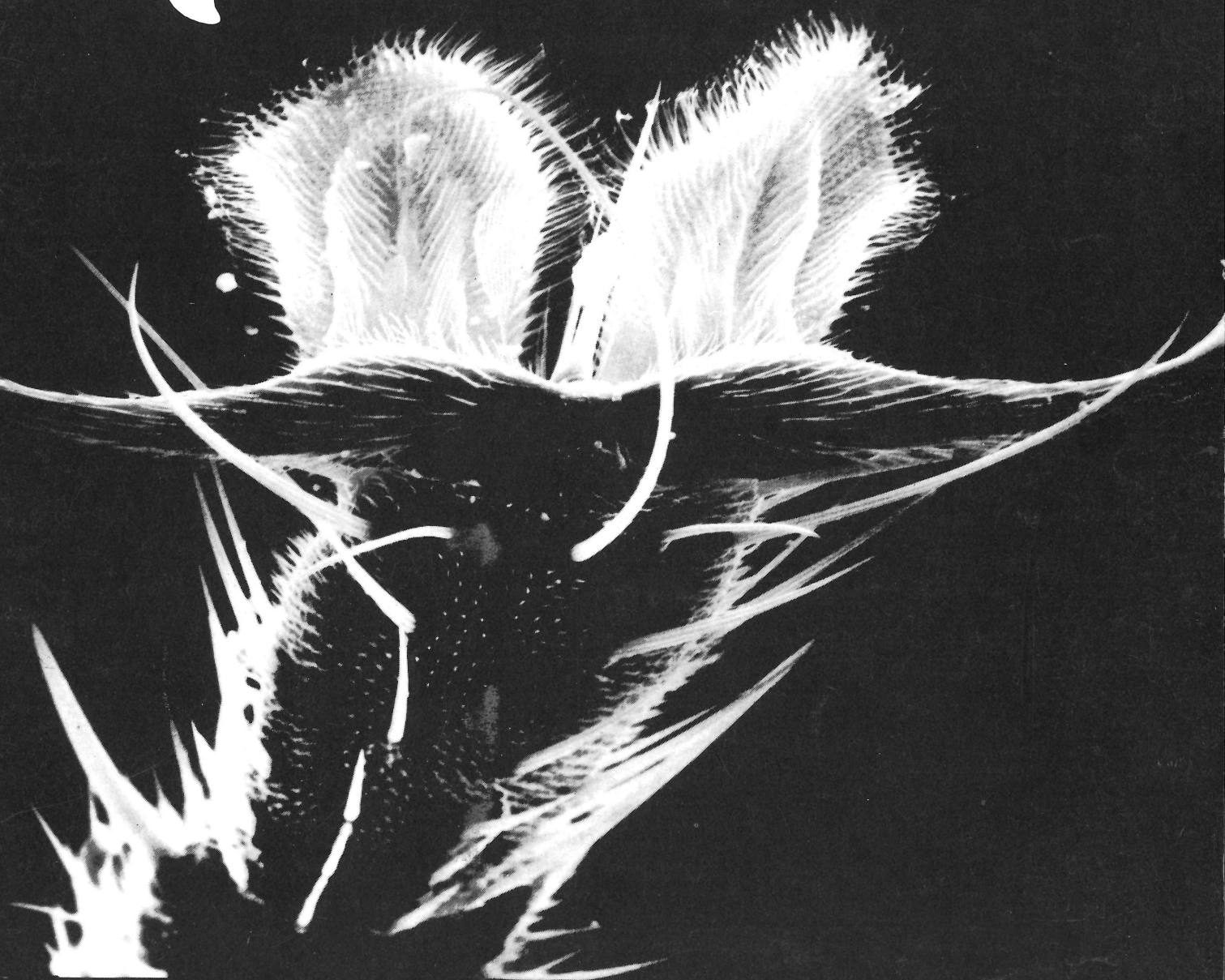




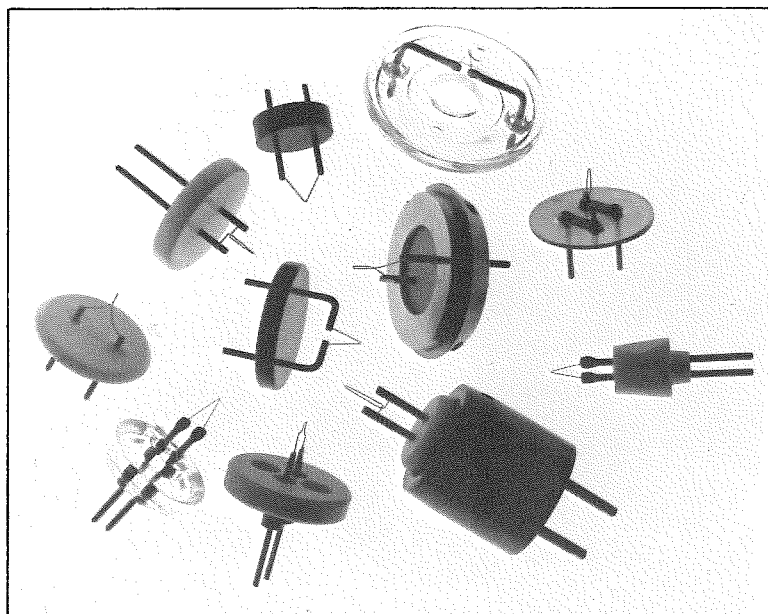
Texas Society for Electron Microscopy

JOURNAL
VOLUME 15, NUMBER 3, 1984



SPECIAL FALL MEETING ISSUE
Abstracts Begin on Page 30

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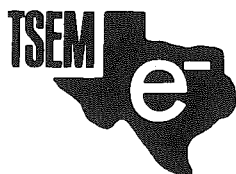
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**TEXAS SOCIETY FOR ELECTRON MICROSCOPY
JOURNAL
VOLUME 15, NUMBER 3, 1984
ISSN 0196-5662**

Randy Moore, Editor

Department of Biology, Baylor University, Waco, Texas 76798

Texas Society for Electron Microscopy

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

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ON THE COVER

A scanning electron micrograph of the pretarsa (foot) and distal tarsomere (lower leg) of *Musca domestica*, the common housefly. 400X. See corresponding story on page 16.

Submitted by Ronald W. Davis, Department of Anatomy, Texas A&M University, College of Medicine, College Station, Texas 77843-1114.

President's Message

With a little luck this issue of TSEMJ will be ready for distribution at our Fall 1984 meeting to be held at the Rodeway Inn in Arlington. This should be a good meeting with special lectures by Beth Gantt of the Smithsonian Institute and Richard Anderson of the UTHSC at Dallas and sessions of contributed papers in both the biological/biomedical and material sciences fields. Howard Arnott was responsible for local arrangements and deserves a big "thank you" from all of us for his work. TSEM is, of course, most fortunate to have people like Howard who are willing to take care of all the behind the scenes details involved with our meetings. Without these people TSEM would be in trouble.

Speaking of meetings, please mark April 11-13, 1985 on your calendar. These are the dates for our Twentieth Anniversary Meeting scheduled at the Menger Hotel in San Antonio. This is shaping up to be a super event that you won't want to miss. Thus far the program includes a special lecture by Keith Porter entitled "Forty years of electron microscopy; a retrospective view" and two special lectures by former TSEM presidents Bill Brinkley and Lea Rudee. Special social events are also being planned including a banquet hosted by none other than our own Jerry Berlin.

Local arrangements are being handled by Bob Blystone (512-736-7231) so if you have suggestions or just want to help with the meeting please contact Bob right away.

At this time I really have little out of the ordinary to report about TSEM. Financially we're in good shape and our membership appears to be holding its own. There's nothing wrong with our Journal that a few more manuscripts won't cure. Our corporate members continue to be faithful and supportive of our efforts. Overall it therefore appears that TSEM is in pretty good shape.

Personally, however, I'm not satisfied with us being in "pretty good shape" and I hope that you're not either. Let's get together and work to make this the best year we've ever had. You can help by attending our meetings, contributing manuscripts and other materials to our Journal, recruiting new members for our Society and sharing your thoughts and ideas about TSEM with the officers.

Sincerely,

Charles W. Mims
President

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 TSEM 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 TSEM, the Journal and its contents, academic or national policies as they apply to TSEM and/or its members and electron 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.

ELECTRON 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 technique and scientific information content.

REGIONAL NEWS

News items should be submitted through the regional editor in your area and made to conform to the standard format used by the regional news section. Regional contributions should be sent to the Regional News Editor. Editorial privilege may be executed for the sake of brevity or to preserve the philosophical nature of the TSEM Journal.

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 Regional News Editor's office.

TECHNICAL SECTION

The Technical Section will publish TECHNIQUES PAPERS, HELPFUL HINTS, and JOB OPPORTUNITIES. The TECHNIQUES 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 in the TSEMJ is restricted to TSEM members or to those whose membership is pending. A membership application form can usually be found in each issue of the TSEMJ. Membership dues are as follows: students \$2.00; regular members \$10.00; Corporate members \$75.00. Individuals who belong to TSEM 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.

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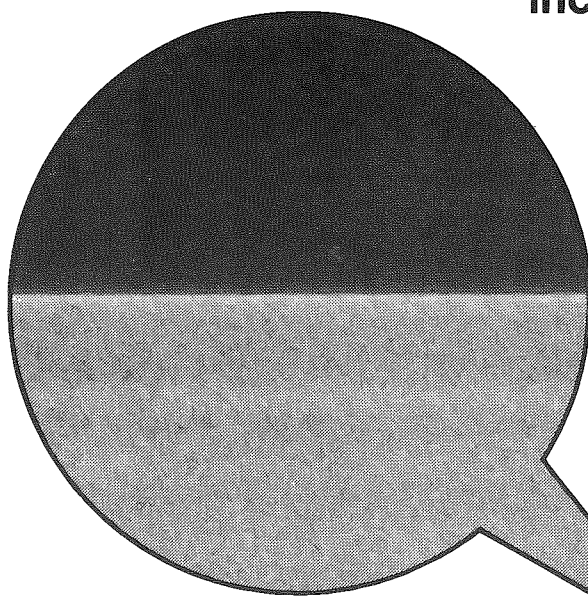
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MINUTES OF THE BUSINESS MEETING

The business meeting of the Texas Society for Electron Microscopy was called to order in room 301 of Rudder Tower on the Texas A & M University campus at 3:55 P.M. April 27, 1984.

President Charles Mims introduced Program Chairman Ernest Couch, who thanked local arrangements chairmen Hilton Mollenhauer and Wayne Sampson, and also Robert Burghart, Dina Gaddy, Rebecca Mathison and Ron Davis for their part in the success of the meeting.

The minutes were read and approved.

The Secretary announced results of the election: Hilton Mollenhauer is President-Elect, Wayne Sampson is Program Chairman-Elect, and Wayne Fagerberg was elected Secretary.

The Treasurer's Report was given and approved. Randy Moore also reported the status of TSEM Journal. Advertising has improved, but more papers need to be submitted by members for continued success of the Journal.

President Mims told the membership that he has agreed to the Executive Council's request that he serve as President a second year, because of the resignation of President-Elect Allen Shannon.

The Fall meeting of TSEM will be held in Arlington, Texas, October 24-26, at the Rodeway Inn on IH 30 near Six Flags Amusement Park. The program is being planned to attract participation of members interested in materials sciences as well as those interested in biology.

The Spring meeting, which will include events to celebrate the 20th anniversary of TSEM, is to be held in San Antonio on April 11-13, 1985.

The meeting was adjourned at 4:15 p.m.

Respectfully submitted,

Elizabeth Root
Secretary

JOB OPPORTUNITY

Electron Microscopy Technician, to study the synaptic connections of neurons in the retina of the eye using electron microscopic immunocytochemistry and autoradiography. At least two years experience, preferably in the nervous system, are required. Experience with the techniques mentioned above would be helpful. Salary is at least \$19,200, depending on experience in electron microscopy. For more information contact David Marshak, Dept. of Neurobiology and Anatomy, The University of Texas Health Science Center, P.O. Box 20708, Houston, Texas 77225, Phone (713) 792-5700.

DISCOUNT JOURNAL SUBSCRIPTIONS

There has been some confusion on just how to subscribe to journals that offer discounts to EMSA members. In order to qualify for the discount, your order MUST be processed through the EMSA Treasurer, with checks payable to EMSA. The current list of journals that offer discounts, and the EMSA member's prices, are as follows:

<i>Bulletin Signaletique</i>	\$ 72
<i>Journal of Microscopy</i>	100
<i>Micron</i>	35
<i>Ultramicroscopy</i>	60

SHORT COURSES AND WORKSHOPS

MONITORING & MAINTAINING THE ELECTRON MICROSCOPE

December 10-14, 1984

Warwick University, England

For further information contact the Administrator, Royal Microscopical Society, 37/38 St. Clements, Oxford OX4 1AJ, phone (0865) 248768/721081

ADVANCED ELECTRON IMAGING TECHNIQUES FOR BIOLOGISTS

December 17-21, 1984

National Institute for Medical Research, London, England

For further information contact the Administrator, Royal Microscopical Society, 37/38 St. Clements, Oxford OX4 1AJ, phone (0865) 248768/721081

UPCOMING MEETINGS

CENTENNIAL SYMPOSIUM ON HIGH RESOLUTION ELECTRON MICROSCOPY

January 7-11, 1985

Arizona State University, Tempe, AZ

For further information contact: Dr. P. R. Buseck, Department of Geology, Arizona State University, Temple, AZ 85287.

20TH ANNIVERSARY MEETING OF THE TSEM

April 11-13, 1985

San Antonio, Texas

(See announcement on page 51)

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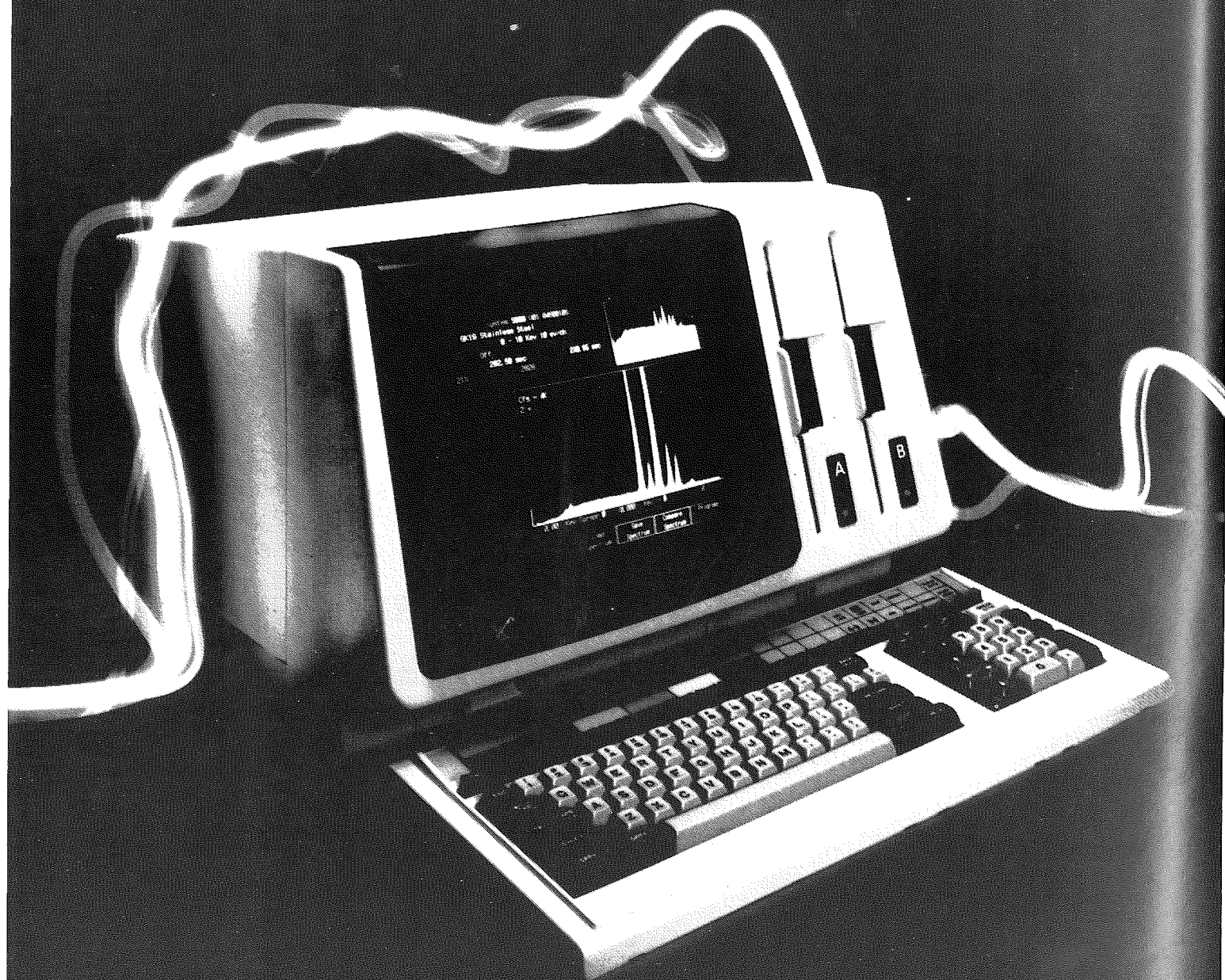
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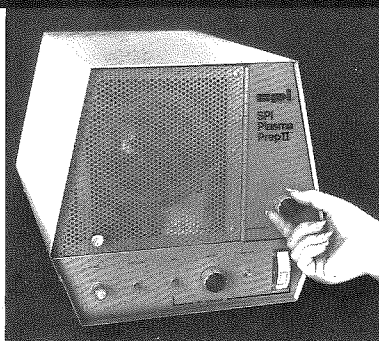
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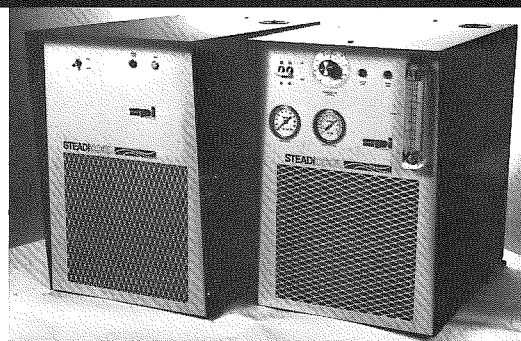
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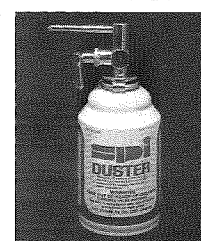
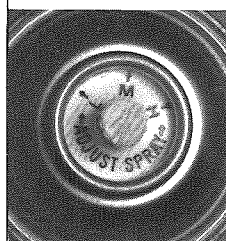
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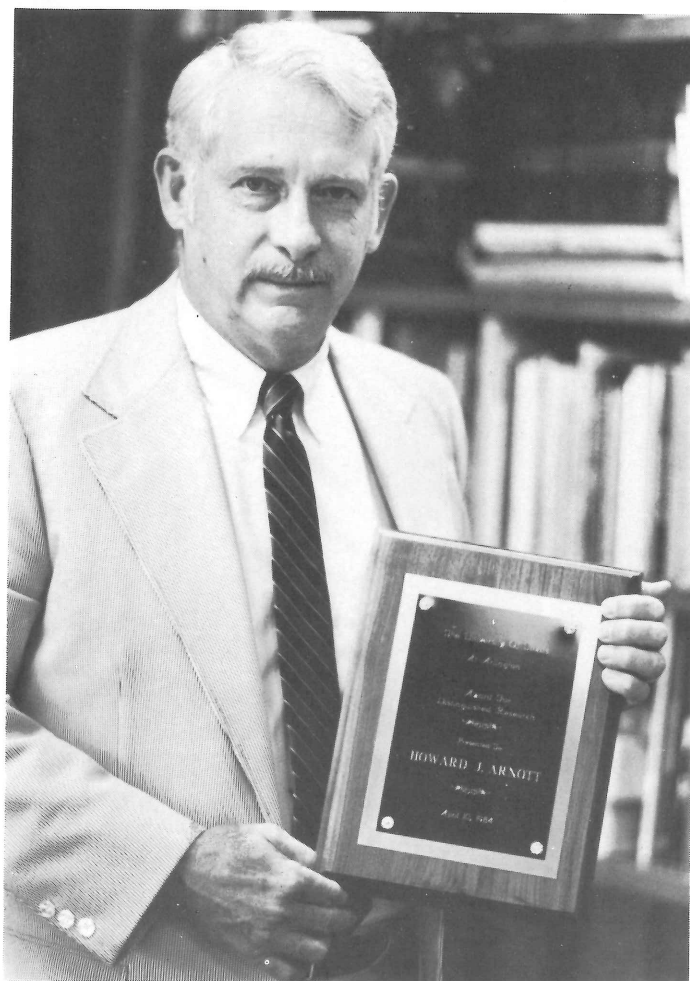
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TSEM SPOTLIGHT



Howard J. Arnott

Ph.D. in Botany, University of California, Berkeley, 1958
Dean of Science and Professor of Biology at UTA, 1974 to present.

