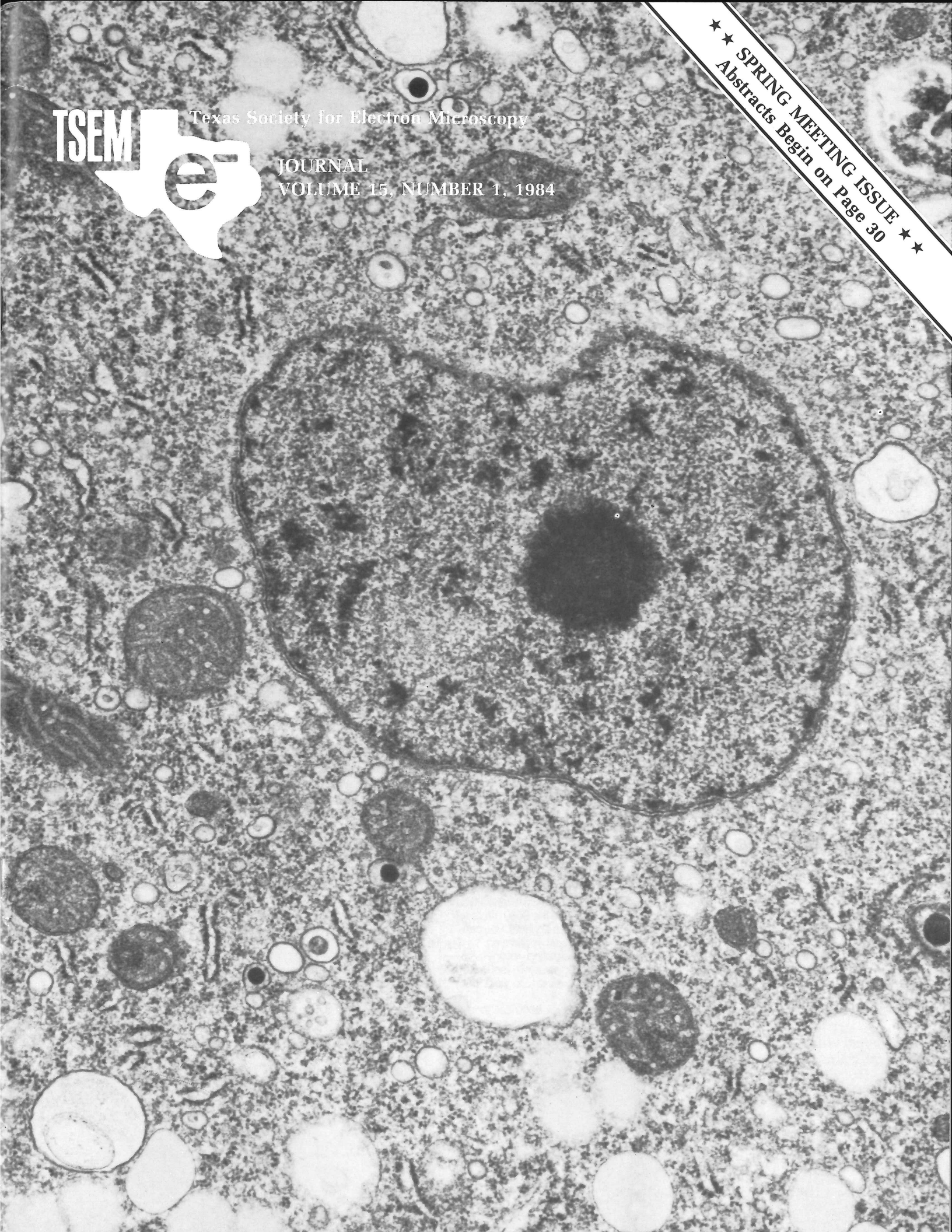
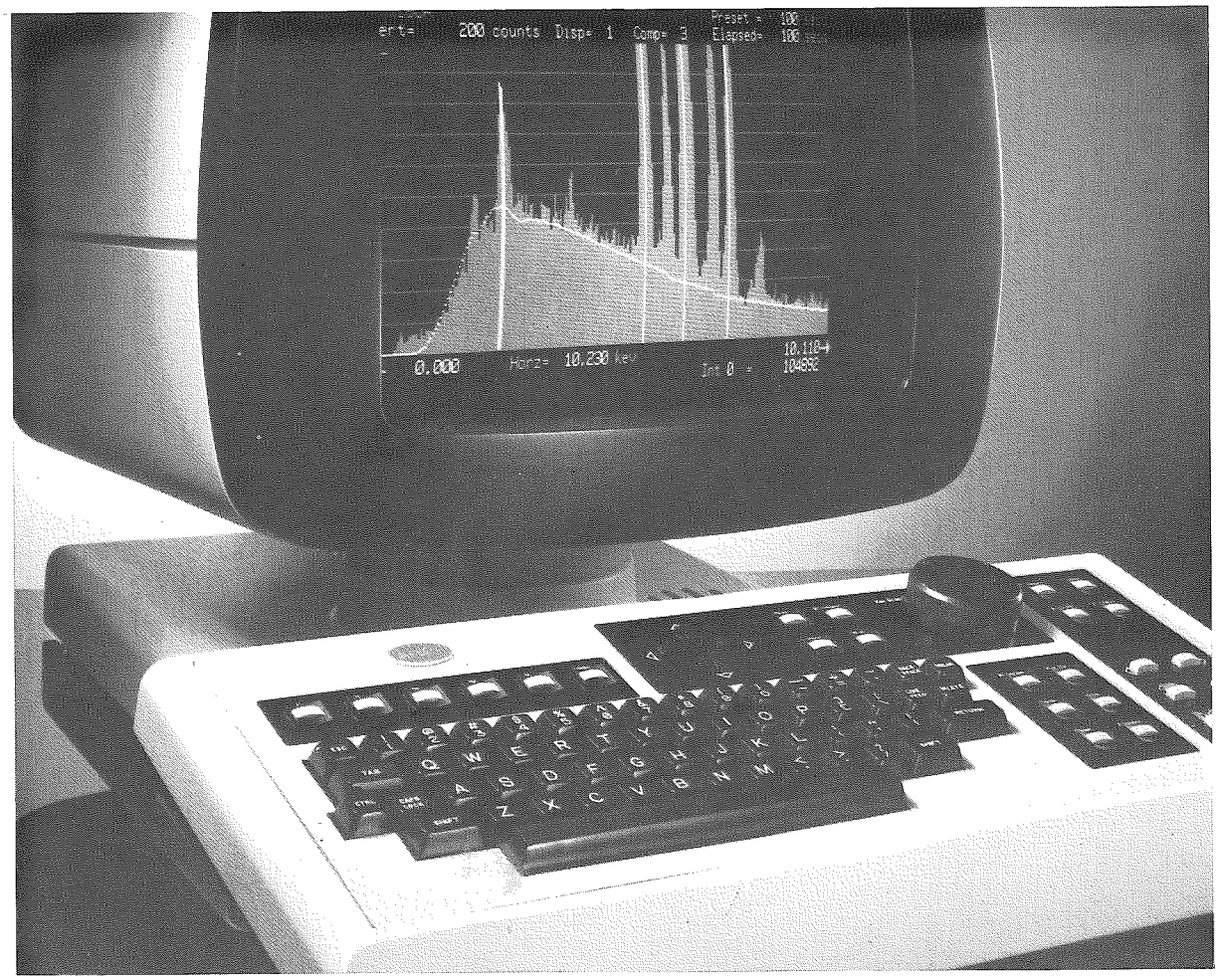


