeting 1997 in Forth Worth while her son was . . . passing by.

Group photo at the Fall Meeting 1997, Holiday Inn Hotel in Fort Worth. From left: David Roberson, Camelia Maier's husband with son George; Jonnell Beaird, former Secretary; Don Smith, President 2000-2001 and his wife Nelia; and Rumpa, wife of Nabarun Ghosh, treasurer Elect, 2000-2001.

Building a Solid Foundation of Commitment in the Scientific Community.

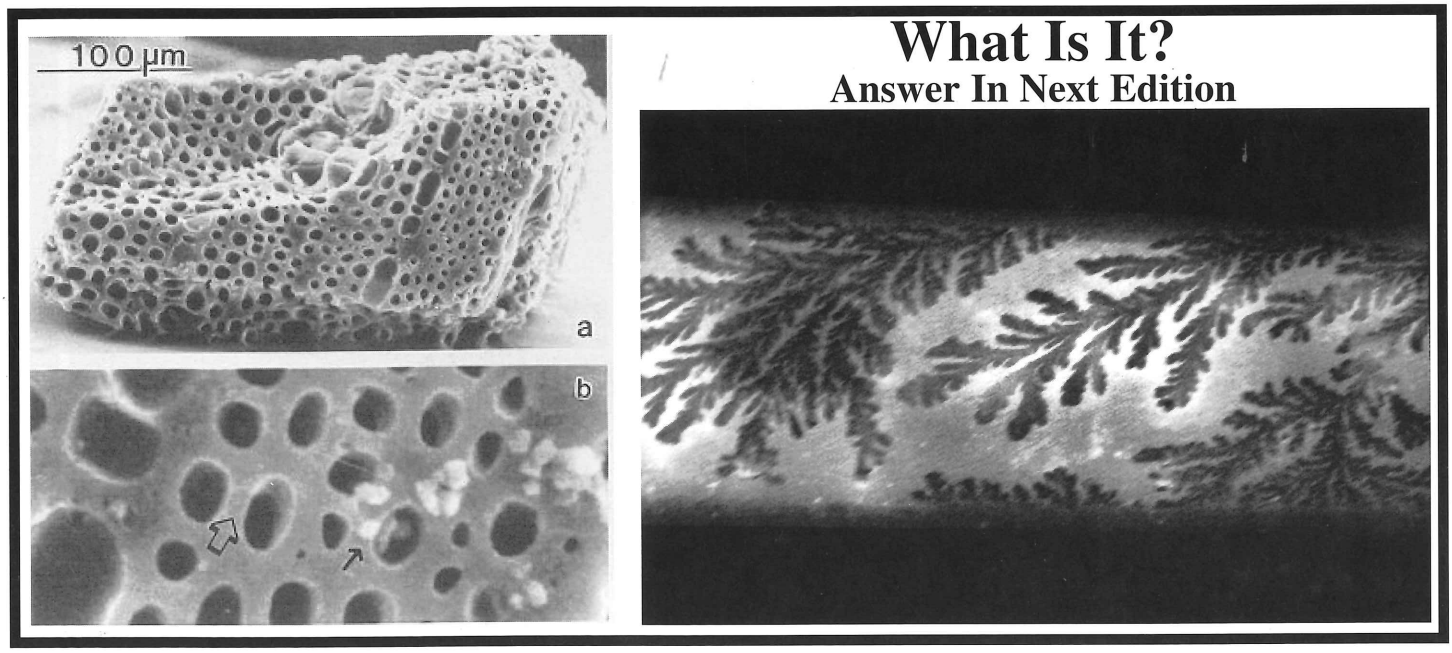


Catalog XII is the answer to every Researchers' (Biological and Materials Science) and Clinicians' needs. We offer a complete line of chemicals, supplies, accessories and equipment for microscopy and histology, as well as general laboratory and biological studies. We now have something for everyone working in a laboratory setting.

Electron Microscopy Sciences

For a copy of our new Catalog XII, please call or write today. • 321 Morris Road • Box 251 • Fort Washington, PA 19034
Toll-free: 1-800-523-5874 • (215) 646-1566 • Fax: (215) 646-8931

Texas Society for Microscopy
University of Texas at Arlington
Department of Biology
Post Office Box 19498
Arlington, Texas 76019
(817) 272-5578



Micrographs on left by Joe B. Dixon; Department of Soli & Crop Sciences, Texas A&M University, College Station, TX 77843.
Micrograph on right by Timothy L. Henry; Department of Biology, University of Texas at Arlington, Arlington, TX 76019.