Major professor for 12 Ph.D. students, 11 masters, 11 postdoctoral students;

Publications: 213 citations (of which about half are published abstracts)

Professional lectures: 184 (some presented by coauthors)

Numerous micrographs and illustrations have been published in books and chapters of others.

My first electron microscopy training began in 1962 at the Dental School of Northwestern where I worked with J.D. Hampton and Ben Rosario. In 1964 I took an NIH postdoctoral position with W.G. Whaley in the Cell Research Institute at The University of Texas at Austin. There, in addition to Whaley, I had many "instructors", including H. Mollenhauer, H. Threm, and M. Dauwalder.

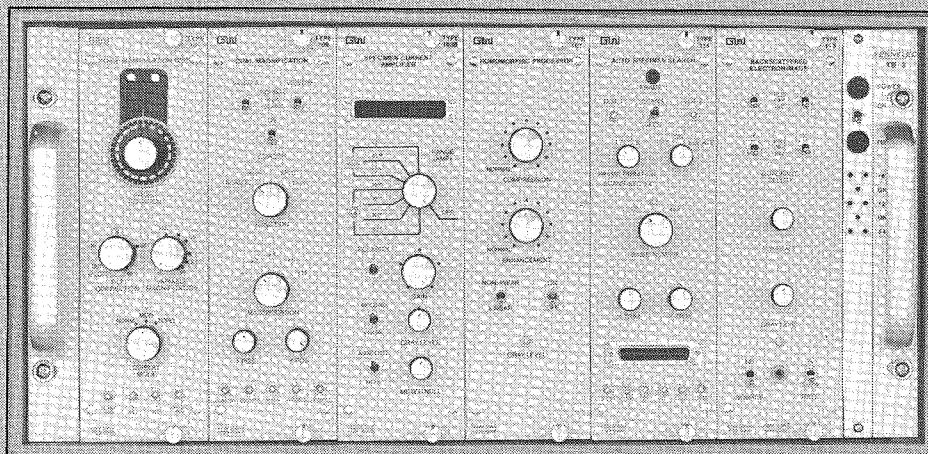
At the present time, in addition to my deanship, I supervise the UTA EM Laboratory which has a transmission and two scanning microscopes; our JEOL JSM-35C with a Tracor/Northern 2000 X-ray analyzer is the mainstay of the laboratory. In addition to administration and research, I

teach General Botany, Cell Biology, and an SEM techniques course, but usually not all at once. While my research is mainly botanical, I have published on the ultrastructure of rats, fish, birds, plant and insect viruses, bacteria, algae, and protozoa. I have also published a few techniques and teaching papers.

My research specialty has centered on crystals in biological systems. With several students I have studied calcium oxalate in many tissues of seed plants and fungi. I also studied protein crystals associated with insect viruses, protein crystals in plant cells, and quinine crystals in the eyes and skin of fishes. At present we are continuing our studies of calcium oxalate crystals in several plant systems. In the past I have also studied plant viruses, eye reflection by noncrystalline systems in fish and birds, and the ultrastructure of seeds and seed germination. In recent years we have also done work on food microstructure, recently completing a study of potato chips. In April 1984, I was honored by receiving the University of Texas at Arlington award for distinguished and sustained efforts in research which consisted of a cash prize and an award plaque (see picture).

As a hobby, I build clocks (grandfather and shelf) and enjoy woodworking. Unfortunately, not all my clocks run well, but they **all look good!**

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TSEM SPOTLIGHT



Charles W. Mims

Charles W. Mims received a B.S. degree in botany from McNeese State University in Lake Charles, Louisiana, in 1966. He did his graduate work in the Department of Botany at the University of Texas at Austin, completing a Ph.D. degree with an emphasis in mycology in 1969. He then joined the faculty at Stephen F. Austin State University in Nacogdoches, Texas, where he is currently Professor and Chairman of the Department of Biology.

Most of Charles' research has concentrated on various aspects of fungal ultrastructure. He is particularly interested in the Myxomycetes, or true plasmodial slime molds, as well as certain plant pathogenic fungi. He is currently involved in a two year, NSF supported, ultrastructural study of members of the basidiomycetous genus *Exobasidium*. To date he has authored or co-authored 30 papers and over 30 abstracts. He is also co-author of the Third Edition of *Introductory Mycology* published by John Wiley and Sons, Inc.

Our current President is active in a number of

professional organizations including Sigma Xi, the Mycological Society of America (MSA) and TSEM. He has served as both Treasurer and President of his local Sigma Xi Club and as Councilor of MSA during 1976-78. He has also served on numerous MSA committees and was the recipient of the 1979 MSA Alexopoulos Prize for Research. Prior to his election as President of TSEM, he was Local Arrangements Person for one of our meetings and Program Chairman for 1979-80.

Charles is married to the former Sandy Davis of Lake Charles, Louisiana. The other members of the Mims' family include of David (13), Shelley (11), and Julie (8) as well as three dogs. Aside from professional activities, most of Charles' time is divided between family activities, sleeping, coaching boys baseball and fishing. It is worth noting that our President is a highly skilled fisherman and has actually had a fish named after him. A "Mims Bass" is any black bass less than 8" in length that has been caught on an artificial lure!

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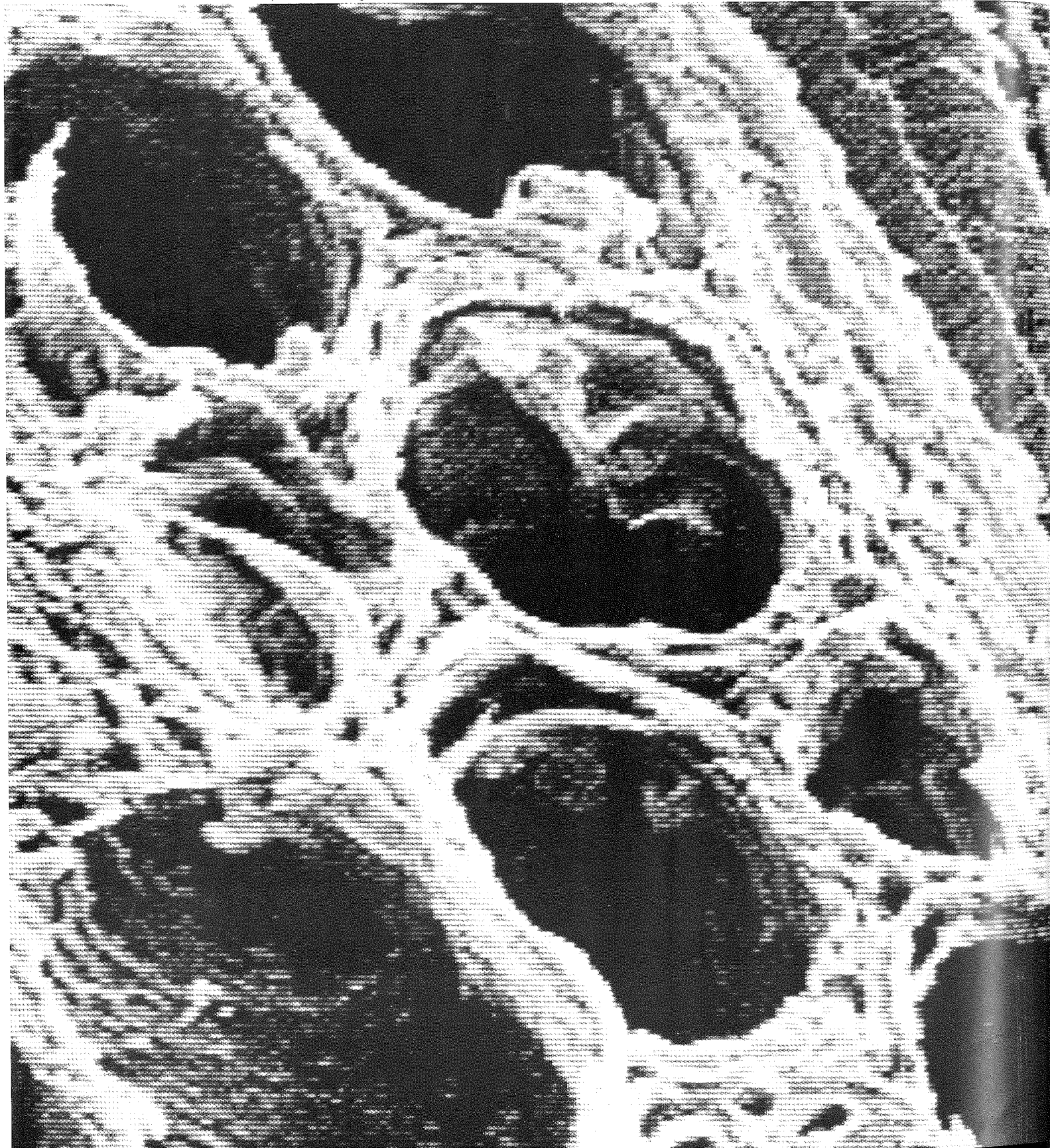
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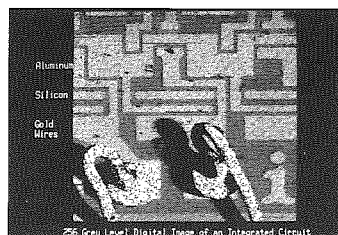
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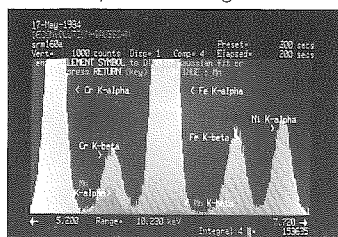
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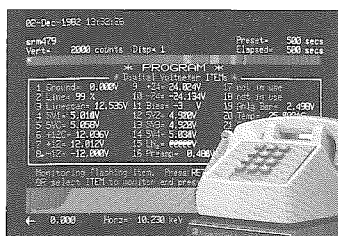
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HOW FLIES AND MOSQUITOES GRASP THE WORLD AROUND THEM

By

Ron W. Davis

Department of Anatomy
College of Medicine
Texas A&M University
College Station, Texas 77843-1114

Have you ever noticed that a fly can walk on a vertical window pane but a mosquito, which is closely related, cannot? I had never paid much attention to this until a friend brought me some flies and mosquitoes that he had raised in culture. He showed me where he had made small horizontal scores in the walls of the culture containers so that the mosquitoes could hang on. He also showed me that this was not necessary for the flies. It was obvious that they had no problem walking on the sides or top of the plastic box they were in. I wondered what the difference was between a fly foot and a mosquito foot.

I obtained samples of laboratory-cultured houseflies and prepared them for scanning electron microscopy. The preparation procedures used were standard except for the initial killing and fixation. I found that if the insects were put into 3% glutaraldehyde or formaldehyde fixative they floated and stayed alive for

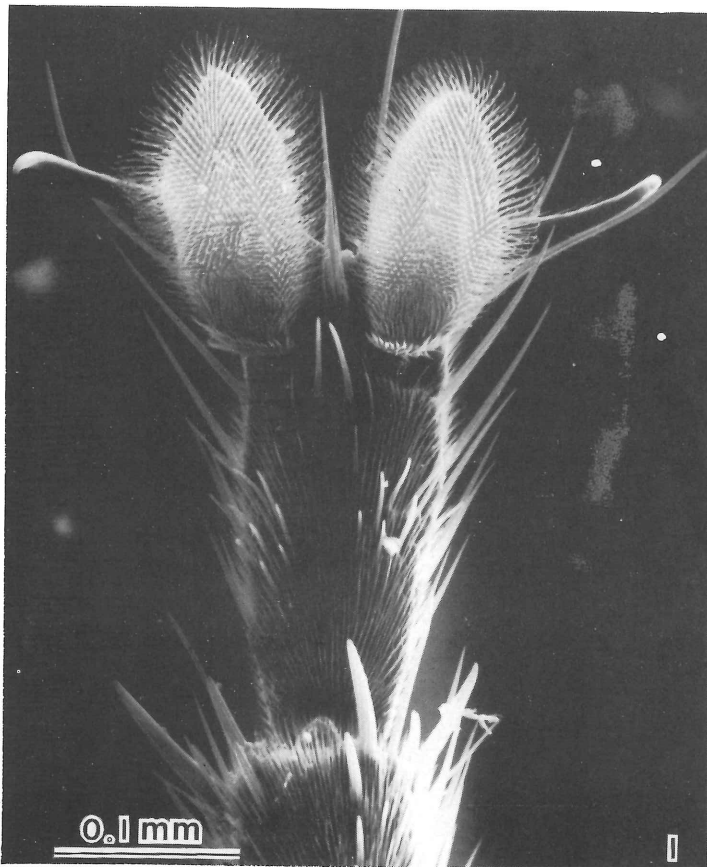


FIGURE 1. The ventral surface of the lower leg (distal tarsomere) and foot (pretarsa) of the housefly. Note the presence of two tarsal claws on either side and joining behind the two pad-like pulvilli. Both large and small hairs cover the leg and body of the fly.

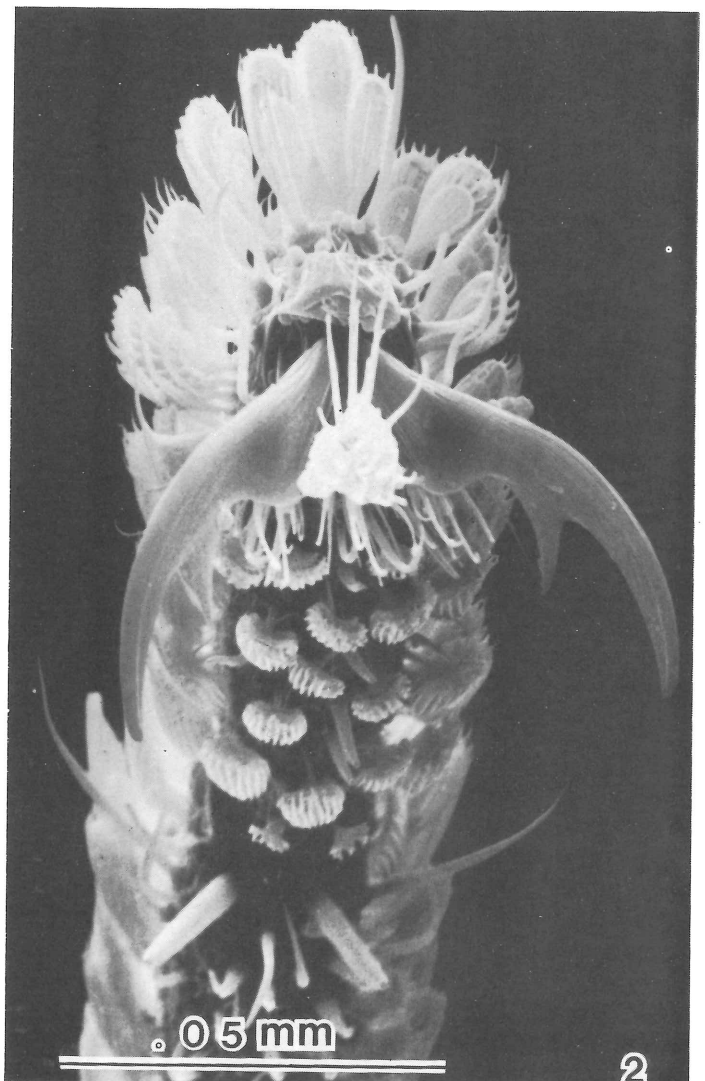


FIGURE 2. The ventral surface of the lower leg (distal tarsomere) and foot (pretarsa) of the mosquito. Note the presence of tarsal claws but the absence of pulvilli. Scales are present on the legs and body of the mosquito. Spiny tactile hairs are also present.

considerable lengths of time. A few drops of Photoflo plus DMSO in 200 ml of fixative wetted and killed the specimens very quickly. Sometimes a small vacuum was used to help remove the air that tended to collect in the body hairs and scales. The specimens were mounted on silver mylar tape and sputter coated in two or more directions before viewing in a scanning electron microscope.