TSEM
Texas Society for Electron Microscopy
JOURNAL
VOLUME 15, NUMBER 1, 1984

★★ SPRING MEETING ISSUE ★★
Abstracts Begin on Page 30



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Contents

TEXAS SOCIETY FOR ELECTRON MICROSCOPY
JOURNAL
VOLUME 15, NUMBER 1, 1984
ISSN 0196-5662

Randy Moore, Editor
Department of Biology, Baylor University, Waco, Texas 76798

Texas Society for Electron Microscopy

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

President's Message 4
Editor's Message 4
Regional News 7
Editorial Policy 7
Treasurer's Report 8
Secretary's Report 8
Article — "The Cytochemical Localization of Glucose-6-Phosphatase in Plant Cells" 11
Article — "The Structure of Graviperceptive Cells in Plant Roots" 16
Short Courses and Workshops 21
Article — "A Gravimetric Formula for Erlandson's Maraglas, D.E.R. 732 Embedding Medium" 26
Abstracts 30
Calendar of Meetings 39
Job Opportunity 39
TSEM Members 42
Job Opportunity 46
Educational Material 46
Technical Note 46
Information for Authors 48
TSEM Application for Membership 51
Corporate Members 51
EMSA Application for Membership 52
Regional Editors 52

ON THE COVER

Transmission electron micrograph illustrating the typical array of organelles found in the plasmodium of the myxomycete **Didymium iridis.** x 35,000. Submitted by Charles W. Mims, Department of Biology, Stephen F. Austin State University, Nacogdoches, Texas.

President's Message

If everything goes as planned this issue of the TSEM Journal will be available in time for our Spring Meeting in College Station and will contain abstracts of the papers to be presented at this meeting. In the future we will also attempt to have an issue of the Journal ready for each of our meetings. To do this, however, the deadlines for receipt of abstracts for each meeting must be met by those individuals planning to present papers. As many of you know we have been somewhat lax about these deadlines in the past, but from now on they will be enforced and late abstracts will not be accepted. Please help those people who plan and implement our meetings by meeting these deadlines.

In regard to our Spring Meeting I would like to thank Hilton Mollenhauer and Wayne Sampson for handling local arrangements for us. We also appreciate the support provided by Texas A&M Medical School. The administration, faculty, staff and students of Texas A&M University have always actively supported our organization and we have had some outstanding meetings in College Station. I'm sure that this year's Spring Meeting will be no exception. I hope that each of you will express your thanks to the representatives of this fine university for their support of TSEM.

Four individuals will go off the TSEM Executive Council following our Spring Meeting. These are Bruce Mackay (Past President), Elizabeth Root (Secretary), Ernest Couch (Program Chairman) and Randy Allen (Graduate Student Representative). These individuals have all worked hard for the good of our Society this past year and deserve our thanks. I would personally like to thank them for their help and support during my term as President. Overall I think TSEM has had a good year and much of the credit goes to those individuals noted above as well as our other fine officers.

This year's Fall Meeting will be in Arlington, Texas. Howard Arnott is handling local arrangements and I'm sure that he would welcome any suggestions you might have regarding the meeting. We are, for instance, particularly interested in having one or more sessions devoted to material/physical sciences. If you know of individuals who are interested in this aspect of electron microscopy please have them contact Howard about this meeting. Their participation in TSEM is very much welcomed and would add much to our Society.

Although I will remain on the Executive Council for another year I will complete my duties as President of TSEM at our Spring Meeting. I have enjoyed my term as your President and appreciate the assistance and support so many of you have provided. In my opinion the overall health of our Society is good at this time. TSEM does, however, have room for improvement and growth. We need to involve many more individuals in our organization, particularly those who are new to Texas. To do this we need the help of all our members. Why not make a commitment to become actively involved in TSEM this year? If we all work together TSEM will continue to be one of the outstanding local EM societies in the nation and something we can all take pride in.

Sincerely,

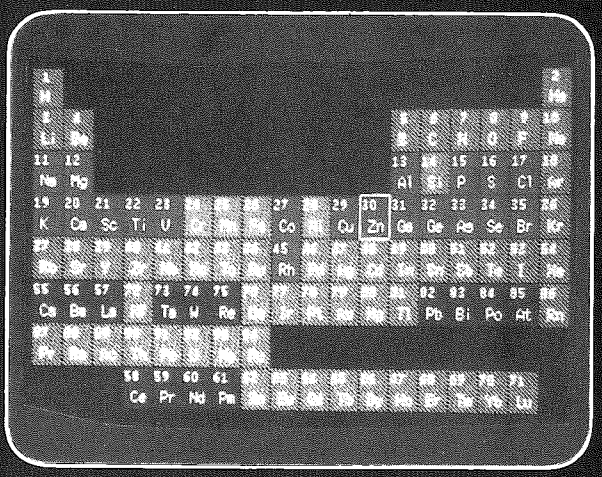
Charles W. Mims
President

Editor's Message

In October of 1983 the Executive Council of the TSEM decided to reinstate the policy of publishing abstracts in an issue of the TSEM Journal and to have this issue of the journal available for distribution at the corresponding meeting of the society. Thus, this issue of the TSEM Journal contains the abstracts of papers presented at the Spring, 1984 meeting of the TSEM in College Station. Only those abstracts received by February 28, 1984 are included. (Note: The deadline for receiving abstracts was February 24, 1984).

An ongoing problem facing the TSEM Journal is securing quality manuscripts for publication. Since assuming the journal's editorship in October of 1983, I have received only four manuscripts, all of which were sent to at least two individuals for peer review. Three of these four manuscripts were accepted for publication and are included in this issue of the journal; the other manuscript is (presumably) being revised in response to reviewers' suggestions. Since TSEM Journal will be published only when quality manuscripts are available, I would encourage each member of TSEM to consider submitting a manuscript for publication in the journal.

