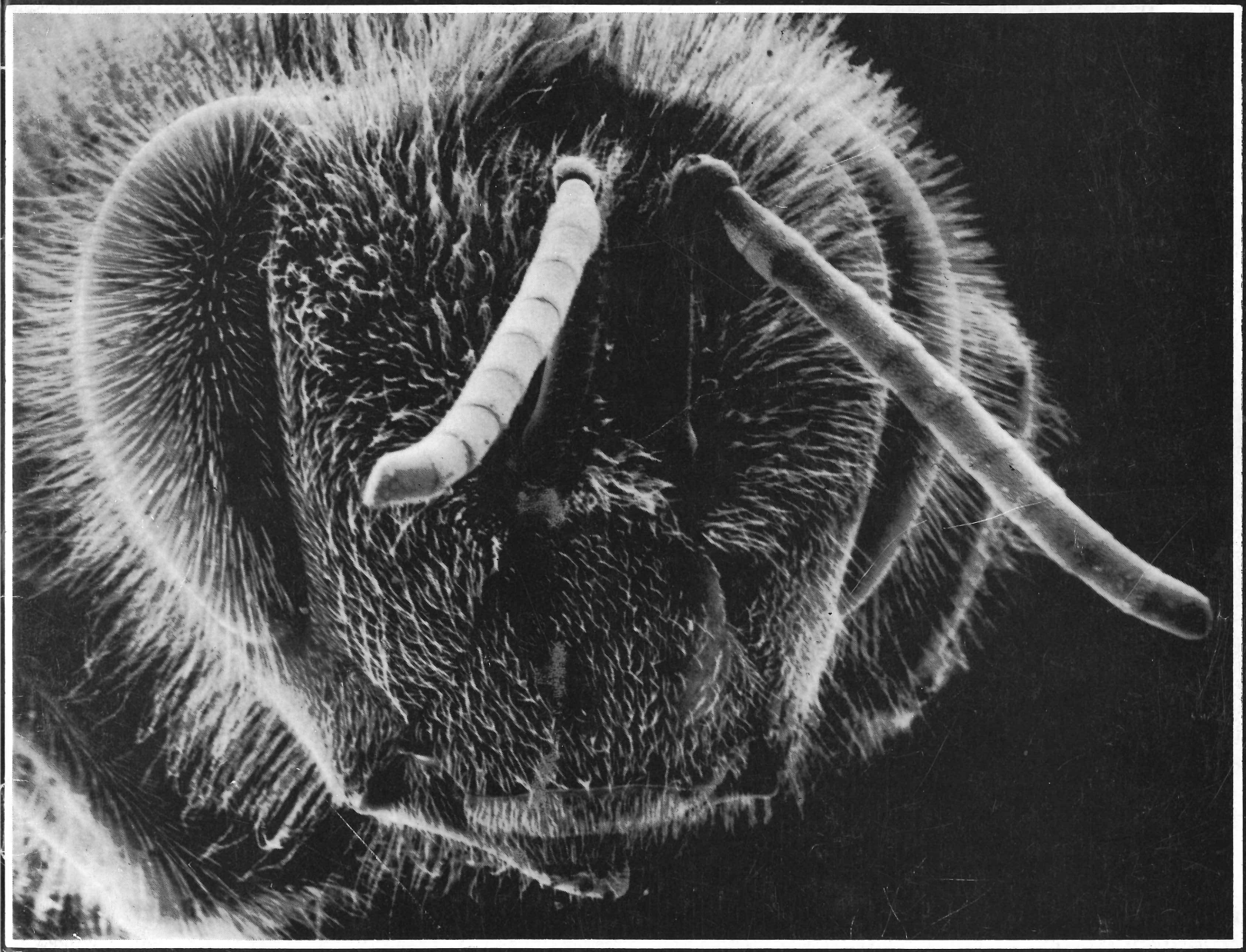
TSEM
Texas Society for Electron Microscopy
JOURNAL
VOLUME 16, NUMBER 1, 1985



# SPECIAL TWENTIETH ANNIVERSARY MEETING ISSUE

Abstracts Begin on Page 65

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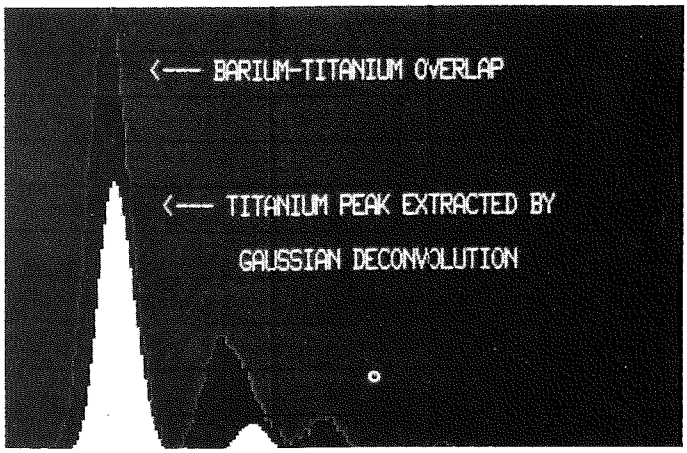


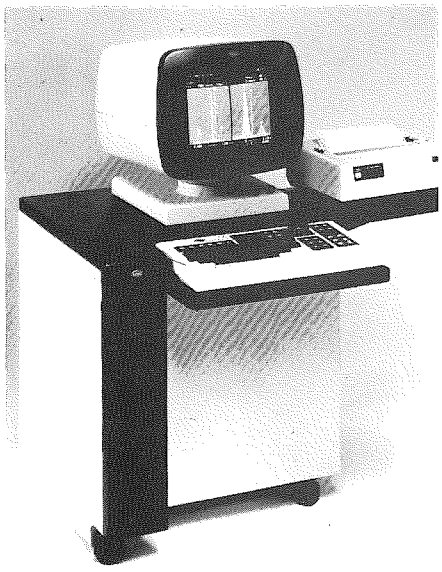

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TEXAS SOCIETY FOR ELECTRON MICROSCOPY
JOURNAL
VOLUME 16, NUMBER 1, 1985
ISSN 0196-5662

*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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### FRONT COVER

A scanning electron micrograph of the head of a common honey bee **(Apis mellifera)**. 40X. See related story beginning on p. 31.

### BACK COVER

A scanning electron micrograph of the posterior of a common honey bee **(Apis mellifera)**. Note the protruding stinger toward the bottom of the micrograph. 37X. See related story beginning on p. 31.

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

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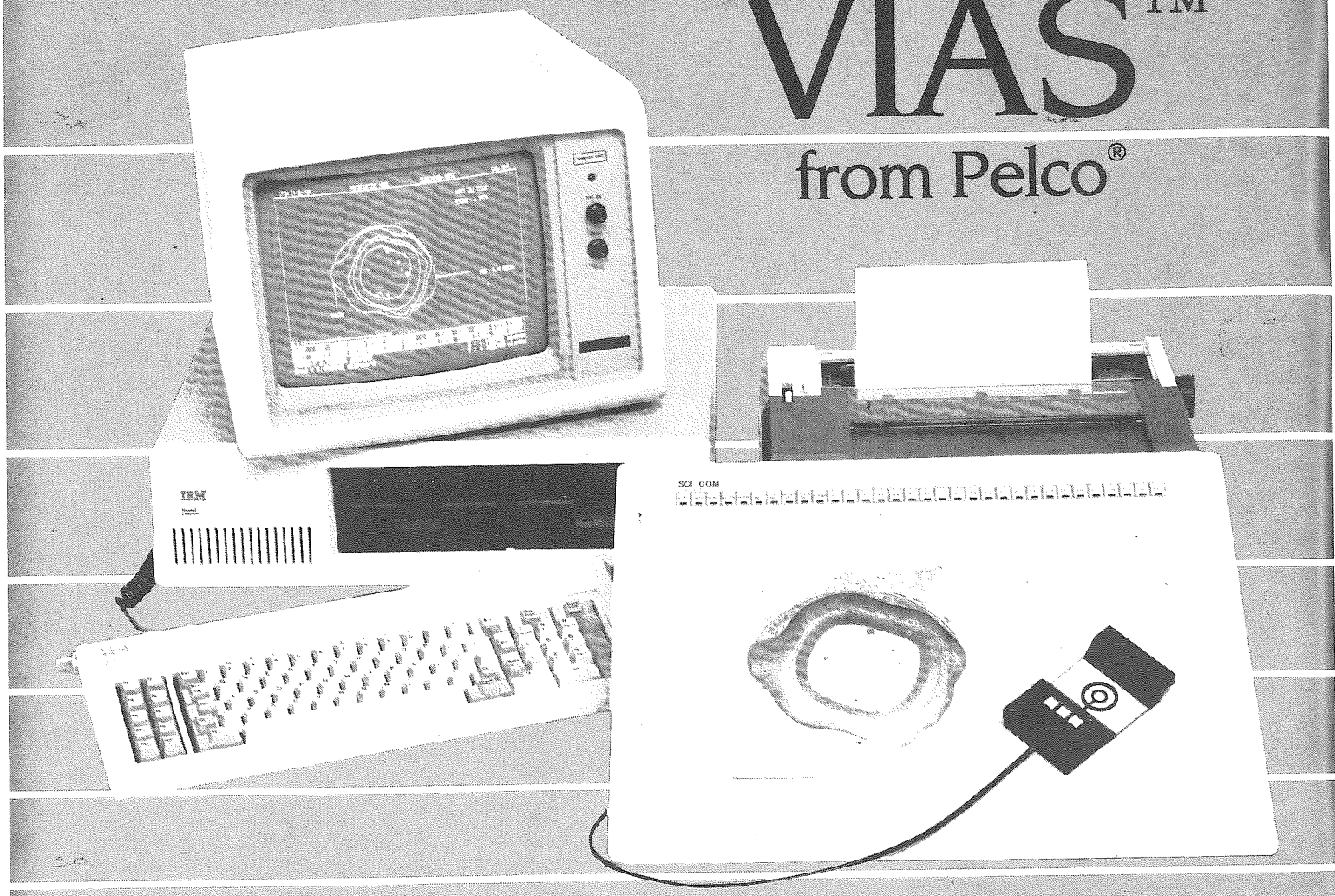


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# President's Message

It is with a combination of excitement and relief that I write this my last "President's Message" to the membership of TSEM. I'm excited because this letter will appear in the special issue of TSEMJ that will be available for distribution at our Twentieth Anniversary Meeting. At the same time, however, I must also confess that I'm relieved that this is the last letter to the membership that I'll have to write. Hilton Mollenhauer will be on the "hot seat" for next year. Good luck Hilton!

In regard to our Twentieth Anniversary Meeting, I'd like to take this opportunity to thank all the individuals who have worked so hard to make this meeting a success. In particular, Bob Blystone needs to be singled out for his work as Local Arrangements Person. Unless one has actually handled local arrangements for a meeting of this size it is difficult to appreciate the amount of time and effort that is necessary to insure that everything works as it should. Bob has certainly done an outstanding job for us and I hope that you will join me in thanking him for his work.

In addition to Bob, Joiner Cartwright, Hilton Mollenhauer, Bob Turner and Jerry Berlin have also contributed greatly to the anniversary meeting. Joiner got all our program announcements out on time, and Hilton helped with many of the important details of the meeting, thereby making my job much easier. Bob and Jerry have been involved in planning the banquet and contacting past TSEM presidents about the meeting.

As most of you are probably aware I am now completing my second year as President of TSEM. I served the extra year as a result of the fact that Allen Shannon was unable to complete his term as President. Although overall I have enjoyed my work this year, I am now firmly convinced that "back to back" terms as President are not desirable either for the individual involved or for the Society. Fortunately for me, however, I had the support of a fine group of officers and I'd like to personally thank them for their support and hard work this year. In particular I'd like to thank Randy Moore and Hilton Mollenhauer who were always available when I needed help and/or advice. Randy is completing his term as Treasurer this year, but will continue as Editor of TSEMJ. Hilton will, of course, begin his term as TSEM President following our anniversary meeting.

Overall I believe that TSEM has had a good 1984-85. Our Fall Meeting was a great success and should provide us with considerable impetous as we celebrate our twentieth anniversary. In particular I hope that a foundation was laid at Arlington for the continued successful interaction of more materials science people with TSEM. As you are aware, we had a special materials science session at Arlington, and have similar sessions scheduled for the anniversary meeting. To insure that this trend continues we must, however, have the support and cooperation of all parties involved. Obviously TSEM would benefit greatly from the active involvement of more of our friends in the materials science field.

During this year the officers of TSEM have worked closely with the Louisiana Society for Electron Microscopy in an attempt to organize a joint meeting in 1985. I'm happy to report that the details of this meeting have been finalized. LSEM will be meeting with us October 31 - November 2, 1985, at the Hilton Hotel in Beaumont, Texas. The Hilton is a beautiful hotel that has only recently opened. Leon McGraw is handling local arrangements for us and has been able to obtain a very attractive package for us. This should be an exciting meeting so I hope that you will atttend and represent our Society by giving a paper. I feel certain that we'll see a good turn out by our friends across the Sabine River.

At our Arlington meeting the Executive Council of TSEM recommended that we extend an invitation for EMSA to hold a meeting in Texas with us acting as host society. I have since extended a formal invitation to EMSA and am awaiting a reply. Hopefully our invitation will be accepted.

In closing I would like to thank our fine Corporate Members for their support of TSEM during my two years in office. They add so much to our Society by their participation at our meetings and also provide considerable financial support for our various events. Please make it a point to personally thank them for their support. Better yet, send your business their way! Your support of them will pay off for everyone in TSEM.

Thank you again for the opportunity and honor to serve as President of TSEM, the **best** local EM society in America!

Sincerely,

Charles W. Mims
President, TSEM

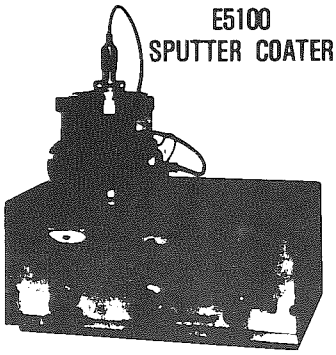
## Make plans now to attend this Fall's Joint-Meeting of the Louisiana and Texas Societies for Electron Microscopy

## OCTOBER 31 - NOVEMBER 2, 1985
## Hilton Hotel Beaumont, Texas

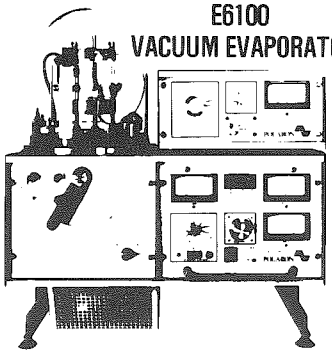
***Meeting information will be sent to all members late this summer.***

# YOUR WINDOW ON THE WORLD OF BETTER ELECTRON MICROSCOPY PREPARATION EQUIPMENT

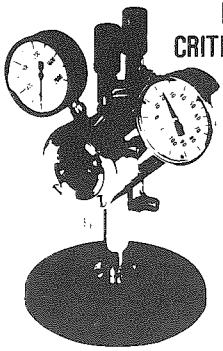
E5100
SPUTTER COATER



E6100
VACUUM EVAPORATOR



E3000
CRITICAL POINT
DRYER



E6000
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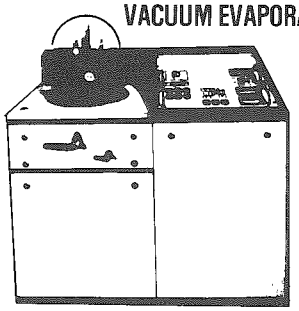

E5050
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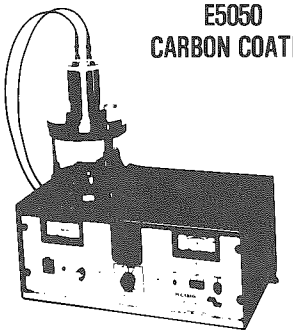
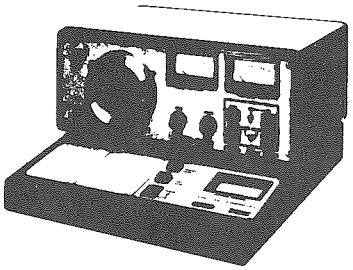
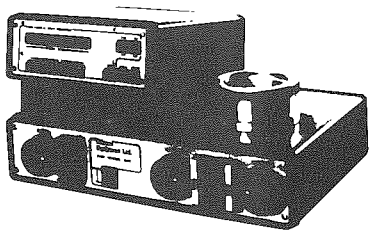


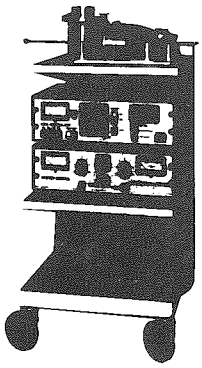
E2000
PLASMA ASHER



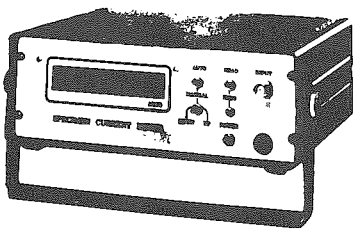
E9300
CP.D TISSUE DEHYDRATOR



E7300
S.E.M. CYRO
SYSTEM



E1100
SPECIMEN CURRENT METER



## FROM POLARON OF COURSE

For information on our complete line,
See us at the T.S.E.M. meeting in San Antonio, TX
or call us toll free on 1-800-524-8200 (except PA)
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# Secretary's Report

When President Mims asked me to take over as secretary after Wayne Fagerberg moved out of state, I knew the position would involve a bit of work. However, I didn't realize that the most difficult task would be maintaining the roster, the list of names and addresses of all 570 + members (or I should say "alleged members") of TSEM. The problem is that I don't know how many of these addresses are still valid. Everything I mail to the membership is sent via 3rd class bulk mail. If it cannot be delivered, it is generally not returned to me as is first class mail. Some school departments or companies will forward third class mail to members who have moved, but they do so at their own expense . . . for a while. So until you tell me that your address has changed, I just keep sending mail into trash cans. I also have a feeling that some people have moved out of state, or have otherwise allowed their memberships to lapse and for one reason or another the secretary has not found out about it. As a result, some of the roster is obsolete.

I have taken two steps to rectify the situation. First, since the roster has become too cumbersome to maintain by hand, I have gone electronic with it. I have designed, and had a friend write, a computer program that makes it much easier to add and delete names, change addresses, print alphabetized address lists or mailing labels sorted by zip code, keep track of whose dues are delinquent, and in general keep this kind of information in order.

The next step is to get the address information updated — to make sure I have your address correct and to drop all outdated addresses. For this I'll need your help. If you haven't already received it, you will soon be getting a questionnaire from me soliciting your correct address plus some demographic data that will tell us a little more about our society. As a result, mailouts will go more smoothly, names won't get lost so easily and we will be able to keep up with dues and records more easily. I will be asking you to, if at all possible, give me your work address rather than your home address. There are a couple of reasons for this. First, we would like to know where our members work and practice electron microscopy. Second, sometimes our corporate members wish to get in touch with members at a particular institution (e.g., to notify them of product demonstration), and they ask me for the appropriate names. If we have only your home address, we won't know that you are at that institution and you might be left out. I will also ask for phone numbers. These will not be given out to other parties unless there is a really good reason. I have had to call members recently and have had a difficult time doing it because I did not have your phone numbers.

Your society has put quite a bit of effort (and in the case of the computer program, some expense) into maintaining the roster. Use this questionnaire to help us update the address information, and we will see that you receive announcements and the journal in a timely manner.

Sincerely,

Joiner Cartwright, Jr.
Secretary

# JOB OPPORTUNITY

**RESEARCH ASSOCIATE IN ELECTRON MICROSCOPY** — The current projects are in the area of Neurobiology and involve the dissection of the intracellular membrane traffic in the assembly of the postsynaptic membrane at the neuromuscular junction. The model systems used are muscle cells and motor neurons in tissue culture, and the molecular interaction between these cells are examined using immunological and immunocytochemical probes. Possibilities for independent research are also available. Salary commensurate with qualifications.

For more information contact: Sherry Bursztajn, Ph.D., Department of Neurology/Neuroscience Program, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030, (713) 799-5991.

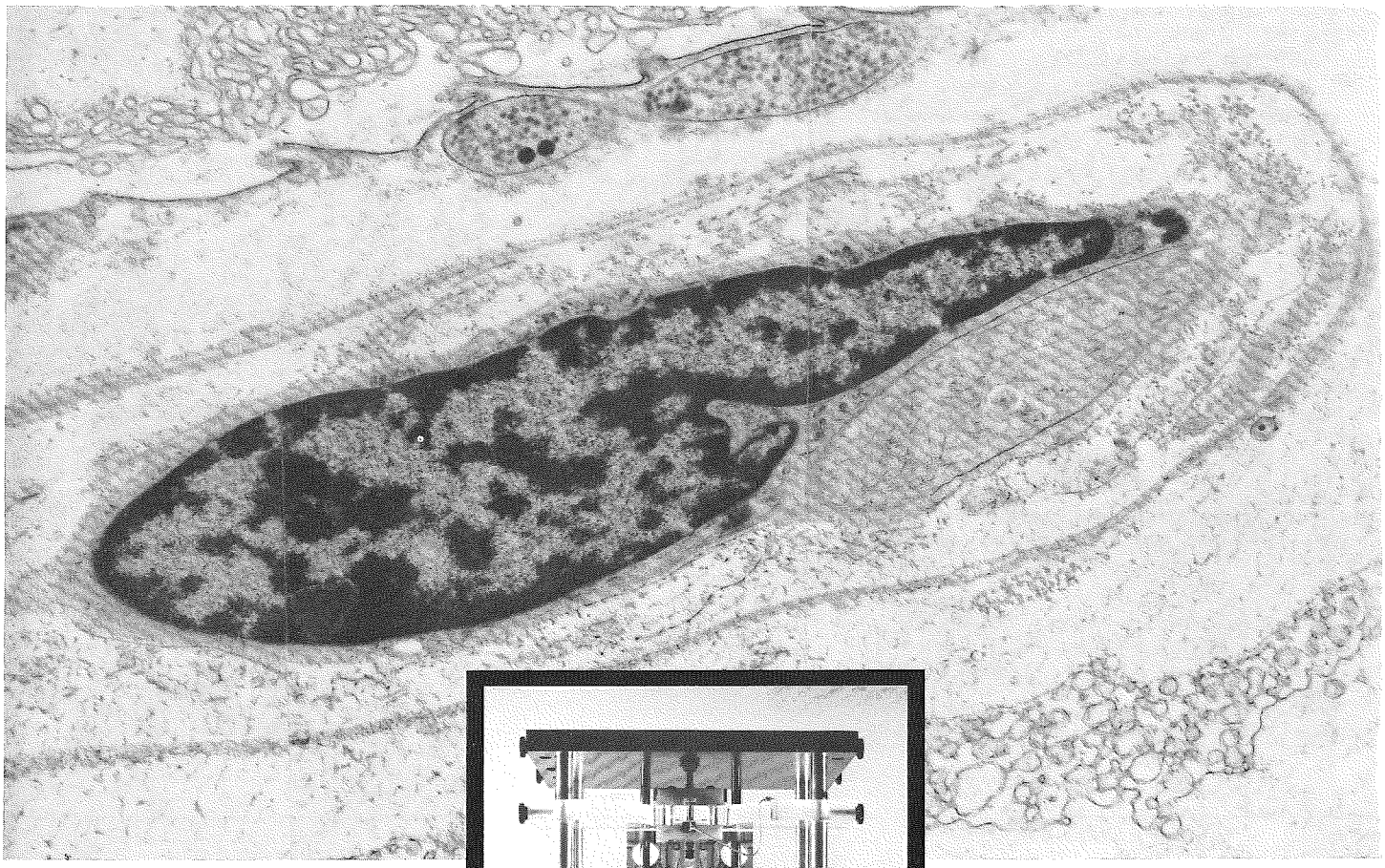
# DISCOUNT JOURNAL SUBSCRIPTIONS

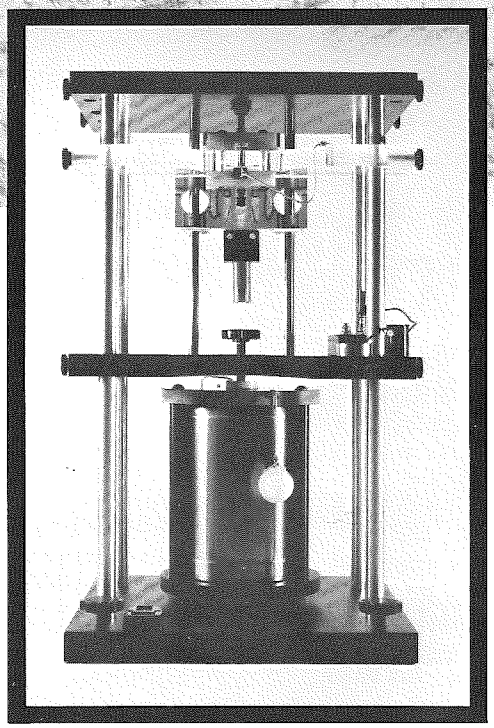
There has been some confusion on just how to subscribe to journals that offer discounts to EMSA members. In order to qualify for the discount, your order MUST be processed through the EMSA Treasurer, with checks payable to EMSA. The current list of journals that offer discounts, and the EMSA member's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

| | |
|---|---|
| *Bulletin Signaletique* | $ 72 |
| *Journal of Microscopy* | 100 |
| *Micron* | 35 |
| *Ultramicroscopy* | 60 |

# "Gentleman Jim"

## Quick Freezing By Bounce-Free Delivery





"Gentleman Jim" is the result of a deliberate attempt to reduce the excitement involved in working with cryogens. This new instrument uses the hydraulic/pneumatic technique of bounce prevention.

Quick freezing requires a high, continuous rate of heat transfer during sample solidification. This can be achieved by firm contact with a copper bar at liquid N temperature; it cannot, however, be achieved by a series of microsecond contacts interspersed with millisecond close approaches (i.e. bounces). By using a hydraulically coupled damper falling with the delivery system, "Gentleman Jim" is able to drastically reduce unwanted bounce.

The micrograph above is from a Torpedine ray electric organ. A "cuff" cell enclosing an axon is located between two electocytes. Electron dense, cholinergic synaptic vesicles are visible in the nerve terminals (x 15,000); 1 micron = 15mm. From the direction in which reticulation develops, one can deduce the direction of freezing.

In conclusion, "Gentleman Jim" is a simple quick freezing device which takes full advantage of liquid nitrogen.

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# Treasurer's Report

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| **ASSETS ON APRIL 26, 1984:** | | |
| Certificate of Deposit No. 64766, Univ. Natl. Bank, Galveston | $ 2,000.00 | |
| Certificate of Deposit No. 10-0475417, United Savings of Texas | 3,081.08 | |
| Certificate of Deposit No. 240-0064030, Republic Bank of Waco | 2,153.26 | |
| Checking Account No. 7914-448-1, Republic Bank of Waco | 3,737.59 | $ 10,971.93 |
| **RECEIPTS:** | | |
| Membership Dues | $ 1,044.00 | |
| College Station Meeting, Registration and Contributions[1,2] | 1,423.00 | |
| Interest | | |
| Certificate of Deposit No. 7914-448-1 | 154.94 | |
| Certificate of Deposit No. 64766 | 67.68 | |
| Certificate of Deposit No. 10-0475417 | 164.57 | |
| Certificate of Deposit No. 240-0064030 | 90.30 | |
| TSEM Journal | | |
| Advertising Revenue | $ 4,500.00 | |
| Subscriptions | 10.00 | |
| Secretarial refund from Wayne Fagerberg | 462.00 | $ 7,916.49 |
| **EXPENSES:** | | |
| College Station Meeting[2] | | |
| Coffee, Cookies, Room Rental | $ 482.18 | |
| Banquet | 825.56 | |
| Mixer | 116.00 | |
| Student Travel | 43.20 | |
| Presidential Expenses | 20.00 | |
| Secretarial Expenses | 1,700.00 | |
| TSEM Journal, Printing Costs for Vol. 15, No. 2[3] | 1,837.00 | $ 5,023.94 |
| **ASSETS ON OCTOBER 25, 1984:** | | |
| Certificate of Deposit No. 64766 | $ 2,000.00 | |
| Certificate of Deposit No. 10-0475417 | 3,245.65 | |
| Certificate of Deposit No. 240-0064030 | 2,243.56 | |
| Checking Account No. 7914-448-1 | 6,375.27 | $ 13,864.48 |

[1]Includes $150.00 donation by EDAX.

[2]Net loss on College Station meeting = $43.94.

[3]Net profit on Vol. 15, No. 2 of TSEM Journal = $263.00.