The difference between a fly foot (Fig. 1) and a mosquito foot (Fig. 2) is interesting. The fly has two small pads called pulvilli — these pads are absent from the mosquito foot. Each pulvillus is covered with small hair-like structures (Fig. 3) that are curved and blunt at the tips (Fig. 4). Glands that secrete a sticky material are present in the epidermis of the pulvilli. This coats the tip of the pulvillus hairs and allows flies to adhere to smooth surfaces.

The claw-like structures are called tarsal claws. They are present on both fly and mosquito feet, and are used to hold on to irregularities on non-smooth surfaces (e.g., a painted wall or ceiling). The stiff-looking hair between the pulvilli of the fly, and the hairs at the base of the tarsal claws of the mosquito, are probably tactile sensory structures. Chemoreceptors are present in the foot area to aid in food recognition.

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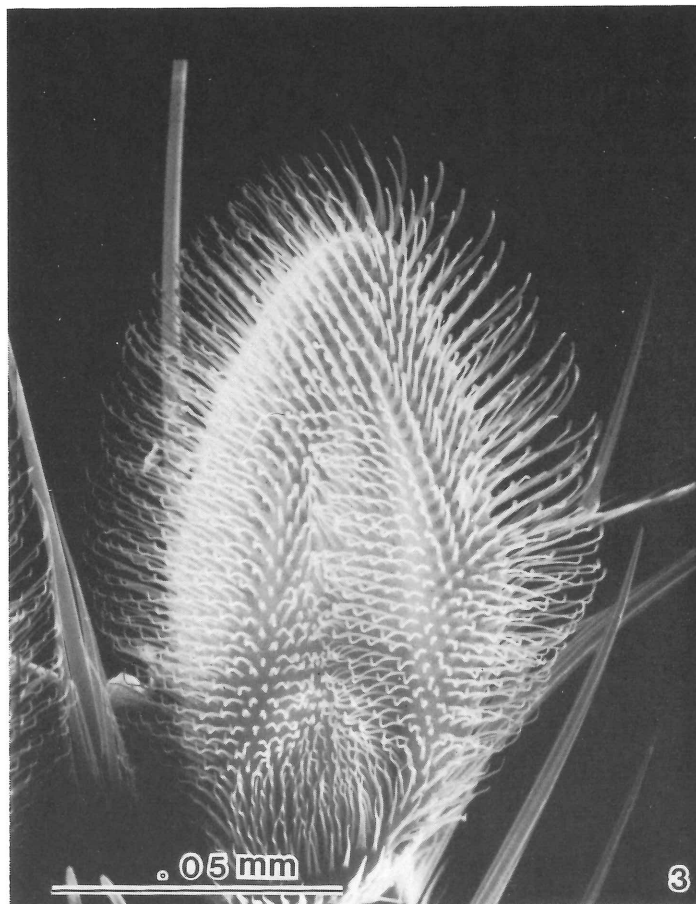


FIGURE 3. A single pulvillus of a fly foot showing the distribution of hair-like spines used in adhesion. Note that the blunted tips of the spines are all curved toward the distal end of the pulvillus.

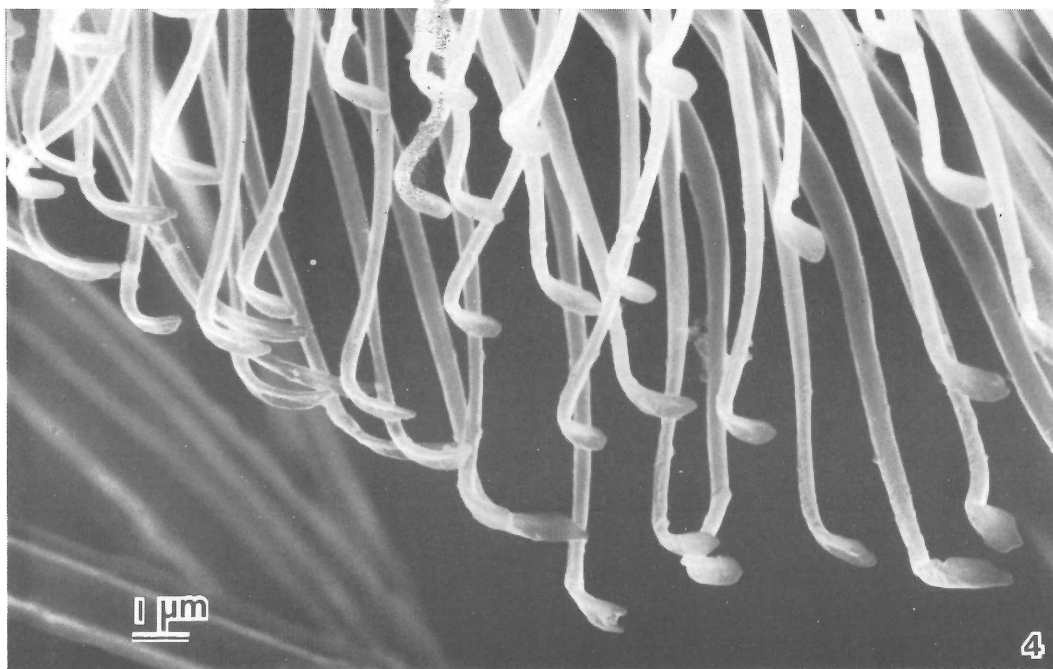


FIGURE 4. A detail of the hair-like spines of the fly pulvillus. Note that the curved and blunted tips are slightly concave.



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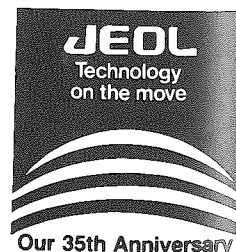
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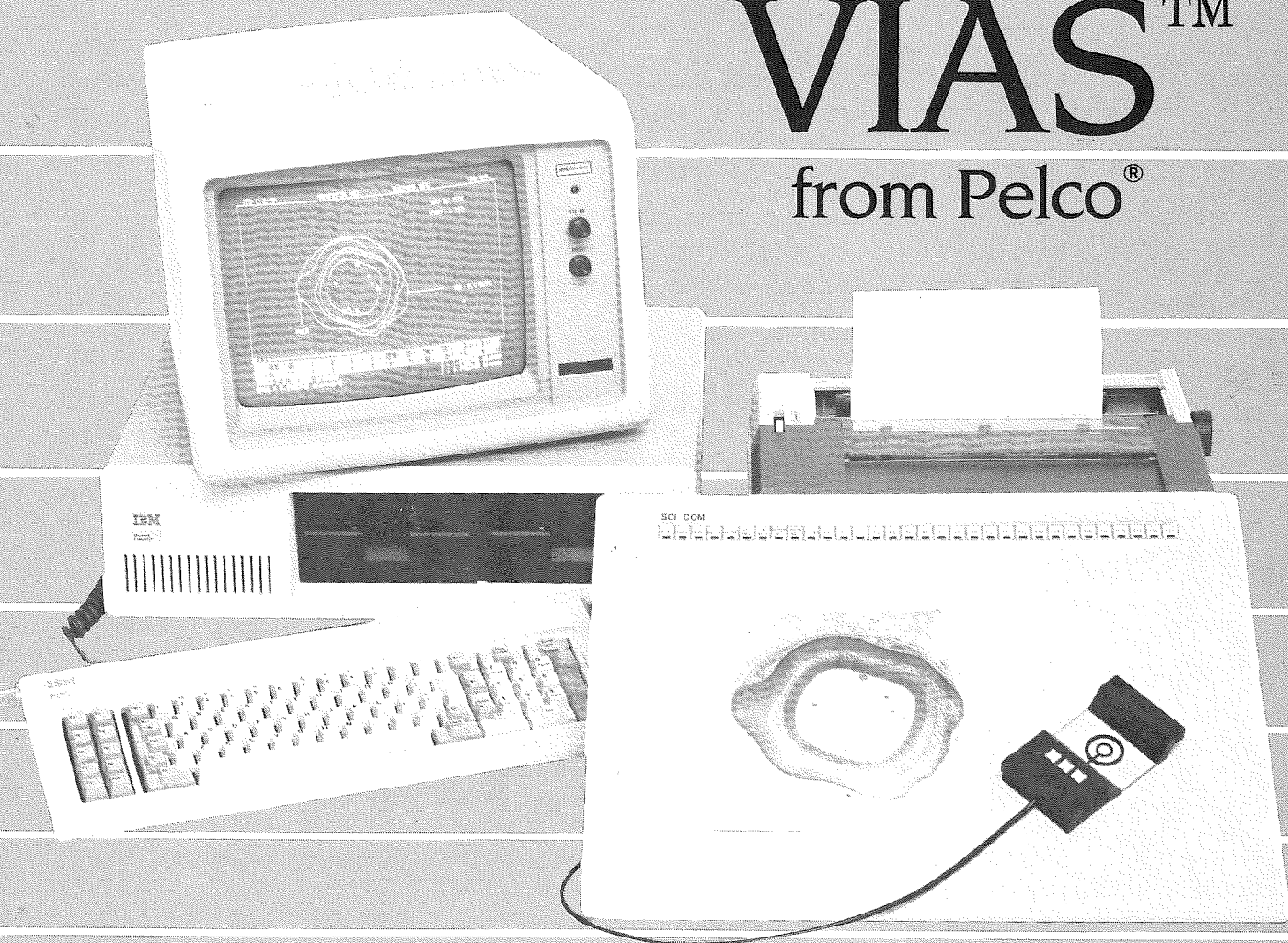
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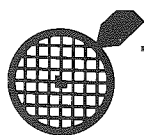


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EFFECT OF PARTIAL NEPHRECTOMY AND OCHRATOXIN A ON RENAL STRUCTURE: A LIGHT AND ELECTRON MICROSCOPE STUDY

By

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ABSTRACT

Ochratoxin A (OA) is a food-borne mycotoxin produced by *Aspergillus ochraceus*. When administered to rats, OA produced focal necrosis of renal tubular epithelial cells, confined mostly to the inner cortex and extending into the subcortical zone at the corticomedullary junction. The cellular changes included redistribution of organelles, loss of endoplasmic reticulum integrity, and reduction in the amount of basal infoldings of plasma membranes. In addition, there was a unique aberration consisting of tightly-packed and interdigitating spherical aggregates of smooth endoplasmic reticulum in numerous proximal tubules. Partially nephrectomized rats showed similar, but substantially enhanced, responses to OA. Thus, partial nephrectomy appears to be a useful procedure for accentuating the effects of nephrotoxic agents and facilitating evaluation and diagnostic procedures. Additionally, these data suggest that impairment of renal function significantly enhances the health hazards of mycotoxins such as OA.

INTRODUCTION

Ochratoxin A (OA) is a naturally-occurring and ubiquitous mycotoxin produced by *Aspergillus ochraceus* and is a contaminant of a variety of foods

for human consumption as well as animal feeds (1). Chemically, OA is a dihydroisocoumarin derivative, (7-carboxy-5-chloro-8-hydroxy-3, 4-dihydro-3R-methylisocoumarin) linked to phenylalanine through the 7-carboxy group (2).

Several studies of the toxic effects of OA have been reported including nephrotoxicity in cultivated renal epithelial cells (3) and induced nephropathy in several species including dogs, pigs, horses, and birds (1,4). Ochratoxin A also has been implicated in Balkan nephropathy, a chronic interstitial nephropathy in humans occurring in a high percentage of rural populations in Bulgaria, Romania and Yugoslavia (4,5) and has recently been reported to be the most prevalent mycotoxin in human foods in the United Kingdom. (6).

This study was undertaken to assess renal cell damage by light and electron microscopy in partially nephrectomized and non-nephrectomized rats exposed to OA. A companion study to determine the toxicokinetics and biochemical effects of OA on partially nephrectomized rats has recently been reported (7,8). The purposes of these studies were to assess the potential health hazards of OA attendant with impaired renal function, and to determine the value of partially nephrectomized animals as models for toxicological studies.

MATERIALS AND METHODS

Ochratoxin A (OA) was obtained from Makor Chemical Ltd (Jerusalem, Israel). Chemical purity (99-100%) was verified via HPLC, TLC, NMR, and MS. Solutions of OA were freshly prepared before use by dissolving the OA in 5% NaHCO_3 in light proof vials.

Sprague Dawley rats (150-190 g) were obtained from TIMCO, Inc. (Houston, Texas). The animals were housed individually in polycarbonate metabolism cages at the Texas A&M Laboratory Animal Resources and Research Facility. They were maintained at 24°C on a 12 hour light-dark cycle. Water and standard laboratory feed were provided *ad libitum*. Rats were acclimatized to their new surroundings for 7 days.

Following acclimatization, a two stage experimental procedure was instituted as follows: a subtotal nephrectomy was done on four rats as described previously (7,8) by removing the right kidney and the lower pole of the left kidney. In another group of 4 animals, the kidneys were exposed but left *in situ*. Post operatively, the animals were allowed to recover for 26 days after which they were divided into 4 groups of two as follows; sham-operated control, partially nephrectomized control, sham-operated and OA-dosed, and partially nephrectomized and OA-dosed. OA dosages were 0.5 mg/kg body weight for 4 days followed by 2.0 mg/kg body weight for 3 days. The OA was administered by intraperitoneal injections of 0.1 ml/100 g body weight. Control animals received only the solvent, 5% NaHCO_3 .

On day 8, all rats were killed using CO_2 asphyxiation and the kidneys or kidney remnants rapidly removed, weighed, and bisected. One half of each kidney or kidney remnant was taken for biochemical assay (7,8). Small pieces of both cortex and medulla from the other halves were prepared for electron microscopy by mincing in a fixative containing 2% glutaraldehyde buffered with cacodylate to pH 7.4. The remaining kidney pieces were placed in buffered 10% formalin in preparation for light microscopic examination.

For electron microscopical studies, the tissues were fixed for 3 hours in 2% glutaraldehyde buffered with 0.1 M cacodylate at pH 7.4 followed by washing for 1 hour in 0.1 M cacodylate buffer at pH 7.4 and post fixation for 1 hour in 1% osmium tetroxide buffered with 0.1 M cacodylate at pH 7.4. All fixatives and rinses were at ice bath temperature (approximately 4°C). The tissues were dehydrated in an ethanol series and embedded in an Epon 812 resin mixture. Thick sections for light microscopy were stained with "Multiple Stain Solution for Frozen Sections" (Polysciences, Inc., Warrington, Pennsylvania). Sections for electron microscopy were stained with uranyl acetate and lead citrate and examined in a Philips EM 300 electron microscope.

RESULTS

By light microscopy, the main features were focal areas of necrosis of tubular epithelium, mostly confined to the inner cortex and extending into the outer medulla at the cortico-medullary junction (Fig. 1). Damage was most severe in the descending straight portions of the proximal tubules and heavily damaged tubules were mainly clustered around relatively normal-appearing collecting ducts (Fig. 1). The glomeruli, interstitium and vessels did not appear affected. Qualitatively, the cellular damage and the distribution of the damaged cells was the same in the partially nephrectomized and OA-treated animals as it was in the OA-only treated animals. However, the foci of tubular necroses were markedly larger in the partially nephrectomized and OA-treated animals than they were in the OA-only treated animals. Cellular necroses and tubule damage were not seen in animals treated only with sodium bicarbonate either with or without the partial nephrectomy.

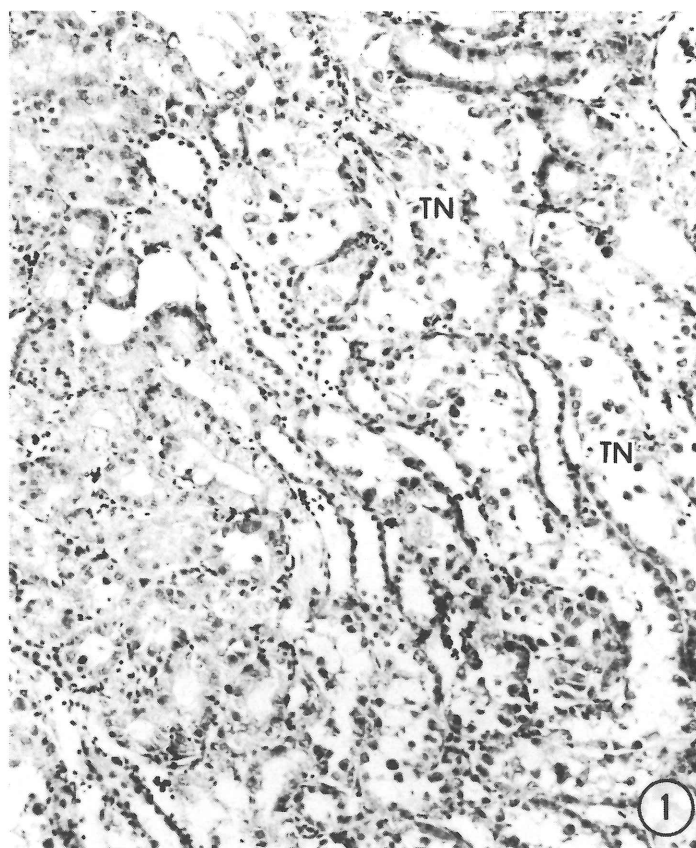


FIGURE 1. Portion of kidney from a partially nephrectomized rat treated with ochratoxin A (OA). Localized areas of tubular necrosis (TN; upper and right portion of micrograph) were confined mostly to the inner cortex with extensions into the outer medulla at the cortico medullary junction. Cellular damage was most severe in the descending straight portions of the proximal tubules. Heavily damaged tubules were often clustered around relatively normal-appearing collecting ducts. X130.

Sham Operated Control: There were no distinguishing features of kidney ultrastructure in this group of animals. Kidney ultrastructure was normal and essentially the same as that reported by Pease (9) and illustrated in standard histology texts (10).

Partially Nephrectomized Control: A mild degree of cellular change was noted at the ultrastructural level within the test animals of this group as compared to that of sham operated controls. Most commonly, the cellular alterations appeared as a mild disorganization of the membranous constituents associated with the cell surface; i.e., the basal infoldings of the plasma membrane were less numerous and less well organized than that of the controls. Both proximal and distal convoluted tubules were affected to about the same extent (Fig. 2).

Ochratoxin-A Treated: Cellular damage varied from a general cytoplasmic disorganization (Fig. 3) to

almost total disruption in a few cells (not illustrated). Principal damage was in the proximal tubules of the cortex and outer medulla with much less damage to the distal tubules (Fig. 3). Most cellular damage was of a general nature; e.g., disintegration of luminal cell plasma membrane and brush border, a redistribution of organelles, loss of endoplasmic reticulum integrity, loss of cell surface area due to fewer and less extensive basal infoldings of plasma membrane, and alterations in form of a few mitochondria (Fig. 3).