Randy Moore
Editor, TSEM Journal



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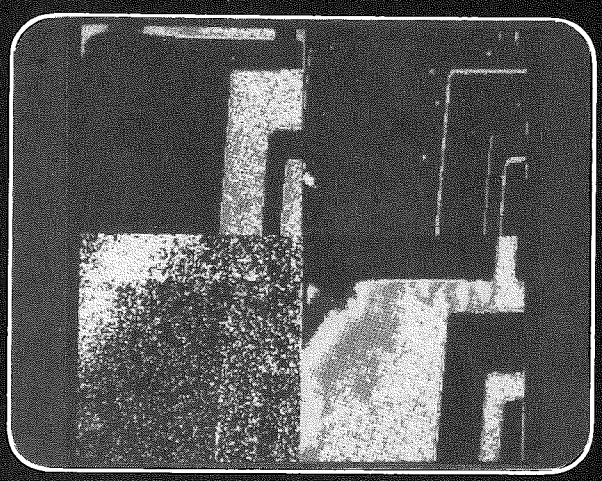

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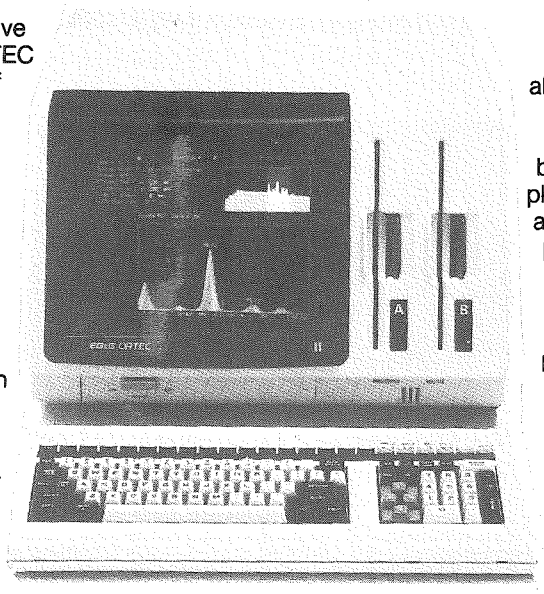

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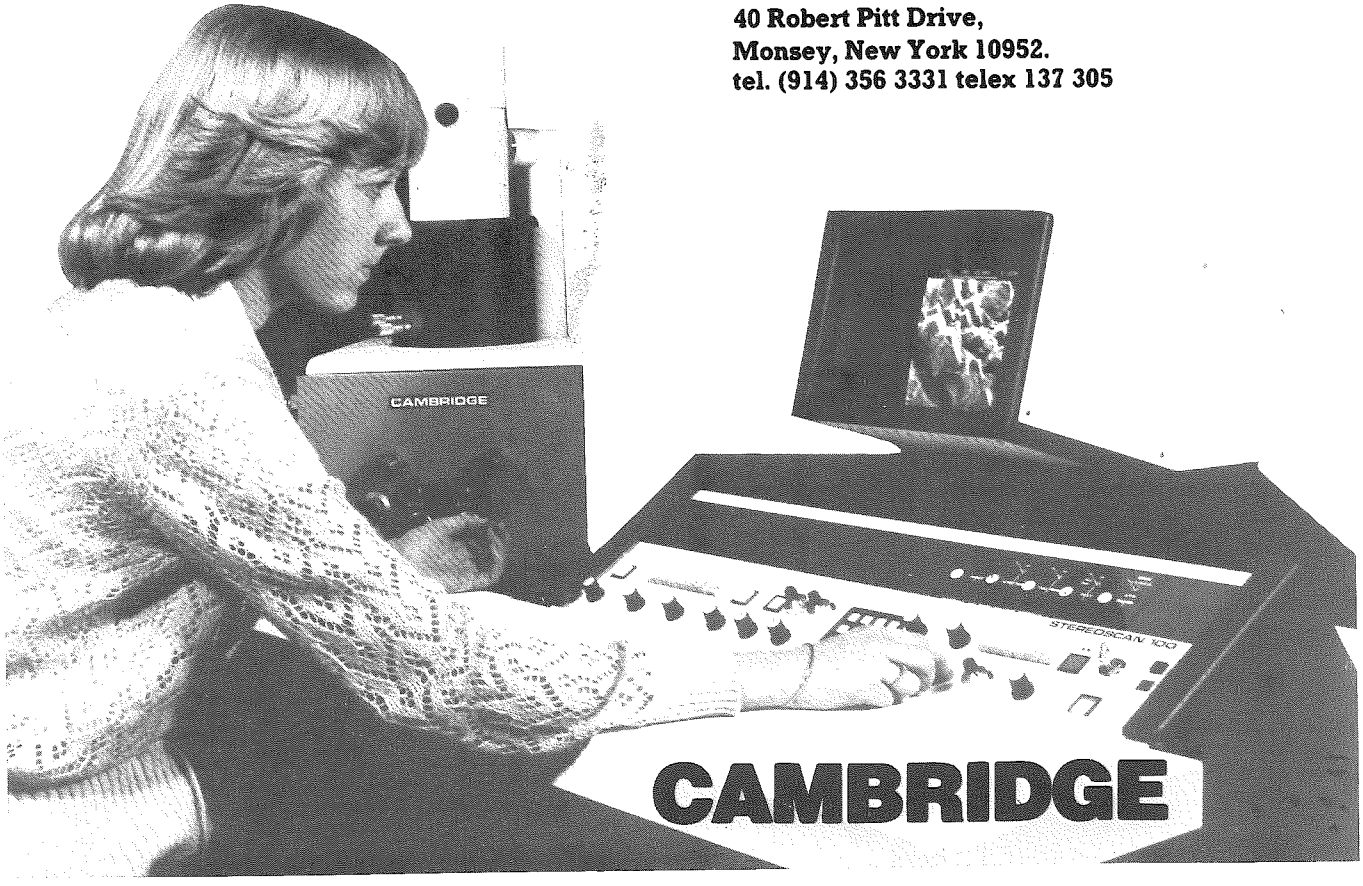

Regional News

TEXAS A&M UNIVERSITY

PUBLICATIONS

Drolesky, R.E., P.J. Holman, T.M. Craig, G.G. Wagner and H.H. Mollenhauer. (1983). Ultrastructure of **Babesia bovis** sexual stages as observed in **Boophilus microplus** cell cultures. Res. Vet. Sci. 34: 249-251.

Morre', D.J., W.F. Boss, H. Grimes and H.H. Mollenhauer. (1983). Kinetics of Golgi apparatus membrane flux following monensin treatment of embryogenic carrot cells. Eur. J. Cell Biol. 30: 25-32.

Sampson, H.W., D.J. Kiessel, L.L. MacKenzie-Graham and I. Piscopo (1983). A cytochemical study of the effect of cholinergic and B-adrenergic stimulation on calcium fluxes of the rat parotid gland. Histochem. 79: 193-203.