Respectfully submitted,

**Randy Moore, Treasurer**
Texas Society For Electron Microscopy

## EMPLOYMENT WANTED

Electron miscroscopist desires employment in the Dallas area. Masters degree in botany. Seven years as an electron microscopist. Two years as manager of a service EM laboratory. Familiar with a wide vareity of instruments and techniques. Has used the Hitachi HU-11 and HS-9, Philips 200, Zeiss 9 and 10, JEOL JSM 35-C with attached energy dispersive X-ray analytical probe, and Blazer's 301 freeze-etch device. Would prefer to work part-time. Contact Jane Ramberg at 405-787-8199 (Tu, Th, evenings and weekends) or 405-271-2031 (M,W,F).

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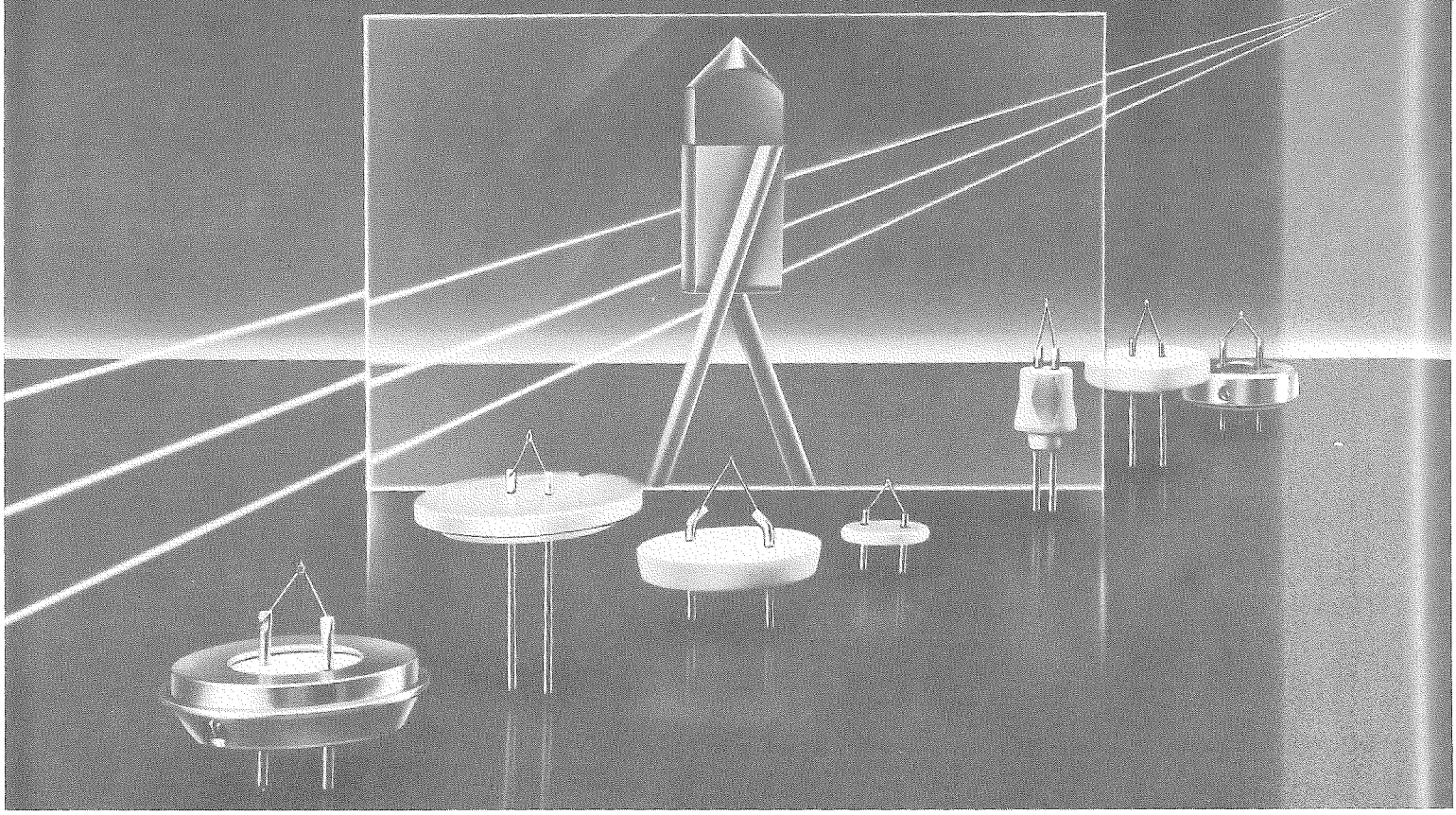

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DENKA manufactures the industry's purest, highest quality $LaB_6$ crystals for use in our cathode tips. We start with crystals larger than any other manufacturer can grow. Our high-quality cathodes represent the pinnacle of precision processing technology and quality control for the precision electronics industry. But quality also shows in the way the DENKA $LaB_6$ Cathode, Model 3, performs on the job.

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**STABILITY.** Thanks to its simple and durable construction, every DENKA $LaB_6$ Cathode, Model 3, offers a stability of better than 3 percent per hour at 1550°C, encouraging long-term test and research uses.

**INTERCHANGEABILITY.** There's no need to buy a new electron microscope just to enjoy DENKA quality. Among the many brands for which the DENKA $LaB_6$ Cathode, Model 3, is available are JEOL, ISI, Hitachi, Philips, Cambridge, Amry, Zeiss, Seimens, and many more.

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# TSEM Minutes

## MINUTES OF THE EXECUTIVE COMMITTEE MEETING

**Arlington, Texas**
**October 25, 1984**

The meeting of the Executive Committee of the Texas Society for Electron Microscopy was called to order by President Charles Mims on Thursday, October 25, 1984 at 6:30 p.m. in the Rush Creek Room of the Rodeway Inn in Arlington, Texas. Committee members present were: President Charles Mims, President Elect Hilton Mollenhauer, Treasurer & Journal Editor Randy Moore, Graduate Student Representative Elizabeth Richardson and Secretary Joiner Cartwright, Jr. Also present were: Jerry Berlin, Bob Turner, Leon McGraw, John Lange and Mary Jane Goad.

The minutes of the previous meeting were circulated and approved.

Future meetings of T.S.E.M. were discussed including the Spring 1985 meeting in San Antonio at which T.S.E.M. will celebrate its 20th anniversary. Calls for papers to be presented will go out the weeks of December 3 and January 7. Abstracts are to be submitted directly to the Journal Editor and must be in his hands no later than February 22, 1985. He will forward copies to the Program Chairman.

Bob Turner made a motion that a bar-b-que be held on Friday night of the San Antonio meetings at Banderra. This special outing would involve an extra charge of approximately $17.00 per person for bus transportation to Banderra and back to San Antonio Friday night and for food at Banderra. The motion was tabled pending input from absent members of the Executive Committee.

Leon McGraw reported on progress with plans for the Fall 1985 meeting in Beaumont to be held jointly with the Louisiana Society for Electron Microscopy on the weekend of October 23. The site and date for the Spring 1986 meeting is yet undetermined. The motion was made and passed that T.S.E.M. would offer to host the meeting of the Electron Microscopy Society of America in 1989.

President Mims recognized Howard Arnott's efforts in the local arrangements for this meeting (Arlington) and especially thanked Mary Jane Goad for her assistance. President Mims then recognized and thanked John Lange for his work in coordinating the participation of the materials scientists in this meeting.

In an emotional moment, John Lange presented President Mims with a teddy bear. The meaning of this remains unclear to the rest of the executive committee. It is uncertain whether President Mims slept with the bear that night.

The Treasurer's report was circulated and approved. Moore reported that six students presenting papers at this meeting applied for reimbursement. These students will be reimbursed the registration fee and half their hotel expense.

Mims reported that Mr. Frank Gibson and Edax Corporation is offering a prize of $100.00 to the student whose presentation is judged best at the meeting. After polling the committee, it was decided to present the prize at this meeting, but postpone a decision to present it at future meetings pending approval of the membership.

A nomination committee, chaired by the Secretary, was set up to nominate people to fill the offices of President Elect, Treasurer and Program Chairman Elect. This committee includes: Joiner Cartwright, Jr., chair; Jerry Berlin; Tom Levesque; Hilton Mollenhauer, and John Lange. Ballots will be mailed to the membership and these officers will be voted on prior to the Spring 1985 meeting in San Antonio.

Nine prospective new members were submitted by the secretary and it was voted to accept them all. They include one corporate member, two student members, and six regular members. President Mims read a letter from Hilton Mollenhauer moving that Dr. Keith Porter be made an honorary member of T.S.E.M. The motion was voted on and passed.

Randy Moore reported that the Journal actually turned a profit of $263.00 this last issue, but is in desperate need of more input and manuscripts from the membership if it is going to be continued.

The meeting was adjourned at 8:30 p.m.
Respectfully submitted,

Joiner Cartwright, Jr.
Secretary

## MINUTES OF THE GENERAL BUSINESS MEETING

**Arlington, Texas**
**October 27, 1984**

The general business meeting was called to order by President Charles Mims on Saturday, October 27, 1984 at 1:00 p.m. at the Rodeway Inn in Arlington, Texas.

President Mims thanked Program Chairman Howard Arnott and Mary Jane Goad for the arrangements of the Arlington meeting. He also recognized and thanked the commercial exhibitors and the members of the panel who selected the student award. Mims then thanked special speaker Grant Anderson for his talk.

The minutes of the previous business meeting were read and approved. President Mims then explained that the previous secretary, Wayne Fagerberg, had moved out of state and that Joiner Cartwright, Jr. had agreed to fill that office.

Hilton Mollenhauer read the Treasurer's Report since Randy Moore had to leave early.

Mims announced that the San Antonio meeting would be held prior to the SEM meetings in Las Vegas in hopes of

attracting people to go. He said that Lee Rudy and Bill Brinkley had agreed to talk and that there would also be two materials science speakers as well. There will also be a technical workshop and various social events. Jerry Berlin is to emcee the banquet.

Jerry Berlin said that he was going to present historical information on T.S.E.M. and asked for information and materials from the membership.

Mims announced that there will be a joint meeting with the Louisiana Society for Electron Microscopy in Beaumont in the Fall of 1985. Leon McGraw is in charge of the local arrangements for this meeting and said it would be at the Hilton Hotel and that there would be a wine & cheese party Thursday evening followed by Las Vegas style entertainment. There will be a seafood banquet Friday evening. Mims called for recommendations for speakers from the membership.

Mims then recognized and commended Randy Moore for his work with the Journal, but said that there was an urgent need for more papers. He said that the Executive Committee had agreed to discontinue the Journal in its present form unless there were more papers submitted. Hilton Mollenhauer reminded the meeting that the Journal is a refereed journal. Bob Burghart suggested that the Journal should ask for review articles. Mims said that although this had not met with support in the past, it could be tried.

Mims announced that a nominating committee, chaired by the secretary, had been set up and would submit nominations for the offices of President Elect, Treasurer Elect, and Program-Chairman Elect. There will, however, be a mechanism for members to submit write-in candidates. Ballots will be mailed to the membership and these officers will be voted on prior to the Spring meeting in San Antonio.

The business meeting was adjourned at 1:30 p.m.

Respectfully submitted,

Joiner Cartwright, Jr.
Secretary

# 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 in 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 philisophical 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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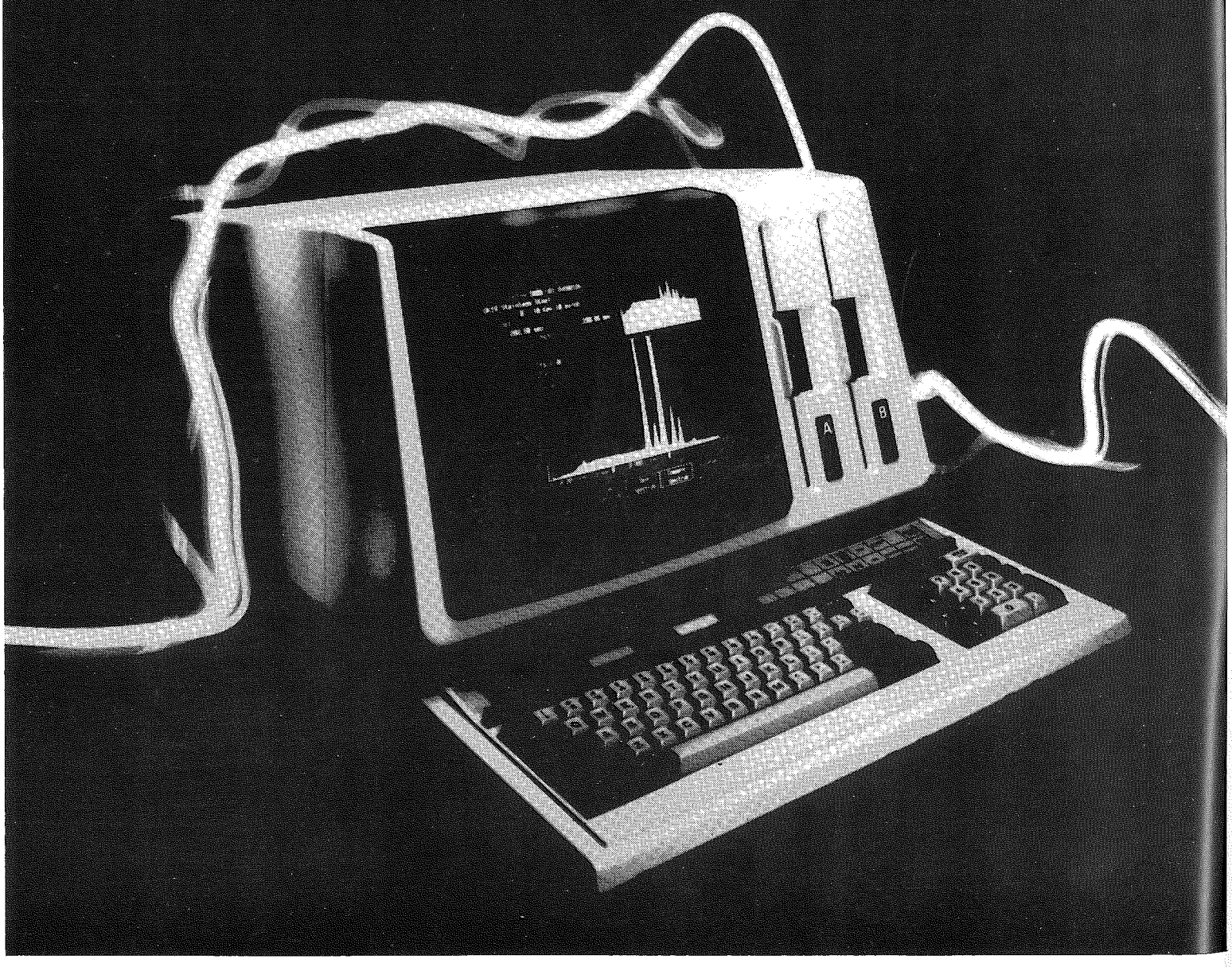
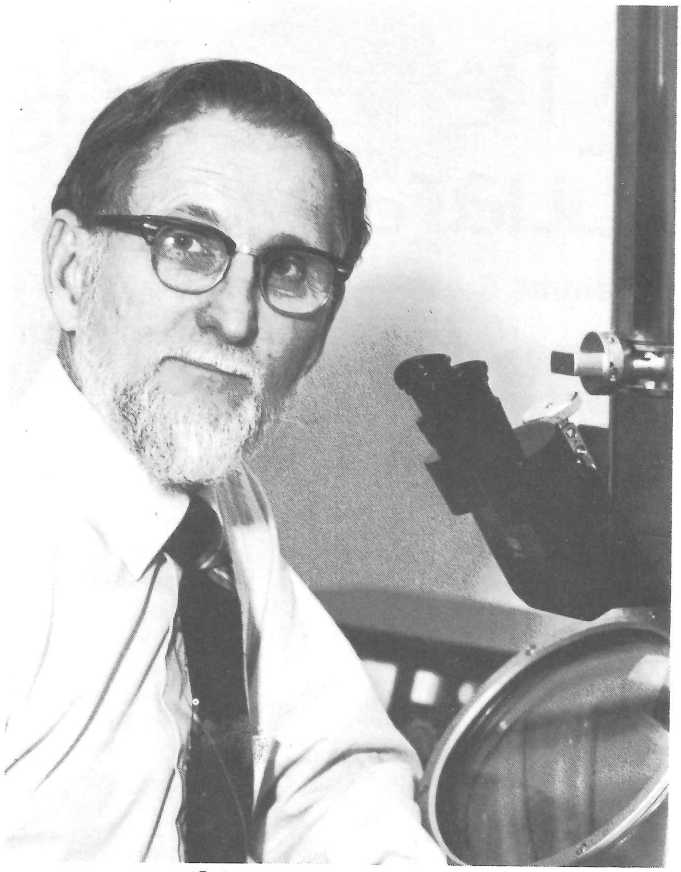


# TSEM SPOTLIGHT



**Hilton H. Mollenhauer**

Hilton H. Mollenhauer received a B.S. degree in electrical engineering from The University of Texas at Austin in 1948 and then took a position as an engineer at Southwest Research Institute in San Antonio, Texas. He returned to The University of Texas at Austin in 1953 and received M.S. and Ph.D. degrees in electrical engineering in 1954 and 1960, respectively. He remained at The University of Texas in the Cell Research Institute until 1965 when he moved to the Charles F. Kettering Research Laboratory in Yellow Springs, Ohio. In 1972, he accepted this present position at the Veterinary Toxicology and Entomology Research Laboratory in College Station, Texas.

Most of Hilton's research has revolved around the problems of cellular secretion, especially as it relates to the structure and function of the Golgi apparatus. In addition, he has maintained a strong interest in developing new techniques useful in TEM. In his current position, he is studying the effects of agricultural chemicals on cells and tissues of livestock. A particualar interest is the interaction between ionophorous feed additives and mycotoxins.

Hilton is associated with a number of professional societies including Sigma Pi Sigma, Sigma Xi, EMSA, ASCB, and TSEM. He has served as program chairman of TSEM and was local arrangements chairman for the last TSEM meeting in College Station. He is on the editorial boards of Protoplasma and Journal of Electron Microscopy Technique.

Hilton is married to the former Barbara Felsing of Austin, Texas. The other members of the Mollenhauer family include Paul (23), John (19), David (18), and two cats. Outside of professional activities, Hilton gardens and builds radio-controlled model airplanes.

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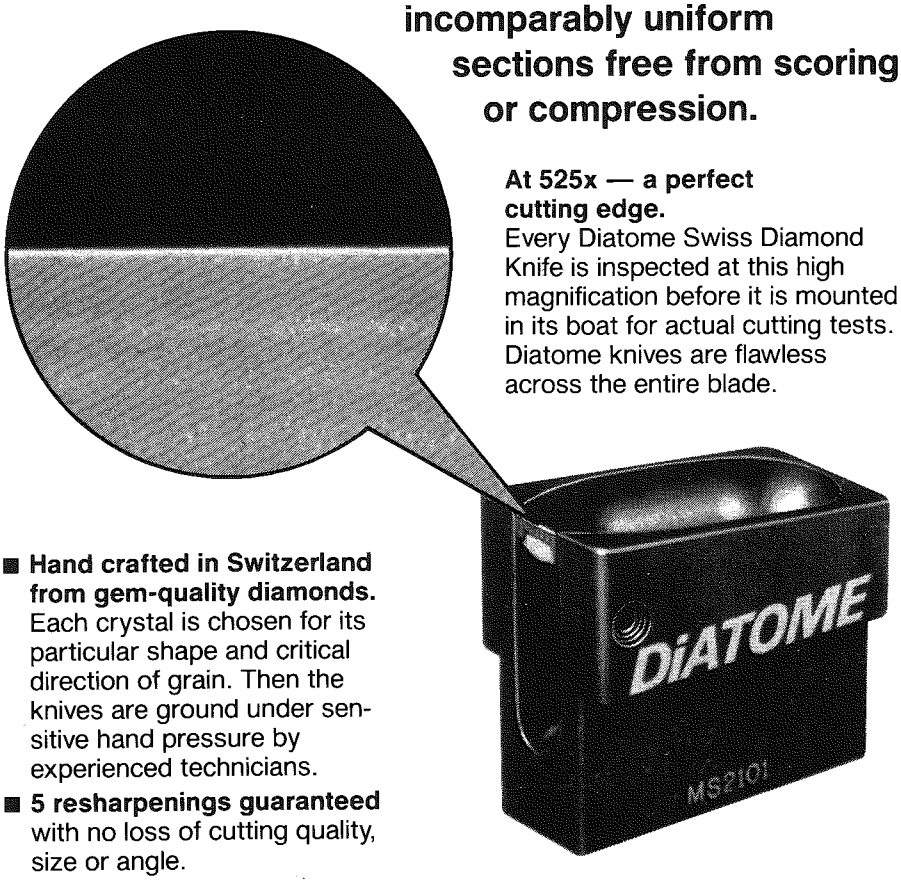


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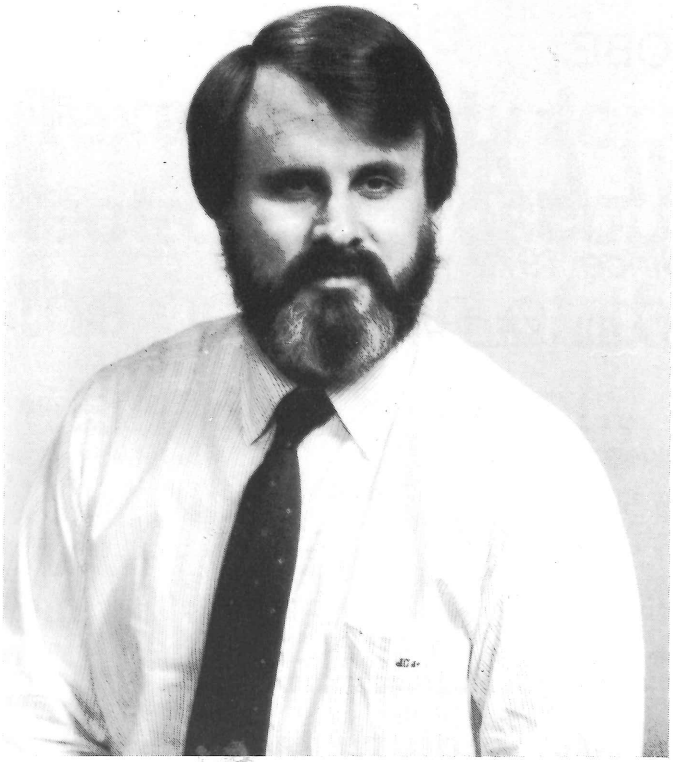

**Joiner Cartwright, Jr.**

I was born in Houston, but was raised in Beaumont. I received a B.A. degree in 1968 from the University of Texas in Austin, majoring in Microbiology. When I got out of college, the draft seemed imminent so I put off going to graduate school and took a job. It was this job, as research technician with Dr. Bill Brinkley at M.D. Anderson Hospital in Houston, that introduced me to electron microscopy. The draft dealt me a safe lottery number and I spent the next three years learning EM in Dr. Brinkley's lab. Our investigations of the mitotic spindle in mammalian cells **in vitro** resulted in two publications.

During this time I decided to return to graduate school. Dr. Brinkley took me with him to the Cell Biology meetings where I met Dr. John M. Arnold from the Pacific Biomedical Research Center of the University of Hawaii who was interested in developmental biology of cephalopod molluscs. Dr. Arnold was looking for a student, so in 1971 I joined the Zoology Department at the University of Hawaii. While I was in graduate school, I spent several years teaching undergraduate General Biology. I also spent most of my summers at The Marine Biological Laboratory at Woods Hole, where I participated in the Physiology and Embryology courses, as well as doing most of my dissertation work on ultrastructural aspects of cellular differentiation in the Atlantic squid, **Loligo pealei**. Besides the dissertation, this work resulted in two publications.

I received my doctoral degree in 1978 and in 1979 came back to Houston where I did a postdoctoral fellowship in Dr. Ann Goldstein's lab in the Department of Medicine at Baylor College of Medicine. I applied for and was awarded Muscular Dystrophy Association Fellowships for two years. While in Dr. Goldstein's lab I studied the cytoskeleton in rat heart muscle cells during development. This work resulted in four publications, presentations at meetings of the American Society for Cell Biology, the Biophysical Society of America, and the Texas Society for Electron Microscopy.

In March of 1984 I accepted the position of director of the electron microscope facilities in the Department of Pathology. This has been a very exciting job as I have had to learn a great deal in a short time. It has been a challenge too. "Special stains" and immunohistochemistry threaten to take some of the diagnostic work away from the electron microscopist. I consider it part of my job to increase the usefulness of EM in diagnostic medicine, especially with such techniques as X-ray microanalysis and immuno-histochemistry at the ultrastructural level. Electron microscopy in clinical diagnostic is my principal responsibility. However, I am also involved in research dealing with asbestos and the effects of asbestos fibers on human alveolar macrophages.

Other than my work, I have strong interests in photography, backpacking, travel (especially the Pacific Basin and the Orient), flying, and Texas history. Until now I have been single. However, that's going to come to a crashing halt in August when I marry Gloria Hui. Gloria is from Hong Kong and came to Hawaii for college and medical school. She just completed her residency in medicine at Baylor and is now doing a fellowship in Cardiology at St. Luke's Hospital in Houston.

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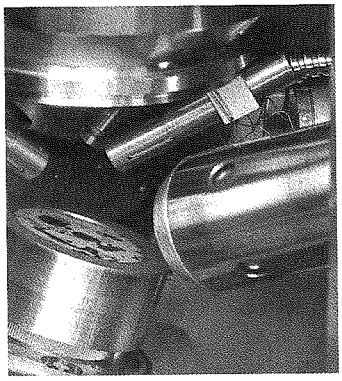

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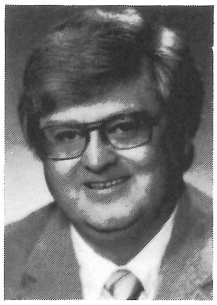
# Past Presidents

## SPOTLIGHT ON TSEM PRESIDENTS

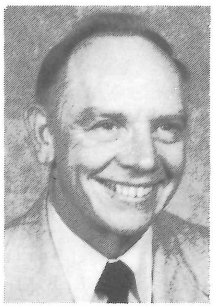
As part of our Twentieth Anniversary celebration an attempt was made to contact all past presidents of TSEM and personally invite them to attend and participate in the Anniversary Meeting in San Antonio. Enclosed with each invitation was a questionnaire designed to obtain updated information about our past presidents as well as some of their thoughts about TSEM. A listing of our past presidents is provided below along with some personal information about those who returned their questionnaires in time to meet the publication deadline for this issue of TSEMJ.

**Bill Philpott, President, 1965** — Current address is the Department of Biology, Rice University, Houston, Texas.

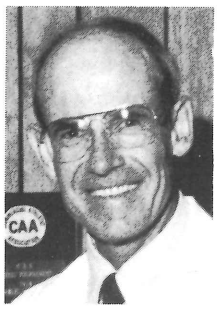
**Lea Rudee, President, 1966** — Lea is currently Dean of Engineering at the University of California, San Diego and Professor of Materials Science. His current research interest involves the use of EM to study the earliest stages of the development of adsorbed layers of plasma protein on artifical surfaces under well characterized flow conditions. As Dean, he has also been actively involved in the development of the Center for Magnetic Recording Research at UCSD which has thus far received commitments of over $9 million from various companies to establish a program in teaching and research related to magnetic recording. Lea had this to say about TSEM. "My most memorable recollection of a TSEM meeting was the first meeting. Bill Philpott and I were members of the same car pool and had both arrived at Rice in the same year to take over electron microscope laboratories in our respective departments. Bill had found the local chapter of EMSA a valuable activity when he was a Post-Doc and suggested we start one. I agreed to help and we called a first meeting. We got a list of names from several sources and found the first attendance to be at least twice what we had expected even in our most optimistic thoughts. I gave a review talk on the then rapidly growing field of the use of EM in the study of defects in crystals. Bill was elected President and I was elected Vice President. Things continued to grow very rapidly during the first two years. It is a source of great satisfaction to have been involved in the creation of an organization that has not only survived, but flourished."



**Daniel K. Roberts, President, 1967** — Dan is Professor and Chairman of the Department of Obstetrics and Gynecology at the University of Kansas School of Medicine in Wichita, Kansas. Dan also holds the rank of Professor in the Department of Pathology at the Medical School and is Chief of Ob-Gyn Service at the Wesley Medical Center in Wichita. According to Dan the Wesley Medical Center has 20 residents and 30 staff physicians. It includes an EM facility of more than 2,000 square feet run by Nola (Bushy) Walker. Dan recalls the following humorous "tidbit" regarding TSEM. "A memorable and somewhat humorous event occurred at the TSEM meeting in Galveston (1967 + yr) when Dr. Ralph Wynn was our guest speaker. We had a cocktail party at a Polynesian place on the way to the causeway. Ralph reminds me every time he sees me that I drove off and left him and he was "forced" to get one of the several young ladies remaining to take him home. I do not remember this but he keeps reminding me."