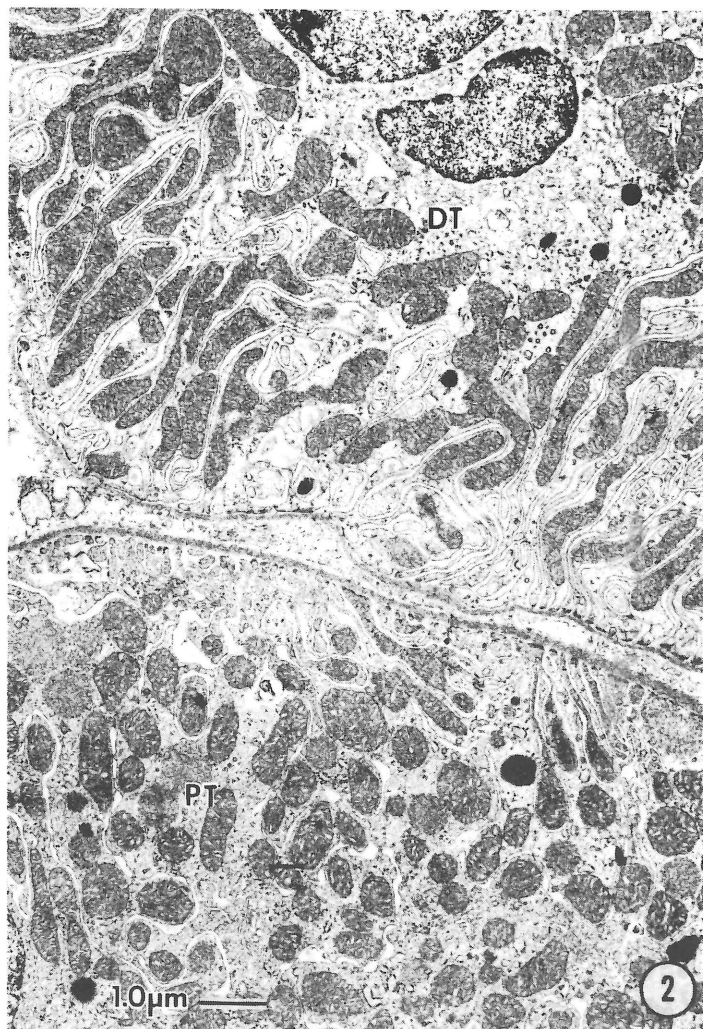


FIGURE 2. Portions of distal (DT) and proximal (PT) convoluted tubules from a partially nephrectomized-only rat showing slight alteration of cellular ultrastructure, particularly in relation to plasma membrane infoldings. Note that both distal and proximal tubules were affected. X7,000.

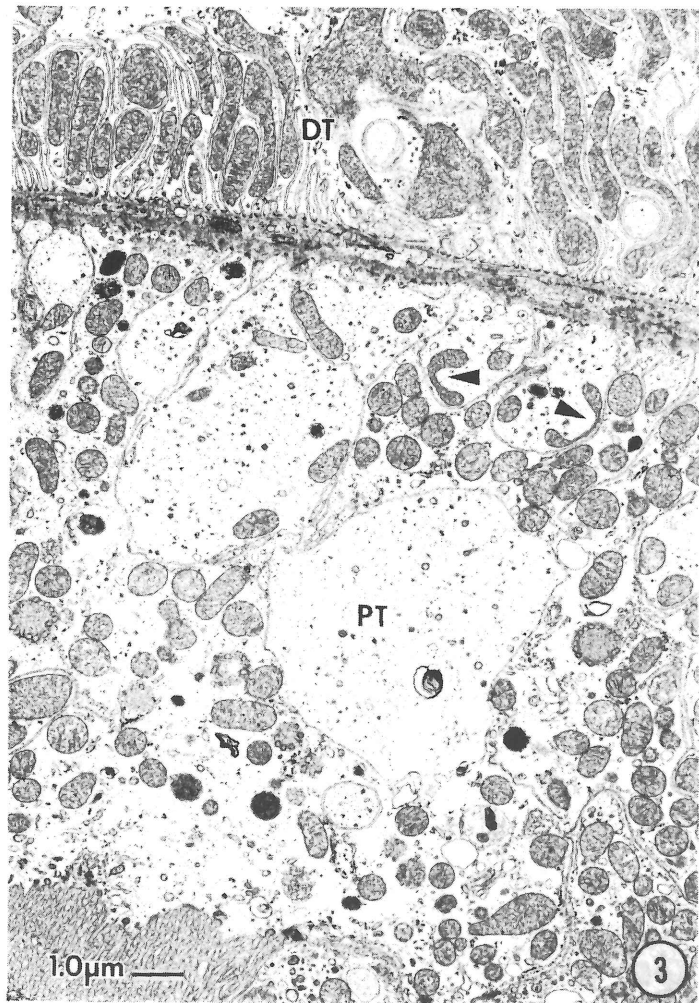


FIGURE 3. Portions of distal (DT) and proximal (PT) convoluted tubules from a rat treated with OA (only) showing marked cytoplasmic damage in the proximal tubule. The cytoplasmic damage consisted primarily of a redistribution of cytoplasmic components, loss of cell surface membrane (i.e. fewer and less extensive invaginations of plasma membrane), mitochondrial shape changes (arrowheads), and development of organelle-free cytoplasmic areas. Aggregates of smooth membrane, like those of Figure 4, were present also but are not illustrated in this micrograph. Changes in the distal tubules were similar to those of the proximal tubules except that they were much attenuated and there were no aggregates of smooth membrane. X6,000.

Vacuolization of cytoplasmic constituents was occasionally observed but was not extensive. A unique aberration consisting of an aggregation of smooth-surfaced membranes was observed in numerous cells of the proximal tubules (see Fig. 4 and accompanying discussion). The aggregated membranes were of tubular configuration and highly interdigitated into a very compact form. No structural aberrations were identified in glomeruli.

Partially Nephrectomized and Ochratoxin-A

treated: The character and distribution of the cellular aberrations in these animals was essentially the same as that of the animals treated only with OA, which are described immediately above, but the extent of damage was markedly greater (compare Fig. 3, 4). The aggregates of smooth membrane were particularly numerous in the partially nephrectomized and treated

animals and many were quite large (3-4 μm in diameter) (Fig. 4). These membrane aggregates were continuous with the rough endoplasmic reticulum at their peripheries (Fig. 5). No structural aberrations were identified in glomeruli.

DISCUSSION

Ochratoxin A (OA) has been established as nephrotoxic fungal agent in a variety of animals, including humans (1,4). Though both proximal and distal tubule effects have been implicated as sites of toxin action (4), most studies suggest that the primary site of toxic action is the proximal tubule (11, 12, 13). As the clearance of OA has been shown to be significantly lower than the GFR (7,8), some of the OA is reabsorbed as it passes down the renal tubules. The principal site appears to be the descending proximal tubules, which also show the most extensive damage. However, we have found that some distal tubule damage is demonstrable in electron micrographs, at least when high levels of OA are administered.

The proximal tubular necroses produced by OA are similar to the classical acute tubular necroses associated with many recognized nephrotoxins such as heavy metals and organic solvents (3), and it is likely that the generalized cellular disruption, vacuolization, membrane disruption, and mitochond-

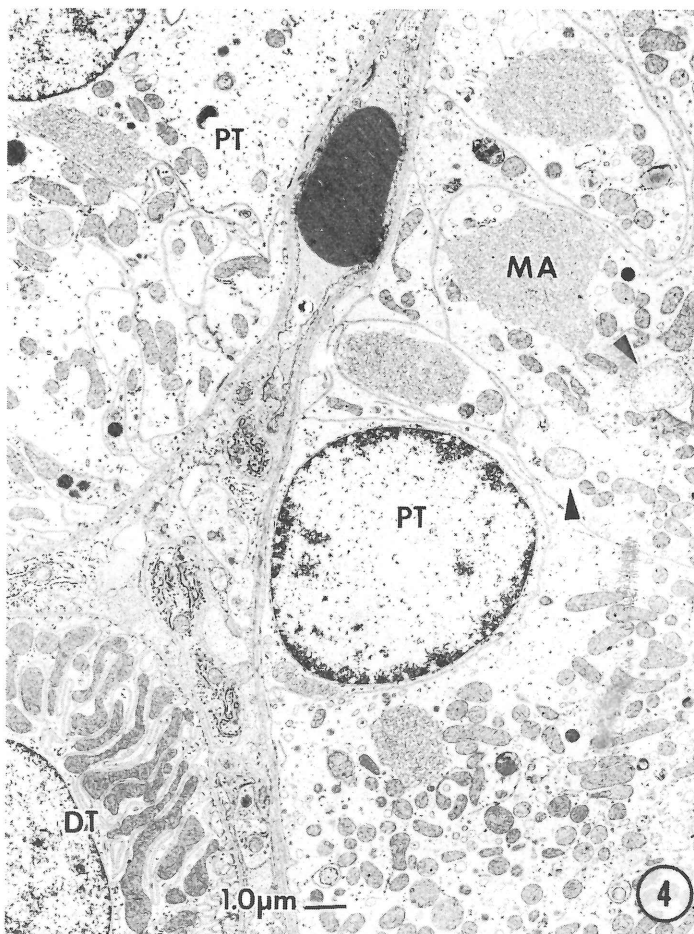


FIGURE 4. Portions of distal (DT) and proximal (PT) convoluted tubules from a nephrectomized rat treated with OA. Generalized cellular damage was similar to that of the treated-only rat (Fig. 3) but much more extensive; i.e., there was more organelle displacement, less plasma membrane invagination and more mitochondrial damage (arrowheads). Additionally, there was a marked increase in the numbers of membrane aggregates (MA). The distal tubules showed only minor cellular aberration. X5,000.

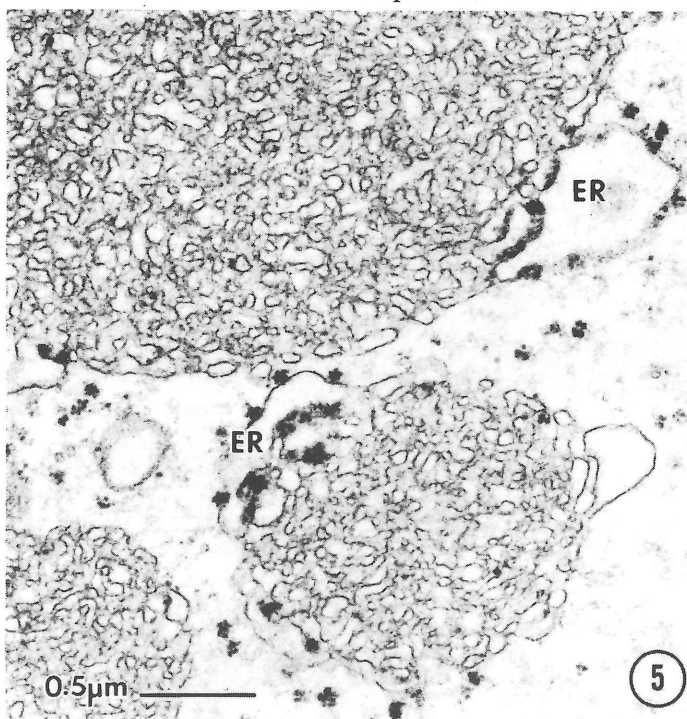


FIGURE 5. The membrane aggregates illustrated in Figure 4 consisted of close-packed masses of smooth endoplasmic reticulum-like membrane connected at their peripheries to rough endoplasmic reticulum (ER). The membrane aggregates were roughly spherical to slightly elongate and ranged up to about 4 μm in diameter. Several membrane aggregates were often present in the same cell. X27,000.

drial changes represent general responses to toxic insult. However, our results indicate other, more specific, aspects of ochratoxicosis, namely the localization of cellular damage primarily to the descending proximal tubules of the inner cortex, and the unique form of the endoplasmic reticulum aggregates. Proliferation of endoplasmic reticulum and formation of "fingerprint" and "myelin-like" bodies are common responses to a variety of toxic agents (14, 15, 16, 17) including mycotoxins and, specifically, OA (11, 12). However, to our knowledge, the compact and interdigitating form of the aggregated membranes observed in our study has not been previously reported in tissues of OA-treated animals.

A primary goal of this project was to evaluate the effects of partial nephrectomy on the cellular and subcellular responses of kidney cells to a toxic insult from OA. We conclude that the localization of tissue damage and the cellular responses to OA are the same whether or not the animal has been partially nephrectomized. However, the extent of cellular damage was much enhanced in the partially nephrectomized model. The localization of the most severe damage to the areas of the descending proximal tubular epithelium may be related to the fact that OA is partially reabsorbed from the glomerular filtrate as it passes down the proximal tubule. Thus, partial nephrectomy appears to be a useful procedure for accentuating the effects of nephrotoxic agents and, thus, facilitating evaluation and diagnostic procedures. Additionally, our results suggest that impaired renal function significantly enhances the health hazard from nephrotoxins such as OA.

ACKNOWLEDGEMENTS

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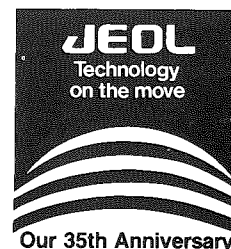


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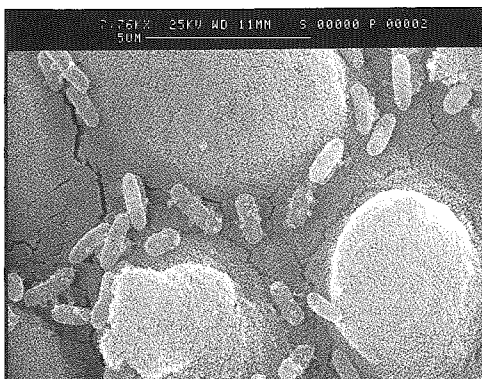
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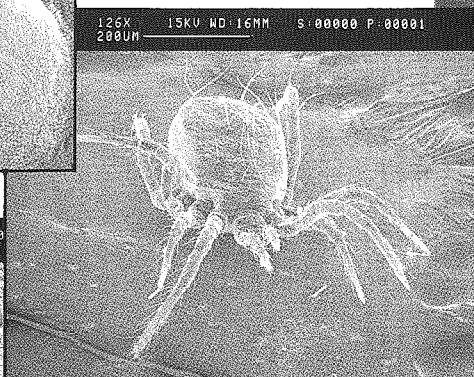
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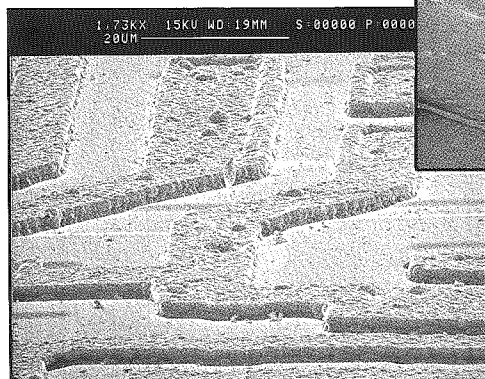
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AVERAGE CHORD LENGTH	8.855E+00 UM
AVERAGE CENTER TO CENTER SPACING	1.621E+01 UM
SURFACE TO VOLUME RATIO	2.469E-01 /UM
EDGE TO EDGE MEAN FREE PATH	7.352E+00 UM
TOTAL TEST LINE	1.350E+04 UM
TOTAL NO. POINTS	3.840E+04
NO. POINTS ON	2.036E+04
NO. FEATURES	6.170E-02/UM

Lineal

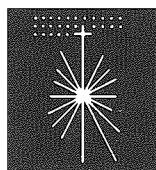
APOPHYLLITE	GLAUCOPHANE	ANDRADITE
ANALCIME	URALITE	MELANITE
THOMSONITE	RIEBECKITE	GLOSSULAR
MORDENITE	CROSSITE	POLYADENALPHE
CLINOPTILOLITE	HEXAGONITE	ALMANDINE
ACMITE	FIBEROUS TALC	SANDINE
DIPSIDE	MUSCOVITE	QLQUOLCLASE
MARZBURGITE	MARIPOSITE	BYTANITE
CASSA	GRANITE	ANDRINETE
HEDENBERGITE	LEPIDOLITE	ANDESINE
PIGEONITE	CHABAZITE	MACROCLINE
SPOUDUMENE	STILBITE	LABRADORITE
HYPERSTHENE	ALUMINA	AMAZONITE
RHODONITE	CALCITE	ALBITE
BUSTAMITE	LIMESTONE	CLEVEANDITE
CUMINGTONITE	DUMORTITE	BIOTITE
GRUNERITE	MAGNETITE	IDEITE
EDENITE	QUARTZ	VERMICULITE

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Abstracts

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BIOLOGICAL SCIENCES

CHARACTERIZATION OF SOME MIMOSOIDEAE (LEGUMINOSAE) GENERA FROM TEXAS. Randy T. Baker, Terry L. Bridges, and L. H. Bragg, Biology Department, The University of Texas at Arlington, Arlington, TX 76019.

Seed coats of seven of the ten Texas genera were examined in this initial mimosoid survey to determine the usefulness of the surface and internal features in characterizing the genera in this subfamily. Not only can the genera (*Albizia*, *Leucaena*, *Acacia*, *Neptunia*, *Desmanthus*, *Pithecellobium*, and *Prosopis*) be separated on the basis of their testa surface ornamentation but the shape of the pleurogram of each is distinctive. Examination of the seeds of these genera in transection also revealed characters which are useful in the separation of these genera. These observed differences warrants further investigation of the remaining genera in this legume subfamily to determine the taxonomic usefulness of these observed characters.