THE UNIVERSITY OF TEXAS AT AUSTIN

PUBLICATIONS — DEPARTMENT OF BOTANY

T. Itoh, R. O'Neil and R.M. Brown, Jr. (1983) The assembly of cellulose microfibrils in selected Siphonocladalean algae. J. Cell Biol. 97:416a.

PUBLICATIONS — CELL RESEARCH INSTITUTE

M. Dauwalder, B. Serlin and S. Roux. (1983) Ca^{45} localization in gravistimulated oat coleoptiles — comparison of antimonate procedures. J. Cell Biol. 97:415a.

K. Wang and R. Ramirez-Mitchell. (1983) Ultrastructural morphology and epitope distribution of titin — a giant sarcomere-associated cytoskeletal protein. J. Cell Biology 97:957a.

OTHER NEWS OF INTEREST TO MEMBERS OF TSEM

Dr. J. B. Longenecker and Dr. Elizabeth Root have received a small grant from NIA entitled, "Nutritional Deficiency and Premature Aging of Brain Cells". Work is being carried out in collaboration with Dr. J B. Kirkpatrick of Methodist Hospital and Baylor University in Houston.

Dr. Daniel Acosta and Dr. Elsie Sorensen have been notified of renewal of their grant, "Hepatotoxicity: an **in vitro** Approach to the Study of Toxicity and Membrane Interactions of Cadmium Using Cultured Rat Hepatocytes" by The John Hopkins Center for Alternatives to Animal Testing.

EDITORIAL POLICY

LETTERS TO THE EDITOR

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

ELECTRON MICROGRAPHS AND COVER PHOTOS

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

REGIONAL NEWS

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

Treasurer's Report

ASSETS ON APRIL 7, 1983:		
Certificate of Deposit No. 91099, Univ. Natl. Bank, Galveston	$ 2,000.00	
Certificate of Deposit No. 10-141345, Houston First Savings	2,713.95	
Checking Account, Forestwood Natl. Bank, Dallas[1]	4,993.54	$ 9,707.49
RECEIPTS:		
Austin Meeting	$ 1,714.00	
Interest		
CD No. 91099	75.00	
CD No. 10-141345	137.78	
CD No. 240-0064030	33.57	
Checking Account	135.34	
Memberships	2,132.00	
Journal Subscriptions and Advertisements	550.00	
Close TSEM Journal Account	1,010.59	
Refund on bonding of A. Shannon	25.00	
Donation to start Student Travel Fund	100.00	
Close 1973 TSEM Account	110.00	$ 6,023.28
DISBURSEMENTS:		
TSEM Journal Printing	$ 3,121.10	
Bonding for R. Moore (Treasurer)	65.00	
Austin Meeting		
Speakers	425.04	
Student Travel	26.45	
Quality Inn	813.41	
Secretarial Expenses	400.00	
Treasurer Expenses	39.02	
Preparation for Tyler Meeting	57.63	$ 4,947.65
ASSETS ON OCTOBER 13, 1983:		
Certificate of Deposit No. 91099, Univ. Natl. Bank, Galveston	$ 2,000.00	
Certificate of Deposit No. 10-141345, Houston First Savings	2,851.73	
Certificate of Deposit No. 240-0064030, Republic Bank of Waco	2,033.57	
Checking Account, Republic Bank of Waco[2]	3,897.82	$ 10,783.12

[1]Includes $50.00 for Paul Enos Memorial Fund.
[2]Includes $100.00 for Paul Enos Memorial Fund and $100.00 for Student Travel Fund.

Respectfully submitted.

Randy Moore, Treasurer

Secretary's Report

Membership in TSEM, as counted in the first quarter of 1984, stands at 451. Of this number, 29 are corporate members, 6 honorary members, 296 regular members and 120 student members. The membership is far from static: new members join us, former members fade away, members change their place of work or residence. During the past two years the net total has increased by about 50 members.

If your address changes, please remember to notify the secretary of TSEM. The new secretary who replaces me will take custodianship of the address list after the April, 1984 meeting. A forwarding address left at the post office will eventually be communicated to TSEM, but not until you have missed getting at least one copy of the Journal, since it is because of undeliverable copies of TSEMJ that the post office sends us your change of address.

My term as secretary has been most rewarding as an opportunity to meet many of you and to become better acquainted with the workings of our organization. TSEM is a fine forum for sharing knowlege and expertise and presents a fine opportunity to get to know others working in electron microscopy. We benefit according to our own efforts. We need to keep writing abstracts, to submit papers to review for the Journal, and to attend meetings. See you at the next meeting.

Elizabeth Root
Secretary

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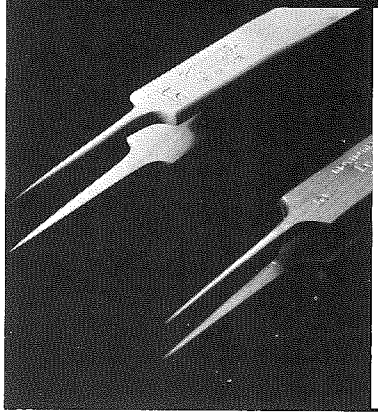

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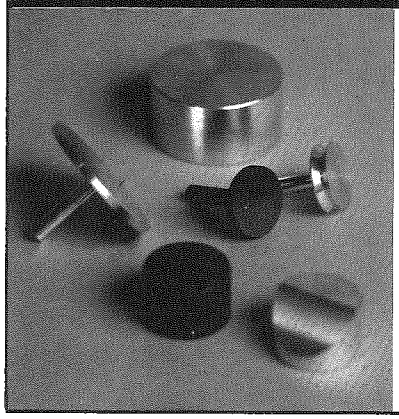