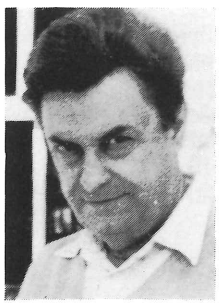
**Don Benefiel, President, 1968** — Don is currently Project Leader for Dow Chemical USA in Freeport, Texas. Don reports that he is still actively involved in EM work with heavy emphasis on microbeam analysis. He is using electron microprobe techniques to solve both research and production problems. Don's most memorable TSEM meeting was held at Gamboa Cay in Galveston. "At this meeting our guest speaker, the eminent Dr. Russell Barnett, was initiated as Witch Doctor of Gamboa Cay, with appropriate ceremony, including transportation around the premises on a "throne" carried by native bearers." His most memorable executive committee meeting was one called to order by Bill Philpott aboard a fishing boat some 75 miles offshore from Galveston.



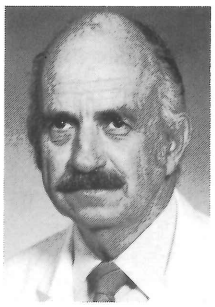
**Bob Yates, President, 1969 —** Bob is Professor and Chairman of the Department of Anatomy at Tulane Medical School in New Orleans, Louisiana. His research interests center on studies of the carotid body at the EM level. He and Dr. I-li Chen are attempting to identify specific nerve endings on carotid body cells using immunocytochemical techniques. Bob is also currently Secretary-Treasurer of the Association of Anatomy Chairman. Bob had this to say about TSEM. "The TSEM is an outstanding organization; it has national visibility due to the interest and enthusiasm of its members. It is always cited and spoken of as **The** example of a local society. There is good leadership among the group as well as excellent student participation. I will always remember the first meeting at Rice University with Dr. Philpott as the organizer. Although all of us who attended were enthusiastic about such a society, I doubt seriously whether anyone suspected that it would emerge as a leading example."

**Joe Wood, President, 1970 —** Joe is Professor of Neurobiology and Anatomy at the University of Texas Medical School in Houston. He returned his questionnaire from Germany where he is on a faculty leave. He is currently involved in EM and cytochemistry of the nervous system. His most memorable TSEM meeting was the first Bandera meeting. He didn't send a picture with his questionnaire but he reports that he is "as handsome as ever"!

**Ward Kischer, President, 1971 —** Ward is currently Associate Professor of Anatomy at the University of Arizona College of Medicine in Tucson. He is involved in both teaching and research and is interested in sequelae of deep injury, scarring and wound healing. His favorite meeting was at the Mayan Dude Ranch — apparently he had to "hide the liquor" to keep it from being stolen.



**Dimitrij Lange, President, 1972 —** As Professor of Biology he is actively involved in teaching and research at the University of Texas at Dallas. His favorite meeting was at Bandera because of the combination of "good science and good company".



**Robert A. Turner, President, 1973 —** Bob is chief of the EM section of Surgical Pathology at Scott and White Clinic in Temple, Texas. Bob writes the following about TSEM: "It has been a personal satisfaction to see and enjoy the growth of a small society to the country's largest. TSEM is not the oldest in the country but without doubt it is the greatest and the most active. Many local societies have patterned themselves from TSEM; especially their newsletters. It's a fond treasure of memories of all the friends and acquaintances I've made through TSEM". Bob has attended **every** TSEM meeting and has fond memories of all. He did have, however, single out a meeting at the Menger as being very special. This meeting had the first commercial exhibits and apparently the hotel's transformer was "blown" as a result of all the equipment. It is also worthwhile to note that Bob was the first president to be elected on a "milk and cookies" platform!

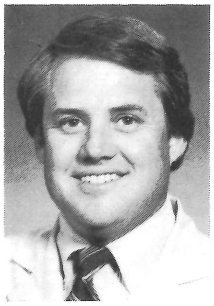
**Terrell R. Hoage, President, 1974 —** Terry is Professor of Biology at Sam Houston State University in Huntsville, Texas. Terry normally teaches 12 credit hours per semester and is Director of the EM lab. His research centers on toxic effects on cell cycle mechanisms. Terry's favorite meeting was the one at Waterwood that included a special panel discussion on grantsmanship.

**Jerry Berlin, President, 1975 —** Jerry is Professor of Biology in the Department of Biological Sciences at Texas Tech University in Lubbock. His major research interests at this time include the initiation and development of the cotton fiber (which is a single cell), the effect of stress (water deficit) on crop plants, the effect of diet and selected minerals on hepatocyte structure, and the use of steriological analysis to study all of the above. Jerry's most memorable meeting was at Waterwood where he and his students shared a three bedroom house and had a ball at the "casino night". This was also the site (according to Jerry, anyway) of his second convincing tennis victory over Ward Kischer who apparently has not challenged Jerry since. Jerry adds "I continue to enjoy the meetings and relish the friendships gained through TSEM. It is an excellent vehicle for graduate student presentations, especially in this day of poster presentations at national meetings."

**Bill Brinkley, President, 1976 —** Bill is Professor and Director of the Division of Cell Structure and Function at Baylor College of Medicine in Houston, Texas. His research involves the cytoskeleton with special emphasis on microtubules, tubulin assembly and mitosis. He and his co-workers are attempting to isolate and characterize MTOCs in eukaryotic cells. Like Terry Hoage, Bill's favorite TSEM meeting was the Waterwood meeting. His comments about TSEM are as follows: "I believe TSEM has evolved into the most successful state EM society in America. It seems to fit the needs of both scientists and technicians — I hope it continues to flourish."

**Larry Thurston, President, 1977** — Larry's address is Rt. 3 Box 288, College Station, Texas 77840.

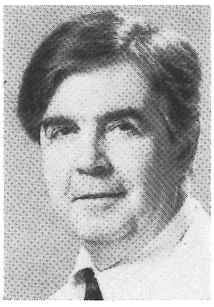
**Ivan Cameron, President, 1978** — Ivan is currently Professor of Cellular and Structural Biology at the University of Texas Health Science Center at San Antonio, where he is active in teaching and research. In particular he is interested in events in the cell cycle, loss of control of cell proliferation as occurs in cancer, and interactions between water, ions and cell architecture. Regarding TSEM, Ivan writes ... "TSEM enables frequent, close and valuable contacts with others interested in cell biology and analytical EM methodology. It is also a good place to meet and recruit new graduate students and research associates. My former graduate students all gave their first reports at TSEM." Ivan also reports that he's seen most of Texas by simply driving to TSEM meetings.



**William B. McCombs, III, President, 1979** — Bill is director of a large multispecialty clinical microbiology and immunology laboratory at Scott and White Clinic in Temple, Texas. He is also Director of the Human Tumor Cell Center at Scott and White. His research interest includes the isolation and characterization of human tumor cell lines. Bill had the following to say about TSEM. "I have nothing but good memories for many TSEM meetings. The times in San Antonio, Arlington, and Bandera are the most memorable. Perhaps the most memorable were those during graduate school. Jerry Berlin would crowd all of his graduate students into cars and would make the "long drive" to different meetings. I say "long drive" since Lubbock is a long way from anywhere. Of these, the most memorable was the 1971 meeting in Arlington. This was my first formal presentation. As a result of this meeting, I was offered a position at Scott and White, where I am presently employed."

**Ann Goldstein, President, 1980** — Ann holds the rank of Associate Professor in the Department of Medicine and Cell Biology at Baylor School of Medicine in Houston, Texas. She is also Director of the Seymour Lieberman Laboratory for Electron Microscopy in the Section of Cardiovascular Sciences. Ann summarizes her research interests as follows. "My major interest is the structure of protein filament assemblies in normal and developing cardiac and skeletal muscle. Using electron microscopy and optical diffraction and reconstruction techniques we have studied the substructure of the Z lattice in intact muscle. We have compared normal and widened Z bands and have defined a common subunit consisting of axial and connecting filaments. Three-dimensional models of the Z band have been built and tested. We have shown that the Z lattice may not be as rigid as previously thought, even at rest length. Our latest model can account for a short range response to tension or torque or both within a myofilament bundle and can accommodate long-range changes in axial interfilament spacing as in shortening or stretching." In regard to TSEM she writes ... "The diversity of our group is a strength. The friendliness and helpfulness of our society create an atomsphere for the fun side of scientific endeavors. Our talented members set standards of excellence and opportunities for public presenation for our student members. Here's to another decade of an even stronger society. Remember — THIS IS TEXAS!"

**Paul Baur, President, 1981** — Paul is owner of Teems Company Plexiglas Fabrications. His address is 3319 Avenue R. in Galveston, Texas. Paul reports that all TSEM meetings were memorable and that he wishes to convey his fondest regard and best wishes to all his TSEM associates. You'll be interested to know that Paul and Carol Baur are the proud parents (as of November 15, 1984) of Brian Blacet Baur. Paul reports that the little guy is "perfect".



**Bruce Mackay, President, 1981** — Bruce is Professor of Pathology at the University of Texas, M.D. Anderson Hospital and Tumor Institute in Houston, Texas. As a surgical pathologist he is involved in routine diagnostic work by light and electron microscopy. His research involves studies on the ultrastructure of human tumors and correlation of the findings with light microscopic, histopathology and clinical features. It is interesting to note that in 1979 (TSEMJ volume 10 (3) page 18) Bruce reported that he was "temporate" — there is, however, no mention of that fact in his current questionnaire!

**Charles Mims, President, 1983 and 1984** — Charles is Professor and Chairman of the Department of Biology at Stephen F. Austin State University in Nacogdoches, Texas. His comments about TSEM are as follows: "TSEM is my favorite professional organization. I've learned a lot about electron microscopy simply by attending our meetings. There always seems to be a open exchange of information between students, technicians, corporate members, beginning microscopists and those using and developing "state of the art" techniques. I've made friends all over the state as a result of TSEM." His favorite meeting was the joint TSEM/LSEM meeting in New Orleans at which Jerry Berlin got sick from drinking the water. This was the same meeting at which President Larry Thurston opened the 8:00 a.m. breakfast-business meeting with the statement "Good evening ladies and gentlemen".

# TSEM'S FIRST TEN PRESIDENTS

(From left to right) Bill Brinkley (6th President), Terry Hoage (10th President), Joe Wood (5th President), Bob Turner, (9th President), Bob Yates (7th President), Demetri Lang (8th President), Don Benefiel (3rd President), Dan Roberts (4th President), Lee Rudea (2nd President), and Bill Philpot (1st President). Photograph and information provided by Bob Turner. Photograph taken in April, 1975 at 10th Anniversary Meeting of the TSEM, Hyatt Regency Hotel, Houston, Texas.

# HONORARY MEMBERS OF TSEM

Russell Barrnett
Donald Duncan
Paul Enos (awarded posthumously)
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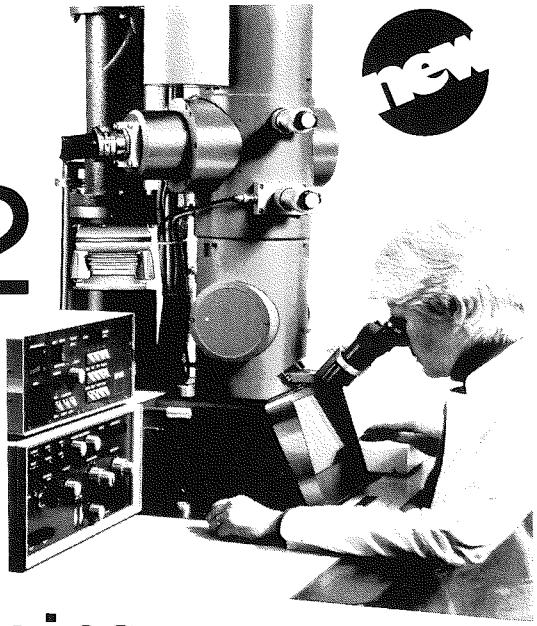


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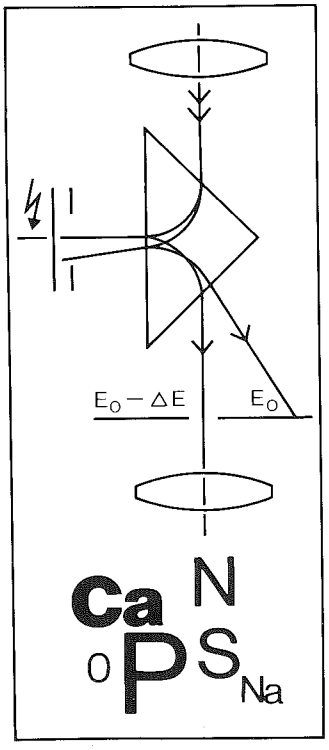


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# TSEM MEETINGS 1965-PRESENT LOCATIONS AND INVITED SPEAKERS

**Spring 1965** — Houston, Rice University — Organization meeting, approved by-laws
**Fall 1965** — Dallas, Statler Hotel — R.W.G. Wyckoff
**Winter 1966** — Houston, Rice University
**Spring 1966** — Galveston, Flagship Hotel — A.C. Van Dorsten
**Fall 1966** — Dallas, Sheraton Hotel — E.B. Bradford, J. Luft
**Spring 1967** — College Station, Holiday Inn (Joint meeting with Texas Academy of Science) - J. Reisner
**Spring 1967** — Galveston, Flagship Hotel — G. Bahr, G. Thomas
**Fall 1967** — Houston, Holiday Inn — F.S. Sjostrand, A.C. Van Dorsten
**Winter 1968** — Dallas, Royal Coach Inn — J. Watson, A.P. Wilska, K.M. Smith
**Spring 1968** — San Antonio, Holiday Inn — M. Moses, A. Crewe
**Fall 1968** — Houston, Rice University
**Winter 1969** — Austin, Villa Capri Motel — P. Marsh, E.C. Ketler
**Spring 1969** — Galveston, Flagship and Gamboa Cay — R. Barnett
**Fall 1969** — Salado, Stagecoach Inn — J. McAlear, A. Crewe
**Winter 1970** — NASA, Nassau Bay Hotel — L. Steere, G. Cocks, L.L. Ross, G. Thomas
**Spring 1970** — San Antonio, Palacio Del Rio — D. Friend, D. Fawcett
**Fall 1970** — Houston, Shamrock Hotel (EMSA)
**Winter 1971** — Galveston, Flagship Hotel — M. Brightman, G. Pappas
**Spring 1971** — Arlington, Six Flags Inn — D. Scarpelli
**Fall 1971** — Houston, Astroworld Hotel — D. Pease, S.J. Singer, N. Feder, A. Seligman
**Winter 1972** — Fort Worth, Sheraton Hotel — First joint meeting with LSEM — K. Porter, R. Fischer
**Spring 1972** — Huntsville, Holiday Inn — J.P. Revel
**Fall 1972** — San Antonio, Palacio Del Rio — H. Fernandez-Moran, J. Watson
**Winter 1973** — New Orleans, Chateau Le Moyne Hotel — Joint meeting with LSEM — H. Fernandez-Moran, W.J. Humphreys, K.R. Lawless, B.M. Siegel
**Spring 1973** — Galveston, Jack Tar Hotel — R. Barnett
**Fall 1973** — Bandera, Mayan Dude Ranch
**Winter 1974** — San Antonio, Menger Hotel — Joint meeting with LSEM — W. Humphreys, R. Barnett
**Spring 1974** — College Station, Texas A&M University — J. Russ
**Fall 1974** — Lake Livingston, Waterwood — J.H. Brown
**Winter 1975** — New Orleans, Delta Towers Hotel — Joint meeting with LSEM — G. Thomas, J. Watson, G. Simon
**Spring 1975** — Houston, Hyatt Regency — A.B. El-Kareh
**Fall 1975** — Bandera, Mayan Dude Ranch — B. Stewart, D. Wherry
**Winter 1976** — San Antonio, Menger Hotel — Joint meeting with LSEM — E. deHarven, G. Thomas
**Spring 1976** — Arlington, Cibola Inn — H. Ris
**Fall 1976** — Temple, Ponderosa Motel/Scott and White Hosital — H. B. Sybers
**Winter 1977** — New Orleans, Monteleone Hotel — Joint meeting with LSEM — J.D. Robertston
**Spring 1977** - Austin, Villa Capri Motel — C. Hackenbrock, D. Harling
**Fall 1977** - Arlington, Rodeway Inn — R. Bolender, H. Smith, E. Underwood
**Winter 1978** — San Antonio, St. Anthony Hotel — Joint meeting with LSEM — M. Ledbetter, J.P. Revel
**Spring 1978** — Lubbock, South Park Inn — M. Brown, F. Low
**Fall 1978** — Nacogdoches, Fredonia Inn — F. Fuller, B. Brinkley
**Winter 1979** — New Orleans, Monteleone Hotel — Joint meeting with LSEM — P. Nakane, H. Ris
**Spring 1979** — Dallas, Marriot Hotel — P. Sterling
**Fall 1979** — San Antonio (EMSA meeting)
**Winter 1980** — Houston, Shamrock Hilton Hotel — Joint meeting with LSEM — M. Beer
**Spring 1980** — Waco, Holiday Inn — J. Wolosewick, E. deHarven
**Fall 1980** — College Station, Texas A&M — J. Johnson, H. Fernandez-Moran
**Spring 1981** — Fort Worth, Kahler Green Oaks Inn — W.O. Milligan, G. Pappas
**Fall 1981** — Corpus Christi, Holiday Inn — Emerald Beach — B. Hardy
**Spring 1982** — Denton, Texas Woman's University — E. Reynolds
**Fall 1982** — Galveston, Galvez Hotel — L. Peachy
**Spring 1983** — Austin, Quality Inn — J. Guyton, H. Fernandez-Moran
**Fall 1983** — Tyler, Sheraton Inn — B. Trump, R. Trelease
**Spring 1984** — College Station, Texas A&M — H. Hagler, C. Fiori
**Fall 1984** . Arlington, Rodeway Inn — E. Gantt, R. Anderson
**Spring 1985** — San Antonio, Menger Hotel — K. Porter, B. Brinkley, L. Rudee, J. Benson, G. Plows

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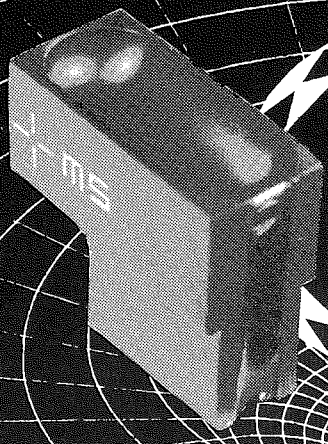


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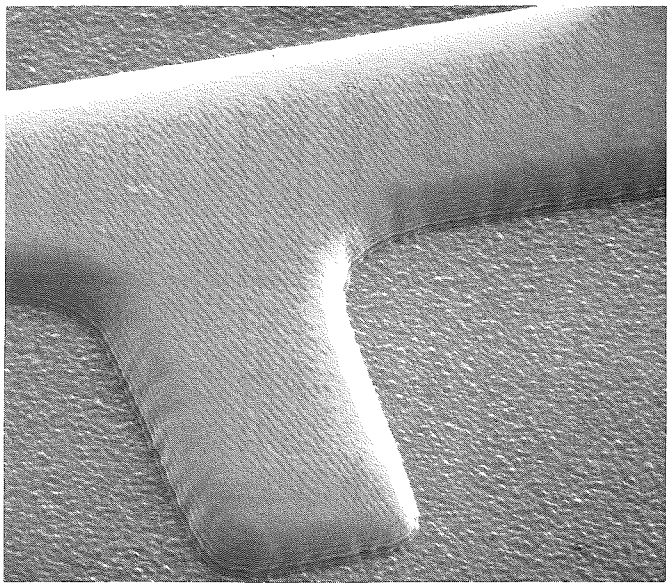
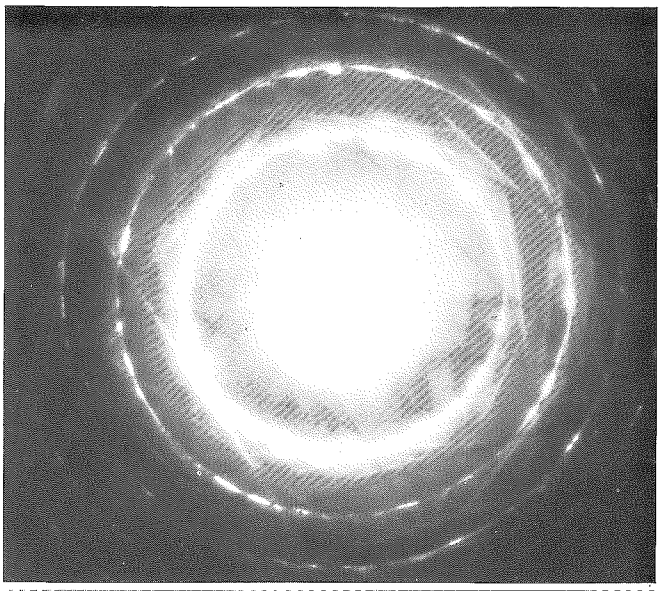
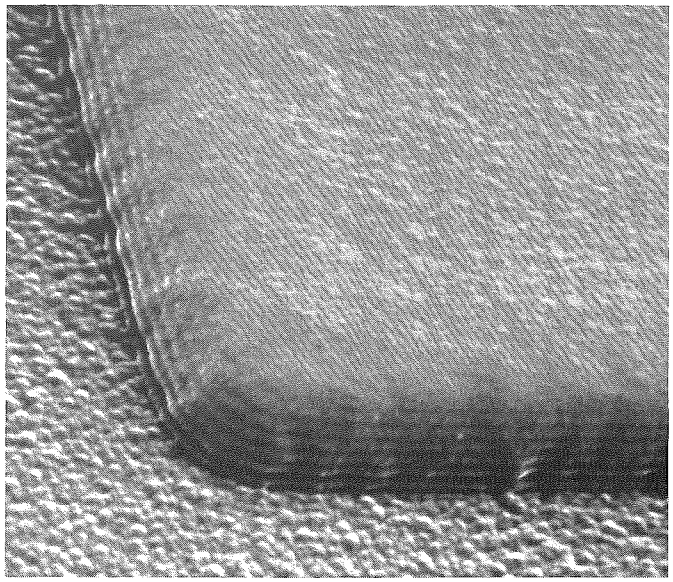
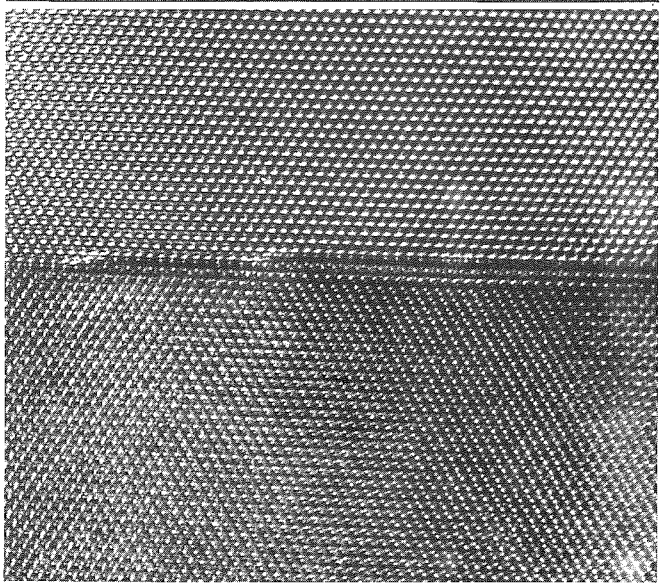






*Standing Wave Observation, magnification 7KX*



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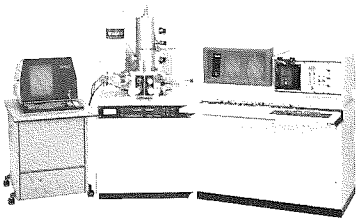

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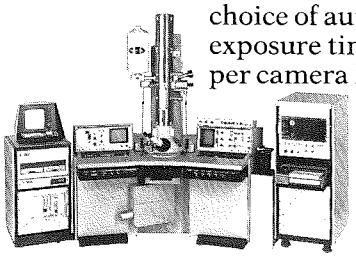

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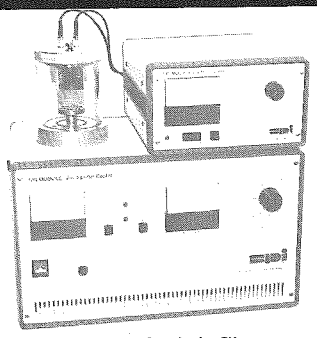

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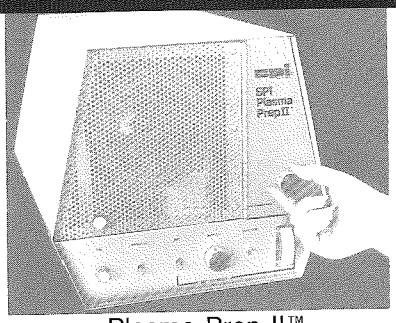


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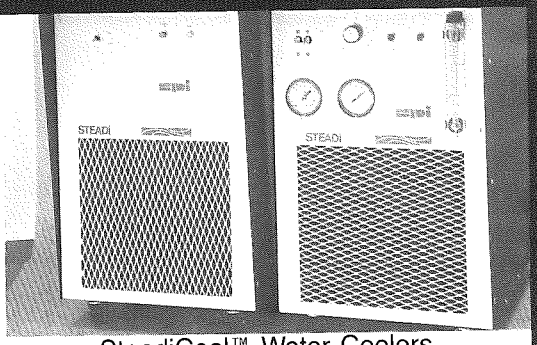


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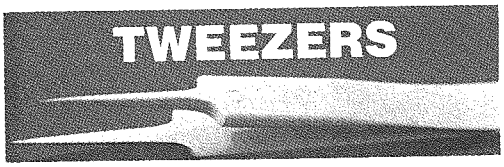

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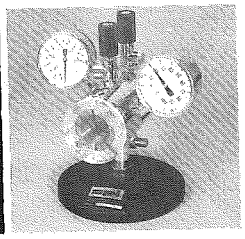

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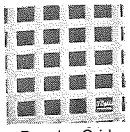

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SECTIONS: The text of each original article and technical report should be divided into four major sections entitled INTRODUCTION; METHODS AND MATERIALS; MATERIALS; AND DISCUSSION.