CALCIUM DEPOSITION ON GAP JUNCTIONS. R.C. Kurten, D. Gaddy and R.C. Burghardt, Dept. Biology, TAMU, College Station TX 77843. Granulosa cells of the mammalian ovarian follicle are conjoined by specialized intercellular contacts known as gap junctions. It is widely accepted that these structures are capable of intercellular traffic of small but metabolically significant molecules and that traffic through these channels can be gated. Experimental evidence that the conductance of at least some gap junctional channels can be gated by the concentrations of certain ions at the cytoplasmic face of the channel has led us to examine the subcellular distribution of calcium (Ca) in regions of junctional contact by x-ray microanalysis. Primary mouse ovarian granulosa cells were cultured at high densities in the presence or absence of FSH and cAMP for 48 hours, washed in PBS and fixed 15 minutes in 2%PFA 3% GTA with 2% lanthanum nitrate as a specific probe for junctional membrane. Ca was then stabilized utilizing the highly sensitive Ca precipitating salt N,N-naphthaloyl hydroxylamine. Following extensive washes the cultures were embedded without subsequent osmication or uranyl acetate en bloc staining. Thick sections were examined in an EDS equipped TEM. Weak Ca signals were detected in unapposed plasma membrane and mitochondria in all treatments examined, consistent with previous observations. In regions of closely apposed plasma membrane not occupied by gap junctions Ca signals were undetectable. In contrast, in all treatments examined gap junctions were found to be consistently associated with continuous subcellular Ca depositions in higher levels than those detected in any other organelle. Further, Ca was never detectable in cytoplasm one probe diameter away from gap junctional membrane. It was not possible to establish significant differences in gap junction associated Ca levels between control cultures and those subjected to FSH or cAMP. It was also not possible to establish significant differences in junction associated Ca levels between different size classes of junctional membrane although physiological considerations suggested differences might be detectable. This study provides valuable baseline data for further studies exploring the role of subcellular Ca in the control of junctional membrane permeability utilizing the sensitive Ca specific compound N,N-naphthaloyl hydroxylamine. (Supported by NIH grant HD-14781)

CHRONIC EFFECTS OF DIETARY COBALT ON TESTICULAR MORPHOLOGY. Hilton H. Mollenhauer, Donald E. Corrier, M. F. Hare, D. E. Clark and M. H. Elissalde, USDA-ARS, Veterinary Toxicology Entomology Research Laboratory, P. O. Drawer GE, College Station, TX 77841.

Adult male rats maintained on a diet containing 230 ppm cobalt were assayed weekly by light and electron microscopy for testicular damage. Testicular congestion became apparent after 35 days of treatment but no degenerative changes were observed until about day 70. From day 70 to the end of treatment on day 98, there was progressive deterioration of testicular morphology and decrease of testicular volume. The decrease of testicular volume was due to degeneration of seminiferous tubules. There was a concomitant increase in the number of cells in the lymphatic vessels surrounding the seminiferous tubules but this was not sufficient to offset the volume changes due to tubule degeneration. Tubule degenerative changes consisted of a thickening of basement membrane, increased packing density of red blood cells in the

veins and arteries, formation of giant cells, loss of central filaments from sperm tails, "vacuolization" of germ cell nuclei, and generalized changes in the subcellular architecture of all cells. Thickening of blood vessel walls also was observed. No cobalt residues could be identified using EDS analyses. The subcellular changes were of a generalized nature and no unique or characteristic changes were observed. These data indicate that testicular degeneration is not a primary response to cobalt. A hypothesis which bears consideration is that the testes become hypoxic due both to blockage of veins and arteries by red blood cells and to changes in permeability of the vascular system and the seminiferous tubules.

SURFACTANTS IMPROVE SECTIONING OF EPOXY EMBEDDING RESINS. Hilton H. Mollenhauer, USDA-ARS, Veterinary Toxicology Entomology Research Laboratory, P. O. Drawer GE, College Station, TX 77841.

Surfactants were found to be extraordinarily effective in improving the sectioning quality of epoxy embedding resins. For this study, the firm or hard resin formulations of Spurr were used and the surfactants were added to the resin mixture in concentrations of up to 10% by weight. Resin formulas other than those of Spurr also appeared promising and will be discussed. Of the many surfactants tested, lecithin (Sigma P3644 Type IV-S from soybean; approximately 40% lecithin) at concentrations of 1-2%, proved most effective. For ease of handling, the lecithin was dissolved in DMAE before adding to the resin mixture. With lecithin concentrations less than 2%, image quality and contrast were similar to those of the parent resin. With lecithin concentrations greater than 2%, glass-cut sections (but not diamond-cut sections) often appeared mottled or had microchatter. Additionally, excessive surfactant gave sections of low image contrast and poor beam stability. However, in spite of these problems, the use of surfactants as embedding resin modifiers is worth consideration. For example, resin blocks containing lecithin are so easy to section that a glass knife can be used to face off a block and, without moving to a new edge, cut dozens of thin sections of good quality. The ability to use glass knives could be of great benefit to students who often cannot afford a diamond knife. Additionally, the ease of sectioning should increase the life expectancy of diamond knives.

ACTION OF ESTROGEN AGONISTS ON UTERINE GAP JUNCTIONS. D. Gaddy, R.C. Kurten and R.C. Burghardt, Dept. Biology, TAMU, College Station TX 77843.

Among the responses of the mammalian uterus to estrogen stimulation is the induction of gap junctions in myometrium and the upregulation of these intercellular contacts in serosa. Coordinated uterine contractile activity during labor is thought to be linked to the sudden appearance of gap junctions in myometrium. The estrogen-induced appearance of myometrial gap junctions in hypophysectomized rats, which are modulated by progesterone and blocked by antiestrogens, was recently reported (Burghardt et al., *Biol. Reprod.*, 30:239; 30:249, 1984). We have further examined the junctional responses of two uterine cell types following administration of a variety of estrogen agonists with different affinities for the estrogen receptor. Daily intraperitoneal injections of diethylstilbestrol, estradiol benzoate, estrone, estriol, 5 α -androstane-3 β ,17 β -diol, zuclophenone citrate or chlorotrianisene were administered in doses ranging from 0.5 μ g to 2500 μ g per day for 5 days and uterine tissues were examined 24 hours after the last injection. While all of these compounds bind and translocate estrogen receptors, 5 α -androstane-3 β ,17 β -diol and the triphenylethylene derivatives were unable to promote the induction of myometrial gap junctions following this chronic stimulation. Increases in junctional contacts between serosal cells were affected by these latter compounds. These studies indicate that the induction of gap junctions in myometrial cells requires chronic high levels of activated estrogen receptors. Such levels occur *in vivo* only during the terminal stages of pregnancy. (Supported by NIH grant HD-14781).

A COMPARISON OF CYSTOLITH STRUCTURE IN SOME MEMBERS OF THE MORACEAE. R.W. Davis, Dept. of Anatomy, College of Med., Texas A&M Univ., College Station, TX 77843.

Samples of leaves from *Morus alba*, *Ficus benjamina*, *F. elastica*, *F. stricta*, *F. carica* and *F. lyrata* were examined with the purpose of comparing the structure of their cystoliths. All the plants examined contained cystoliths that were attached by a stalk to the apical and external cell wall of the lithocyst that contained them. In one species, *F. carica*, cystoliths were also found in epidermal hairs.

Specimens were processed for light and electron microscopy by standard techniques. Median longitudinal sections were obtained of stalked cystoliths from all the above species. Median longitudinal sections of the cystolith hairs were not obtained and only off-median sections were examined.

All the cystoliths examined, including the cystoliths in hairs were surrounded by a thin layer of cytoplasm and were extra cytoplasmic. Calcium and silicon were observed in the cystoliths as well as other elements including Al, Mg, P, Cl, Mn and Zn. Most of the cystoliths observed could be divided into at least 3 distinct zones, the stalk, a dilated stalk end and a main body. The stalk and dilated stalk end appeared high in silicon content while the main body of the cystolith appeared high in calcium. Some areas were found where both calcium and silicon seemed to be present. The presence of other elements was variable.

Representative light and transmission electron micrographs and x-ray spectra of cystoliths will be presented.

PRESENCE OF ISLANDS OF SILICON IN THE ADAXIAL LEAF CUTICLE OF *FICUS lyrata*. R.W. Davis, Dept. of Anatomy, College of Med., Texas A&M Univ., College Station, TX 77843.

A preliminary investigation was performed to determine the distribution of silicon deposits in leaves of the ornamental plant *Ficus lyrata*. During this study it was observed that small deposits of silicon were present in the adaxial cuticle but absent in the abaxial cuticle. These deposits appear as islands in the cuticular matrix but probably are continuous with cell wall material that extends into the cuticle and forms a reticulate pattern at the cuticle-cell wall interface. A reticulate area is also present at the abaxial cuticle-cell wall interface but little or no silicon could be detected visually or with energy dispersive x-ray analysis.

Other members of the Moraceae have been observed to have silicon in their epidermis but it has been present as a layer lying along the cuticle-cell wall interface. I am aware of only one other report describing silicon in the cuticle proper of any plant.

All specimens were container grown and maintained as houseplants. Samples were collected and processed by standard methods.

Electron micrographs and x-ray spectra will be shown of silicon islands in leaves of *Ficus lyrata*. These will be compared with silicon deposits in the leaves of several other members of the Moraceae.

STRUCTURE AND LOCATION OF PLASTIDS IN COLUMELLA CELLS OF A CAROTENOID-DEFICIENT MUTANT OF *ZEa MAYS*. C. Edward McClelen, Randy Moore, and James D. Smith. Department of Biology, Baylor University, Waco, TX 76798, and Department of Soil and Crop Science, Texas A&M University, College Station, TX 77843.

We have used optical and electron microscopy to study the structure and location of plastids in columella cells of vp-9 mutants of *Zea mays*, which are carotenoid-deficient. Plastids in columella cells of some of the mutants lacked or contained significantly reduced amounts of starch. However, these plastids did sediment to the lower portion of the columella cells. These results indicate that the sedimentation of amyloplasts in columella cells is not necessarily due directly to their increased density resulting from increased starch content.

ANALYSIS AND IMMUNOCYTOCHEMICAL LOCALIZATION OF THE ILS STORAGE PROTEIN OF SUNFLOWER EMBRYOS. R.D. Allen and C.L. Nessler, Dept. of Biology, Texas A&M University, College Station, TX 77843.

The globulin storage protein of sunflower seeds exists as a hexamer with a molecular weight of about 300,000 daltons. This protein has been purified by gel filtration chromatography and its subunit composition analyzed by SDS polyacrylamide gel electrophoresis. The subunits are heterogeneous, with a molecular weight range of 50 to 60 kd. Each subunit consists of two smaller polypeptides linked by disulfide bridges. Rabbit antiserum was raised against purified sunflower seed storage protein and its specificity demonstrated by western blot analysis. These antibodies were used for ultrastructural localization of the ILS seed protein in developing sunflower embryos. Immunocytochemical reactions were carried out on epoxy embedded sections and bound antibodies were visualized with *Staphylococcus* protein A-colloidal gold. Gold particles were most numerous on protein bodies and on electron dense deposits within vacuoles.

CYTOCHEMICAL LOCALIZATION OF ADENYLATE CYCLASE IN CORTICAL CELLS OF ROOTS OF *ZEa MAYS*. C. Edward McClelen and Randy Moore, Department of Biology, Baylor University, Waco, TX 76798.

Adenylate cyclase catalyzes the synthesis of 3',5'-cyclic adenosine monophosphate (cAMP) from adenosine triphosphate. We have localized adenylate cyclase in cortical cells of primary roots of *Zea mays* using 5'-adenylylimidophosphate (AMP-PNP) as a substrate. Tissues fixed for 1 to 2 hours in buffered glutaraldehyde were incubated for 30 to 60 minutes in a medium containing 0.5 mM AMP-PNP, 2 mM MgSO₄, 4 mM Pb(NO₃)₂, 8% dextran, and 80 mM Tris-maleate buffer, pH 7.4 at 30 C. Adenylate cyclase was cytochemically localized in the nucleolus, nucleus, vacuoles, and along the plasmalemma of cortical cells of *Zea* roots.

STRUCTURAL ASPECTS OF THE INHIBITION OF GRAVITROPISM BY PRIMARY ROOTS OF *ZEa MAYS* BY CHLORAMPHENICOL. Randy Moore, Department of Biology, Baylor University, Waco, TX 76798

Primary roots of *Zea mays* seedlings germinated and grown in 0.1 mM chloramphenicol (CMP) were significantly less graviresponsive than roots germinated and grown in distilled water. Elongation rates of roots treated with CMP were significantly greater than those grown in distilled water. Caps of control and CMP-treated roots possessed extensive columella tissues comprised of cells containing numerous sedimented amyloplasts. I could not detect any significant differences in the ultrastructures of columella cells in control and CMP-treated roots. These results indicate that the reduced graviresponsiveness of CMP-treated roots is not due to reduced rates of elongation, the absence of the presumed gravireceptors (i.e., amyloplasts in columella cells), reduced amounts of columella tissue, or significant changes in the structure of graviperceptive cells. These results are consistent with CMP altering the production and/or transport of effectors which mediate gravitropism.

ULTRASTRUCTURE OF COLUMELLA CELLS IN PRIMARY ROOTS OF *ZEa MAYS* TREATED WITH FLURIDONE. Robert Stoker, Randy Moore, and James D. Smith, Department of Biology, Baylor University, Waco, TX 76798, and Department of Soil and Crop Science, Texas A&M University, College Station, TX 77843

We treated seed and seedlings of *Zea mays* with Fluridone, a pyridinone herbicide that inhibits the biosynthesis of carotenoids and abscisic acid. Since abscisic acid is believed by some to be the effector that mediates root gravitropism, we analyzed the ultrastructure of columella (i.e., graviperceptive) cells in Fluridone-treated roots in order to determine if treatment with Fluridone correlates with any major changes in cellular structure. Columella cells in Fluridone-treated roots possessed numerous sedimented amyloplasts, and were characterized by ultrastructures similar to those of control roots. These results suggest that treating seedlings of *Zea mays* with Fluridone has no major effect on the structure of columella cells.

CELLULAR INTERACTIONS DURING THE FORMATION OF APPROACH GRAFTS IN SEDUM TELEPHOIDES. Randy Moore, Department of Biology, Baylor University, Waco, TX 76798

Approach grafts between cut internodal surfaces of Sedum telephoides are characterized by extensive cellular interdigitation at the graft interface. Adhesion and callus proliferation, but not tissue interdigitation, occur when these surfaces are separated by a porous or impermeable barrier. Graft partners adhere to barriers via the deposition and subsequent polymerization of cell wall materials. Cells adjacent to the barriers had protoplasmic ultrastructures similar to those not contacting barriers. The outer walls of these cells were of uniform thickness and lacked plasmodesmata. These results suggest that diffusible substances alone cannot account for the differentiation of plasmodesmata between contacting cells.

STEREOLOGICAL ANALYSIS OF SPERMATOGENESIS IN THE LIVERWORT PETALOPHYLLUM RALFSII (WILS.) NEES ET GOTT. Steven E. Ehlers and Dale M. J. Mueller. Texas A&M University, College Station, Texas 77843.

The androgonial cells in this liverwort undergo a remarkable cytological transformation to produce the mature sperms. Within each cell, this process includes: 1) the degeneration and loss of the chloroplast, 2) the formation of a single amyloplast, 3) the morphogenesis and condensation of the nucleus, 4) the de novo development of the locomotive apparatus (two flagella and the underlying multilayered structure) and 5) the loss of extraneous cytoplasm. The relative volume of the cell components is compared for the various stages in this development. This metamorphosis entails the streamlining of the cell from a cuboidal vegetative initial through spherical antheridial cells to linear, mature sperms.

THE INTERNAL CUTICLE OF LEAVES FROM LOW CONDUCTING STRAINS OF COTTON (GOSSYPIUM HIRSUTUM). J.D. Berlin and J.E. Quisenberry. Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409 and USDA-ARS, Route 3, Lubbock, TX 79401.

Cotton like most land plants, wastes water. More water moves through the plant than is actually needed to support vegetative and reproductive growth. This movement of water, called transpiration, is ordinarily controlled by stomatal and cuticular resistances at the leaf-air interface. The stomatal component is the major (95%) avenue for water loss in cotton. A search for drought-tolerant or water-use efficient germplasm from several hundred strains in the World Cotton Collection identified strains having opposite extremes with respect to stomatal conductance. The low conducting strains appeared to have a thicker internal cuticle surrounding the sites of internal evaporation in the leaf than did the high conducting strains. Specifically, the low conducting strains had a ruthenium red-positive cuticle covering the inner cell wall of the upper epidermis and the upper portion of the palisade cells. A similar internal cuticle surrounded the spongy mesophyll and the inner cell wall of the lower epidermis in leaves of both low and high conducting strains. This anatomical difference could be important in promoting more water-use efficient lines of cotton. Supported in part by Cotton Incorporated and by the Plant Stress Institute.

MUSCLE FIBER DEGENERATION AND REGENERATION INDUCED BY PROLONGED WEIGHT-LIFTING EXERCISE IN THE CAT. C. J. Giddings, W. B. Neaves, and W. J. Gonyea, The University of Texas Health Science Center at Dallas.

The morphological characteristics of the flexor carpi radialis muscle (FCR) were examined from cats trained to perform weight-lifting exercise to receive a food reward. Untrained sex- and weight-matched cats served as controls. Following training (ave. 52 wks), the cats were anesthetized and the long insertion tendon of the FCR was surgically isolated. The tendon was attached to a tension transducer and the muscle was set at its optimal length. The muscle was perfused with 2% glutaraldehyde in 0.1M cacodylate buffer.

Small bundles of fibers were teased from trained and control muscles and embedded in Epon. Using spaced serial sections, the morphological features were examined throughout the fiber length. Fibers from trained muscle showed a higher incidence of degenerative changes such as disrupted sarcolemma, pyknotic nuclei, vacuolation, and disorganization of contractile elements. In addition, the degree of degeneration was found to vary along the length of a fiber. Trained muscle possessed both larger and smaller fibers than did control tissue. The smallest fibers observed in trained muscle were considered to be regenerating or "new" fibers since no degenerative changes were observed within them. "Satellite-like" cells were observed in trained muscle. Such cells resembled satellite cells but also contained developing myofilaments. Since evidence of degeneration-regeneration was observed in control muscle but at a lower frequency, it was postulated that weight-lifting exercise accelerates muscle fiber turnover in the cat FCR. Supported by N.I.H. AM 17615.

COMPUTER ASSISTED STEREOSCOPY. Arthur Cole, Physics Dept., University of Texas System Cancer Center, Houston, Texas 77030

Computer processing of stereo-pair images has applications in diagnostic radiology, electron microscopy, and other imaging systems. One stereo pair can provide information to reconstruct planar (tomographic) or non-planar sections of the specimen. The information may be displayed as either a two or a three dimensional image seen from any selected viewpoint. Stereo-pair images may introduce ambiguities and constraints. The initial designs for image scanning and computer procedures are intended to minimize those constraints. A first requirement is that parallax measurements (displacements of an object viewed in left and right images) be made accurately. Thus, to extract maximal information, positional accuracies of 1 part per 50,000 for electron micrographs and 1 part per 5,000 for X-radiographs are required. This is as much as 100 times the accuracy of video systems. Secondly, the huge data content of 10¹⁰ bits and 10⁸ bits for EM and X-ray images must be reduced to usable proportions. Signal algorithms were designed to retain essentials but greatly compress the data. Thirdly, procedures used to select image pair correlates (image points from left and right images that correspond to the same object point) should involve reasonable computation times. Simplified algorithms use signal, contour, and near neighbor pattern comparisons in a sequence of decision steps. Finally, display should be convenient and retain image resolution. Proposed algorithms improve image sharpness and present any selected portion of the data from any selected viewpoint although no pixels are displayed for uniform density regions since no stereo information exists there. The result is similar to that for an exposure compensated image.