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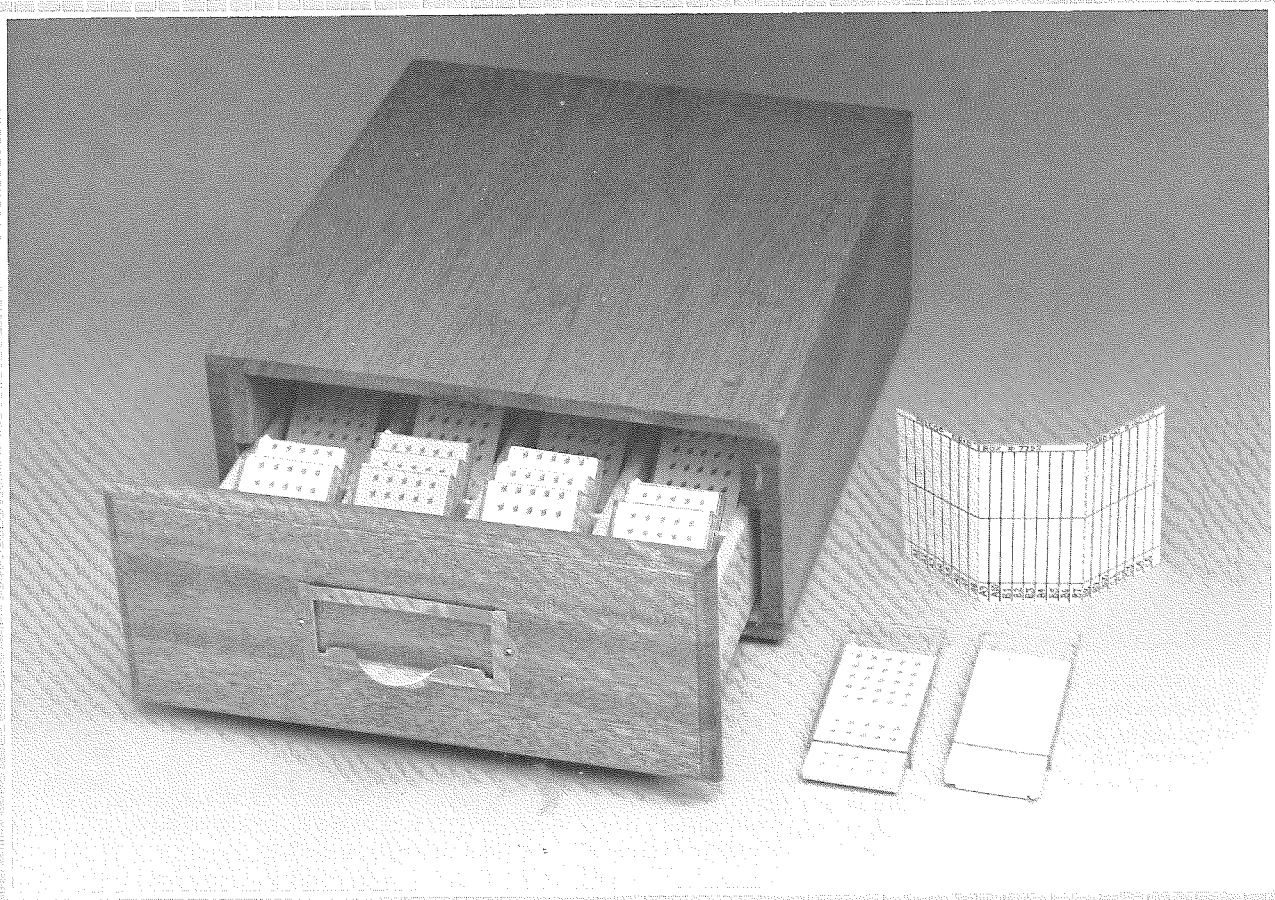

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THE CYTOCHEMICAL LOCALIZATION OF GLUCOSE-6-PHOSPHATASE IN PLANT CELLS

By

C. Edward McClelen and Randy Moore

Department of Biology
Baylor University
Waco, Texas 76798

Phosphatases comprise a large group of enzymes that hydrolyse phosphate esters to yield a product and inorganic phosphate. The cytochemical localization of phosphatases is relatively simple, and represents the activities of a broad spectrum of phosphatases present in the tissue and active at the chosen pH (1).

Glucose-6-phosphatase (G-6-Ptase) (E.C. 3.1.3.5.) is an acid phosphatase with a pH optimum of 6.5 (2). G-6-Ptase is a magnesium-activated enzyme that catalyzes the dephosphorylation of glucose-6-phosphatase to yield glucose and inorganic phosphate:

$$\text{glucose-6-phosphatase} + H_2O \xrightarrow[\Delta G^{0\prime} = -4.02 \text{ kcal mol}^{-1}]{Mg^{++}} \text{glucose} + P_i$$

Since the plasmalemma is more permeable to glucose than to glucose-6-phosphate, G-6-Ptase has been suggested to be involved in the extracellular transport of glucose (3). Although the cytochemical localization of G-6-Ptase has been studied extensively by zoologists with both light (4) and electron microscopy (5-9), very few studies have utilized plant tissues (10).

PROTOCOL FOR GLUCOSE-6-PHOSPHATASE LOCALIZATION IN PLANT CELLS

The cytochemical localization of G-6-Ptase is based on the capture of inorganic phosphate relased during dephosphorylation of glucose-6-phosphate. Exogenously added lead ions react with the liberated phosphate, forming an insoluble precipitate of lead phosphate at the site of enzymatic activity (11). Gomori (12) and Takamatsu (13) developed this method of enzyme localization independently. A more extensive discussion of the so-called Gomori reaction as it applies to the cytochemical localization of phosphatases is presented elsewhere (14).

Our protocol for G-6-Ptase localization in plant cells is modified from that used in studies of animal cells (2):

1. Fix cubes of tissue (approximately 1 mm^3) in 2% glutaraldehyde in 0.10 M Tris-maleate buffer (pH 6.5) for 15 to 30 min at room temperature. G-6-Ptase is inactivated by formaldehyde and long fixation times in glutaraldehyde (15).
2. Section the tissue into slices 50-100 μm thick.
3. Wash the tissue for 15 to 30 min in 0.10 M Tris-maleate buffer, pH 6.5.

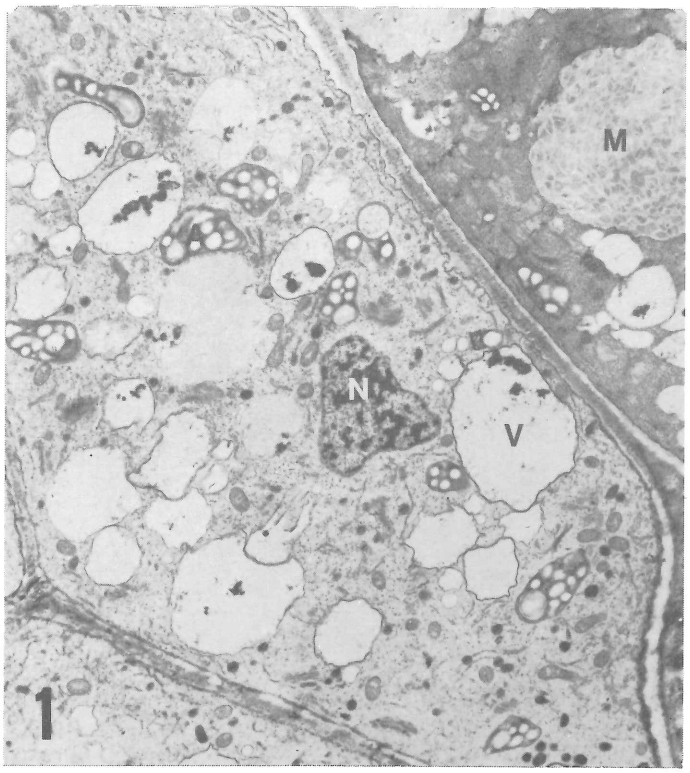


FIGURE 1. Representative control section of a peripheral cell from a **Zea** root cap. N = nucleus; V = vacuole; A = amyloplast; M = mucilage. x 6,400.