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(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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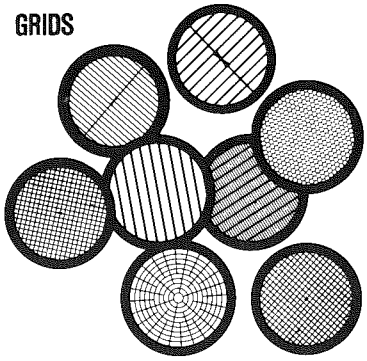

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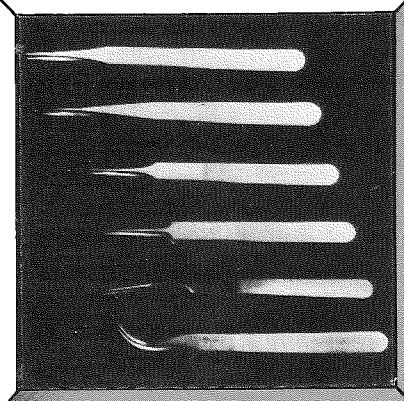

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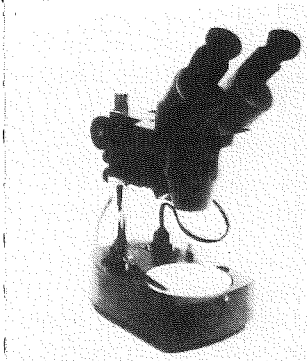

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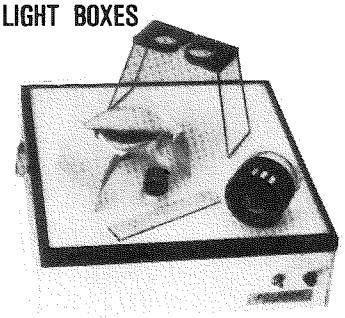

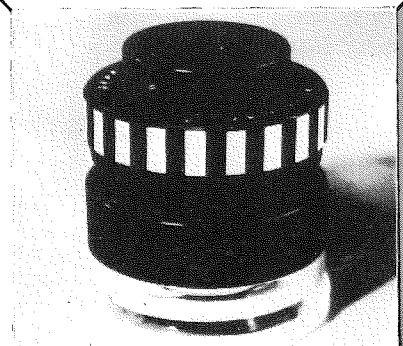

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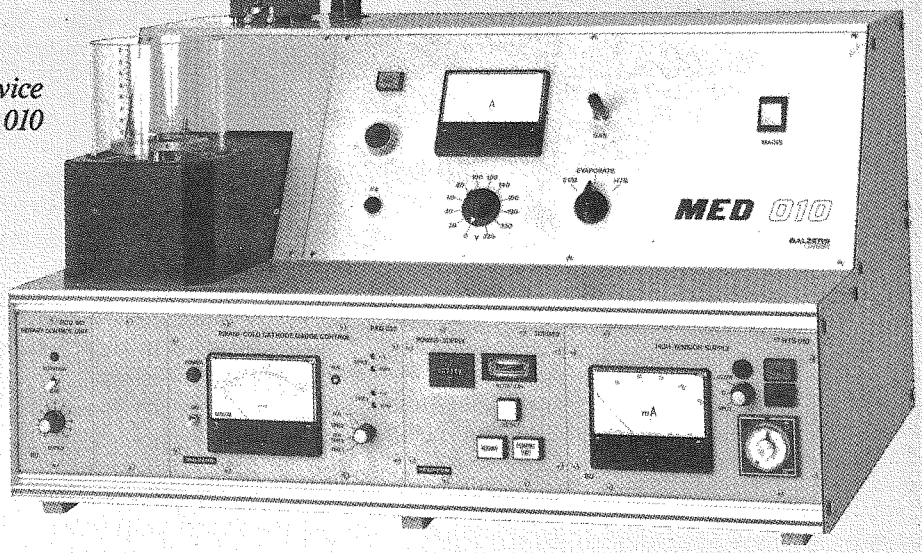


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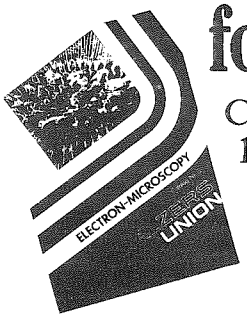


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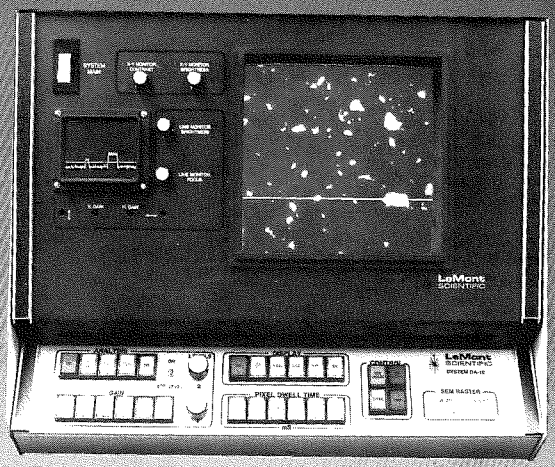

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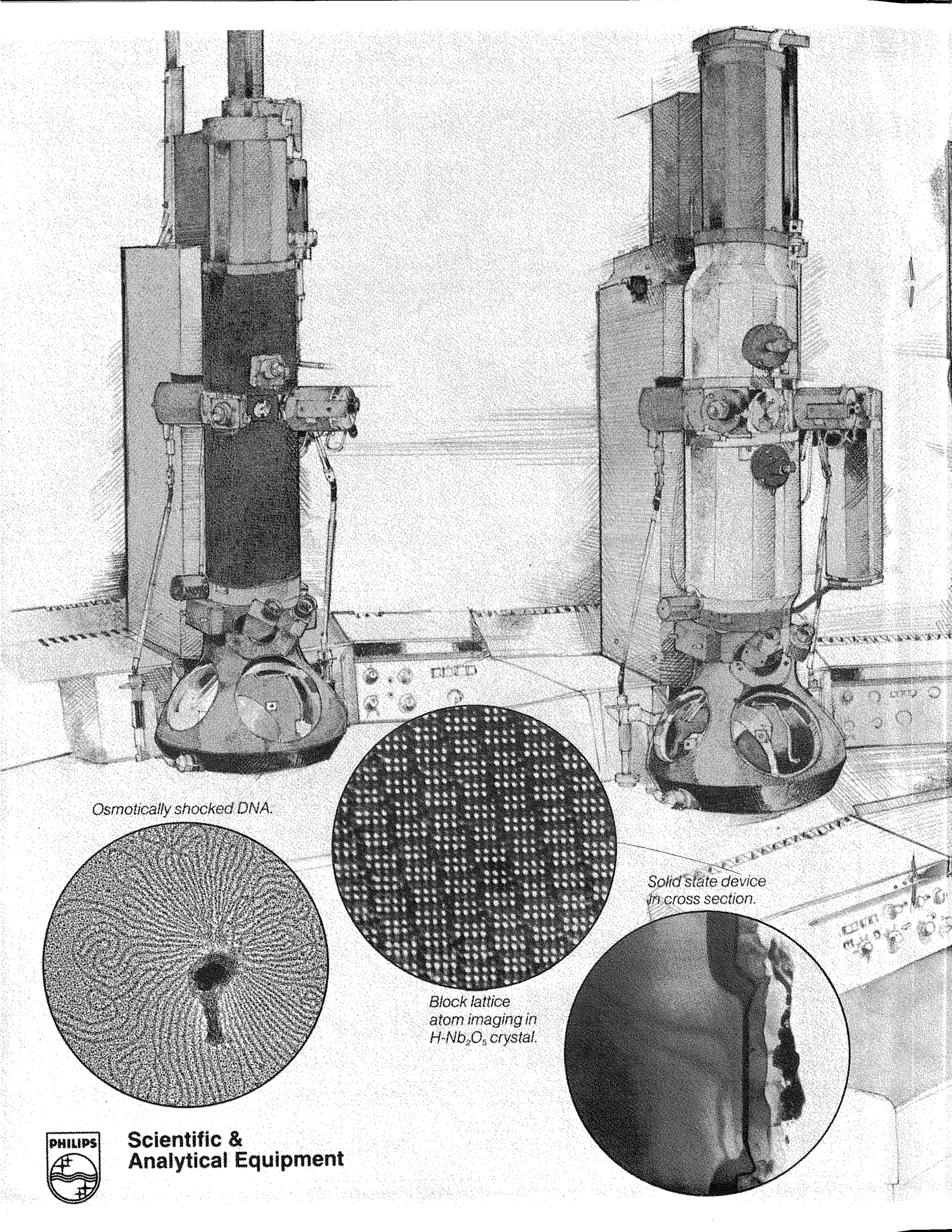

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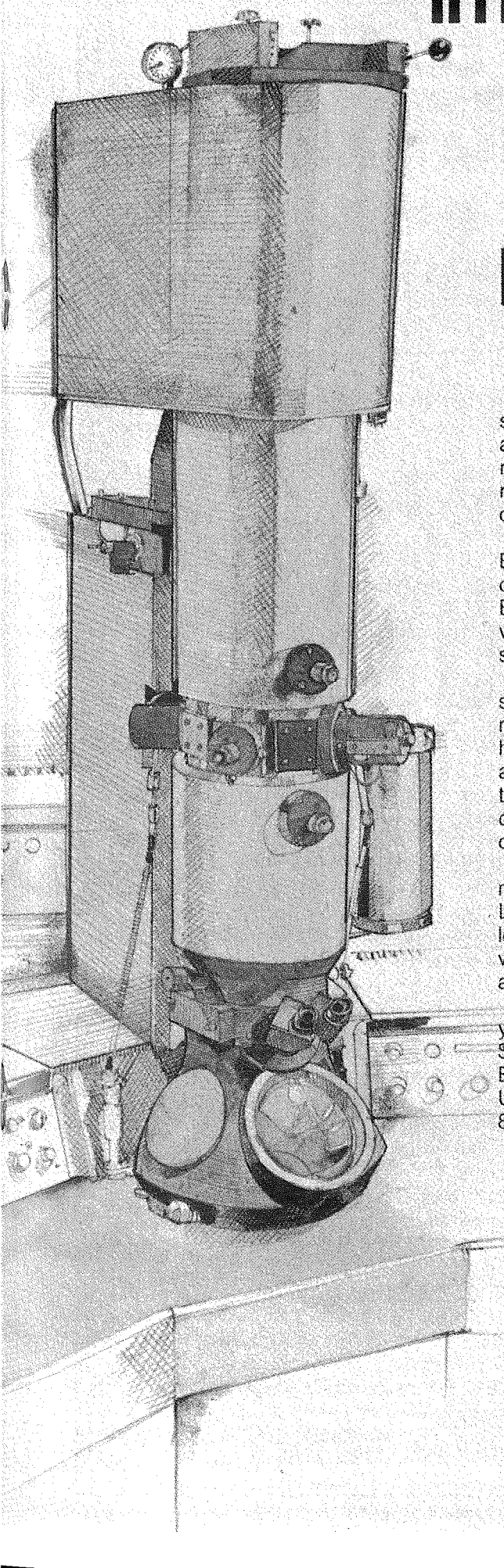

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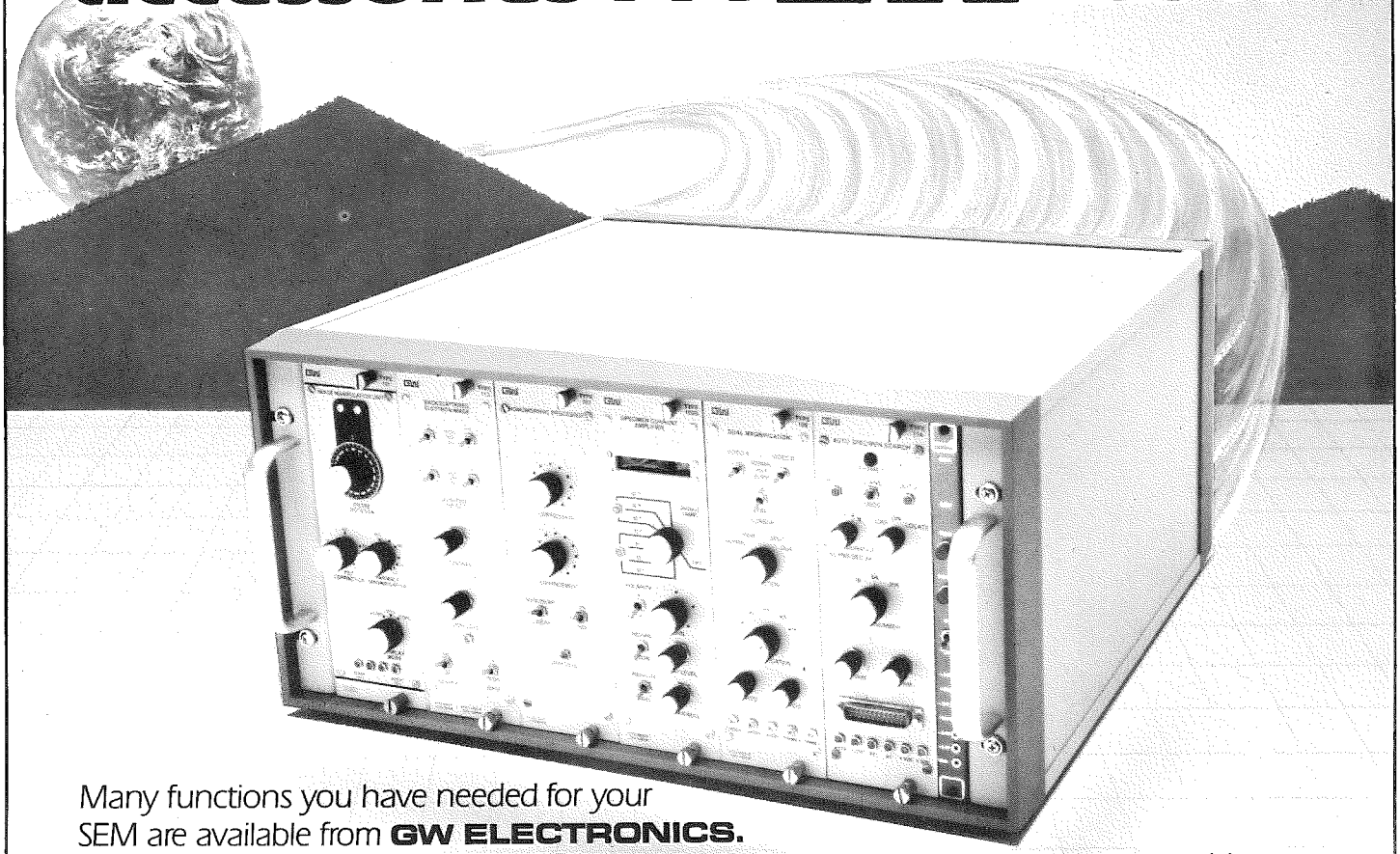

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# DEVELOPMENT OF THE TRICHOME SHIELD OF THE BROMELIAD *TILLANDSIA RECURVATA*

By

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## INTRODUCTION

According to Carlquist (1), few species of angiosperms are truly globrous. The epidermis is usually ornamented with structures called hairs or trichomes (2). Plant hairs develop from epidermal tissue, grow, and differentiate into structures with a variety of morphologies and functions. Trichomes range from simple unicellular structures to complex multicellular outgrowths. Functions attributed to trichomes include reduction of transpiration by reducing evaporation (3), temperature regulation by reducing the absorption of radiant energy or by increasing dissipation after absorption (4, 5), chemical and mechanical defense from predators and pathogens (6, 7, 8), water and mineral loss by exudation or evaporation (9), and water, mineral, and nutrient absorption (10, 11).

Since the early days of light microscopy these minute structures have been popular subjects for morphological studies and there has been much speculation as to their adaptive significance. Although much of the descriptive work was completed by the turn of this century, the fine structure of plant hairs (especially those that serve some obvious function) is still an active area of study (12).

Trichomes of atmospheric bromeliads have been a favorite area of investigation among anatomists, physiologists, ecologists, and taxonomists interested in epiphytes. Trichomes of atmospheric bromeliads function in thermal regulation and are absorptive structures (13, 14, 15, 10, 16, 17). Bromeliad trichomes reaches their highest degree of specialization in the genus **Tillandsia**, where the presence of elaborate peltate trichomes has allowed atmospheric species to invade the most exposed and nutrient depauperate areas of the epiphytic habitat.

This study focused on the ontogeny of trichomes which play such an important role in the ecology of atmospheric tillandsias. **Tillandsia recurvata** (ball moss) was chosen as the model because the seminal study of bromeliad trichome development was carried out using **T. usneoides** (Spanish moss), a plant with similar habitat requirements and considered taxonomically close to **T. recurvata** (18).

Seeds of **T. recurvata** were obtained from field pollinated plants. Coma hairs were trimmed from the seeds, and the seeds were washed with 20% Clorox for five minutes prior to being rinsed and placed on autoclaved nutrient agar in sterile petri dishes (19). Seeds were germinated under a photoperiod of 13 hours light and 11 hours dark. After a minimum of ten days, seedlings were dissected (the remaining coma hairs, radicle, and cotyledon were removed) and the first few true leaves fixed and prepared for SEM or TEM according to standard procedures.

## TRICHOME DEVELOPMENT

The first and second leaves produced by **T. recurvata** seedlings possess completely developed trichomes by the time the leaves emerge from the seed. Developing trichomes were also observed on the third and subsequent leaves. The development of the absorptive trichome in **T. recurvata** appeared to follow a sequence similar to that observed by Billings in **T. usneoides** (18), and is as follows:

1. Initiation of the external developmental sequence first becomes apparent as a single cell appears to bulge from the surrounding epidermal cells. This cell appears to be separated from the surrounding epidermis (Figs. 1a, 2a, 3a). This initial cell will undergo a series of divisions ultimately resulting in the development of a mature absorptive trichome.
2. The first sequence of cell divisions results in four cells. These cells enlarge and protrude further from the leaf surface (Figs. 1b, 2b).
3. Periclinal divisions of the four cells then produce two series of four cells: four inner cells surrounded by four outer cells (Figs. 1c, 2c, 3b).
4. Cells of the outer layer divide anticlinally so that the four inner cells are eventually surrounded by eight cells. Each of the eight cells then divides periclinally giving rise to two series of eight cells that surround the four central cells.
5. Each cell of the outer layer undergoes an anticlinal division resulting in concentric rings of eight and 16 cells around the four central cells (Figs. 1d, 4a).
6. The penultimate series of cell divisions are periclinal divisions of the outer series of 16.
7. One or more anticlinal divisions of the outermost layer of cells follows. This outermost layer of cells elongates to form the wing of the trichome (Figs. 1e,

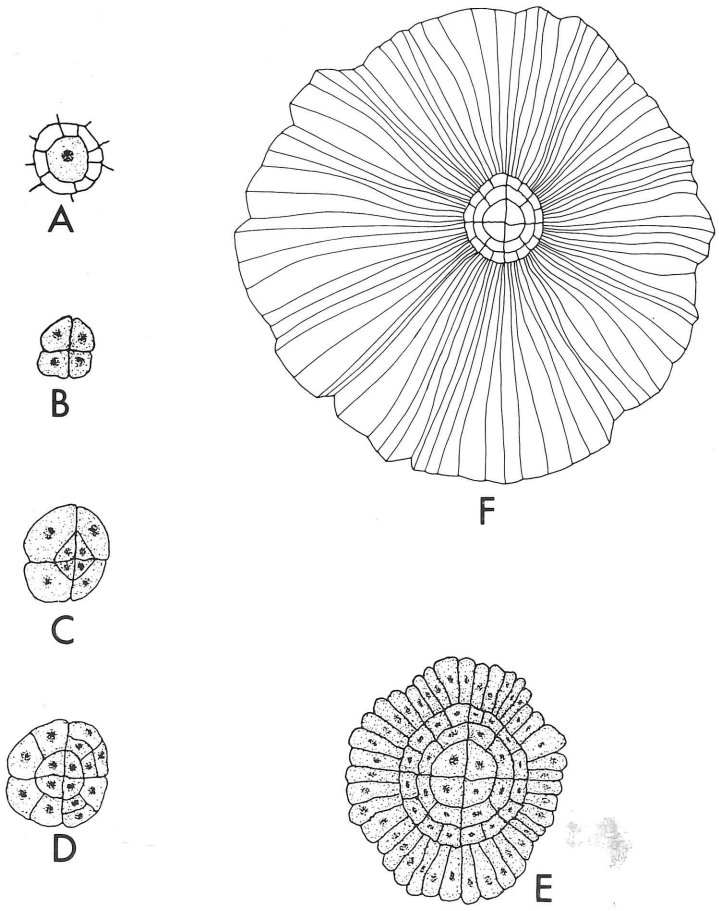


**FIGURE I:** Summary of the developmental sequence of the trichome shield in **T. recurvata** beginning with a single cell (A) and ultimately resulting in the completed winged shield (F).

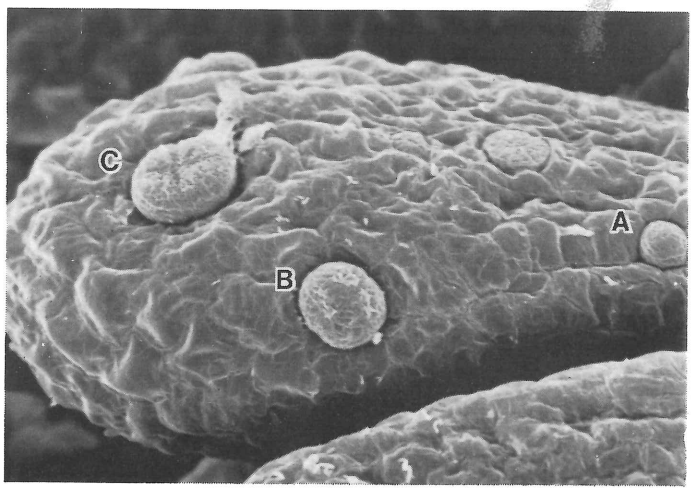


**FIGURE 2.** The third leaf of a **T. recurvata** seedling showing developing hairs at the one cell (A), four cell (B), and eight cell (C) stage.

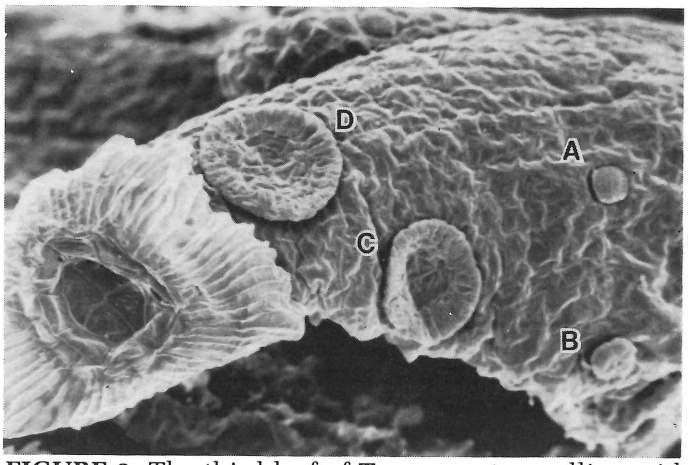


**FIGURE 3.** The third leaf of **T. recurvata** seedling with developing trichomes at the one (A), and four cell (B) stages. Two trichomes (C, D) show cells of the wing beginning to elongate.

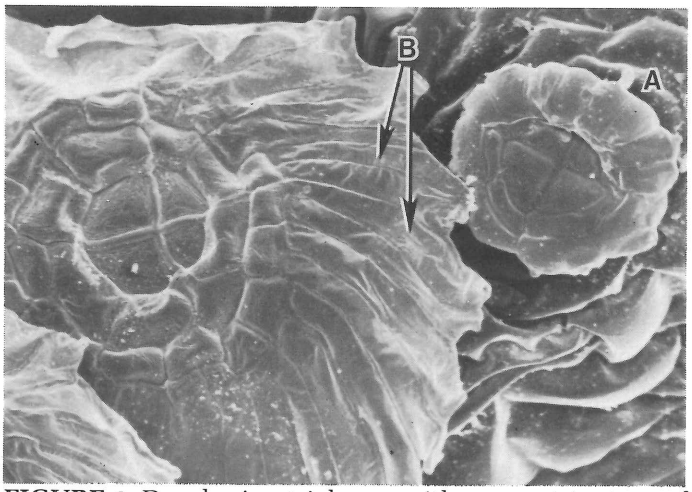


**FIGURE 4.** Developing trichome with concentric rings of eight and 16 cells around the four central disc cells (A), and elongating cells of the wing (B) on a more mature hair.

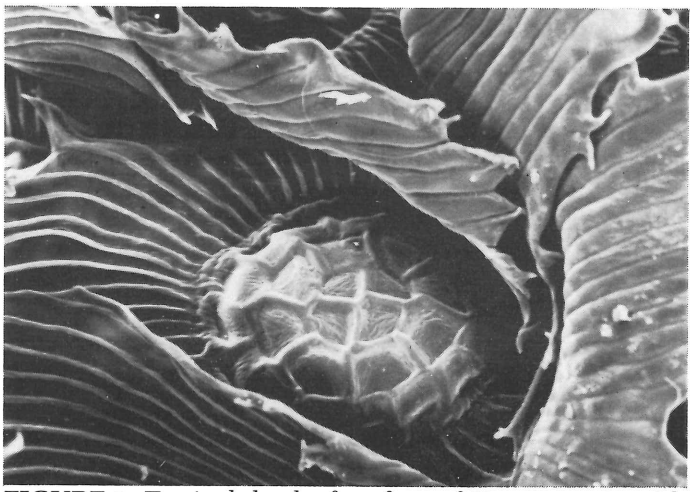

**FIGURE 5.** Typical dry leaf surface of **T. recurvata** with trichome wings flexed upward.

3c, 3d, 4b).

8. After extensive cell wall deposition and cutinization of the uppermost layer of the cell wall, a contoured fit of upper and lower cell walls results. Following the final stages of trichome development the cell contents are lost from the shield cells which constitute the surface portion of the hair.

**SO WHY ALL THE INTEREST IN THESE HAIRS?**

When the tillandsioid trichome is dry, the reflexed wing (Fig. 5) makes the plant surface highly reflective, producing the silvery, lusterous appearance of many atmospheric bromliads. The upward flexure of the wing occurs when the contoured upper and lower cell walls of the central disc and rings cells are closely appressed (Fig. 6). Desert populations of **T. fasiculata** reflect between 42% and 47% of the visible light striking their adaxial surfaces. Tank-forming bromeliads which lack the highly specialized tillandsoid trichomes, such as **Cataopsis nutans** and **Guzmania lingulata,** and are, in general, found in mesic, shaded situations, reflect no more than 28% of the light striking their surfaces (13). Infrared radiation is also reflected, thus reducing heating of leaves in exposed situations. Leaves of **T. flexuosa** and **T. circinnata** with intact trichome cover have been shown to be 23.8% and 27.8%, respectively, cooler than plants whose trichomes are removed (14, 18).

Trichomes covering the surface of atmospheric bromeliads absorb water and nutrients (13, 15, 10, 15, 17). The trichomes are one-way water conduits to the mesophyll, allowing for rapid uptake of water, minerals and organic solutes obtained from precipitation and runoff. (Fig. 7) (13). When water strikes the surface of an atmospheric bromeliad, the water spreads across the hydrophyllic leaf surface and into the disc and ring cells of the shield (i.e., surface portion) of individual hairs. The upper walls of cells of the shield rise as the interior of these dead cells fill with water. Due to the shape of the upper and lower walls of the disc and ring cells, the leaf of the trichome unfolds and lays parallel to the surface of the leaf as water enters the shield. The action of expansion of the lumina of the shield cells and downward flex of the wing is believed to produce a minute suction, drawing fluids into the shield (10, 20).

Water as well as inorganic minerals and amino acids are quickly incorporated into the living, densely cytoplasmic, stalk cells in atmospheric tillandsias (21) (Fig. 8). Complex infoldings of the plasma membrane of the dome cell and stalk cells have been observed. These infoldings have suggested to some authors pinocytosis as the mechanism for incorporation of solutes into the dome cell (22, 23).

As the leaf surface dries, water is lost from the shield and the lateral walls collapse. The thick upper cell walls of the central disc and ring cells, which are cutinized only on their upper surfaces, fall into the epidermal concavity of the trichome, resulting in a tight closure between the upper, living, dome cell of the trichome stalk and the external environment (22).

At first glance, a dense cover of absorptive trichomes seems to be the perfect answer to the challenges of the epiphytic habitat, those challenges being drought and extreme nutrient paucity. The trade-offs for atmospheric bromeliads are low light use efficiency and severely reduced gas exchange

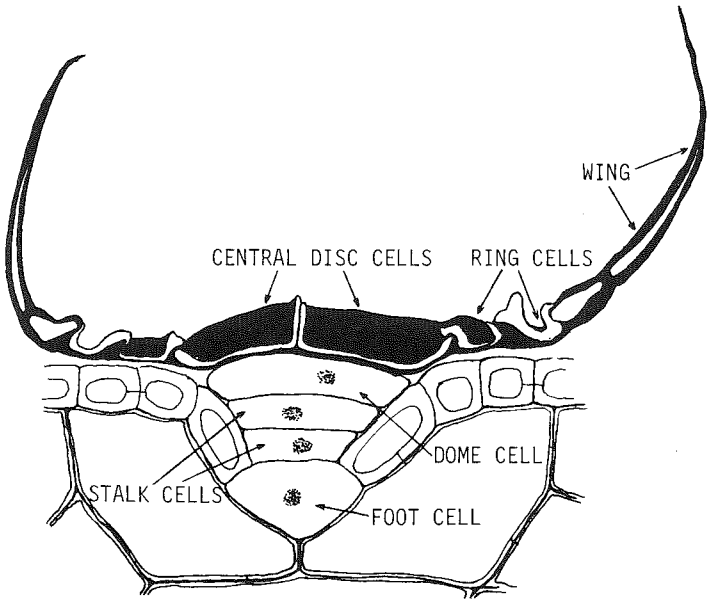


**FIGURE 6.** Median section through a tillandsioid trichome in the dry condition.

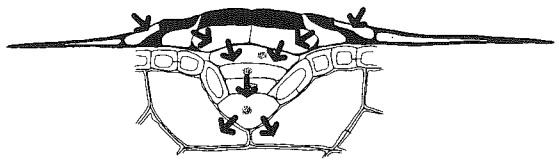

**FIGURE 7.** Median section through a tillansioid trichome in the wet condition showing the path followed by water into the mesophyll.

when the trichome is wet and the wing closely appressed to the leaf surface (24). The atmospheric tillandsias are found, to a greater extent than any other group, in the most exposed portions of the epiphytic habitat, these sites being (in general) both well illuminated and dry.