VARIATIONS IN THE ENDOPLASMIC RETICULUM OF TUMOR CELLS.

Bruce Mackay and Fadi Abdul-Karim, Dept. Pathology, University of Texas Cancer Center, M.D. Anderson Hospital, Houston, Texas 77030.

A broad range in the quantity and appearance of the endoplasmic reticulum can be encountered in human tumors. At the two extremes are the abundant parallel aggregates of well differentiated exocrine adenocarcinomas and the sparse, slender, serpentine cisternae of many lymphomas. Two uncommon types of variation will be discussed. In the first, peculiar complexes of the membranes of the reticulum and ribosomes are formed. The best known is the so-called ribosome-lamellar complex that can be seen in approximately 50% of hairy cell leukemias, and infrequently in other types of leukemia. We have observed an unusual variant of this theme, in which the membranes are concentrically rather than spirally configured, in two cases of monocytic leukemia and a soft tissue neoplasm of the chest wall of undetermined cell type. The other type of variation of the endoplasmic reticulum is characterized by an accumulation of material within the cisternae. The material can take the form of filaments, microtubules, or amorphous aggregates. Filaments can be seen in proliferative disorders of fibroblasts including some fibrosarcomas and malignant fibrous histiocytomas. Microtubules are present in approximately 10% of melanomas, and in a rare variant of extraskeletal myxoid chondrosarcoma. Amorphous electron-dense material accumulates within the endoplasmic reticulum in occasional liposarcomas and it may be homogeneous or undergo focal condensation to create bizarre formations.

ACETAMINOPHEN EFFECT ON MOUSE PANCREATIC ISLETS

A.M. Andrews, H. Han Hsu and J.A. Hinson, National Center for Toxicological Research, Jefferson, AR 72079

Acetaminophen (paracetamol) is a widely used effective analgesic and antipyretic drug which is a remarkably safe agent when used in therapeutic doses. When acetaminophen was administered to mice there was a drastic increase in blood insulin levels (3X at 3 hours, 11X at 8 hours). To determine any ultrastructural changes of the pancreatic islet cells, male B6C3F₁ mice were dosed with acetaminophen, 500 mg/kg. The animals were anesthetized with ether at 3, 4.5, 6, 8 and 20 hours then perfused with paraformaldehyde - glutaraldehyde at each time interval. Progressive ultrastructural changes in the pancreas were observed only in the Beta cells of the groups studied. The prominent changes were pronounced intercellular spaces, cytoplasmic vacuolation, damaged membranes of cytoplasm, secretory granules and other organelles, and finally breakage of outer nuclear membrane. The ultrastructural changes indicated that the selective damage to the Beta cells was in direct relationship to the increase in insulin levels.

SEED COATS OF SELECTED CASSIA SPECIES (LEGUMINOSAE). Louis H. Bragg, Biology Department, The University of Texas, Arlington, TX 76019.

Seeds of several species of Cassia from Texas were examined with SEM for differences that may aid in further establishing their taxonomic status. Differences were noted in their particular surface patterns. Surface depressions or pits when present were located on different areas of the seeds. Trans-sections of the seeds revealed additional differences among the examined species. Such distinguishing characters between these limited species provides a basis for a more extensive study within this genus.

STEREOLOGICAL ANALYSIS OF THE PALISADE CHLOROPLASTS OF HIGH AND LOW CHLOROPHYLL STRAINS OF COTTON. S.R. Short-Russell and J.D. Berlin. Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409.

Chemical analysis of the World Cotton Collection revealed four strains having extremes in chlorophyll content--T-231 and T-766 had an average of 37 and 40 ug chlorophyll/cm² leaf area, respectively, while T-166 and T-125 had 27 and 25 ug chlorophyll/cm², respectively. The purpose of this investigation was to compare the morphology of these strains. The low chlorophyll plants were taller and produced lighter green leaves. Light microscopic studies indicated a correlation between leaf thickness and chlorophyll content--the high chlorophyll lines had thicker leaves and taller palisade cells. Stereological analysis of electron micrographs of the palisade cells revealed no significant differences in the chloroplast fractional volumes between the varying strains. T-231, one of the high chlorophyll strains, had significantly fewer but larger chloroplasts per cell than the other three strains. T-125, T-766 and T-166 appeared to have approximately the same sized chloroplasts, but T-125, a low chlorophyll strain, had fewer chloroplasts per palisade cell. Studies are under way to quantitate the amount of chlorophyll contained in each chloroplast. The long range goal of this investigation is to develop a more efficient cotton plant. Supported in part by Cotton Incorporated and by the Plant Stress Institute at Texas Tech University.

ULTRASTRUCTURE OF LOPHODINIUM POLYLOPHUM: A PRELIMINARY REPORT. J.M. Chesnick and S.V. Carty, Dept. Biology, Texas A&M University, College Station, TX 77843

For the first time living material of the dinoflagellate *Lophodinium polylophum* has been collected and described. SEM and light microscopy revealed distinctive thecal ridges overlaid by a pattern of hexagonal plates. Using propionic acid-lactic acid-oracein stain a C-shaped nucleus was identified.

Observation of ultrastructure of the cell revealed an interesting internal organization. Cell features included an amphiesma composed of at least 2 lower membranes lying adjacent to the cytoplasm, thecal plates, and a densely staining thin layer above the plate material; numerous giant mitochondria in intimate contact with chloroplasts; many discoid chloroplasts, thylakoids occurring in 3's; a large pusule occupying the central portion of the cell; trichocysts with a crystalline core and terminal fibrils; golgi and starch granules. Efforts are currently being made to isolate and grow *L. polylophum* in culture for further studies concerning life history and cellular processes.

ULTRASTRUCTURE OF THREE SPECIES OF EXOBASIDIUM GROWN IN PURE CULTURE. E. A. Richardson and C. W. Mims, Department of Biology, S. F. Austin State University, Nacogdoches, TX 75962.

Members of the fungal genus *Exobasidium* are all plant pathogens attacking a variety of wild and cultivated flowering plants. For this study three species were isolated from the infected tissues of their hosts and grown in pure culture on nutrient media. The three species isolated were *E. vaccinii* Wor. from lowbush blueberry, *E. japonicum* Shirai from cultivated azalea, and *E. camelliae* Shirai from *Camellia*. Samples of each fungus were subsequently removed from the culture media and prepared for examination with either TEM or SEM. Ultrastructural features of cultured material were then compared with those of material produced on host tissues.

In culture the colonies of both *E. japonicum* and *E. camelliae* were more "yeast-like" in appearance than those of *E. vaccinii*. All three species produced thin-walled hyphae although those of *E. vaccinii* were more highly branched and much more extensive than those of either *E. japonicum* or *E. camelliae*. Septa were present in the hyphae of all three species although they were irregularly spaced. Typical cellular organelles were present along with distinctive, electron-dense structures of an unknown nature. All three species produced tremendous numbers of long, slender conidia. These arose both from the hyphae as well as from other conidia and appeared to be similar to those produced in the wild on host tissues.

DEVELOPMENT OF THE ABSORPTION TRICHOMES OF TILLANDSIA RECURVATA. J. A. Matos, Dept. of Biology, Stephen F. Austin State University, Nacogdoches, Texas 75962.

Absorptive trichomes densely cover the shoot system of *T. recurvata* (Bromeliaceae) in the mature plant. The trichome cover functions in water and nutrient uptake as well as in light reflection. Young seedlings have a sparse trichome cover which becomes increasingly dense as the plant matures.

Development of trichomes begins while the young leaves are still contained within the leaf sheath of the seedling. Each trichome develops from a single epidermal cell. The stalk of the trichome develops first by a series of periclinal cell divisions. A number of anticlinal divisions of the outermost cell of the stalk occur resulting in four central disk cells. Eventually four concentric rows of cells are produced around the central disk, the last of which, after a number of additional cell divisions, forms the conspicuous wings characteristic of bromeliad trichomes. Mature trichomes are present by the time that the first leaves emerge, some 10-15 days after germination. Trichome development continues, and by 15-20 days after germination the young leaves have a full trichome cover. Ultrastructural aspects of this developmental process using SEM and TEM will be presented.

CALCIUM OXALATE CRYSTALS IN *GEASTRUM*. H. J. Arnott and K. D. Whitney, Department of Biology, The University of Texas at Arlington, Arlington, TX.

Basidiocarps of a *Geastrum* species were collected in plant litter beneath the branches of *Pinus edulis* and *Juniperus osteosperma*, in connection with our study of leaf litter fungi. The individual star-shaped basidiocarps are about 1 inch in diameter and have a large upright spore case. The surface of the spore case is covered with crystals of calcium oxalate, similar to those reported by Horner and Tiffany for *G. minus* (Amer. J. Botany 71 (5, part II): 31-32, 1984). Unlike those seen by Horner and Tiffany, the large bipyramidal crystals observed in our material are mostly unweathered. These crystals are covered and sometimes penetrated by peridial hyphae. A second type of crystalline body was also found abundantly on the endoperidial surface, interspersed with the bipyramidal crystals. These were composed of many radiating plates, the plates often arranged like the blades of a Japanese fan.

THE USE OF PROCESS ART IN TEACHING ULTRASTRUCTURAL ANALYSIS. H. J. Arnott, Department of Biology, University of Texas at Arlington, Arlington, TX 76019.

Extracting data from electron micrographs represents a challenge for both the student and teacher. Although there is much interest in quantitative methods, often involving computers and sophisticated pattern recognition techniques, the basic element of ultrastructural analysis still involves careful examination of electron micrographs. Students can be taught to examine micrographs in a considerate and thoughtful manner through the assignment of electron micrographs as subjects for written reports. Another teaching technique is process art, the subject of this paper. Process art is art in which the process of creating the "art work" is the "art work," and the people creating the "art work" are a part of it. The strategy used in this gambit is to involve the students in a micrographic analysis which becomes a component of a finished art work that can be exhibited and used as a part of the pedagogy. To accomplish this a micrograph is cut into enough equal parts so that each student has one "submicrograph." Each student receives a 4 x 6 in. white card and double stick tape with which to attach his submicrograph to the card. Students are given 20 minutes to write an analysis of the micrograph using the remaining card space and to date and sign the card. The instructor then attaches the analyses to a poster board in an appropriate geometric fashion thus completing a piece of process art. If several of these art works are produced as the course unfolds, the evolution of both individual and class analytical skills can be traced and documented. The technique can function effectively as both a diagnostic tool and a mechanism for stimulating student interest.

A NEW CULTURE SYSTEM FOR INVESTIGATING ULTRASTRUCTURAL ASPECTS OF SILICEOUS SCALE LAYER BIOGENESIS IN CHRYSOPHYCEAN ALGAE. C. D. Sandgren and S. A. Hall, Department of Biology, University of Texas at Arlington, Arlington, TX 76019.

Members of the Mallomonadaceae (algal class Chrysophyceae) produce layers of highly ornamented siliceous scales that cover the entire cell. These scales are presumed to have conservative, species-specific morphologies. They are produced via a highly organized intracellular assembly process mediated by golgi vesicles. A new culture system will be described in which scale biogenesis in *Synura petersenii* Korsh. can be artificially manipulated leading to dramatic variations in scale morphology and the number of scales present on cells. It is ultimately possible to: 1) completely shut off scale synthesis resulting in populations of physiologically active scale-free (or "naked") cells, and then 2) to reactivate this biosynthetic pathway so that a completely new layer of normal scales is produced within a matter of hours. Various stages in the processes of scale layer loss and scale layer regeneration will be illustrated with SEM micrographs. The significance of this experimental system for studying golgi-mediated biogenesis of extracellular products as well as for studies of chrysophycean systematics will be discussed.

ULTRASTRUCTURE OF THE INTERFACE BETWEEN A MISTLETOE AND ITS HOST. James D. Mauseth, Dept. Botany, University of Texas, Austin TX 78713

The mistletoe *Tristerix aphyllus* (Loranthaceae) infects *Trichocereus chilensis* (Cactaceae) in central Chile. *Tristerix*

aphyllus is an endoparasite, that is, it exists completely embedded in its host's body, only the flowers emerging for reproduction. The endophytic portions of the parasite consist of uniseriate or multiseriate strands of cells that abut the host tissue intimately. The contact interface between host and parasite consists of a thin host wall and an unusually thick parasite wall. The interface wall of *T. aphyllus* does not show any modifications to facilitate absorption of materials from the host: there are no labyrinthine ingrowths nor are there plasmodesmata. Similarly, the cytoplasm of these interface cells does not appear to be different from that of other parenchyma cells.

ULTRASTRUCTURAL ANALYSIS OF HUMAN SAPHENOUS VEINS OBTAINED DURING CORONARY ARTERY BYPASS GRAFT SURGERY. D. A. Hay and A. J. Roberts, Dept. Biology, Stephen F. Austin State University, Nacogdoches, TX 75962.

Coronary artery bypass graft (CABG) surgery is currently one of the surgical procedures most frequently performed in the United States. It is also the subject of considerable controversy. The central issue is the questionable long-term patency of the grafted segments. Post-operative angiographic studies indicate early and/or accelerated occlusion of many of these vessels. Segments obtained during CABG surgery were prepared for TEM and SEM analysis. Samples were then examined for such signs of injury as endothelial denudation, intimal and medial edema and platelet aggregates. The damage to control segments was generally limited to less than 10% of the total luminal surface, whereas implants on occasion exhibited injury to more than 50% of the endothelial surface. Such injuries may have occurred during the "handling" of the saphenous veins, i.e., during their surgical exposure, isolation, removal and storage prior to anastomosis. Whether these injuries may be related to or responsible for the premature occlusion of saphenous vein grafts is presently under investigation.

An SEM STUDY OF THE LICHEN *UMBILICARIA PENNSYLVANICA*. H. J. Arnott and Linda Lopez, Department of Biology, University of Texas at Arlington, Arlington, TX 76019.

Specimens of *Umbilicaria pensylvanica* Hoffm. (the rock tripe) were collected on rocks in the woods surrounding the JEOL office in Peabody, Essex County, Massachusetts. These plants were found in great abundance growing on the surface of large granite boulders which were common in the area. The plants were dry and brittle and could be dry fractured or fractured in LN₂. When viewed from above with the SEM the surface is composed of many polygonal areas about 100 to 200 μ m across. Fractured sections viewed in the dissecting microscope show compact surface zones, a thin green zone (algal) just below the upper layer, and a central white zone. The surface layers when viewed by SEM reveal extremely compact hyphae "cemented" together by an extracellular matrix; this zone contains very few airspaces which are 2.5 μ m in diameter when present. The white zone is composed of elongate hyphae of two types and separated by a large airspaces 10 to 20 μ m in diameter. Some hyphae are smooth, 2 to 4 μ m in diameter with thick walls. Other hyphae, slightly thicker in diameter, appear to be encrusted; both types have a small lumen and relatively thick walls.

HEXADECANE-INDUCED INTRACELLULAR MEMBRANES AND ULTRAMEMBRANE-BOUNDED LIPID-LIKE BODIES IN *ACINETOBACTER* CLW. Randall E. Dukes and James R. Stewart, Dept. Biology, University of Texas, Tyler, Texas 75701.

Acinetobacter strain CLW is an atypical member of a mono-specific genus in two attributes: it has a high G+C Mole percent and it synthesizes the lipid reserve poly- β -hydroxybutyrate (PHB). When grown with hexadecane as the sole carbon source, intracellular membranes (trilamellar) are present and electron transparent lipid-like bodies enclosed in ultramembranes are numerous in mid-log and stationary phase cells. Spectrophotometric measurements indicate that PHB is present in hexadecane-grown cells but not in amounts sufficient to account for the tremendous number of lipid-like bodies that the cells possess. The contents of the lipid-like bodies will be investigated. Comparative ultrastructural and spectrophotometric studies of crude-oil-grown cells are in progress.

CALCIUM OXALATE CRYSTAL PRODUCTION IN TWO MEMBERS OF THE MUCORALES. M. D. Powell and H. J. Arnott, Department of Biology, University of Texas at Arlington, Arlington, TX.

Calcium oxalate crystals are observed in association with the sporangia of *Mucor hiemalis* and *Rhizopus oryzae*. Crystal morphology in both species is consistent from generation to generation and their appearance on the sporangial surface occurs at specific times during the growth cycle. Morphology of the individual crystals varies from simple crystals consisting of single spines in *M. hiemalis* to complex crystals with twin spines on a common base in *R. oryzae*. During the growth of both species there appears to be an initial period where a layer of cell wall material covers the crystals followed by their eruption on the surface of the sporangia. The consistency of morphology and development suggests some form of biological control. Features of crystal development are compared and possible mechanisms are explored.

ELECTRON AND IMMUNOFLUORESCENT MICROSCOPY OF ISOLATED FROG URINARY BLADDER EPITHELIAL CELLS STIMULATED BY CALCIUM IONOPHORE AND ANTIDIURETIC HORMONE. A.J. Mia, L.X. Oakford and T. Yorio, Dept. Life Sciences, Bishop College, Dallas, TX 75241 and Depts. Anatomy and Pharmacology, Tx. Coll. Osteop. Med., Ft. Worth, Tx 76107

Calcium plays a key role in the regulation of the hydro-osmotic response of antidiuretic hormone (ADH) in amphibian epithelia. Isolated frog urinary bladder cells treated with the calcium ionophore, A23187, or ADH were found to have a loss of cell polarity with respect to apical and basal-lateral membranes. Microvilli were distributed around the entire cell surface as compared to the intact epithelium in which microvilli are only seen at the apical barrier. There is an indication that these morphological changes are associated with a redistribution and realignment of microfilaments and microtubules as revealed by fluorescent microscopy. Most dissociated cells labeled for actin and tubulin using fluorescein isothiocyanate (FITC) show a weak fluorescein labeling at the cell boundary. Ultrathin sections viewed in the transmission electron microscope also suggest that these cytoskeletal components undergo redistribution from the deeper region of the cytosol to one at the cell periphery. SEM studies reveal that some dissociated cells show membrane proliferation and a formation of elongated microvilli over the cell surface when exposed to A23187. The microfilament system is an essential component to the water flow response of ADH. The alterations in intracellular calcium by the ionophore could alter the microfilament-microtubule assembly-disassembly process thus modulating the actions of ADH.