4. Incubate the tissue sections in the following media for 30-60 min at room temperature:

Stock solutions	ml of stock solution	final conc. in incubation medium (mM)
A. 0.2 M Tris-maleate, pH 6.5	6	60
B. 0.02 M lead nitrate (CO_2 free)	3	3
C. distilled, deionized water	11	
D. disodium glucose-6-phosphate	30 mg	4
sucrose (optional)	1.5 g	

Note: Mix stock A with C, then divide into two equal portions. Slowly mix stock B with one portion of the A-C solution. Mix stock C with the other portion of the A-C solution. Mix the resulting two solutions (while stirring) and filter any precipitate.

5. Wash the sections for 15 minutes in 0.1 M Tris-maleate buffer, pH 6.5.

6. Postfix some of the tissue in 2% osmium tetroxide (in 0.1 M Tris-maleate, pH 6.5), dehydrate in ethanol, and embed in Spurr's epoxy (16). Process the other tissues without postfixation in osmium tetroxide.

Controls: There are three control treatments for G-6-Ptase cytochemistry: (i) boil the tissue for 5 minutes before incubation, (ii) incubate the tissue in a media lacking glucose-6-phosphate substrate, (iii) in-

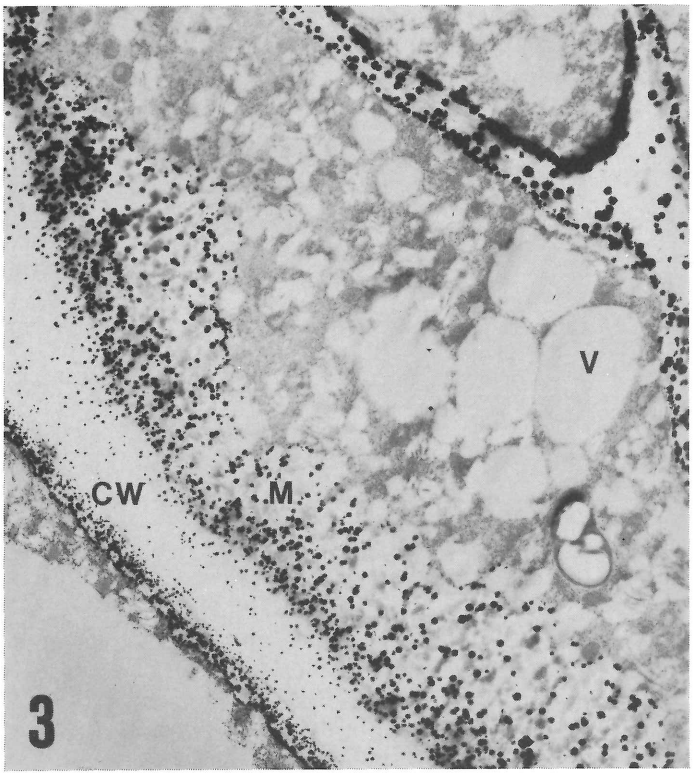


FIGURE 3,4. Cytochemical staining of mucilage (M) and cell wall (CW) in an inner peripheral cell. V = vacuole. Figure 3, x 7,400; Figure 4, x 22,400.

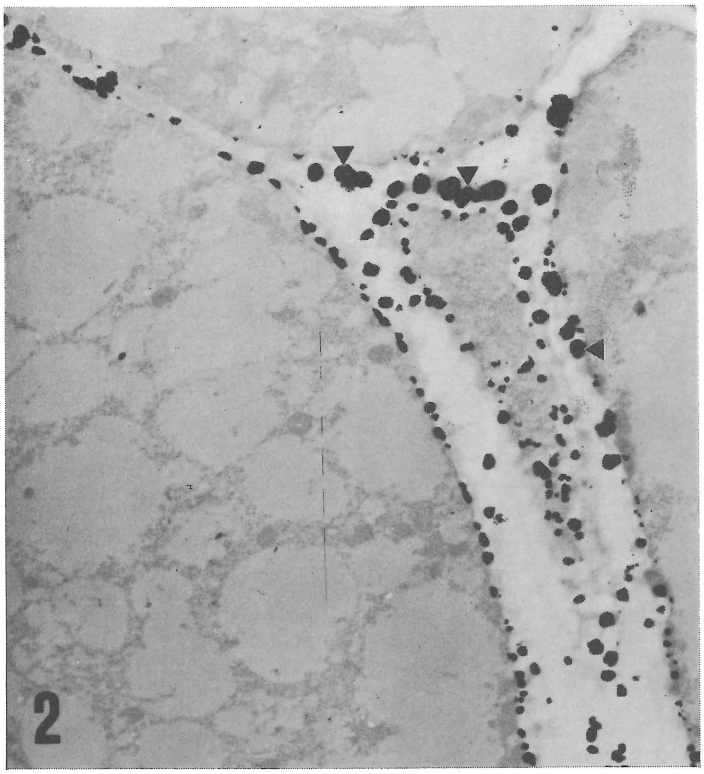
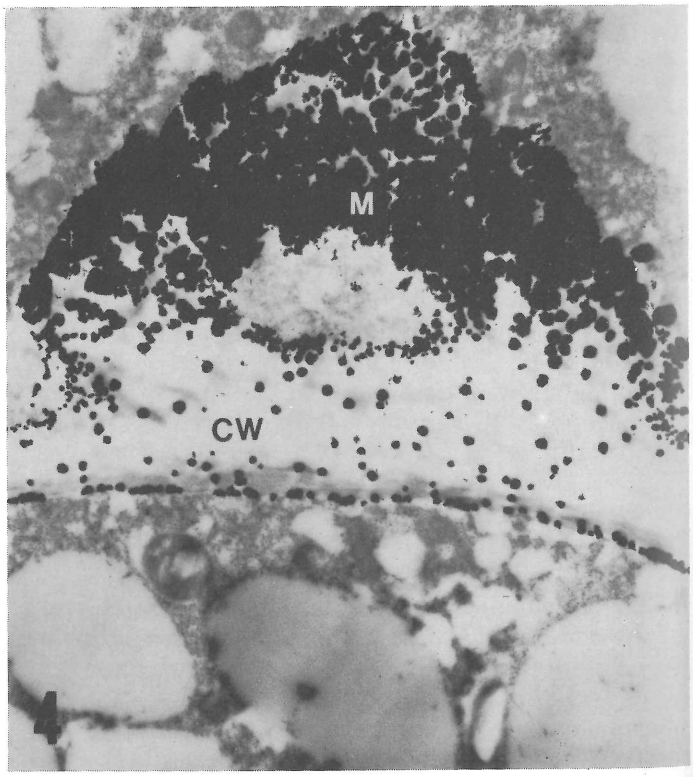


FIGURE 2. Cytochemical staining of the plasmalemma and cell wall in a columella cell of **Zea**. Arrowheads indicate reaction product. CW = cell wall. x 10,200.