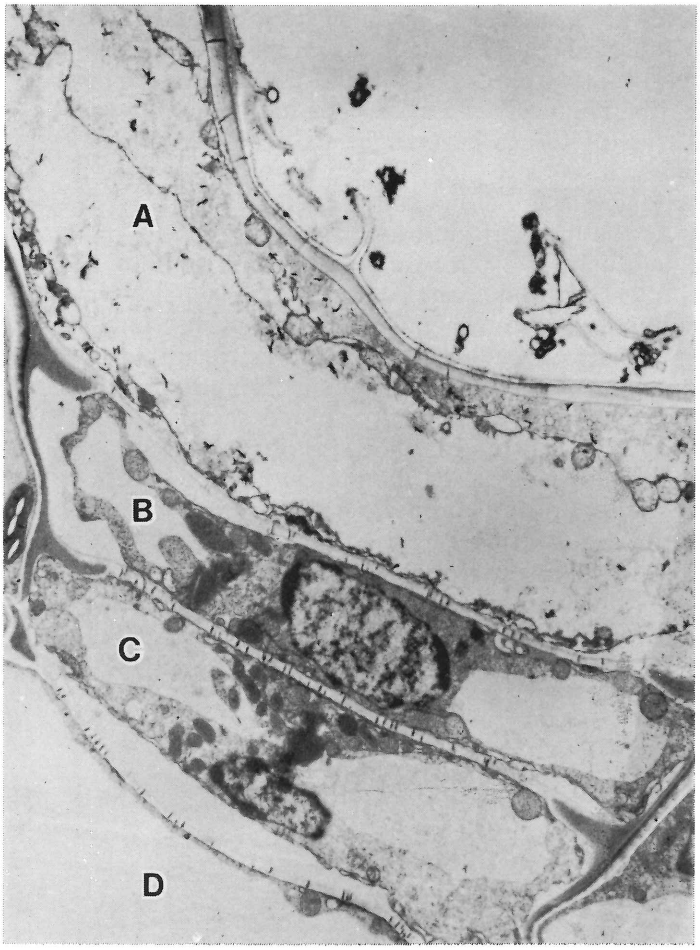


**FIGURE 8.** Densely cytoplasmic cells of **T. recurvata** trichome stalk showing the dome cell (A), two stalk cells (B, C), and a portion of the foot cell (D).

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# ELECTRON MICROSCOPY IN LATIN AMERICA: OPPORTUNITIES FOR VISITS

There are considerable problems for electron microscopists in the Third World. They need our special consideration and help to overcome the difficulties of their situation. One of the most direct ways of providing such help is by working with them in their own countries; this is a rewarding experience for both parties and usually an enjoyable one.

At this time, two laboratories are in need of help in establishing new research programs, with funding available to bring in a visitor for a period of a few months.

**1. Bogota, Columbia.** The Universidad Nacional de Columbia has a JEOL 100B with a STEM unit. It is now run by physicists (who have the skill to maintain it) but is used for both biological and materials applications. They are in need of someone to help them develop specimen preparation techniques for materials science and to guide them through some materials science applications.

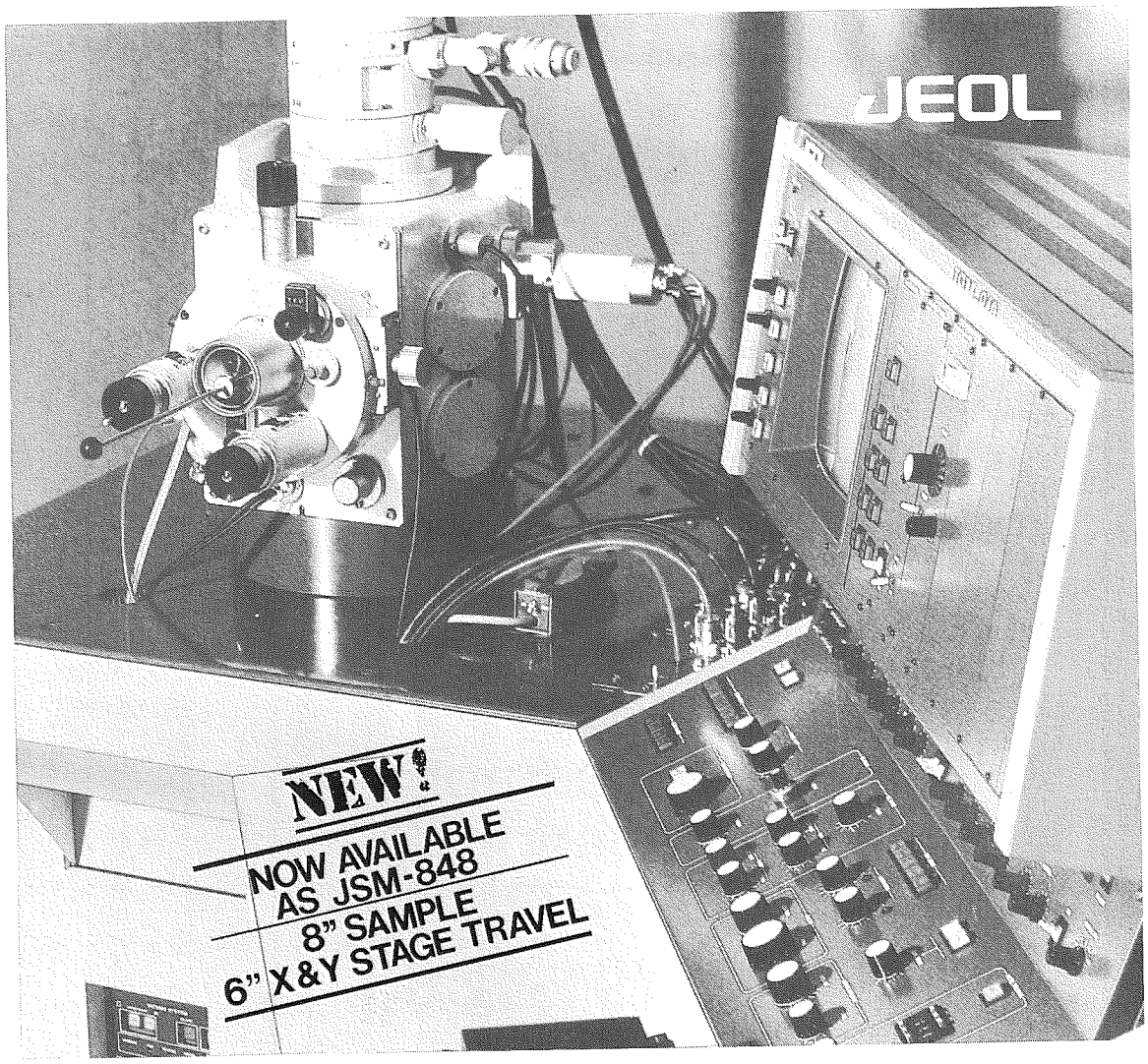
Contact: Rafael Buritica, Deparamento de Fisica, Universidad Nacional, Apartado Aereo 91060, Bogota, Columbia.

**2. Santiago, Chile.** The Physics Deparment of the Universidad de Chile has a Philips EM 300 that is currently being fitted with a condenser-objective lens. They will have funding for a visitor in 1985. They are looking for someone who will be able to help them with an ion miller they are designing for in-house construction (they do not have funds to buy one) and to help them establish a program of symmetry determination by convergent-beam methods. This will complement the successful x-ray diffraction program in the same laboratory.

Contact: Oscar Wittke, Departmento de Fisica, Universidad de Chile, Casilla 5487, Santiago, Chile.

In both cases the person should be a post-doctoral level or higher. He or she would need to be adaptable and be prepared to work under conditions quite different from those prevailing in the United States or Europe. On the other hand, these visits could make a very significant contribution to the development of microscopy in the countries in question.

If you are interested in further information please contact me: Alwyn Eades, Materials Research Laboratory, University of Illinois, 104 S. Goodwin, Urbana, Illinois 61801 (217) 333-8396.

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# ELECTRON MICROSCOPY OF CLAY MINERALS

By

**James L. McAtee, Jr.**
**Department of Chemistry**
**Baylor University**
**Waco, TX 76798**

## INTRODUCTION

Electron microscopic study of clay minerals began soon after the commercial introduction of electron microscopes. Some of the earliest investigations of clay minerals by electron microscopy were by Ardenne (1), Eitel (2), Middel (3), Humbert (4), Shaw (5), and Marshall (6). From these early studies a great deal of information was obtained about the shape and size of clay minerals. Early investigators soon found, however, that the study of clay by electron microscopy was frought with problems. Since most clays have water of hydration, adsorbed water, and so called structural water (structural OH), mounting clay minerals in the electron beam typically resulted in dehydration, desorption, and even breakdown of the structural water to the point that images of the clay minerals were constantly changing. Some of the early work involved replica techniques, particularly for those studies involving the textural arrangements of the clay. An early technique developed by Bradley (7) used a single-stage, pre-shadowed or self-shadowed carbon replica method. As better techniques have been developed for the preparation of clay samples and improvements in transmission electron microscopes so that the beam intensity could be reduced, many of the early problems have been eliminated, and the direct observation of clay minerals by transmission electron microscopy is now done routinely.

Following the development of the scanning electron microscope, workers used it to largely replace the replica methods for the observation of clay texture and freshly fractured clay surfaces. Utilizing the greater depth of field of the SEM (i.e., compared with a light microscope), much information can be gained by using the SEM in the study of both relatively pure clay minerals and clays found in soils, rocks and minerals.

## TEM OF CLAYS

### Mutual adsorption of clays and hydrous oxides

Smectite clays have a high surface area (about 800 $m^2/g$). Clays adsorb many different substances, including inorganic to organic materials. X-ray diffraction studies of natural clays have demonstrated that many samples contain hydrous oxides mixed with the clay. The mutual adsorption of clay minerals and colloidal hydrous oxides have been studied by electron microscopy in order to gain a greater understanding of their properties and how these properties might affect the rheology of dispersed clays (8,9). Adsorption of hydrous oxides by smectites, montmorillonite and hectorite depends on the nature of the exchangeable cations associated with the clay surfaces and the relative concentrations of clay and hydrous oxide. The particles of both sodium and calcium forms of montmorillonite and hectorite bend or curl in the presence of adsorbed hydrous oxide, and the degree of curling is dependent upon the amount of adsorbed hydrous oxide. Examples of such curling are show in Figure 1.

### Clay dispersions at various pH

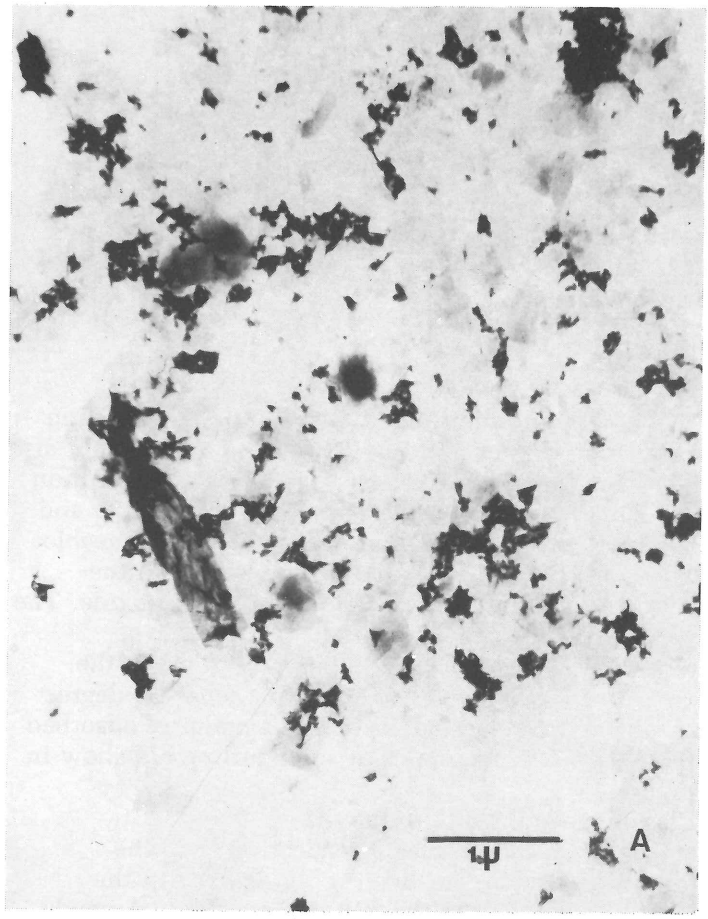
One of the major uses for smectites is as the viscousifier for rotary drilling fluids used in the petroleum industry. The pH of these fluids depends largely on other additives and on the type of formation being drilled. The pH of these fluids may vary widely. Montmorillonite dispersions at pH 1 to 13 were examined by electron microscopy utilizing the adsorption of clay on a film of cytochrome c. (10). Electron micrographs obtained by this preparation method were much more definitive for the individual particle-particle interaction than one could deduce from standard drop or spray preparation techniques commonly in use.

### Clay studies at elevated temperatures

Some interesting studies of kaolin, montmorillonite, mica, and talc have involved clays examined by hot-stage electron microscopy. This investigation was instigated to learn more about the structural and morphological changes that the clays undergo during heating from room temperature to 1000°C (11, 12, 13). One of the most interesting observations was that there was significant structural reorganization of kaolinites immediately following the dehydroxylation of the clay at 600°C. X-ray diffraction evidence on these same clays had not indicated such a drastic amount of reorganization at temperatures this low.

### Critical point drying of clays

A significant advance in sample preparation of clay minerals was made by the use of critical point drying. These were particularly adaptable to organic-clay complexes (14, 15). Figure 2 illustrates several examples of clay complexes that were prepared by critical point drying. The most striking contrast between critical point and air dried samples is in the



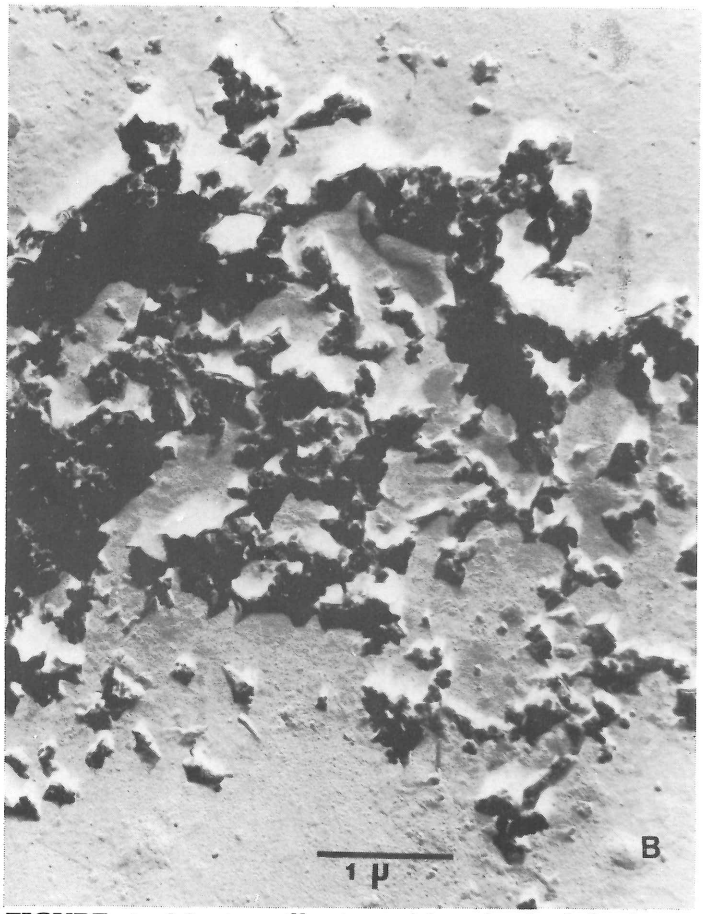




**FIGURE 1:** Montmorillonite with adsorbed hydrous chromium oxide. A. Partially flocculated. B. Flocculated.

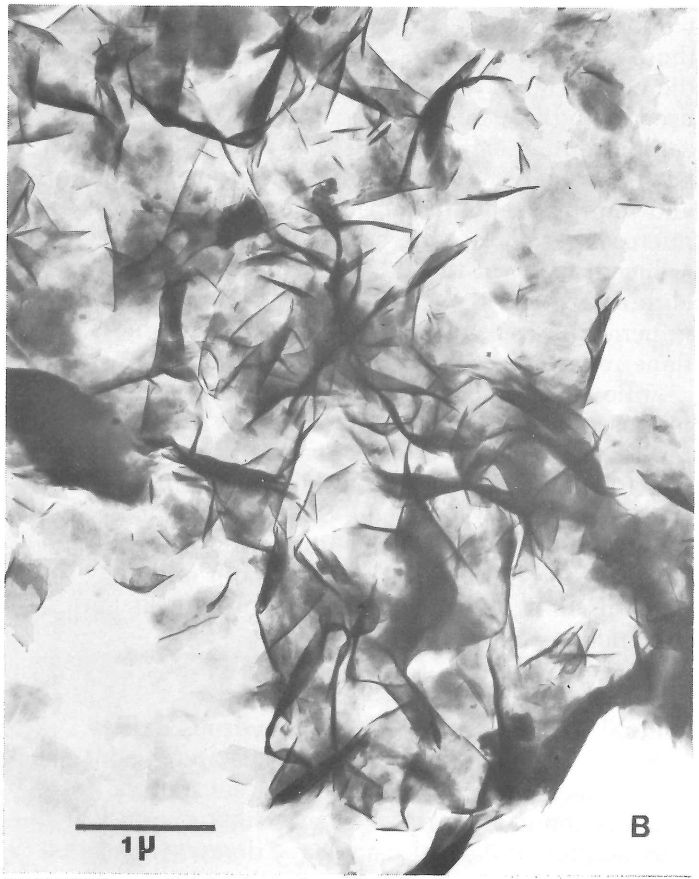




**FIGURE 2:** Critical point dried organo-smectites. A. Laurylammonium montmorillonite. B. Benzylammonium montmorillonite.

degree of curling of the clay sheets. In critical point dried samples, curling was extensive, whereas there was no such curling in the same samples prepared by air drying. The morphology of organo-smectites prepared by critical point drying depended upon the type of and the amount of exchanged organic cation. The amount of curling observed in electron micrographs was associated with the hydrophobicity of the organo-clay complex: the greater the hydrophobic nature of the complex, the greater the amount of curling.

**Freeze-drying of clays**

In order to better observe the changes in curling and aggregation of clay platelets, to show better the shapes of individual clay particles, and to preserve the delicate morphology of aggregates, a freeze-drying technique was developed employing a special sample holder. The holder was designed so only one side of a carbon-coated grid was allowed to be exposed to the clay suspension during the freezing and drying process (16). Electron microscopic examination of samples prepared by this method depicted the subtle morphological differences among a group of clays and showed a greater number of particles than those prepared by other techniques (Figure 3).

**Modified freeze-drying-shadowing of clays**

The most recent method employed in my laboratories to enhance the study of individual clay particle morphology and particle-particle interactions has been the development of a low-temperature freeze-drying-shadowing method. The method gives much more information regarding the interaction of the clay particles in the dispersed state (17). After a carbon-coated sheet of mica is placed on a cold stage, the sample is sprayed onto the carbon film, freeze-dried, shadowed with a heavy metal, and a second carbon film deposited. The sample is not disturbed or moved during the preparation process. A sketch of the apparatus used for this method is shown in Figure 4. Electron micrographs obtained by this method of preparation are illustrated in Figure 5. It will be noted in these micrographs of clay that they show a pronounced ''stringing'' or end-to-end association of the particles. This type of association agrees quite well with current theories related to the viscosity of clay dispersions. Currently this method of sample preparation is being applied to a variety of clay dispersions including those with changes in exchangeable cations and pH and of organo-clays dispersed in a variety of organic fluids.


**FIGURE 3:** Electron photomicrograph of hectorite prepared by simple freeze drying.

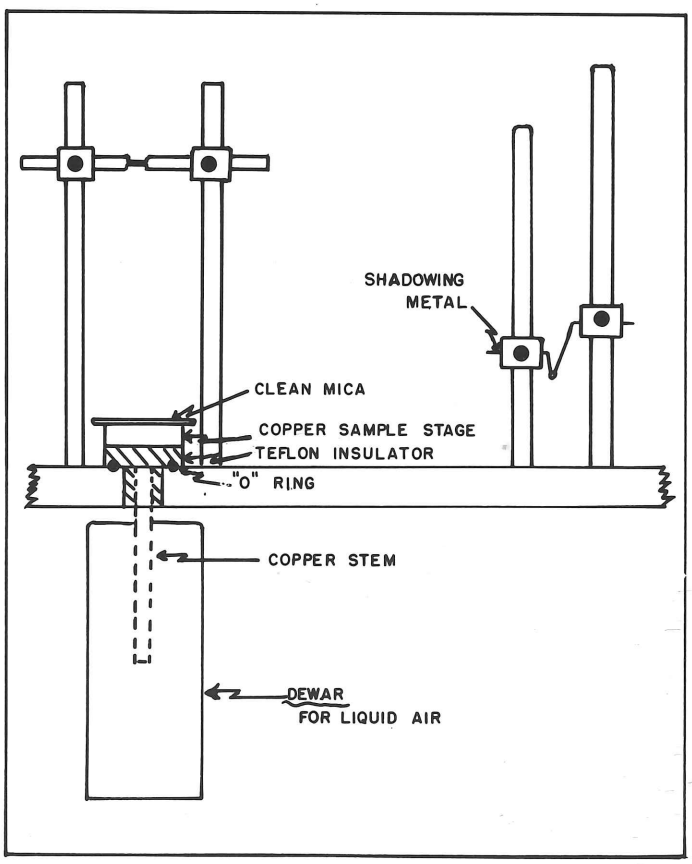



**FIGURE 4:** Sketch of freeze drying apparatus.

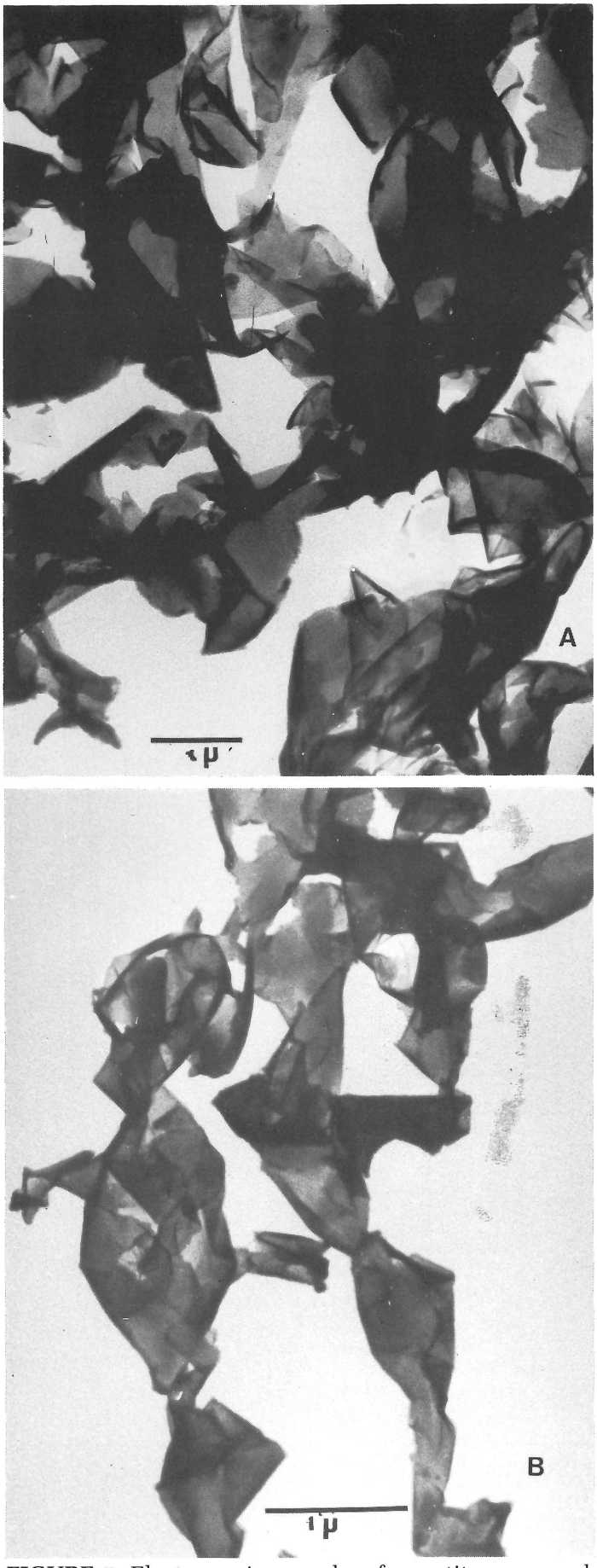



**FIGURE 5:** Electron micrographs of smectites prepared by modified freeze drying.


## ACKNOWLEDGEMENTS

Appreciation is expressed to the Robert A. Welch Foundation for extensive support of the many graduate and undergraduate students that have participated in the electron microscopy study of clays at Baylor University.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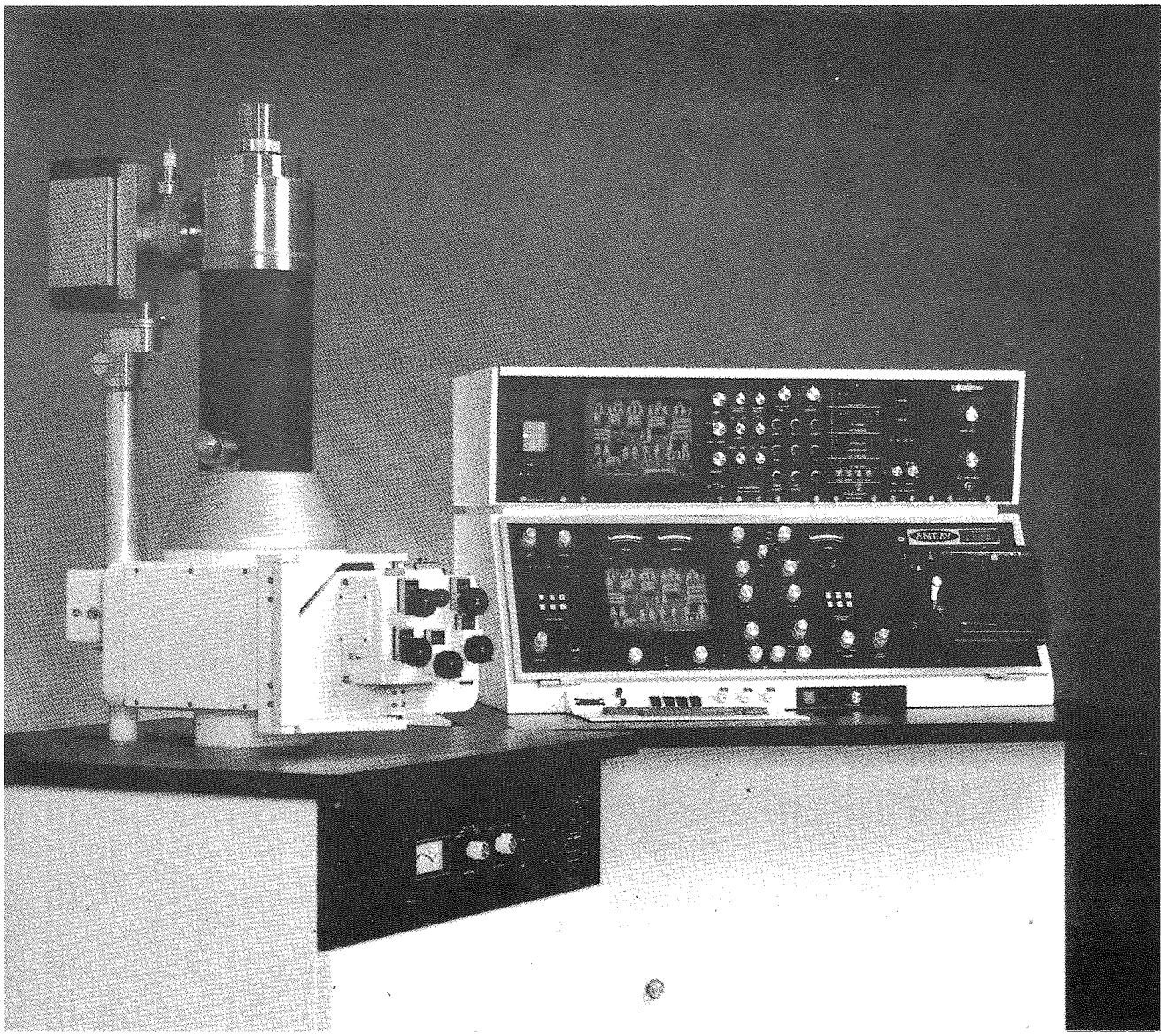