MATERIAL SCIENCES

THE APPLICATION OF ENERGY DISPERSIVE X-RAY SPECTROSCOPY AND SCANNING ELECTRON MICROSCOPY TO THE ELECTRONICS INDUSTRY. Alan B. Weckerling, Director, Spectrum Laboratories, 2602 Electronic Lane, Suite 606, Dallas, Texas 75220. (214) 353-9150.

The Scanning Electron Microscope and Energy Dispersive X-ray Spectrometer have become indispensable tools for the electronic industry. Typical applications include failure analysis, production machinery calibration, plating thickness and problem solving, competitive comparisons, and process control confirmations. Several unusual as well as typical cases will be discussed.

SCANNING ELECTRON MICROSCOPY FOR THE PETROLEUM INDUSTRY. Alan B. Weckerling, Director, Spectrum Laboratories, 2602 Electronic Lane, Suite 606, Dallas, Texas 75220. (214) 353-9150.

The proper characterization of core samples for clay types and mineral chemistry is of vital economic importance to the petroleum industry. Once the minerals and clays present in a core sample have been identified by Scanning Electron Microscopy and Energy Dispersive X-ray

Spectroscopy, large amounts of money and effort are expended to complete a well or stimulate it for increased production. Improper chemical injection into a well will result in decreased production, no increase in the production rate, or a complete shutdown of the well.

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D.C. Benefiel, Analytical Services, Dow Chemical U.S.A., Freeport, TX 77541 (409) 238-1075

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THE WORKHORSE SEM IN THE MATERIALS LABORATORY

Craig C. Garrison, Analytical Services, Dow Chemical U.S.A., Freeport, TX 77541

The surface and Solid State Chemistry laboratory at Dow Chemical's Texas Operations facilities recently acquired a new scanning electron microscope (SEM). Careful consideration was given to the present and future role of the SEM at the time that this purchase was made. The instrument was equipped with EDX and solid state backscatter detectors, computer controlled stage axes and beam positioning. These options allowed the deferral of a large amount of work to overnight sessions during which the instrument works unattended. Also, new capabilities in elemental characterization and image analysis were added. The demand for SEM time has steadily risen in the two years since we obtained this instrument and we see future applications which will tax this time even further.

PHYSICAL CHARACTERIZATION OF SEMI-INSULATING PARTIALLY OXIDIZED SILICON (SIPOS). R.F. Pinizzotto, Ultrastructure, Inc., 1850 N. Greenville Ave - 140, Richardson, TX 75081, R.A. Bowling and J.A. Keenan, Texas Instruments, P.O. Box 225936, M.S. 147, Dallas, TX 75265, and Y.H. Kwark, Stanford University, Stanford, CA 94305.

Semi-insulating partially oxidized silicon films are presently being considered for use in high speed, high power bipolar device contacts. The stoichiometry and microstructure of these films can be varied over a wide range. One can thus tailor the electronic properties of the contacts by changing the bandgap, Fermi level, etc. We have performed detailed microstructural evaluations of several types of SIPOS films using transmission electron microscopy, Auger electron spectroscopy, electron spectroscopy for chemical analysis and Rutherford backscattering spectroscopy. TEM showed that the as-deposited material was homogeneous and amorphous. After annealing, the SIPOS consisted of small, isolated regions of crystalline silicon surrounded by SiO₂. There was no evidence of preferred orientation. Increasing the anneal temperature was found to increase the silicon particle size. The AES and ESCA measurements confirmed the TEM results. There were two distinct peaks that corresponded to pure Si and to Si in an oxide environment. The RBS data showed that the films were uniform in gross stoichiometry throughout their thickness. However, the silicon / oxygen ratio was found to vary with anneal conditions, indicating that the films were reacting with oxygen impurities in the supposedly pure N₂ anneal ambient. It will be shown that the results of the various characterization techniques are complementary, and that multiple approaches are needed to fully characterize a materials system.

APPLICATIONS OF THE SCANNING ELECTRON MICROSCOPE AND ENERGY DISPERSIVE X-RAY SPECTROMETER IN A COMMERCIAL LABORATORY. Alan B. Weckerling, Director. Spectrum Laboratories. 2602 Electronic Lane, Suite 606, Dallas, Texas 75220. (214) 353-9150.

The Scanning Electron Microscope (SEM) and attached Energy Dispersive X-ray Spectrometer (EDS) is utilized in a surprising variety of cases. The technology is essentially non-destructive which makes it the method of choice in many forensic applications where the sample is either impossible to replace or is protected by court order. SEM/EDS is used to analyze small samples, expensive materials, or total unknowns. A typical day's work might include an automobile accident, analysis of cement, assaying of precious metals, electronic component failures or quality control, inclusions in metal samples, verifying a whale's tooth, fire damage evaluation, core samples from an oil well, and particle size determinations.

SCANNING ELECTRON MICROSCOPY STUDY OF GOLD EMBRITTLEMENT IN SOLDERING PROCESSES. J. L. Marshall, Advanced Manufacturing Technology, Motorola, Inc., 5555 N. Beach St., Ft. Worth, Texas 76137.

When gold-plated leads are soldered with tin-lead, the brittle bimetallic of gold-tin develops. The development of this bimetallic in solder joints is studied by scanning electron microscopy/energy dispersive X-ray. The characterization and recognition of potentially gold-embrittled solder joints (both internally and externally), which may develop into poor electrical contacts, are explored.

CONVERSION FROM OIL DIFFUSION TO CRYOGENIC PUMPING OF AN SEM

A.B. (Sonny) McKinney, Jr., SEM Lab Engineer, Texas Instruments, Incorporated, Stafford, Texas
A Cambridge model S4-10 SEM was converted from an oil diffusion pumped vacuum system to a cryogenic pumped system. The mechanics of the conversion are discussed as well as necessary modifications to the automatic vacuum sequencing logic. Sample micrographs are included to illustrate the problem of vibration generated by the pumps and how we overcome the difficulty.

LOW VOLTAGE SEM OF INSULATING MATERIALS.

Marylyn Hoy Bennett and John F. Truett, Texas Instruments Dallas, Texas 75265

It has long been recognized that low voltage operation (less than 5KV) of the SEM eliminates sample charging of insulating surfaces and increases topographical contrast. However, the problems of low source brightness, increased chromatic aberration, and increased difficulty in secondary electron signal collection have been major limitations to low voltage observation. Recent improved microscope designs and sources have removed these limitations, and now the task remains to find the proper instrument parameters to survey various insulating surfaces.

In the semiconductor industry, fabrication of shrinking geometries on electronic devices has gone beyond the resolving power of the light microscope and necessitated the use of the SEM as an inspection tool. Many process steps employ insulating material for patterning, and it is important to observe wafers at different steps to characterize or inspect these steps. It is imperative to do this in a nondestructive manner so as to return the surveyed wafers back to the process line. Conventional SEM survey would require a conductive coating, thus removing wafers from the process flow and reducing lot yield. Only by using low voltage operation can this problem be avoided.

In our laboratory, we experimented with photoresist and PMMA, which are two common insulating surfaces in our industry. Using a scanning electron microscope with low voltage capability, we have varied instrument parameters and arrived at operating conditions for nondestructive survey. This method now provides us a simple and routine survey which will be presented.

OBSERVATION OF MAGNETIC DOMAIN STRUCTURES IN THE SEM

A.C. Campbell, MS&E Dept. University of Texas, Austin, TX 78712

The magnetic domain structure of a cobalt based permanent magnetic alloy has been observed in the SEM using type I magnetic contrast. Type I magnetic contrast is due to the Lorentz force interaction between low energy secondary electrons and the demagnetizing field outside the surface of the specimen.

Since the highest magnetic trajectory contrast occurs for a small number of low energy secondary electrons, two approaches were used to increase the type I contrast level. A low beam voltage of 5 to 10 KV was used to enhance the fraction of low energy secondary electrons. The second technique was to place a grounded aluminum cap between the specimen and the detector. The grounded cap served to suppress the higher energy secondary and backscattered electron signal, which increased the fraction of low energy secondary electrons in the total detected signal.

The domain structures observed were indicative of magnetization parallel to the c-axis (easy axis) of the sample, which was known to be nearly parallel to the surface of the specimen film. Various light and dark contrast ripples were observed in the domain structure. The ripple effect is explained by the fact that the c-axis of some grains was tilted a few degrees away from being parallel to the film surface. The tilt produced varying levels of closure flux along the surface of the film. Other film samples and domain structures are currently being examined with this technique.

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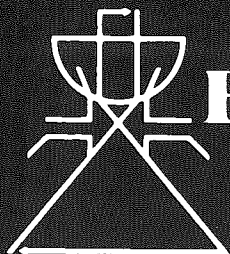
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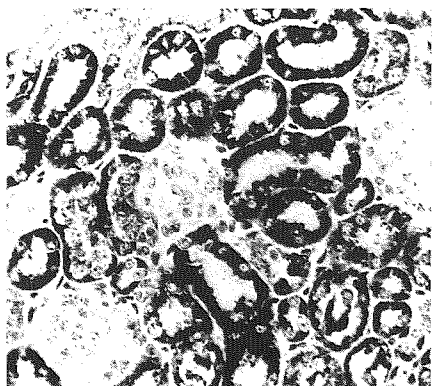
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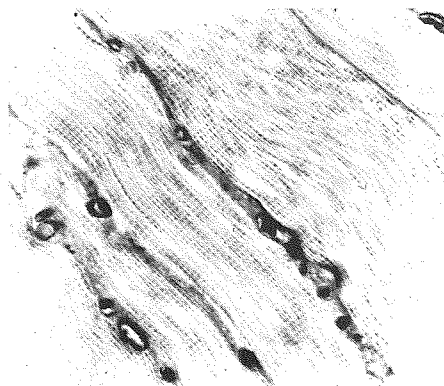
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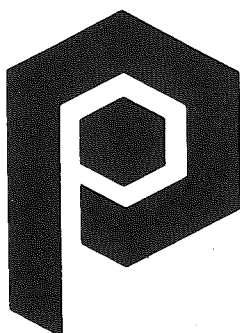
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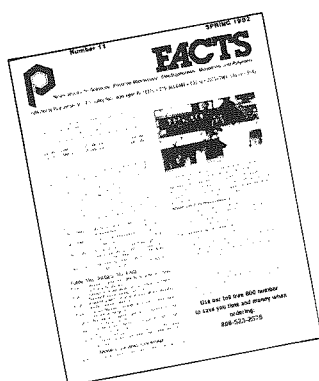
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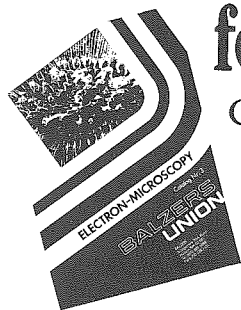
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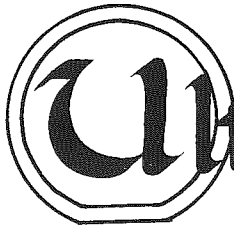
1. **Introduction to the Zeiss 9 Electron Microscope.** Details of the construction of the Zeiss 9S TEM. Produced by Carl Zeiss, Inc. (24 min)**
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9. **Critical Point Drying** (videotape or 16-mm film). A film by Walter Humphries providing both theory and practical advice. Produced by the University of Georgia, 1977. (22 min)**
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12. **Autoradiography.** Lecture by Albert Jones of University of California, San Francisco, on the theory of autoradiography at both light and EM levels. Produced by EML, UCB.† (Part A, 60 min; Part B, 6 min)**
13. **Autoradiography.** A laboratory demonstration by Ted Apple of the techniques discussed in Tape 12. Produced by EML, UCB.† (32 min)**
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15. **Glycol Methacrylate Embedding for Light Microscopy.** A laboratory demonstration of GMA embedding, sectioning and staining, by Richard Moe of the Botany Dept., UCB. Produced by EML, UCB.† 1980. (60 min)**
16. **Interpreting Transmission Electron Micrographs Three-Dimensionally.** A brief introduction to the conceptual problems involved in relating the two-dimensional micrograph to cell structure. Produced by Mark Pederson of the University of California, San Francisco, Scientific Illustration Curriculum. (6 min.)**
17. **Introduction to Freeze Fracture.** A discussion and demonstration of technique (using a Balzers 360 m) by Caroline Schooley of the EML, UCB staff. Produced by EML, UCB.† 1982. (80 min)**
18. **Critical Point Drying.** Theory and practice of critical point drying, by Albert A. Bartlett, Univ. Colorado, Boulder, 1975. (36 min)
19. **Weak-Beam Electron Microscopy.** 1977 EMSA tutorial covering both the theory and practice of this technique by John B. VanderSande, Massachusetts Institute of Technology. (50 min)
20. **A Lecture on Electron Channeling.** A useful lecture on the application of this technique to a number of problems in materials science by David Davidson, Southwest Research Institute, 1978. (48 min)
21. **Preparation of Macromolecules for Transmission Electron Microscopy.** An EMSA tutorial lecture by Henry S. Slayter, Harvard Medical School. (47 min)
22. **Preparation of Support Films for Transmission Electron Microscopy.** A lecture and demonstration by David G. Pechak, Bowling Green State University, 1980. (16 min)
23. **Basic Optics in Scanning Electron Microscopy.** A basic description of the design and operation of a scanning electron microscope, by Richard E. Crang, University of Illinois, Urbana, 1980. (40 min)
24. **Biological Procedures in Electron Microscopy** (videotape or 16-mm film). A brief history of light and electron microscopy followed by a detailed presentation of specimen preparation of biological material. The design and operation of a basic electron microscope completes the presentation, by Richard E. Crang, University of Illinois, Urbana, and Jack A. Ward, Bowling Green State University, 1971. (40 min)
25. **Smaller Than Life — The Riddle of the Virus** (16-mm film). College-level study explains the state of research on viruses and how close we have come to solving the riddle. Time-Life, 1966. (39 min)
26. **Electron Microscopy: Principles and Practices.** 34-mm slides or 10 filmstrips with cassette tapes covering a number of aspects of electron microscopy specimen preparation, x-ray microanalysis, etc., by Richard E. Crang, University of Illinois, Urbana, and Jack A. Ward, Bowling Green State University, 1975. (25 min/film strip)
27. **Operation of the JEOL JEM 100C, 100CX Transmission Electron Microscope.** David Cummings of the EML, UCB.† staff teaches alignment procedures and basic operating techniques. Produced by EML, UCB.† 1982. (Part A, 60 min; Part B, 15 min)**

40. **Gas-Solid Interactions.** A lecture by E.A. Kenik, Oak Ridge National Laboratory. From the 1982 HVEM Summer Institute, Argonne National Laboratory. (Pt. 1, 40 min; Pt. 2, 40 min)

- 51. Scanning Electron Microscopy. Pt. 1: Procedures for Viewing a Specimen.** A lecture on SEM procedures produced by Elizabeth Mathews of San Joaquin Delta College. 1982. (14 min)

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PURPOSE: The goal of the TSEM Journal is to inform members of the society and the Journal's readers of significant advances in electron microscopy, research, education, and technology. Original articles on any aspect of electron 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 Electron Microscopy. Acceptance by this Journal of advertisements for products or services does not imply endorsement. Manuscripts and related correspondence should be addressed to Randy Moore, Editor, TEXAS SOCIETY FOR ELECTRON MICROSCOPY JOURNAL, Department of Biology, Baylor University, Waco, Texas 76798.

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 EM techniques. It is understood that the submitted papers will not have been previously published. Accepted manuscripts become property of the TEXAS SOCIETY FOR ELECTRON MICROSCOPY JOURNAL 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.

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MANUSCRIPT PREPARATION: Manuscripts should conform with the following guidelines:

FORMAT: Submit an original and two copies of the entire manuscript, typed, double-spaced, on 8½ 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:

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

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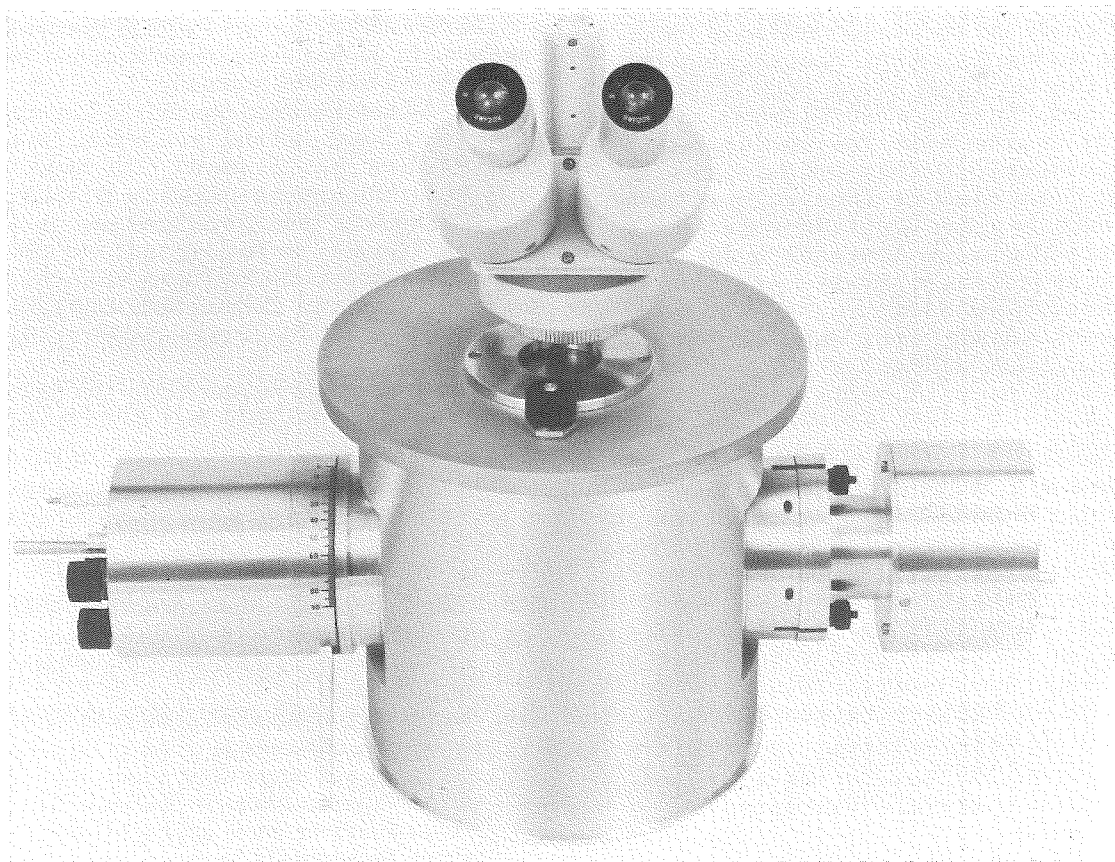
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An interesting question therefore, is what effect do ions or electrons of even very low energy have on the mobile sodium and potassium ions contained in biological materials? Similarly one should consider the sensitive large molecular chains in polymers or the intricate structure of DNA in chromosomes. In electron microscopy and other analytical and diagnostic processes, the whole field of organics may be questioned, if charged particles are used in the investigating process.