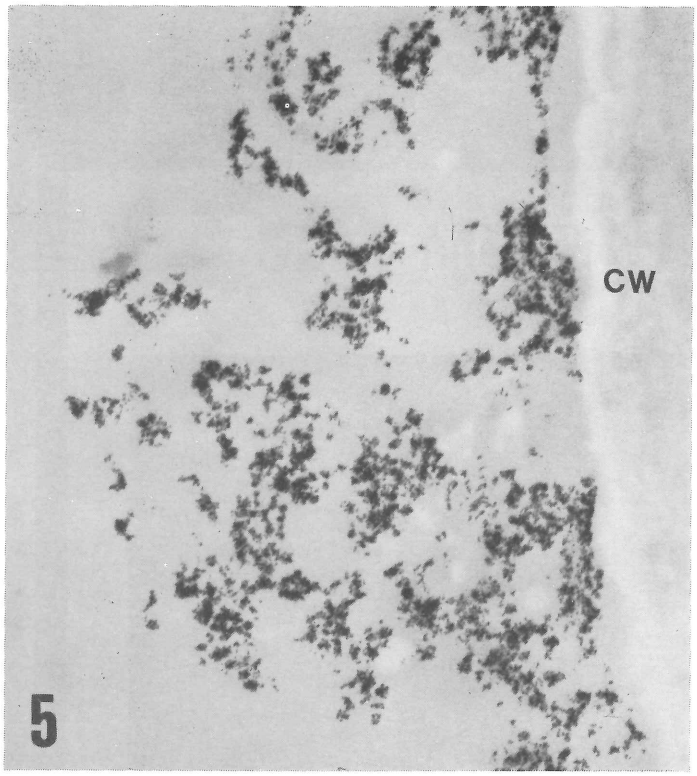


FIGURE 5. Glucose-6-phosphatase staining was restricted to the cytoplasm in the outermost peripheral cells. CW = cell wall. x 23,700.

cubate the tissue in media containing the substrate and 0.01 M sodium fluoride, a phosphatase inhibitor. Other compounds reported to be inhibitors of G-6-Ptase include D-glucose, ammonium molybdate, and the oral antidiabetic drug Orinase (17).

Cytochemical staining for G-6-Ptase according to the above protocol is consistently specific for the designated enzyme and gives precise ultrastructural localization. Enzymatic precipitate is absent in controls (Fig. 1). Sections that were photographed for this paper were not counterstained so as to allow for positive identification of staining due to enzymatic activity.

GLUCOSE-6-PHOSPHATASE IN PLANT CELLS

G-6-Ptase is a specific marker for the endoplasmic reticulum in animal cells (9, 18). This localization is in marked contrast to that observed in plant cells (11). Hall (11) localized G-6-Ptase in the plasmalemma and tonoplast of cells in roots of **Beta vulgaris.** In root caps of corn (**Zea mays**), G-6-Ptase localization is similar to that reported by Hall (11), but the localization changes during cellular differentiation. In columella (i.e., graviperceptive) cells located in the center of the cap, relatively small amounts of G-6-Ptase staining are associated with the plasmalemma (Fig. 2). As columella cells differentiate into peripheral cells, enzymatic staining is associated with mucilage and, to a lesser extent, the cell wall (Fig. 3,4). The outermost peripheral cells (i.e., cells that have completed mucopolysaccharide secretion) are characterized by small amounts of G-6-Ptase staining present only in the cytoplasm (Fig. 5). Thus, G-6-Ptase activity begins to increase in columella cells located in the center of the cap (i.e., cells not directly involved in mucilage secretion) and peaks in cells located nearer the periphery of the cap (i.e., cells reponsible for mucilage secretion). At the completion of mucilage secretion, G-6-Ptase activity is minimal and is restricted to the cytoplasm. Since glucose is present in mucilage secreted by **Zea** root caps (19), these results suggest that G-6-Ptase is involved in the production and/or secretion of mucilage by root caps of **Zea mays**. More research is needed to further document the site of G-6-Ptase activity in plants, especially in light of the apparent dichotomy between the site of activity in animals as compared to plants.

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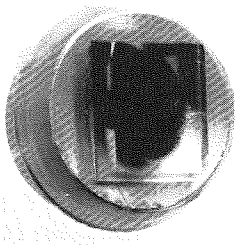

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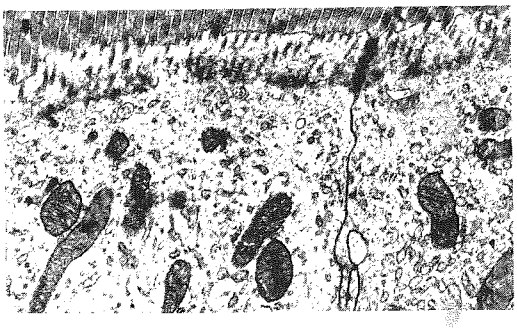

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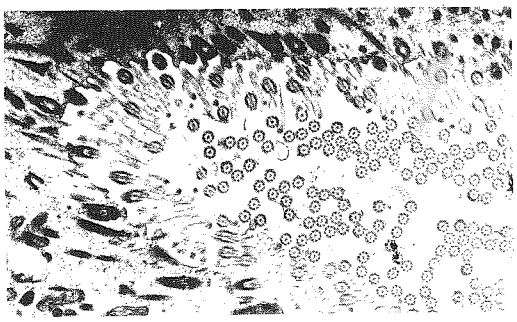

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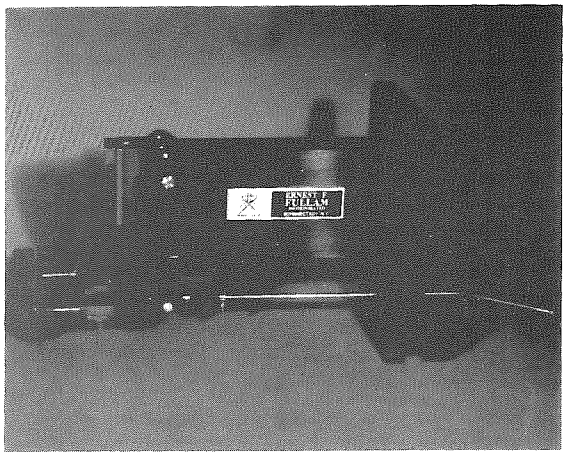