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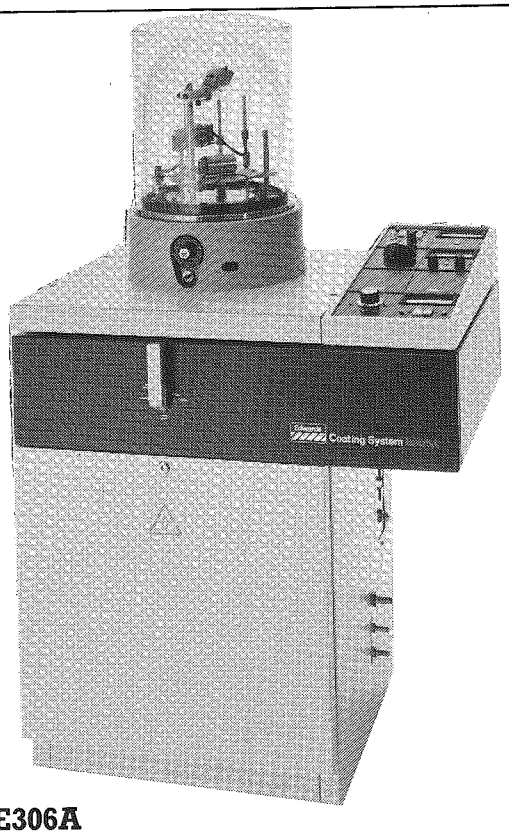

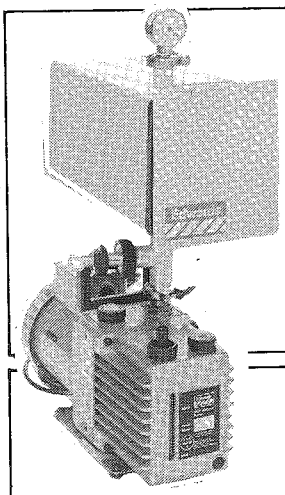

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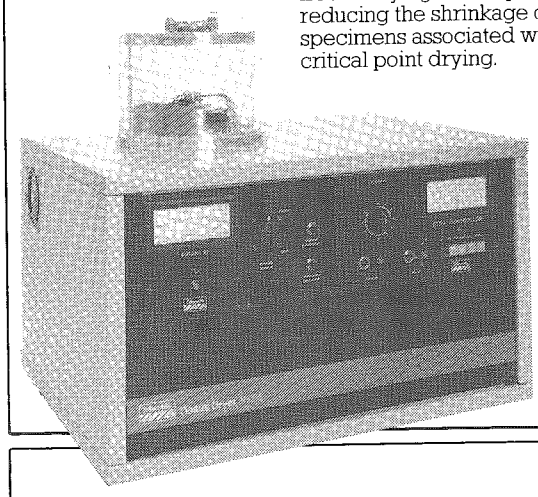

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# STRUCTURAL ASPECTS OF GRAFT INCOMPATIBILITY BETWEEN *BRASSICA OLERACEAE* AND *LYCOPERSICON ESCULENTUM*

By

**Randy Moore**
**Department of Biology**
**Baylor University**
**Waco, TX 76798**

## ABSTRACT

Broccoli **(Brassica oleraceae)** cells underwent cellular senescence when grafted to tomato **(Lycopersicon esculentum)**. This senescence (1) was not nearly as pronounced as that which occurs in some other incompatible heterografts, and (2) was characterized by loss of the primary vacuole, a decreased staining intensity of the cytoplasm, loss of membrane integrity, vesiculation of the cytoplasm, and death of the cell. Dead cells of **B. oleraceae** collapsed and formed a necrotic layer at the graft interface that separated the two graft partners. By 20 days after grafting, the necrotic layer of dead **Brassica** cells was 2 to 3 cells thick. Cells of **Lycopersicon** appeared healthy throughout graft development. Similar responses in both broccoli and tomato occurred in **Brassica/Lycopersicon** (i.e., scion/stock) and **Lycopersicon/Brassica** heterografts, as well as in approach grafts between plants with intact roots. Symplastic continuity, cellular interdigitation, and vascular redifferentiation were not observed at any stage of graft development. The tensile strengths of heterografts between broccoli and tomato peaked at approximately 13 g breaking weight (BW)/mm$^2$ graft area (GA) at 5 d after grafting, while compatible autografts in broccoli and tomato were characterized by a maximal tensile strength of approximately 160 g BW/mm$^2$ GA by 15 d after grafting. These results suggest that graft incompatibility between broccoli and tomato may be due to a toxin(s) originating in tomato that moves into adjacent broccoli cells and there elicits the cellular incompatibility response.

## INTRODUCTION

Plant grafting is a horticulturally and economically important technique. Grafting has been used for a variety of purposes, including the improvement of disease resistance, obtaining special forms of growth, perpetuating or speeding production of certain plants, and studying the transmission of viral diseases (Mahlsted and Haber 1957; Hartmann and Kester 1975; Hartmann, Flocker and Kofranek 1981). Grafting has also been used by non-horticulturists as a tool for basic botanical research (e.g., transmission of the flowering stimulus — see Lang, Chailakhyan and Frovola 1977).

Although events associated with the formation of a compatible graft union are becoming more clearly defined (Stoddard and McCully 1979, 1980; Moore 1981, 1984a; Moore and Walker 1981a, c; Parkinson and Yeoman 1982; Jeffree and Yeoman 1983), little is known about the mechanisms underlying graft incompatibility (see reviews by Moore 1981, 1983a). Indeed, a biochemical basis for graft incompatibility is known for only two graft combinations, those being between (1) pear and quince (Gur, Samish and Lifshitz 1968), and (2) peach and almond (Gur and Blum 1973). Similarly, our understanding of the cellular events associated with graft incompatibility is equally rudimentary, since there have been only three reports of the ultrastructural changes that occur in response to incompatible grafting (Moore 1982a, 1984b; Moore and Walker 1981b). In spite of their small number, however, studies of graft incompatibility have yielded significant results, one of the most notable being that graft incompatibility between pear and quince can be overcome by injecting ferrous polysulphide into the graft interface (Gur 1972).

In this paper I report the ultrastructural changes associated with the development of graft incompatibility between **Brassica oleraceae** (broccoli) and **Lycopersicon esculentum** (tomato). These structural changes are interpreted relative to a corresponding study of the development of tensile strength of the graft union.

## MATERIALS AND METHODS

**Plant material and growth conditions** — Plants of broccoli **(Brassica oleraceae)** and tomato **(Lycopersicon esculentum)** were grown in a mist chamber under a daily regime of 16 h of light and 8 h of dark at a constant temperature of 25° C. Light was provided by a combination of incandescent and fluorescent lamps at an intensity of 45 klx. Plants were watered regularly with a modified Hoagland solution (Hoagland and Arnon 1938).

**Grafting procedures** — Broccoli and tomato were grafted by two different methods: (1) conventional grafts, and (2) approach grafts. All grafted plants were kept it the mist chamber under the conditions described above.

Conventional grafts between broccoli and tomato

were made according to methods described previously by Moore and Walker (1981a). The diameter of the graft unions at the time the grafts were established ranged from 2.4 mm to 3.8 mm. The diameter of the graft unions when the plant material was prepared for microscopical observation or determination of tensile strength ranged from 2.4 mm to 4.5 mm. A collar of latex tubing used to support the graft was removed immediately prior to preparing the tissue for microscopical observation or determination of tensile strength of the graft union.

Approach grafts were made by longitudinally cutting and then reuniting young expanding internodes. Parafilm was used to hold the cut surfaces together.

**Microscopy** — Grafts at various developmental stages were excised and immediately fixed in 2 % glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 6.9) for 3 h at room temperature. After three 10 min rinses in buffer, the tissue was postfixed in a 2 % solution of buffered osmium tetroxide for 2 h at room temperature. The grafts were then dehydrated in a series of ethanol solutions and embedded in Spurr's epoxy (Spurr 1969). Ultrathin sections were stained with uranyl acetate and lead citrate prior to electron microscopical observation. Adjacent 1 $\mu$m sections were stained with Toluidine Blue O for light microscopical observation.

**Measurement of tensile strength** — Measurements of the tensile strength of graft unions were made in a manner similar to that described previously (Lindsay, Yeoman and Brown 1974). Values for the tensile strengths of the graft unions are reported as the force required to separate the two graft partners (i.e., g breaking weight) per unit contact are (i.e., mm$^2$ graft area).

## RESULTS

More than 50 heterografts of **Brassica/Lycopersicon** (i.e., scion/stock) and **Lycopersicon/Brassica** were made in this study. An unsuccessful graft between the stock and scion resulted in every instance. In micrographs of these heterografts (i.e., Fig. 2, 3), the **Brassica** partner is denoted by "B", while the **Lycopersicon** partner is denoted by "L".

**Internodal cells of Brassica and Lycopersicon** — Internodal cells of **Brassica** and **Lycopersicon** are shown in Fig. 1a and 1b, respectively. Both types of cells possessed (1) a layer of granular cytoplasm which contained the usual compliment of cellular organelles, and (2) a large central vacuole. Encircling the cells was a primary cell wall.

**The Brassica/Lycopersicon heterograft** — By 5 d after grafting, proliferation of callus cells at the graft interface began in both graft partners (Fig. 2a, asterisk denotes severed vascular bundle at site of original cut). Cellular divisions to produce these callus cells were typically oriented parallel to the graft interface. The points of contact between callus cells of the graft partners were (1) surrounded by numerous in-

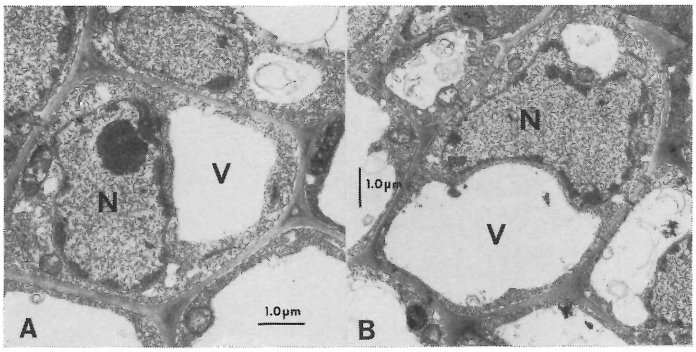


**FIGURE 1.** Internodal cells of **Brassica** (Fig. 1a) and **Lycopersicon** (Fig. 1b). Both types of cells possess a large primary vacuole (V) surrounded by a layer of densely staining cytoplasm containing the usual compliment of cellular organelles. N = nucleus. (a) X 14100. (b) X 11200.

tercellular spaces (Fig. 2a-c; arrows denote graft interface), and (2) indentifiable by the thickened cell wall shared by the contacting cells (Fig. 2d-f). A densely staining material was typically located between the contacting cell walls (Fig. 2d-f). **Lycopersicon** cells at the graft interface appeared healthy, and were characterized by the presence of a large primary vacuole that was surrounded by a layer of densely staining cytoplasm (Fig. 2d-f). Adjacent cells of **Brassica** exhibited early signs of senescence, such as a reduced staining intensity of the cytoplasm, the occurrence of flocculent material throughout the cytoplasm, and replacement of the large primary vacuole by smaller vacuoles (Fig. 2d, 3). Numerous dictyosomes were occasionally present adjacent to the portion of cell wall contacting the neighboring **Lycopersicon** cell (Fig. 2f). Despite the senescent appearance of **Brassica** cells, most organelles (e.g., mitochondria) appeared viable at 5 d after grafting (Fig. 2e, f).

At 12 days after grafting, a darkly staining necrotic layer separated the two graft partners (Fig. 2g, h; NL = necrotic layer). The necrotic layer was composed of remnants and collapsed walls of dead cells (Fig. 2h). **Lycopersicon** cells adjacent to the graft interface continued to appear healthy, and were characterized by the presence of a densely staining layer of cytoplasm that surrounded a large primary vacuole (Fig. 2h). Bordering cells of **Brassica** were characterized by the absence of any membrane-bound organelles (Fig. 2h).

At 20 days after grafting, there continued to be a necrotic layer at the graft interface that separated **Brassica** from **Lycopersicon** (Fig. 2i, 3a). Cells of **Brassica** and **Lycopersicon** adjacent to the necrotic layer had appearances similar to those described above for 12 day-old grafts. **Brassica** cells in the process of collapsing (and thus contributing to the necrotic layer that separated the graft partners) were characterized by the presence of densely staining remnants of vesiculated cytoplasm (Fig. 3a).

**The Lycopersicon/Brassica heterograft** — Develop-

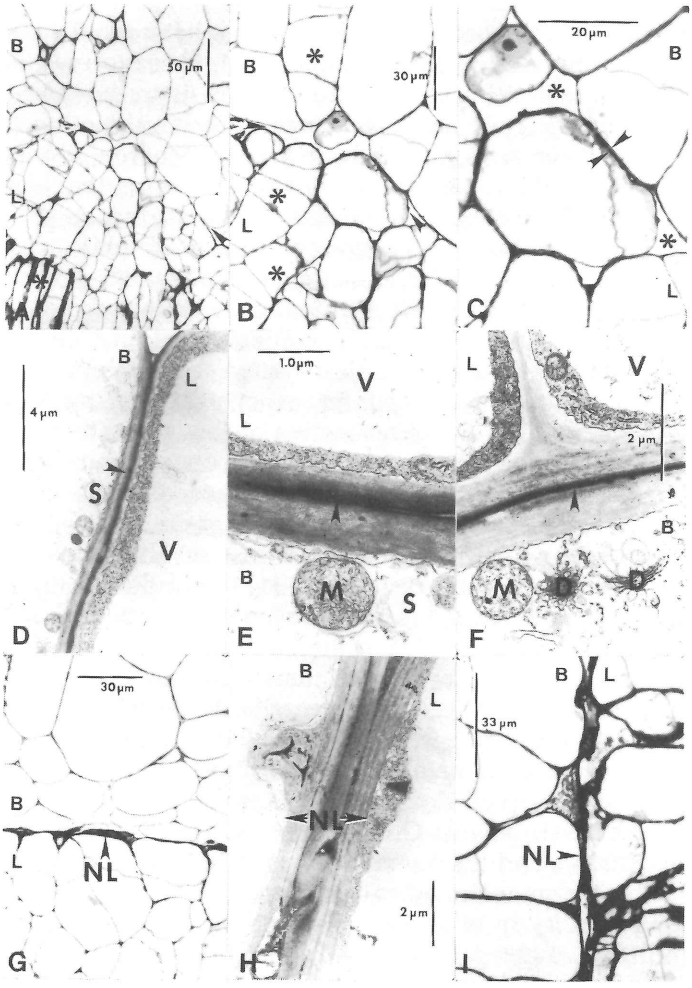


**FIGURE 2.** Development of the **Brassica/Lycopersicon** heterograft. In all micrographs, **Brassica** cells are denoted by "B" and **Lycopersicon** cells are denoted by "L". (a)-(f). The graft interface at 5 d after grafting (a). Arrowheads denote the graft interface between broccoli and tomato. The graft interface was composed of callus cells contributed by both partners. * = severed vascular bundle at site of original cut. X 400. (b). Callus cells were typically oriented in files perpendicular to the graft interface (*). X 740. (c). Areas of contact between callus cells of broccoli and tomato (arrowheads) were characterized by the presence of numerous intercellular spaces (*). X 1500. (d)-(f). A densely staining material (arrowhead) was present between the contacting cell walls of broccoli and tomato. Tomato cells appeared healthy and were characterized by the presence of a large primary vacuole (V) surrounded by a densely staining layer of cytoplasm. Adjacent broccoli cells were characterized by a reduced staining intensity of the cytoplasm and the presence of small vacuoles (S). Numerous dictyosomes (D) were occasionally present in broccoli cells at the graft interface. M = mitochondrion. (d) X 7100; (e) X 20000; (f) X 15000. (g), (h). The graft interface at 12 d after grafting. A necrotic layer (NL) consisting of collapsed cell walls and cellular remnants separated the graft partners. Note the absence of organelles in the broccoli cell shown in (h). (g) X 720; (h) X 10000. (i). The graft interface at 20 d after grafting. A necrotic layer (NL) continued to separate the callus cells of broccoli and tomato. Note the reduced staining intensity characteristic of the protoplasm of broccoli cells. X 1200.

ment of the **Lycopersicon/Brassica** heterograft was similar to that of the **Brassica/Lycopersicon** heterograft. At 5 days after grafting, **Lycopersicon** cells adjacent to the graft interface appeared healthy, and were characterized by the presence of a densely staining layer of cytoplasm that surrounded a primary vacuole (Fig. 3b). **Brassica** cells (1) were characterized by a reduced staining intensity of the cytoplasm, (2) possessed flocculent material throughout the cytoplasm, and (3) lacked a primary vacuole. Organelles in **Brassica** cells appeared viable (Fig. 3b).

A necrotic layer consisting of 2 to 3 collapsed cells developed at the graft interface between 12 (Fig. 3c, d) and 20 d (Fig. 3e, f) after grafting. **Lycopersicon** cells adjacent to the graft interface maintained a healthy appearance, while **Brassica** cells underwent a slow senenscence that eventually led to their death and collapse at the graft interface.

I did not observe symplastic continuity, cellular interdigitation, or vascular redifferentiation across the graft interface at any stage of graft development in heterografts between **Brassica** and **Lycopersicon.** Heterografts typically separated by 30 to 40 days after grafting.

**Approach grafts** — The interface of a 20 day-old approach graft between rooted plants of **Brassica** and **Lycopersicon** is shown in Fig. 3g and 3h. A continuous necrotic layer composed of collapsed walls and cellular remnants separated the two graft partners. **Lycopersicon** cells bordering the necrotic layer appeared healthy, while adjacent **Brassica** cells appeared senescent (Fig. 3h).

**Tensile strength** — The development of tensile strength in **Brassica/Lycopersicon** and **Lycopersicon/Brassica** heterografts is shown in Fig. 4. For comparative purposes, graphs depicting the development of tensile strength of compatible autografts in **Lycopersicon** and **Brassica** are also shown. All data points represent the mean ($\pm$ standard deviation) of at least six independent trials. The tensile strength of a 20 day-old approach graft was 5 $\pm$ 3g BW/mm$^2$ GA.

## DISCUSSION

**Cellular responses** — The response that characterized graft incompatibility between **Lycopersicon esculentum** (tomato) and **Brassica oleraceae** (broccoli) was cellular necrosis. This necrosis (1) was quite slow (as evidenced by a necrotic layer of only 2 to 3 col-

lapsed cells at 20 d after grafting), and (2) was restricted to the broccoli partner of the heterograft. Similar observations of differential responses to incompatible grafting (e.g., cellular necrosis in one partner, with a significantly milder response in the other partner) have been reported previously (Moore and Walker 1981b, 1983; Moore 1984b), indicating that the cellular responses associated with graft incompatibility may vary considerably in the stock and scion. Also, since **Lycopersicon** cells remained healthy throughout graft development, there was no mutual rejection by the graft partners as might be expected if a mutual recognition system were operative.

Necrosis of broccoli cells in response to grafting with tomato occurred irrespective of the orientation of the stock and scion (i.e., **Brassica/Lycopersicon** or **Lycopersicon/Brassica).** Necrosis of broccoli cells also occurred at the interface of approach grafts between plants with intact root systems. These results suggest that cellular necrosis in broccoli (1) is not due to desiccation of tissues resulting from severance of the vascular system, and (2) maybe due to a toxic substance(s) that moves from tomato into broccoli and there elicits the cellular incompatibility response. Supporting this suggestion are the facts that (1) the presence of toxic metabolites in members of the Solanaceae is well documented (Turova, Seifula and Belykh 1961; Nishie, Gumbmann and Keyl 1971; Roddick 1974), and (2) the movement of toxic metabolites has been demonstrated to be responsible for graft incompatibility in other systems (Gur et al. 1968; Gur and Blum 1973; Moore and Walker 1983).

Graft incompatibility between certain plants appears to be dependent on the orientation of the graft partners. For example, the **Prunus salicina/Prunus domestica** heterograft is successful, while the **P. domestica/P. salicina** combination is not (Hartmann et al. 1981). The behavior of these reciprocal grafts has been suggested to be due to a predominantly one-way flow of toxic metabolites (Hartmann and Kester 1975) (in this case, downward from the **P. domestica** scion into the **P. salicina** rootstock), as might be envisioned to occur in the phloem. This type of tissue interaction does not occur in the heterograft between broccoli and tomato, since incompatibility resulted no matter how the stock and scion were arranged. These observations suggest that the toxin(s) presumed responsible for graft incompatibility between broccoli and tomato are diffusible substances whose movement is not restricted to the vascular tissue. A similar mechanism (i.e., diffusion of toxins) has been suggested to be responsible for graft incompatibility in other systems (Gur et al. 1968; Moore and Walker 1983; Moore 1984b).

The broccoli/tomato heterograft is only the third system in which the ultrastructural aspects of graft incompatibility have been reported. The cellular changes associated with the necrosis of broccoli cells in response to grafting with tomato were similar to

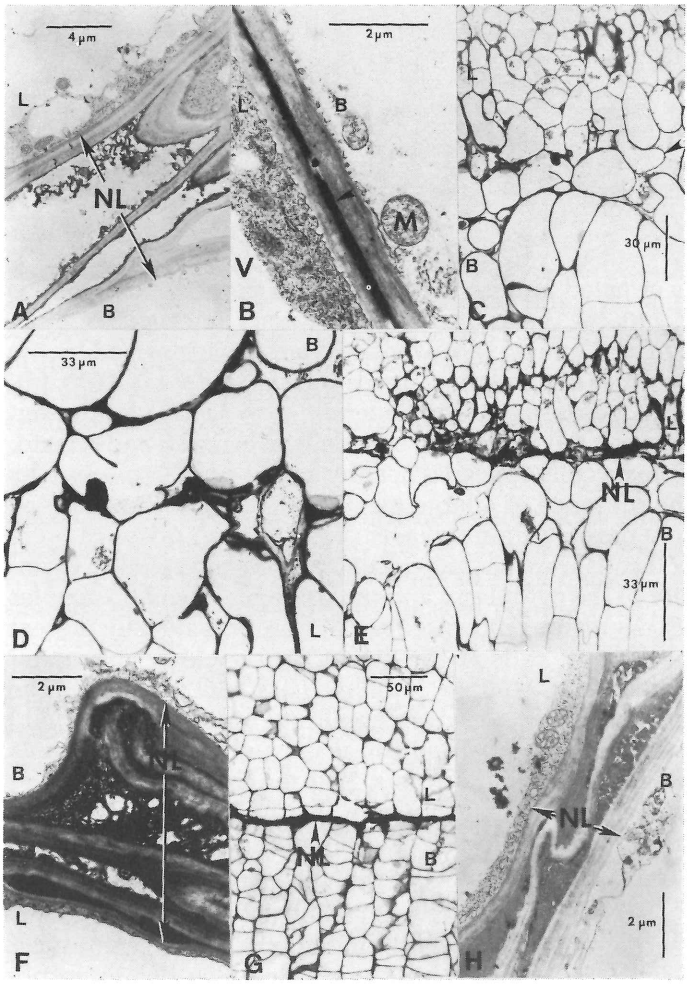


**FIGURE 3.** In all micrographs, **Brassica** cells are denoted by "B" and **Lycopersicon** cells are denoted by "L". (a). The interface of the **Brassica/Lycopersicon** heterograft at 20 d after grafting. Healthy tomato cells were separated from senescent broccoli cells by a necrotic layer (NL) consisting of collapsed cell walls and cellular remnants. X 4600. (b)-(f). Development of the **Lycopersicon/Brassica** heterograft. (b). The graft interface at 5 d after grafting. Tomato cells appeared healthy and were characterized by the presence of a densely staining layer of cytoplasm surrounding a primary vacuole (V). Broccoli cells appeared senescent and lacked a primary vacuole. A densely staining substance (arrow) was present between the contacting cell walls. M = mitochondrion. X 15000. (c), (d). The graft interface between broccoli and tomato at 12 d after grafting. (c) arrowheads denote graft interface. X 710. (d) X 1200. (e), (f). The graft interface at 20 d after grafting. A necrotic layer (NL) consisting of collapsed cell walls and cellular debris separated the graft partners. Broccoli cells bordering the graft interface continued to appear senescent, while tomato cells remained healthy. (e) X 1300; (f) X 10000. (g), (h). The interface of a 20 d old approach graft between broccoli and tomato. A necrotic layer (NL) separated the two graft partners. (g) X 400; (h) X 10000.

those reported for both other incompatible grafts that have been investigated (Moore and Walker 1981b; Moore 1984b). These structural similarities are correlated with the fact that graft incompatibility in each of these systems has been suggested to be due to the involvement of toxins (Gur et al. 1968; Moore and Walker 1981b, 1983). Also, the structural similarities of graft-induced cellular necrosis (reported here and elsewhere — see Moore and Walker 1981b; Moore 1984b) with that accompanying wounding (Shaw and Manocha 1965; Jones, Graham and Ward 1975; Favali, Conti and Bassi 1978; Niki, Yoshida and Sakai 1978) support the suggestion that cellular necrosis may be a defense mechanism used by plants in a variety of situations (Moore and Walker 1981b).

Although the cellular responses associated with graft incompatibility are similar to those which occur in response to wounding (e.g., periclinal cellular divisions, accumulation of dictyosomes at the graft interface — see discussions in Moore and Walker 1981a, b), wounding is not a prerequisite for the expression of graft incompatibility (Moore and Walker 1983; Moore 1984b). That is, cellular incompatibility is a unique response that is only fortuitously superimposed on the initial wound response associated with establishing a graft (Moore and Walker 1983).

Necrosis of broccoli cells resulted in the formation of a necrotic layer at the graft interface of heterografts between **Brassica** and **Lycopersicon.** However, this necrosis was not nearly as pronounced as that which occurs in some other incompatible heterografts. For example, the necrotic layer in heterografts between **Sedum** and **Solanum** was composed of up to 10 collapsed cells by 14 d after grafting (Moore and Walker 1981b), while that of a heterograft between **Brassica** and **Lycopersicon** was typically composed of only 2-3 collapsed cells by 20 d after grafting (Fig. 3a). Nevertheless, the necrotic layer between **Brassica** and **Lycopersicon** was continuous across the graft interface by 20 d after grafting and was never ruptured to establish direct contact between cells of the stock and scion. The presence of a necrotic layer (1) is typical of incompatible grafts (Buck 1954, 1971; Fletcher 1964), and (2) serves to insulate the stock from the scion. This insulation ultimately results in starvation of the scion and its eventual separation from the stock. It is interesting to note that graft incompatibility between certain members of the Solanaceae (1) apparently does not involve cellular necrosis, and (2) is characterized by the absence of vascular redifferentiation (Yeoman et al. 1978).

Vascular redifferentiation did not occur in callus cells of either graft partner at the graft interface. A similar absence of vasular redifferentiation has been reported for other incompatible grafts (Moore and Walker 1981b) and has been suggested to be due to the absence of a direct cellular interaction between graft partners (Moore and Walker 1981b).

**Development of tensile strength** — The development of tensile strength in the **Brassica/Lycopersicon** heterograft was indistinguishable from that characteristic of the **Lycopersicon/Brassica** heterograft. This functional similarity in graft development is not surprising, however, given the structural similarities between the development of the two grafts.

Grafts between broccoli and tomato initially adhered, even though the partners were incompatible. Similar observations have been made previously for other grafts (Yeoman et al. 1978), and support the suggestion that adhesion of the stock and scion is not directly related to graft compatibility or incompatibility (Moore and Walker 1981a, b). The initial adhesion of graft partners has been suggested to be the result of a cellular wound response that does not involve cellular recognition (Moore and Walker 1981a).

The tensile strength of incompatible heterografts between broccoli and tomato peaked approximately 5 d after grafting at a value of approximately 13 g BW/mm$^2$ GA. This value agrees well with those reported for other incompatible heterografts between herbaceous tissues (Moore 1983b) as well as autografts that have been experimentally manipulated so as to delay certain aspects of development (Moore 1984a). These results therefore support the suggestion that the cellular wound response responsible for the initial adhesion of graft partners contributes approximately 12 g BW/mm$^2$ GA to the tensile strength of graft unions between herbaceous tissues (Moore 1983b). More comprehensive discussions of the contributions of structural events that occur during graft formation to the development of tensile strength are presented elsewhere (Lindsay et a. 1974; Moore 1982b, 1983a, b; McGarry and Moore 1983).