Obviously an electron microscope must use electrons and the interpretation of the image is made with due allowance being given for induced negative charges in the specimen as a result of the electron beam; but what about the specimen preparation techniques — for example when ions are used?

In TEM examination of ceramics, semiconductors, oxides, nitrides etc., ion beam milling is a well established technique. This important specimen preparation method is now being replaced with Fast Atom Milling. Specimen defects due to ion beam charge can now generally be disregarded. Further, the use of efficient cryo — “cool” stages — means that any heating effect of the atom beams can be drastically reduced if not eliminated.

For SEM applications a whole new field of specimen preparation becomes possible by the imaginative use of Fast Atom beams. Both selected small areas and large areas of specimens can be etched by neutral particles at carefully controlled angles. It is feasible gently to remove layer by layer of delicate organic and inorganic structures, clean surface contaminants or even atom mill to more deeply embedded structures within the specimen. The controlled and gentle atomic charge free etching process can be used for a whole variety of SEM applications. In fact, results of users to date indicate that the Fast Atom etching technique may well present some truly exciting possibilities in the use of the SEM.

These etching techniques can be carried out in purpose built machines, small modules or can even take place in situ within the electron microscope. Some new very small and powerful Atom sources have been developed which allow their direct incorporation into the specimen chamber. The neutral beam can etch insulating materials as effectively as conducting specimens.

The rapidly growing Semiconductor research and on line diagnostic investigating technologies are another important application for the Fast Atom techniques. The Saddle Field source can, in fact, be operated for long periods with many reactive gases, heavy halogen vapours etc. allowing reactive etching of, for example, silicon with respect to its compounds or other semiconductors and alloys, or allowing materials such as indium phosphide to be etched without stoichiometric imbalance. With non-inert gases, the beam may be molecular or contain radicals but still remain largely uncharged.

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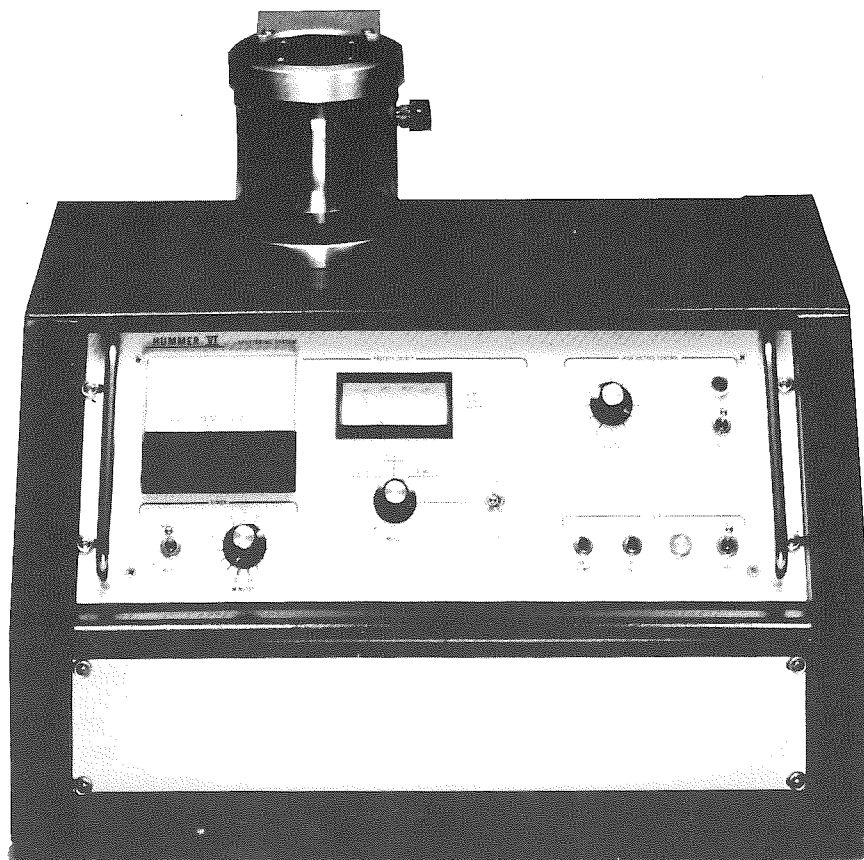
Thin films of gold, platinum, tungsten or heavy metal alloys produced by this technique become conducting at a few atomic thicknesses, which is essential if the latest high resolution SEM and STEM instruments are to be fully exploited.

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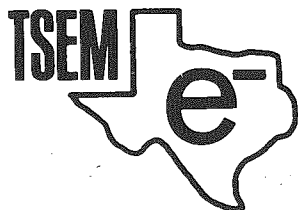
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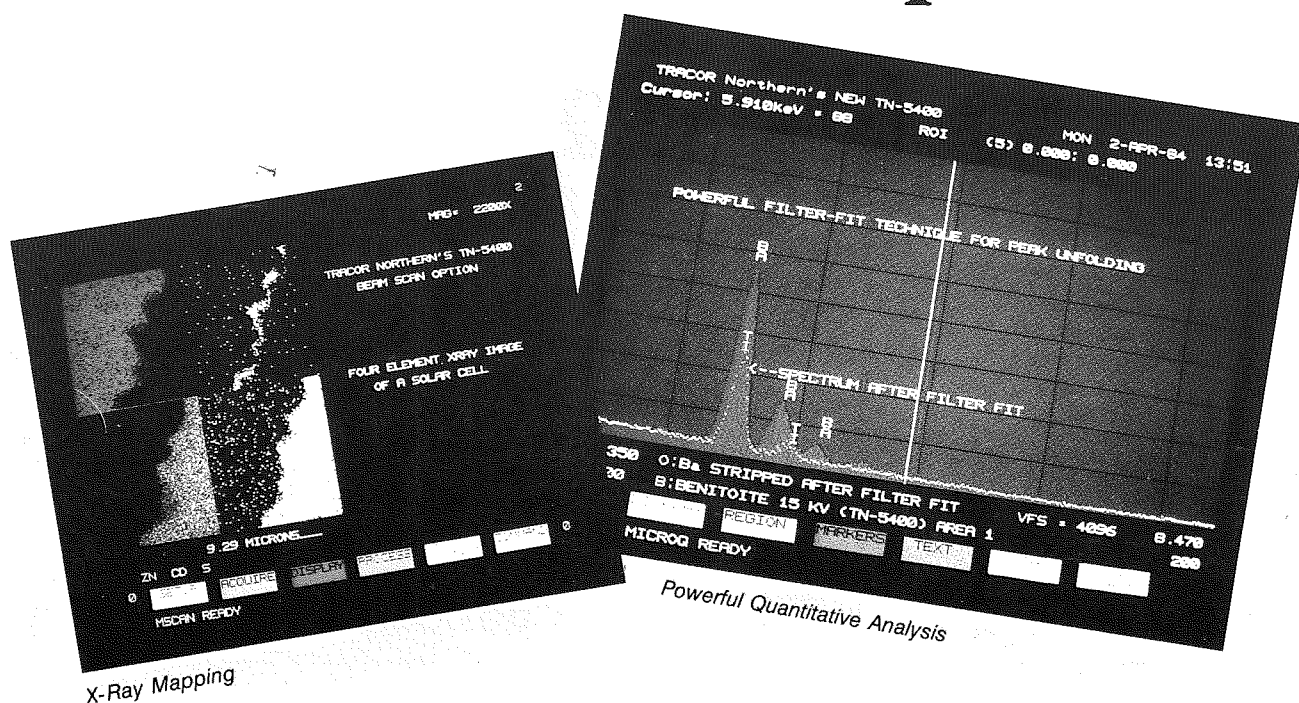
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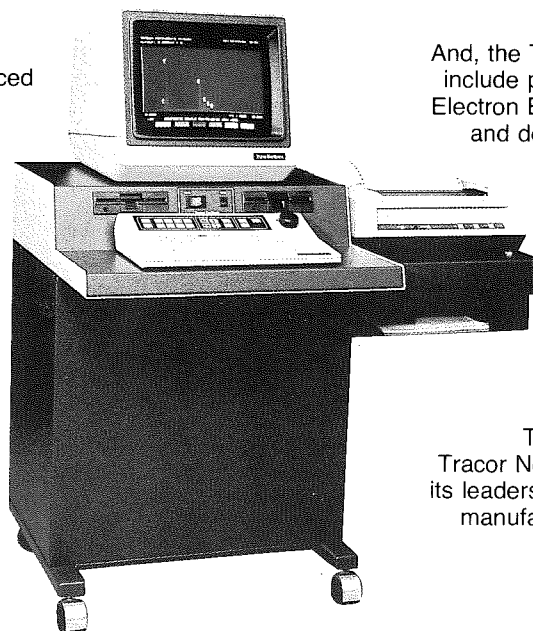
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Signature of EMSA Member making nomination

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Action _____

Remarks _____

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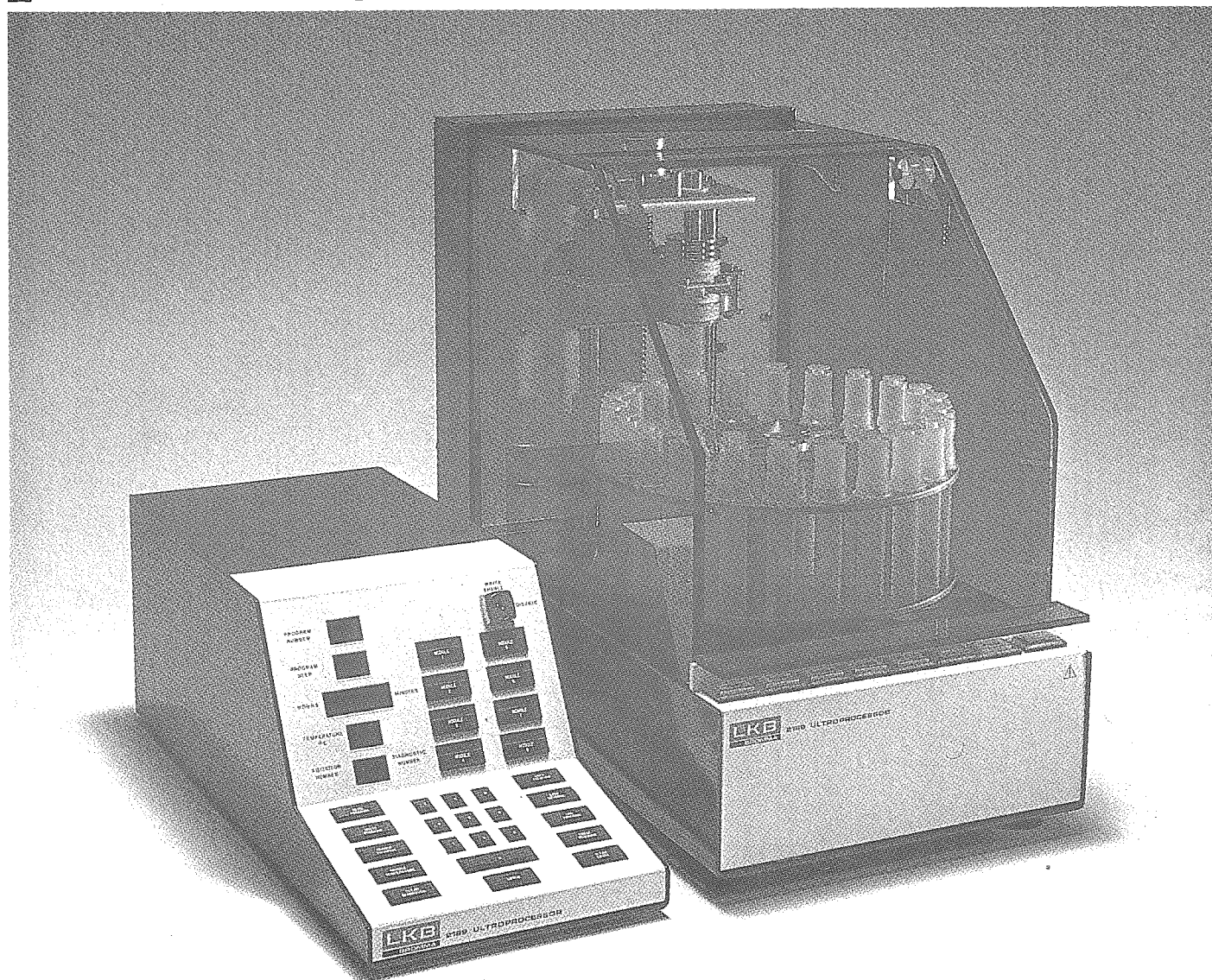
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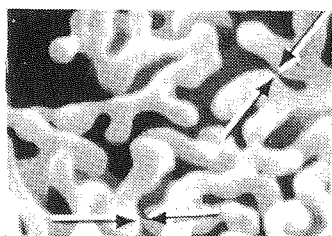
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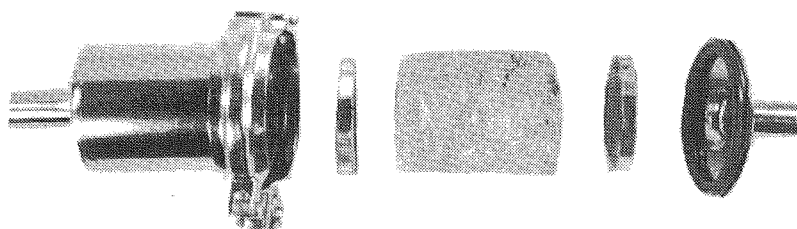


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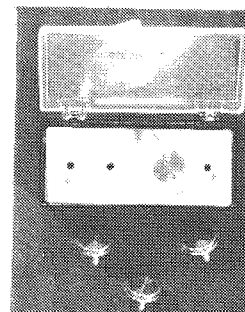
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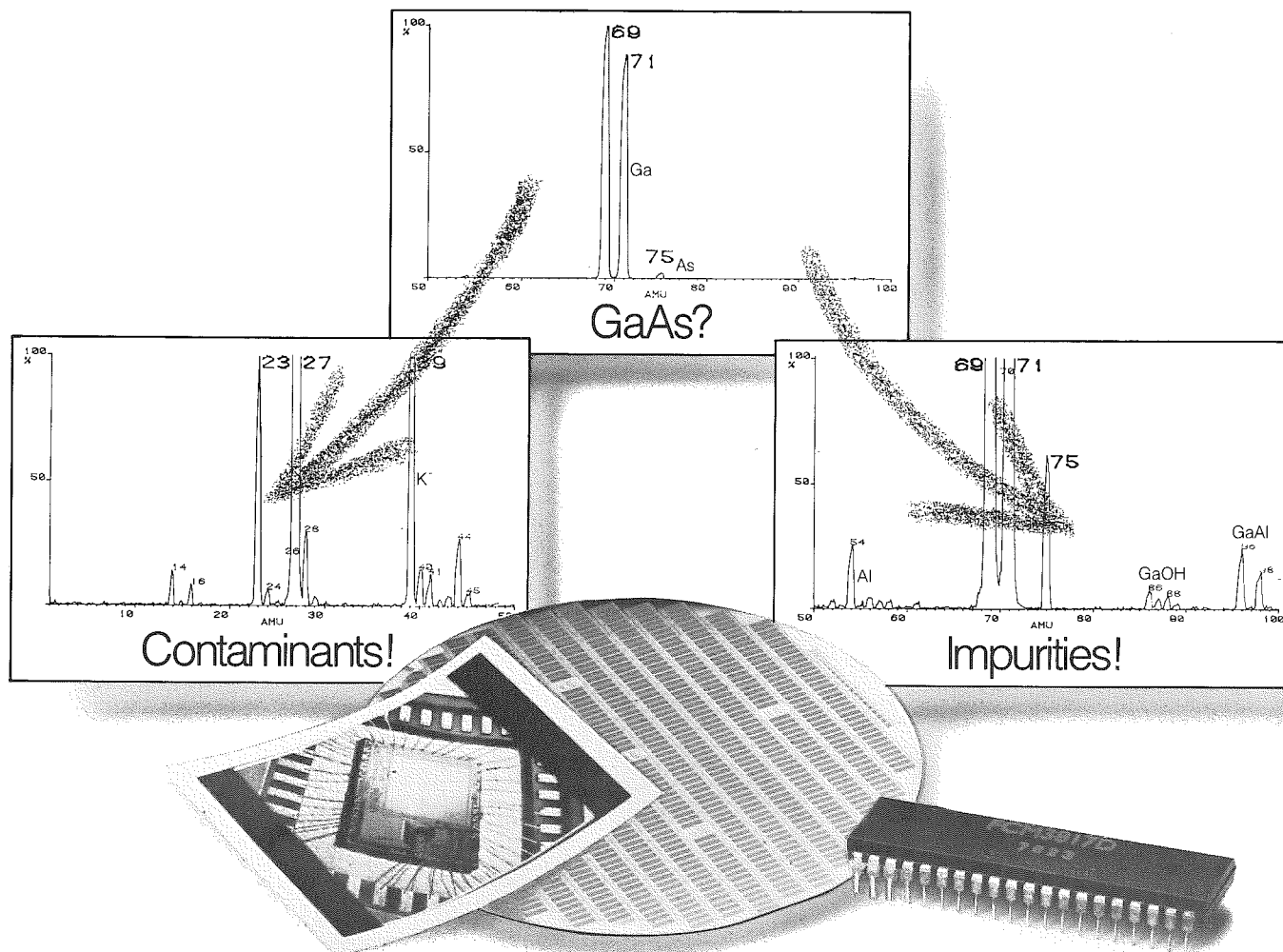
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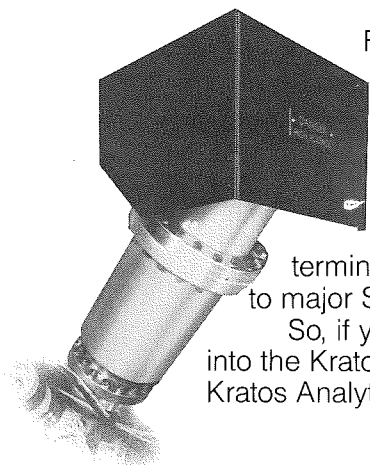
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