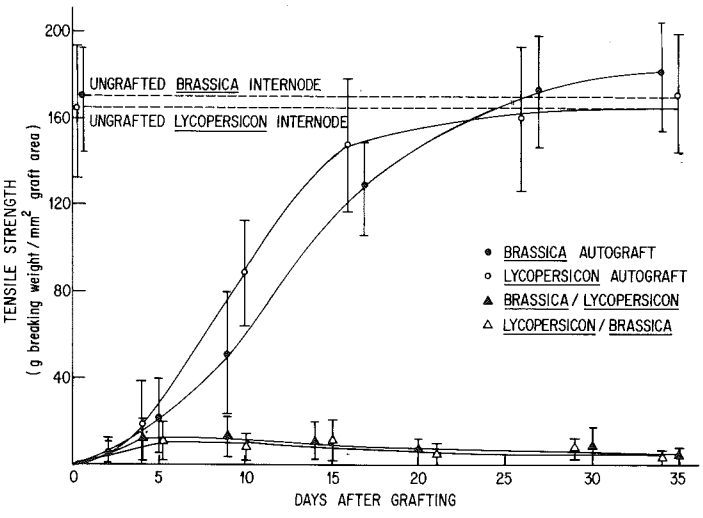


**FIGURE 4.** The development of tensile strength in various grafts involving **Brassica** and **Lycopersicon.** All values are expressed as the force required to separate the graft partners (i.e., g breaking weight) per unit graft area (mm$^2$ graft area). Variation is expressed as ± standard deviation.

The development of tensile strength in compatible autografts in tomato and broccoli was similar to that described previously for other autografts (Moore 1982b, 1983b; McGarry and Moore 1983). In all systems investigated to date, the tensile strength of the fully developed autograft approximates that of a comparable ungrafted internode (see discussions in Moore 1982b, 1983b).

**Graft development** — The three developmental stages of a compatible graft have been suggested to be (1) adhesion of the stock and scion, (2) interdigitation of callus cells at the graft interface, and (3) redifferentiation of vascular tissue across the graft interface (Moore 1983a). In the incompatible heterograft between broccoli and tomato, the stock and scion did adhere, but neither interdigitation of callus cells nor vascular redifferentiation occurred. Therefore, incompatibility in this system is expressed at an early stage of graft development. This is in contrast to certain other incompatible heterografts (e.g., pear/quince) in which graft incompatibility is expressed after each of these developmental events (i.e., adhesion, callus interdigitation, and vascular redifferentiation) has occurred. However, it is also important to note that the expression of graft incompatibility is not necessarily restricted to any one of these "stages" of graft development (Moore 1984b), and that cellular incompatibility associated with graft incompatibility does not require direct cellular contact (Moore 1984b).

## ACKNOWLEDGEMENTS

This research was supported by Grant No. PCM-8207933 from the National Science Foundation. The author thanks Steve Ransom for his excellent technical assistance.

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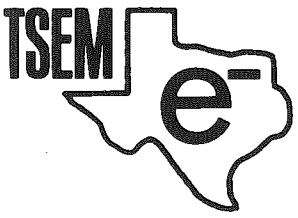


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## BIOLOGICAL SCIENCES

QUANTITATIVE ULTRASTRUCTURE OF APICAL MERISTEMS VARYING IN GROWTH RATE, MORPHOGENIC ACTIVITY, AND PHYLOGENY. James D. Mauseth, Dept. of Botany, University of Texas, Asutin, Tx. 78713.

Dormant short shoot apices of Opuntia polyacantha were cultured under three conditions: cytokinin and high sucrose to stimulate the formation and rapid growth of a leafy long shoot; cytokinin and no sucrose (slow growth of a leafy long shoot); gibberellic acid and high sucrose (rapid growth of a spiny short shoot). These meristems, and also dormant (uncultured) ones, were analyzed by stereological, ultrastructural techniques. By comparing meristems growing with cytokinin but with or without sucrose, correlations between metabolic rate and apical ultrastructure were studied; comparison of leaf-producing and spine-producing meristems permitted examination of correlations with morphogenic role; comparison with published data for four other species permitted study of phylogenetic effects, and comparison with dormant apices revealed information about meristem activation. Ultrastructure varied according to each condition: metabolic rate, morphogenic activity and species can be distinguished by quantitative methods. Apical ultrastructure is most strongly correlated with rate of growth such that apices of differing species resemble each other if growing at similar rates, whereas apices of a single species differ markedly if growing at differing rates or if performing different morphogenic activities. Hyaloplasm is an excellent indicator of metabolic rate; mitochondria, nuclei, and vacuoles are not.

THE ROLE OF VITAMINS IN THE PREVENTION OF GOUTY ARTHRITIS. C. M. Martinez and D. Feldman, Bioengineering Program, Texas A&M University, College Station, TX. 77843.

Approximately 0.2% of the total population (5% of all arthritic patients) in the United States. are afflicted with gout. Although it is believed that acute gouty arthritis is caused by the deposition of monosodium urate (MSU) crystals, neither the mechanism of crystallization nor the pathogenesis of the disease is fully understood. Some investigators have suggested that the size and configuration of the MSU crystals are responsible for cellular lysis.

Crystallization of MSU has been linked to excessive uric acid concentrations in body fluids (hyperuricemia). The fact that only 17% of hyperuricemic individuals exhibit symptoms of gouty arthritis indicates that other factors besides hyperuricemia influence MSU crystallization. Therefore, treatment of gouty arthritis should be based on the prevention or inhibition of MSU crystallization. This investigation is to examine substances normally found in synovial fluid and observe their effects on MSU crystallization.

Monosodium urate was crystallized in vitro from standard solution and from solutions containing substances normally found in synovial fluid. Vitamins, especially riboflavin, were found to inhibit the number and size of MSU crystals in the various media prepared. Clinical in vivo studies are now being contemplated to determine if the paucity of these compounds in the synovial fluid triggers gout in hyperuricemic individuals.

MORPHOLOGICAL SURVEY OF SELECTED GENERA OF EQUINE CYATHOSTOMES. M.T. Suderman[1], T.M. Craig[1] and A.J. Neumann[2], [1]Dept. of Veterinary Microbiology and Parasitology, CVM-TAMU, College Station, Texas 77843 and [2]Dept. of Biology, TAMU, College Station, Texas 77843.

Recent studies indicate that cyathostomes (Strongylidae: Cyathostominae) may be a causal factor in verminous equine colitis and digestive malabsorption (Bueno et al., 1979; Jasko and Roth, 1984). Cyathostome parasitism is a major contributor to nematode burdens in horses, however, identification of these parasites is difficult due to a lack of an agreed nomenclature and information concerning their life cycles. The definitive work on cyathostome taxonomy is a survey of cyathostomes by Litchenfels (1975) using light microscopic techniques.

Seven genera of equine cyathostomes found in the ileo-caeco-colic region were selected for comparison and analysis of morphological characteristics between Nomarski DIC and scanning electron microscopy. Of special taxonomic interest were the buccal coronal leaves, reproductive organs, and sensory structures. Comparison of Nomarski and SEM micrographs should allow the veterinary researcher to better identify these nematodes.

VASCULAR CASTING: APPLICATIONS FOR THE STUDY OF THE VASA VASORUM AND OTHER MICROVASCULAR BEDS. J.T. Hansen and A. Guzman, Dept. Cellular and Structural Biology, University of Texas Health Science Center, San Antonio TX 78284

Examination of the microvasculature of various tissues with the light microscope often yields only limited information, even when preparations have been labelled with monastral blue B, horseradish peroxidase, lead chromate, carbon particles, or colored gelatin. Many of these "labelled" preparations suffer from incomplete filling of the smaller vessels due to poor diffusion of the injected substances. Moreover, using light microscopy alone, one often cannot discern vessel detail or the three-dimensional configuration of the microvasculature. We have used a low viscosity commercial methacrylate resin (Mercox; sold in kit-form by Ladd Research Industries, Inc.) which completely fills even the smallest capillaries. The resulting corrosion casts then can be examined in the SEM, which permits greater resolving power and the advantage of viewing the specimen in its three-dimensional state. Mercox can be injected and polymerized within 5-10 min following vascular perfusion of the tissues with EM fixatives. The selected tissues are macerated in sodium hypochlorite, washed in water, dried, mounted, coated, and viewed in the SEM. Examples of several microvascular preparations and an outline of the detailed methodology will be presented. (Supported by NIH grant HL-31320 and AHA grant 83 733).

A MOTORIZED DUPONT-SORVALL MT-1 ULTRAMICROTOME. T. Caceci Dept. of Veterinary Anatomy, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843

A DuPont-Sorvall MT-1 ultramicrotome, normally a manually-operated instrument, was equipped with a small, high-torque DC motor. The motor and control circuitry permitted automatic operation at continuously-variable cutting speeds between one and five sections per minute. The motorization package is easily removable, and does not require any modifications to the MT-1, which can be reconfigured for manual operation whenever desired. It was found that for smooth operation, the connection between the motor output shaft and the handwheel spindle of the MT-1 must be rigid and perfectly coaxial and that the motor should be isolated from the frame of the ultramicrotome. The handwheel was removed and the motor was connected directly to the handwheel spindle by means of a cylindrical coupling and a specially-modified #10-32 TPI machine screw. This provided rigidity. The motor was mounted on a small wooden stand with provision for vertical adjustment, which allowed for proper of the motor shaft and the handwheel, and eliminated vibrations. Ultrathin sections were cut from tissue blocks embedded in Epon-Araldite, Spurr, and Maraglas epoxy resins with good results. Glass knives were used. The conversion does not offer many of the convenient operating features of more sophisticated ultramicrotomes but a laboratory which has an MT-1 on hand may find this an economical substitute for a new instrument. In teaching labs a motorized MT-1 would allow students to learn both manual and automated sectioning techniques at low cost. It would also be useful if large numbers of survey sections for LM are to be cut, as in pathology laboratories. This would avoid tying up time on a more sophisticated instrument.

DIAGNOSTIC ELECTRON MICROSCOPY: A REAPPRAISAL. B. Mackay, Dept Pathology, University of Texas M. D. Anderson Hospital and Tumor Institute, Houston TX 77030.

On the occasion of an important anniversary, it is appropriate to look back and ahead and reflect on the evolution, current status and future role of electron microscopy. In the field of diagnostic medicine, the technique underwent a period of development during which there was considerable speculation and controversy over its potential, followed by two decades of intensive application which did much to determine its true worth. It became apparent that the practical benefits of ultrastructural examination of diagnostic tissues are limited, but in a number of specific areas electron microscopy has been recognised to have an important role. The most extensive application is in the identification and classification of tumors, where the full potential of ultrastructural studies has not yet been realised. Because of the broad range and complexity of morphology that exists among human neoplasms, examination of considerable numbers of examples of each type is necessary in order to define diagnostic criteria and determine degrees of overlap in fine structure among different tumors. The application of transmission electron microscopy in the study and diagnosis of human tumors is likely to continue at its present level during the coming decade, but because of current economic restraints, implementation in a hospital setting requires selective use based upon an awareness of its values and limitations relative to those of other diagnostic methods including conventional light microscopy, histochemistry, and immunocytochemistry.

LIVER AND PANCREAS CHANGES IN SELENIUM-EXPOSED FISH. E.M.B. Sorensen, Dept. Pharmacol./Toxicol., University of Texas, Austin, TX 78712

Lepomis species were collected upon numerous occasions from two lakes (in North Carolina and in Texas) containing abnormally elevated concentrations of selenium in the water and sediments as indicated from repeated chemical analyses by several state agencies. The hepatopancreas (liver and associated, disseminated exocrine pancreas) was processed for a number procedures including high voltage and conventional transmission electron microscopy, optical microscopy, and neutron activation analysis. The hepatocytes of redear and green sunfish which accumulated the highest levels of selenium were characterized by central or focal necrosis, granular cytoplasm, fatty infiltration, Kupffer cell hyperplasia, increased numbers of lysosome-like structures, and/or reduced quantities of rough endoplasmic reticulum and glycogen particles. These nonspecific changes were considered evidence for hepatocyte destruction. Exocrine pancreas cells were disorganized architecturally and showed reduced rough endoplasmic reticulum, proliferation of smooth endoplasmic reticulum, and increased cisternal space compared with reference fish collected from selenium-deplete areas. Hepatocyte destruction and concomitant exocrine pancreas cell hyperplasia or hypertrophy probably resulted in the markedly increased relative areas occupied by pancreas cells compared to that of hepatocytes.

SELENIUM-INDUCED NEPHROTOXICITY IN FISH. E.M.B. Sorensen, Dept. Pharmacol./Toxicol., University of Texas, Austin, TX 78712.

Lepomis microlophus and L. cyanellus were collected upon several occasions from Martin Lake, east Texas, and Belews Lake, North Carolina over a period of three years to access the impact of excess levels of selenium from electrical generating station operations. The mesonephros from a number of individuals from these areas and from reference, selenium-replete areas was processed for ultrastructural and histopathological examination using standard methodology. Glomeruli from reference fish were similar to the mammalian counterpart except for lamination, occasional effacement, and the presence of nucleated erythrocytes in the capillary loops. Glomeruli from fish which had accumulated abnormally high levels of selenium (as determined by neutron activation analysis) had increased numbers of mesangial cells and greater quantities of mesangial cell matrix — which are diagnositic of proliferative glomerulonephritis. This condition resulted in permeability changes within the renal corpuscle and was characterized (in these selenium-exposed fish) by uremia. Those fish accumulating the highest levels of selenium showed the most severe changes.

A SCANNING ELECTRON MICROSCOPIC STUDY OF IN OVULO AND SOMATIC IN VITRO EMBRYOS OF COTTON (GOSSYPIUM HIRSUTUM). S.R. Short-Russell, Norma L. Trolinder, J.D. Berlin and J.R. Goodin, Dept. of Biological Sciences, Texas Tech Univ., Lubbock, TX 79409-4149.

Scanning electron microscopy (SEM) was employed to detect anomalies in embryos of Gossypium hirsutum obtained from tissue culture methods. Somatic in vitro embryos were obtained from tissue culture of calli derived from hypocotyl of 3 day old seedlings. These were compared to in ovulo embryos taken from green house grown plants of the same variety. Each of the major stages of development from globular to mature embryo were obtained from both sources and processed for SEM using standard techniques. The only gross morphological differences found were due to the space limitations placed on the embryos during development: (1) in tissue culture, several embryos may develop adjacent to one another and become inseparable, whereas in vivo embryos are isolated by the ovule, (2) in vitro, the cotyledons are not restricted by the ovule and do not undergo the extensive folding that is seen in ovulo embryos, and (3) the globular stage in vitro sometimes becomes larger than that of in ovulo. In our study, we could detect no differences in the general shape, appearance, epidermal surfaces, or the degree of differentiation of embryos at the same stage of development taken from the two sources. Supported by Cotton Incorporated and the Plant Stress Institute and Water Resources Center at Texas Tech University.

Microcasting: A Technique to Study Soft Tissue Ingrowth in Porous Biomaterials Using the Scanning Electron Microscope. T.D. Estridge and D.S. Feldman, Bioengineering Division, Department of Industrial Engineering, Texas A&M University, College Station, Texas 77843.

Porous biomaterials have been used for many different applications, including fixation or stabilization of various implantable devices, tissue ingrowth to create blood compatible surfaces, scaffolding to help the body repair or regenerate tissue, and prevention of epidermal downgrowth in percutaneous devices. One of the most common soft tissue porous biomaterial is dacron velour. A well developed vascular system is needed in porous biomaterials to insure implant stability by tissue ingrowth. One factor that can affect vascularity and thus tissue ingrowth is implant preparation (cleaning and sterilization). In this study, the vessel ingrowth into dacron velour for implants sterilized by steam, ethylene oxide (EtO), or radio frequency glow discharge (RFGD) was examined.

The vascular ingrowth was shown by a microcasting technique, specifically designed for the animal model and materials employed. The blood of the animal was replaced by polymethylmethacrylate. After the implants were removed, the surrounding tissue was macerated with potassium hydroxide. The resultant vascular ingrowth can be visualized with a dissecting microscope, a light microscope, or a scanning electron microscope.

Preliminary results have shown that vascular response was greatest for EtO sterilized implants followed by steam sterilized implants then RFGD sterilized implants. Surface alterations caused by sterilization procedures appear to affect the initial tissue response and consequently determine the ultimate success of the implant.

**CEROID-LIPOFUSCINOSIS IN THE GOAT. R.W.Storts[1], J.W. Templeton[1], K.R. Pierce[1], R.A. Fiske[2], P.J. Luttgen[3], C.L. Hall[3], M.S. Frey[1], Dept. of Vet. Path.[1], Texas Vet. Med. Diag. Laboratory[2], Dept. Sm. An. Med. & Surgery[3], Texas A&M University, College Station, TX 77843.**

**Ceroid-lipofuscinosis, which is a progressive degenerative disorder of the central nervous system that occurs in several animal species and man, was detected for the first time in a Texas herd of Nubian goats. Clinical signs, which occured as early as four months of age in a total of nine animals studied, included: a tendency for development in females of coarse facial features that lacked feminine characteristics, stiffness of gait, tremor, ataxia, deterioration of body condition, ruminal tympany (bloat), and paresis. Affected animals continued to eat and drink until the terminal stages of the disease. Signs also were characterized in some animals by remissions, which lasted for varying periods, and exacerbations. The disease is apparently genetically determined by an autosomal recessive mode of inheritance. Microscopic lesions were characterized by a**

**swelling of neurons in the central nervous system and peripheral ganglia that resulted from an accumulation of a granular material that was eosinophilic following hematoxylin-eosin staining, PAS and acid-fast positive, and autofluorescent. Neuronal degeneration was also detected. Important ultrastructural lesions were characterized by a prominent accumulation of membranous profiles within the neuronal cytoplasm that was interpreted as being ceroid-lipofuscin in nature.**

ULTRASTRUCTURAL CHANGES IN THE SKIN OF CATTLE EXPERIMENTALLY INFESTED WITH PSOROPTES OVIS. P.C. Stromberg[1], W.F. Fisher[2], F.S. Guillot[2] and M.S. Frey[2], Dept. of Veterinary Pathology, Texas A&M University[1], College Station Tx 77843 and USDA Livestock Insects Laboratory[2], Kerrville Tx 78028

Nine Hereford calves experimentally infested with Psoroptes ovis were maintained in stanchions for nine weeks. Eight calves developed florid, progressive exudative dermatitis. Ultrastructural observations of superficial skin from infested calves included capillary endothelial hypertrophy and separation with resultant severe edema fluid accumulation, degranulating mast cells, eosinophils, lymphocytes and plasma cells. There was separation of dermal collagen bundles and fibers and leakage through the basement membrane into the epidermis. Although some epithelial cells had hydropic degeneration, the most frequent change was separation of epidermal cells in the stratum spinosum by fluid accumulation between cells. Desmosomal junctions were not separated, but appeared to tear off the adjacent cell membranes. Fluid accumulation between cells in the stratum corneum was of lesser volume, creating a laminated appearance. Keratin, serum, exfoliated epidermal cells, bacteria and active phagocytic cells were observed in the scab on the skin surface.

ULTRASTRUCTURAL COLOCALIZATION OF 5HT AND BOM IN ENDOCRINE CELLS FROM HUMAN FETAL LUNG. J. M. Hoffpauir and R. D. Dey, Dept. of Cell Biology, UTHSCD, Dallas, TX 75235.

Endocrine cells in the airway epithelium of human fetal lungs are known to contain an amine, 5-hydoxytryptamine (5HT), and a peptide, bombesin (BOM). However, the exact endocrine cell type that contains 5HT and BOM has not been described at the ultrastructural level. This investigation provides immunocytochemical evidence that 5HT and BOM are stored in a single cell type, the $P_1$ cell. Airways from human fetal lungs were fixed in 4% formaldehyde and embedded in lowicryl. Thin sections were incubated either in anti-5HT antiserum (diluted 1:3000) or in anti-BOM antiserum (diluted 1:600) and then labelled in affinity purified goat anti-rabbit IgG-FITC-gold complexes (18nm). For colocalization, thin sections were incubated on each side with a different antiserum, then incubated in two different sizes of IgG-gold complexes (8 and 30nm). Controls consisted of absorption of the primary antiserum with an excess (100µg) of either 5HT or BOM. 5HT and BOM were observed in the DCVs of $P_1$ cells. It was apparent from serial sections that 5HT and BOM were stored in the same $P_1$ cells. Labelling did not occur when the anti-5HT antiserum was absorbed with 5HT or when the anti-BOM antiserum was absorbed with BOM. Sections labelled for 5HT on one side with large gold particles and for BOM on the other side with small gold particles revealed that 5HT- and BOM-immunoreactivity was often located in the same DCV. These results demonstrate that 5HT and BOM are stored in $P_1$ endocrine cells of human fetal lung. Furthermore, a single DCV may contain both 5HT and BOM.

IN SEARCH OF THE PERFECT FORMVAR FILM. R.W. Davis, Dept. of Anatomy, College of Med., Texas A&M Univ., College Station TX 77843.

Using Formvar films is something that EM people typically try to avoid if they can. Most feel they are nothing but trouble. If the films release from the slide they very likely have dirt or holes at the one place where you want to take a micrograph. There are times, however, when using a film is desirable or unavoidable. Although I have not solved all the problems associated with Formvar films, I have found a method that consistently produces strong, fairly clean films with very few holes. Briefly the method is:

1) Heat a clean glass microscope slide in a 70°C oven for 2-5 minutes.
2) Using a clean Pasteur pipet, drop .15-.25% (w/v) Formvar in ethylene dichloride with 10% (v/v) absolute methanol added, onto the warm horizontally held slide.
3) Rock the slide back and forth sideways to distribute the Formvar solution. Do this quickly before the slide has a chance to cool or much of the solution to evaporate.
4) Quickly pour off the excess solution and hold the slide vertically inside the 70°C oven until it is dry.
5) Strip the film, place grids and pick it up as usual.
6) Store the film and grids in a clean covered container.

The main differences between this technique and others is: 1) The Formvar solution is pipetted onto the slide 2) the slide is first heated and then allowed to dry in a heated environment. Both of these seem to be necessary. The advantage of pipetting the solution is unknown. Heated drying is believed to be necessary to counter the cooling effect of evaporation and subsequent condensation of water droplets which cause holes. Visual aids demonstrating the technique will be presented.

ON THE CARE OF CLEANING OF POST STAIN DISPENSERS. R.W. Davis, Dept. of Anatomy, College of Med., Texas A&M Univ., College Station, TX 77843.

One time or another everyone has trouble with post stain percipitates. This can be compounded in a central EM facility where numerous people may use the same stock solutions. The following method has been useful for dispensing staining solutions and cleaning the dispensers.

Post stain solutions are made up in the normal way. They are poured into separate 60 ml disposal plastic syringes and excess air is expelled. The open end of the syringe is fitted with a 25 mm clear plastic swin-loc type filter holder. I use polycarbonate filters with a .2 um pore size on all stain and rinse water syringes. Cellulose acetate filters can not be used, as they develop cracks. Aqueous uranyl acetate and Reynold's lead citrate have been stored on the general counter top for several months with apparently little adverse effect. Uranyl acetate in alcohol can be stored in a dark refrigerator between uses to avoid having it go bad.

The advantage of this method is that stains are always ready for use and there is little chance that the solutions will be contaminated. A disadvantage is that the tips of the filter holders eventually build up a percipitate and periodically need cleaning.

The filter holders can be cleaned by removing them from the syringe and completly disassembling them. Soak the pieces in ca. 60% nitric acid, rinse well with water, reassemble, add a filter and replace on the syringe. This will remove even the densest accumulations of lead and uranium. The nitric acid can be stored in a plastic bottle and repeatedly used. Cleaning takes about 5 minutes. If percipitate problems persist it is usually because 1) the filter is cracked or not in the holder right 2) the filter holder tip is dirty 3) the problem does not lie with the stain.

PREPARATION OF GUINEA PIG AIRWAYS FOR ELECTRON MICROSCOPY. M.L. Davis, J.O. Ford, and R.F. Dodson, Department of Cell Biology/Environmental Sciences, The University of Texas Health Center at Tyler, Tyler, TX 75710

Guinea pigs have had wide use as in vivo models for studies of pneumoconioses and other respiratory disorders related to inhaled substances. These animals have been shown to respond to a number of respiratory insults in a manner similar to humans. The present investigation arose from a need to properly preserve guinea pig conducting airways for electron microscopy and to select similar levels of these tiny airways for morphometry from several different individuals in both control and experimental studies of the lung. Hartley guinea pigs of both sexes were anesthetized and perfused via the right ventricle with 0.1M PIPES buffered 3% glutaraldehyde (pH 7.3). A bilateral nephrectomy for drainage was performed at the initiation of perfusion to insure that the respiratory system received both right and left side perfusion. Prior to perfusion, the lungs were inflated via a tracheal cannula to 25cm $H_2O$ and maintained in this state throughout the procedure. Following removal of the lungs, heart and trachea, microdissection was performed to select airways and airway bifurcations for electron microscopy. The dissection was performed with the aid of small surgical instruments and a stereomicroscope equipped with a ringlight fiberoptic light source. Tissue samples were fixed in PIPES buffered glutaraldehyde overnight at 4°C. The tissues were osmicated, en bloc stained with uranyl acetate, dehydrated in ethanol and embedded in Spurr's epoxy resin. These fixation and collection methods provided very satisfactory preservation of airways for both light and electron microscopy.

A MORPHOMETRIC ANALYSIS OF THE REDISTRIBUTION OF ORGANELLES IN COLUMELLA CELLS OF HORIZONTALLY-ORIENTED ROOTS OF ZEA MAYS. Randy Moore and C. Edward McClelen, Department of Biology, Baylor University, Waco, Texas 76798

In order to determine what structural changes in (the presumed) graviperceptive cells are associated with the onset of root gravicurvature, we have quantified the redistribution of organelles in columella cells of horizontally-oriented, gravi-responding roots of Zea mays cv. Yellow Dent. Root gravicurvature began by 15 min after reorientation, and did not involve significant changes in the 1) volume of individual columella cells or amyloplasts, 2) relative volume of any cellular organelle, 3) number of amyloplasts per cell, or 4) surface area or cellular location of endoplasmic reticulum. Sedimentation of amyloplasts began within 1 to 2 min after reorientation, and was characterized by an intensely staining area of cytoplasm adjacent to the sedimenting amyloplasts. By 5 min after reorientation, amyloplasts were located in the lower distal corners of columella cells, and, by 15 min after reorientation, overlaid the entire length of the lower cell wall. We did not detect any consistent contact between amyloplasts and any cellular organelle at any stage of gravicurvature. Centrally-located nuclei moved to the proximal ends of columella cells by 15 min after reorientation. I did not detect any major redistribution of vacuoles, mitochondria, or dictyosomes that correlated with the onset of root gravicurvature. These results indicate that amyloplasts and nuclei are the only organelles whose movements correlate positively with the onset of gravicurvature by primary roots of this cultivar of Z. mays.

ULTRATSRUCTURE OF THE BASAL BODY REGION IN THE ZOOSPORE OF ALLOMYCES. G.R. Aliaga and J. Pommerville, Department of Biology, Texas A&M University, College Station, TX 77843.

The aquatic fungus, Allomyces macrogynus, reproduces asexually by the formation of motile zoospores which possess a posterior tinsel-type flagellum anchored to the basal body (kinetosome). Because the three-dimensional structure and orientation of the associated striated rootlet with respect to the basal body is poorly understood, this ultrastructural study was undertaken in an effort to determine more precisely the three-dimensional structure and orientation of these structures. Electron micrographs show that the rootlet is located next to the proximal region of the basal body which contains the characteristic cartwheel structure. In longitudinally-sectioned material, the zoospore rootlet comprises three electron dense bands, each band being separated by a lighter area composed of loosely packed fibrillar material. The width of the three rootlet bands extends no further than the length of the cartwheel region. The outer band of the rootlet is adjacent to the basal mitochondrion, which forms an invagination partially surrounding the rootlet. Zoospores sectioned transversely in the region of the rootlet have three dark bands. The rootlet band adjacent to the basal body contains short repeating extensions that link the rootlet to the basal body. Published reports of the three dimensional structure of the rootlet and the basal body differ with our interpretation of the overall structure of the rootlet and especially with regard to the orientation of the rootlet to the basal body. By understanding the structure of the rootlet and its orientation with respect to the basal body, the function of the flagellar rootlet may be more easily elucidated.

TIMING AND CYTOLOGICAL STUDIES ON GAMETOGENESIS IN ALLOMYCES MACROGYNUS. T. Sewall and J. Pommerville, Biology Department, Texas A&M University, College Station, TX 77843.

Studies on timing and cytological events during gametogenesis in the water mold, Allomyces macrogynus, were conducted in order to gain a more precise understanding of the poorly understood process of cytoplasmic cleavage. Experiments were conducted using gametothalli grown in droplets of culture medium. Sporulating gametangia produced in this way exhibit a high degree of developmental synchronony. Timing of gametogenic events such as flagellum formation, cytoplasmic cleavage, nuclear cap formation, and gamete release was determined by examining samples prepared at five min intervals for TEM using standard fixation methods both with and without the membrane stain, potassium ferricyanide. Within 5 min after induction, the developmentally-arrested lipid crown stage began to disappear and flagellum formation began. Cleavage furrows were seen initially between flagella but later the furrows radiated into the cytoplasm thereby delineating uninucleate gametes. After ribosomal aggregation occurred forming the nuclear cap, gametes were released 45 min from induction when the pore plugs were discharged. These preliminary observations using a synchronized system will enable us to conduct further studies on the origin of the cleavage membrane and the mechanism of gamete formation.

DIAGNOSTIC ELECTRON MICROSCOPY ON FINE NEEDLE ASPIRATION BIOPSIES. M. Steglich, R. Katz, N. Sneige, B. Mackay, Dept Pathology, University of Texas M. D. Anderson Hospital and Tumor Institute, Houston 77030.

The use of thin needle aspiration biopsies of tumors for diagnosis has burgeoned in the past few years. The technique is convenient for the patient in that it can be performed with less delay or cost than a surgical biopsy, and it allows rapid interpretation by light microscopy since the smears are available for study within minutes. An experienced pathologist will provide a specific diagnosis in over 90% of cases in which positive material is obtained. In the hands of a skilled operator, the procedure provides sufficient material for light microscopy, flow cytometry and electron microscopy, and in problem cases it is important to have a specimen available for ultrastructural study. The yield of suitable material for transmission electron microscopy from a fine needle aspirate depends on a number of factors including the cellularity of the specimen, presence of necrotic debris, and content of blood and inflammatory cells, and in order to harvest the maximum number of well preserved, undistorted cells, proper handling and processing are critical. The technique used to concentrate the specimen must not induce artefactual alterations in the tumor cells, and when a mixture of cells is present it should selectively isolate the neoplastic cells. From our study of a series of cases, we will discuss the advantages and shortcomings of different methods, including the artefacts that each may cause, and examples of the diagnostic contributions will be illustrated.

DISASSEMBLY OF PLANT DICTYOSOMES AND MITOCHONDRIA BY PHOSPHOTUNGSTIC ACID AND SODIUM CHLORIDE: AN ULTRASTRUCTURAL STUDY. Hilton H. Mollenhauer and D. James Morré, USDA-ARS, Veterinary Toxicology and Entomology Research Laboratory, P. O. Drawer GE, College Station, TX 77841 and Dept. of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, IN 47907.

Phosphotungstic acid (PTA) and sodium chloride (NaCl) react rapidly with isolated dictyosomes to release cisternae from the dictyosome stack. PTA and NaCl apparently react with the substances of the intercisternal region that maintain dictyosome form. The released cisternae show a flattened central region and a fenestrated and/or tubular peripheral region. Intermediate stages of unstacking can be preserved by stabilizing the sample with glutaraldehyde at selected times following initiation of treatment. When this is done, remnants of the intercisternal region, as well as intercisternal elements, are visible on the flattened surfaces of the cisternae. PTA and NaCl solubilize some proteins; whereas, glutaraldehyde stabilizes proteins thus suggesting that the stabilizing structures of the intercisternal regions are, at least partly, proteinaceous. Intercisternal substances must be important to dictyosome functioning since they maintain both the separation of cisternae and dictyosome polarity. Solubilization of the intercisternal substances may represent a useful approach for understanding the mechanisms for maintenance of dictyosome form. PTA also will break down mitochondria leaving cristae intact. In the bean root, these cristae appear as short, sausage-like, tubules attached to remnants of inner mitochondrial membrane.

ULTRASTRUCTURE OF PYLORIC CAECA IN THE BLACKBASS MICROPTERUS SALMOIDES. W. A. Monroe and D.A. Hay, Department of Biology, Stephen F. Austin State University, Nacogdoches, Texas 75962.

The fine structure of pyloric caeca in the blackbass Micropterus salmoides appears to be similar to that found in the duodenal region of the small intestine of higher vertebrates. Villi are broad, fold-like projections (plicae). Cells lining the lumen are typically absorptive, exhibiting a brush boarder of microvilli and a glycolyx; adjacent cells possess junctional complexes. Mucus-secreting goblet cells are present but are infrequent. Beneath the basal surface of the absorptive columnar cells are small cells with dense, granular cytoplasm and long, highly attenuated processes

that appear to extend between and to wrap around adjacent columnar cells. Cross-sections of these processes approach the lumen, but do not penetrate the junctional complexes. Such cells may serve a secretory function. Between the inner and outer layers of the muscularis externa are postganglionic nerve cell bodies similar to those of Auerbach's plexuses in higher vertebrates. On the basis of our observations pyloric caeca appear to complement normal intestinal absorption.

ULTRASTRUCTURAL OBSERVATIONS ON THE SPINDLE POLE BODY IN THE FUNGUS EXOBASIDIUM CAMELLIAE VAR. GRACILIS. E.A. Richardson and C.W. Mims, Dept. of Biology, S.F. Austin State University, Nacogdoches, TX 75962.

A spindle pole body (SPB) is a nucleus associated organelle that appears to function as a microtubule organizing center in fungi lacking centrioles. Recent ultrastructural studies have revealed structural differences in SPBs of fungi belonging to different taxonomic categories. As a result it appears that SPB structure may be a useful phylogenetic marker. In this study TEM was used to examine SPB structure in E. camelliae var. gracilis in the hope that such data will help to clarify the taxonomic position and phylogenetic affinities of the genus Exobasidium.

In most thin sections of early prophase meiotic nuclei of Exobasidium the SPB appears simply as an electron-dense, globular structure closely associated with the nuclear envelope. As meiosis progresses the SPB eventually becomes inserted into the nuclear envelope where it appears to be involved in spindle formation. From the data currently available it appears that the SPB of Exobasidium is intermediate in structure between SPBs described for rust fungi (Uredinales) and those described for smut fungi (Ustilaginales).

STEREOLOGICAL QUANTITATION OF HEPATIC PEROXISOMES AND LYSOSOMES AFTER HYDROGEN PEROXIDE TREATMENT.
A. Chowdhury and E. W. Hupp, Dept. of Biology, Texas Woman's University, Denton, Texas 76204.

The ultrastructure of hepatic peroxisomes and lysosomes of rats was studied at 2, 7 and 21 days after treatment with five intraperitoneal injections of 1.8 ml of 1.5% hydrogen peroxide. Optimum cytochemical localization of acid phosphate activity was obtained by using a modified Gomori technique (Microscopic Histochemistry: Principals and Practice, Chicago, University of Chicago Press, 1952). Localization of peroxidatic activity of catalase in peroxisomes was demonstrated by the application of the modified alkaline 3,3' diaminobenzidine tetrahydrochloride (DAB) method of Novikoff and Goldfischer (J. Histochem. Cytochem. 17:675-680, 1969). Quantitation of morphometric variation of lysosomes and peroxisomes was performed by using stereological technique. With predetermined precision, unbiased representative micrographs were taken from each section. The volume, surface and numerical densities on each micrograph were estimated by counting points on a test grid superimposed on each micrograph. None of the parameters (volume, surface and numerical densities) showed any significant difference at the 0.05 level between treated, control and untreated control. The toxic effects of hydrogen peroxide on ultrastructural morphology will be described.

ELECTRON MICROSCOPIC (EM) IDENTIFICATION AND QUANTIFICATION OF LIPID. John R. Guyton, M.D., and Thomas A. Schifani. Baylor College of Medicine, Houston, Texas.

In thin sections of tissue processed routinely for EM, neutral lipid appears as round, electron-lucent or homogeneous gray spaces. However, the identification of lipid is not always satisfactory, since vesicles with aqueous centers and open spaces outside of cells may not be distinguished from lipid. We have gained experience with 2 techniques for enhancing the retention and visibility of lipid in tissue prepared for EM. Addition of potassium ferrocyanide to osmium tetroxide during secondary fixation enhances the electron density of membranous, but not neutral lipid. The use of an osmium-thiocarbohydrazide-osmium sequence greatly enhances the electron density of osmiophilic structures and provides satisfactory visualization of neutral lipid in non-atherosclerotic regions of human aorta. Quantitative stereologic determination of lipid volumes is hindered by the lack of well-developed stereologic theory for a diverse particle population with sizes extending down to near zero. Locational analysis of extracellular neutral lipid in normal human aortic intima showed that within the plane of section, 75% of lipid (area fraction) resided in droplets adjacent to or within elastic membranes. Considered 3-dimensionally, a substantially higher fraction of lipid must be localized adjacent to elastic membranes.

Electron microprobe studies on the extent of ion adsorption in open-cytoplasm. I.L. Cameron[1], J.T. Hansen[1], K.E. Hunter[1] G.M. Padilla[2], [1]Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, Tx. and [2]Physiology Department, Duke University Medical Center, Durham, North Carolina.

Are monovalent ions totally free in solution within a living cell? To gain information on the mechanisms involved in the establishment and maintenance of subcellular gradients of Na, K, Cl and other elements in the unicellular flagellate, Euglena gracilis, we turned to the ultracentrifugal stratification of its intracellular contents in unruptured and viable cells. The centrifugation was done in 5% dextran at 100,000g for 1 hr. Stratified and non-stratified Euglena were cryofixed for energy dispersive X-ray microanalysis of Na, K, Cl and other elements in thin freeze-dried cryosections. A number of significant elemental concentration differences were found between chloroplast, nucleus, paramylon and open-cytoplasm in the non-stratified cells. Stratification caused several ions to redistribute. For example, we observed a significant increase in K and Cl in the nucleus which was correlated to the condensation of chromatin. Also Cl, but not Na, decreased significantly in the region of cytoplasm that was cleared of organelles and of observable cytochemical enzyme activity. We conclude from the data that more than half of the Cl in open-cytoplasm was adsorbed to the material that was removed by ultracentrifugation. Thus, it appears that a close association of at least one ion, Cl, to ultracentrifugable material is involved in maintenance of the measured Cl concentration in the open-cytoplasm of the non-stratified cell.

POLYSTYRENE MEMBRANES AND DIRECT-VIEW ELECTRON MICROSCOPY FOR THE STUDY OF INTACT CULTURE CELLS, A. Cole, E. Armour and R. Langley, Physics Dept., Un. of Texas System Cancer Center, Houston, TX 77030

Thin polystyrene membranes on nylon electron microscope grids were used as substrates for cell attachment and growth in culture. The cells were fixed in glutaraldehyde and critical point dried. Observations and micrographs were made using a real-time direct-view stereo transmission electron microscope system developed by A. Cole. Although internal structures of compact rounded CHO cells of 10μm diameter were not resolvable using the 100 KeV system, surface structures at the cell peripheries were shown to contain many cell processes of 0.1μm diameter and up to 2μm length. Fine structure of the cell processes was resolved. Internal structures of growing cells which flattened to less than 2μm were imaged in three dimensions. The cytoplasmic matrix contained thin (≈10nm) and thick (~20nm) fibrils. Mitochondria (0.3 to 0.7μm) were surrounded by a low density shell of 0.1 to 0.2μm thickness which consisted of thin fibrils (~10nm) connecting the mitochondrial surface to the cytoplasmic matrix. The function of this mitochondrial "halo" is being investigated. Our new techniques allow straightforward study of normal or aberrant cell structure at any stage of the cell cycle using intact cells which have not been modified by trypsinization, centrifugation, embedding, sectioning etc. Thick regions can be imaged using higher voltage stereo systems.

CALCIUM INVOLVEMENT IN SQUID IRIDESCENCE, R.T. Hanlon and K.M. Cooper, The Marine Biomedical Institute, The University of Texas Medical Branch, Galveston, TX 77550-2772.

Topical application of acetylcholine (ACh) causes iridophores in the dorsal mantle skin of Lolliguncula brevis to become iridescent (J. Cell Biol., 99, 386a, 1984). We studied the possible involvement of $Ca^{2+}$ in the expression of this iridescence. ACh ($2.75 \times 10^{-7}$M) caused the appearance of red

to gold iridescence in iridophores of excised dermal layers when it was applied along with $Ca^{2+}$ (10mM) in the artificial sea water bathing medium. Treatment with ACh in medium lacking $Ca^{2+}$ resulted in the brief appearance of red iridescence, but it was not maintained. The calcium ionophore A23187 ($10^{-5}$M), when applied in the presence of $Ca^{2+}$ (without ACh), caused the appearance of iridescence of red, gold, and sometimes green and blue colors. Verapamil ($2x10^{-4}$M), a calcium channel blocker, inhibited to a great degree the effect of ACh + $Ca^{2+}$ on these cells. Our study suggests that $Ca^{2+}$ may be involved in the in vitro production of iridescence in these iridophores. (Supported in part by NIH grant DHHS RR 01024 to R.T.H.).

THE MORPHOLOGY AND HABITAT OF THREE FUNGUS BEETLES ASSOCIATED WITH THE SPOROPHORES OF THE RUSTY CONK, Phellinus gilvus. J.P. Shields, J.W. Bing and D.L. Kulhavy. Department of Biology and School of Forestry, Stephen F. Austin State University, Nacogdoches, Texas 75962.

Stage 4 (late stage) sporophores of Phellinus gilvus were collected from dead hardwoods in East Texas and examined for insect associates. Insects were identified and placed in categories descriptive of their relationship to the fungus: Mycetobionts (obligatory fungal inhabitants), Mycetophiles (facultative fungal inhabitants), or Mycetoxenes (chance visitors). Three species of mycetobiontic Coleoptera (beetles) were chosen for detailed scanning electron microscopy (SEM) of their external morphology because they required the fungus for nourishment, shelter, and a breeding site. The SEM revealed that the external morphology of the beetles (two in the family Ciidae, Ennearthron aurisquamosum and Malacocis brevicolis, and one in the family Bostrichidae, Endecatomus rugosus), was similar. The structure of the legs and large numbers of setae located over the entire surfaces as well as a streamlining of the overall body shape illustrate the adaptation of these species to the fungal habitat. Sexual dimorphism includes size differentiation in M. brevicolis and protuberances on the head and thorax in E. aurisquamosum. Males of both species had pubescent fovea on the first abdominal segment.

STEREOLOGICAL ANALYSIS OF SPERMATOGENESIS IN THE LIVERWORT FOSSOMBRONIA FOVEOLATA RADDI. 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 metamorphesis entails the streamlining of the cell from a cubiodal vegetative initial through spherical antheridial cells to linear, mature sperms.

WATER STRESS EFFECTS ON THE PALISADE CELLS OF COTTON STRAINS EXHIBITING VARIABILITY IN LEAF TURGIDITY. Berlin, J.D. and J.E. Quisenberry, Department of Biological Sciences, Texas Tech University, Lubbock, Texas and USDA-ARS, Plant Stress and Water Conservation Unit, Lubbock, TX 79409.

Two cotton (Gossypium hirsutum L.) strains have been identified as having extreme phenotypic expressions of leaf turgidity when grown under water deficit conditions: T25 retained turgid leaves whereas T169 wilted. Shoot biomass did not differ between the two strains under irrigated conditions, but the nonwilting T25 produced significantly more biomass than did T169 under water deficit conditions. A stereological analysis comparing the palisade cells of these two strains was undertaken to identify what cellular and subcellular mechanisms might explain the observed differences in how the plants respond to water stress. The fractional volume of palisade cell per leaf was a consistent .71 to .74 regardless of strain or growing conditions. Cell volume was not altered by stress in T25, but was significantly reduced ($P < 0.01$) in T169. The number of palisade cells per leaf was significantly reduced in both T25 ($P < 0.05$) and T169 ($P < 0.001$). In the nonwilting T25, the mean volume densities for chloroplast starch granules and mitochondria were significantly reduced ($P < 0.001$) whereas chloroplast lipid bodies were significantly increased ($P < 0.001$). In contrast, T169 exhibited reductions in mean volume densities for total cytoplasm, chloroplasts, starch granules and chloroplast lipid bodies and an increased volume density for the central vacuole. In addition, the central vacuole of many palisade cells contained phenolic materials. Supported by Cotton Incoporated and the Plant Stress Institute and the Water Resources Center at Texas Tech University

# MATERIAL SCIENCES

STATISTICS AS A TOOL IN QUALITY ASSURANCE. Mary Lou Percy, Quality Assurance. Texas Instruments. Austin, Texas 78769.

The Tracor Northern 2000 has proved to be a valuable diagnostic tool in the printed wire board shop. However, the question arises--how accurate is this instrument? The process limits have been calculated for both day-to-day fluctuation and variability in a given sample over a short period of time. High and low percentages of Sn were measured from Sn/Pb alloys previously analyzed using wet chemistry techniques. The application of statistics generated by the EDX to a real life plating operation will be demonstrated.

ORIENTATION DEPENDENCE OF XRAY PRODUCTION IN SINGLE CRYSTALS OF InP. L. Rabenberg, Mechanical Engineering Department and Materials Science and Engineering Program, University of Texas, Austin TX 78712.

Current technology now makes it simple to acquire rocking beam scanning transmission channelling patterns from single crystals. Using digital control of the rocking coils of a JEOL 1200 EX TEM-STEM, it is possible to acquire EDS spectra as a function of incident electron beam orientation. Dynamic electron diffraction within the specimen modulates the electron wave front, concentrating its intensity near planes of In atoms or P atoms alternately. The patterns of elastic transmitted electrons and x-ray production are directly comparable.

SCANNING ELECTRON MICROSCOPY STUDY OF SOLDERING TO COPPER-NICKEL. J. L. Marshall, Advanced Manufacturing Technology, Motorola, Inc., 5555 N. Beach St., Ft. Worth, Texas 76137.

The solder process to copper is well understood and stands on a wealth of copper-tin metallurgical data. By contrast, soldering to copper-nickel is poorly understood -- an adequate phase diagram for the copper-nickel-tin system does not exist. In the present study, the soldering process to copper-nickel is investigated using scanning electron microscopy/energy dispersive X-ray analysis. The various phases of copper-nickel-tin that develop during soldering are identified and their respective roles in the overall process are evaluated. The extension of the copper-tin and the nickel-tin phase diagrams to develop a copper-nickel-tin phase diagram is partially successful and can explain the development of the observed phases.

SAMPLE PREPARATION FOR CROSS-SECTIONAL TRANSMISSION ELECTRON MICROSCOPY. R.F.Pinizzotto, F.Y.Clark and M.L.Jarvis, Ultrastructure, Inc., 1850 N. Greenville Ave - 140, Richardson, TX 75081.

Cross-sectional transmission electron microscopy (XTEM) is now a part of the standard repertoire of most analytical TEM laboratories. Sample preparation for XTEM is a skill that is only perfected by diligent practice. However, there are standard procedures that can be used to maximize one's ability to obtain electron transparent interfaces in a minimum amount of time. This paper will review the principles and practices of XTEM sample preparation. The procedures used for integrated circuit applications will be described from wafer scribing to final ion milling. Variations of the technique to other types of materials will be discussed.

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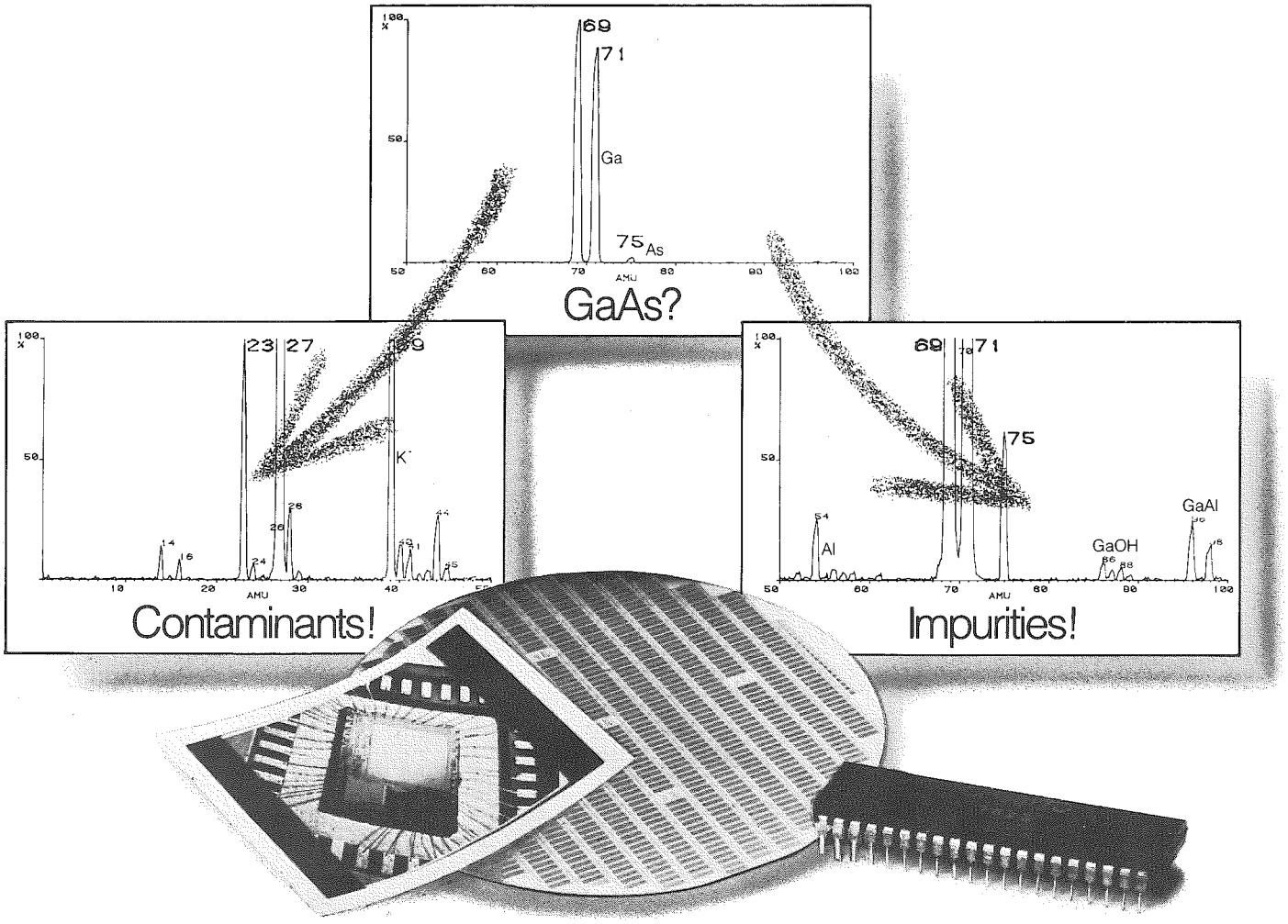


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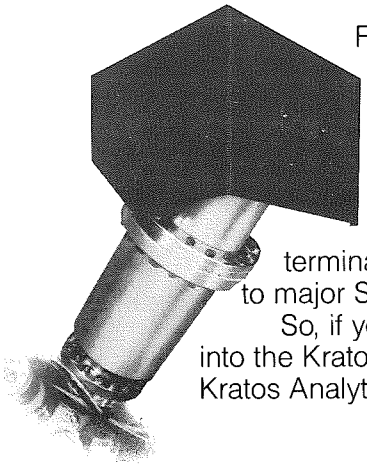

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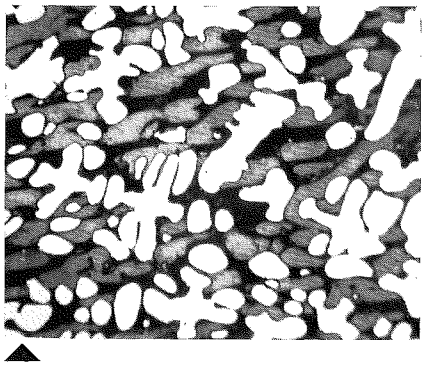

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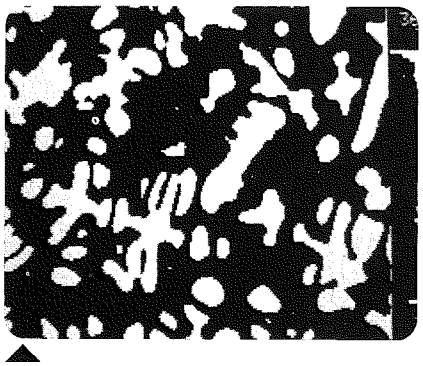

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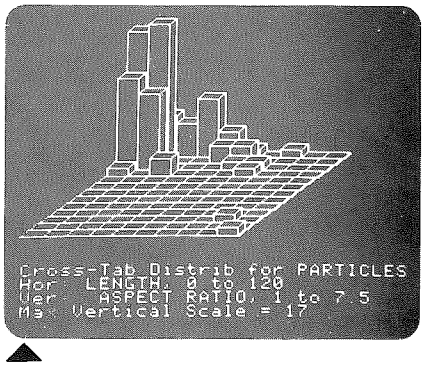


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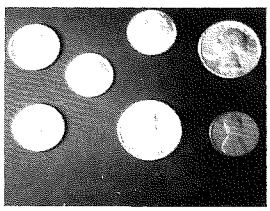

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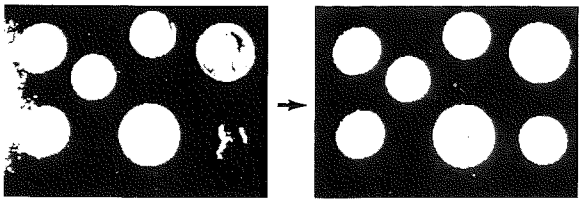

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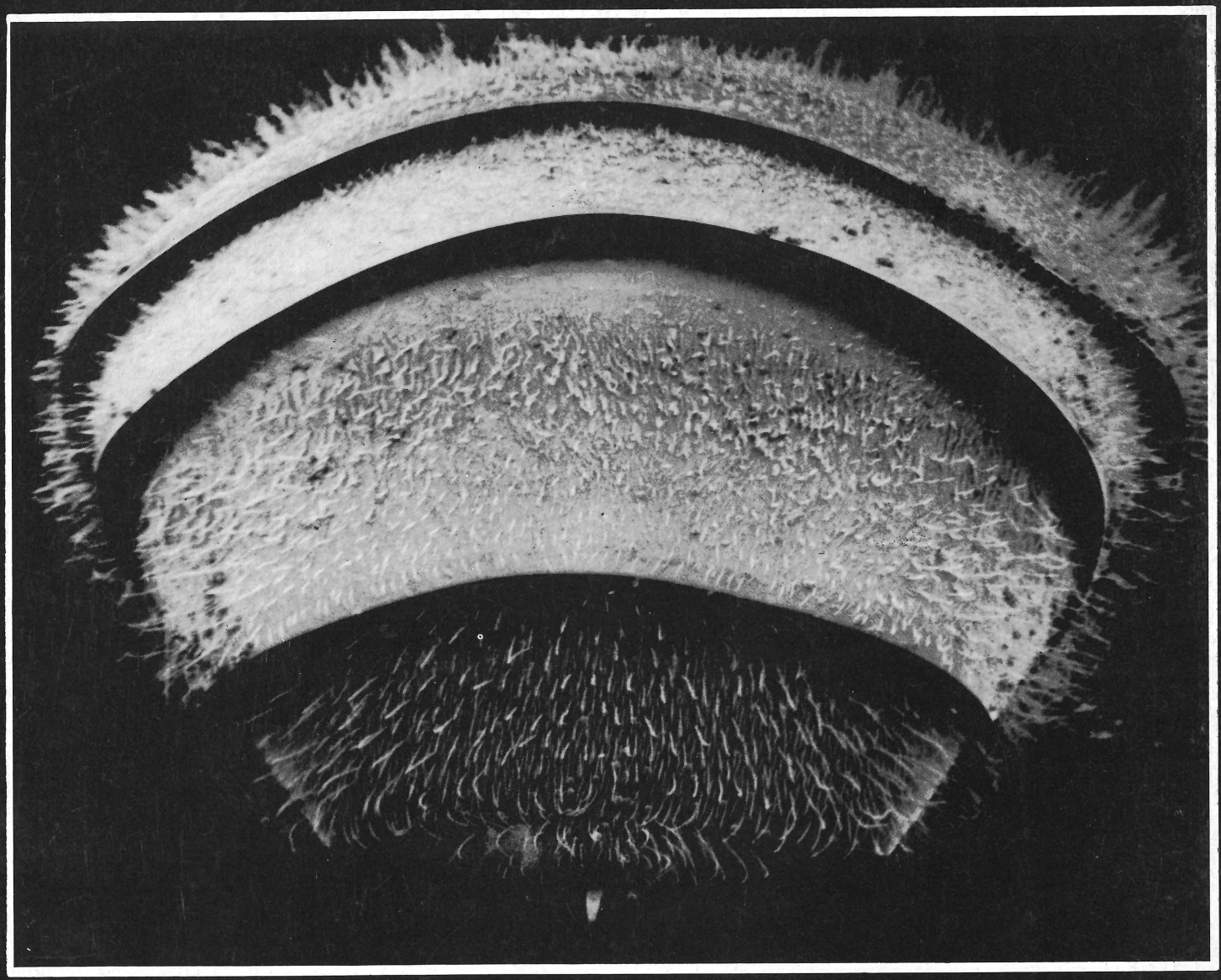

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