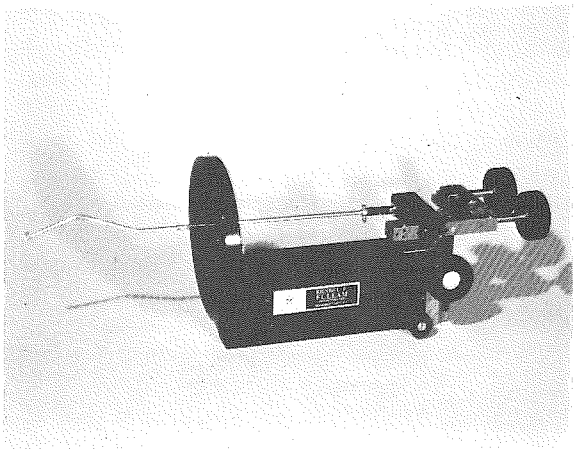

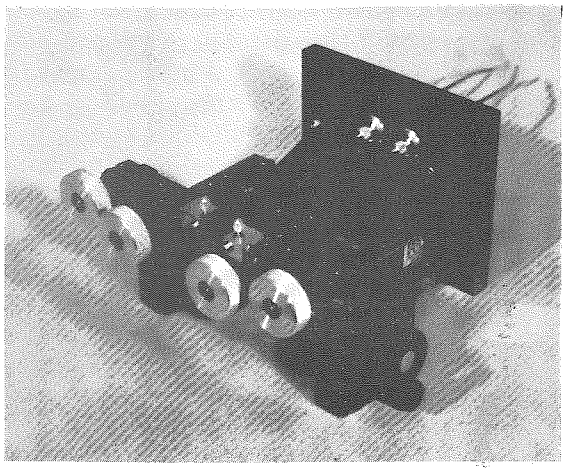

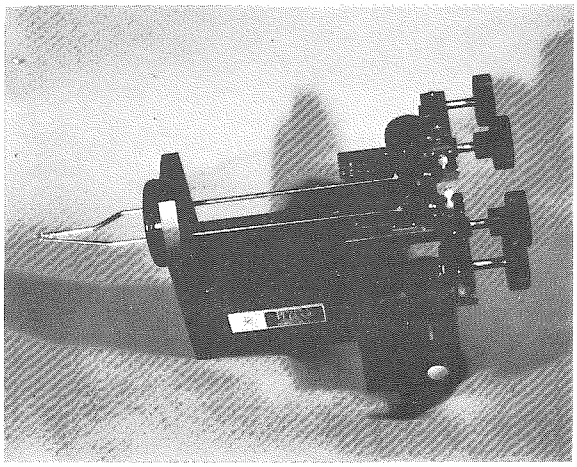

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The Structure Of Graviperceptive Cells In Plant Roots

By

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Baylor University
Waco, Texas 76798

The quest to understand how plant roots perceive and respond to gravity began over a century ago. One of the earliest investigators was Charles Darwin, who noted that removal of the root cap abolished the gravitropic responsiveness of primary roots (1). This observation has been confirmed several times (see citations in reference 2), and indicates that the root cap is the site of graviperception by plant roots.

The cap of a primary root of corn (**Zea mays**) is shown in Fig. 1. The cap is comprised of several cell types, each having a unique structure and function (3,4). For example, the calyptrogen is located immediately adjacent to the root proper, and is the meristem responsible for forming the root cap (3,4).

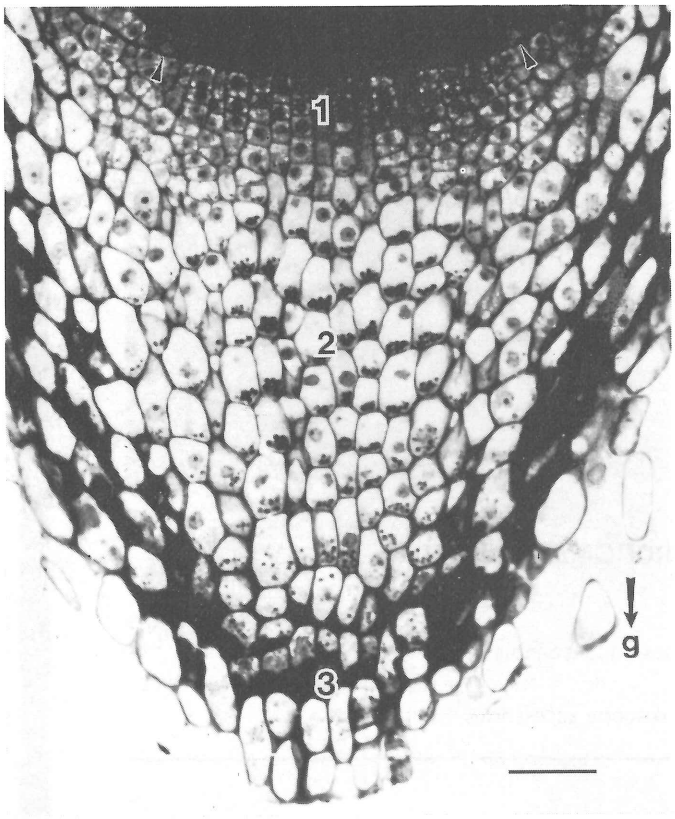

FIGURE 1. Median longitudinal section of a cap of a primary root of **Zea mays**. The cap is comprised of (1) calyptrogen cells, (2) columella statocytes, and (3) peripheral cells. Arrowheads indicate junction with root proper. Gravity vector (g) is indicated by arrow. Scale bar = 50 μm. (from reference 4)

Peripheral cells, located at the edge of the cap, are specialized to produce and secrete mucilage to aid the root in moving through the soil (3,4). In the center of the root cap is the columella tissue, named for the fact that its cells are typically arranged in longitudinal "columns" (Fig. 1). Columella cells are the cells believed to be responsible for graviperception by plant roots (5).

DIFFERENTIATION OF COLUMELLA CELLS

Columella cells differentiate from calyptrogen cells (Fig. 2). The calyptrogen is composed of 4 to 5 layers of meristematic cells arranged in longitudinal files perpendicular to the cap junction (Fig. 2). Calyptrogen cells range in shape from cuboidal to flattened in the plane parallel to the cap junction. Prominent nuclei are located in the center of calyptrogen cells and are surrounded by a densely staining cytoplasm that contains the usual compliment of cellular organelles. Mitochondria, plastids, and components of the endomembrane system are distributed randomly in calyptrogen cells.

Calyptrogen cells elongate in the plane of the root axis as they differentiate into columella cells (Fig. 2). The most striking features of this differentiation of

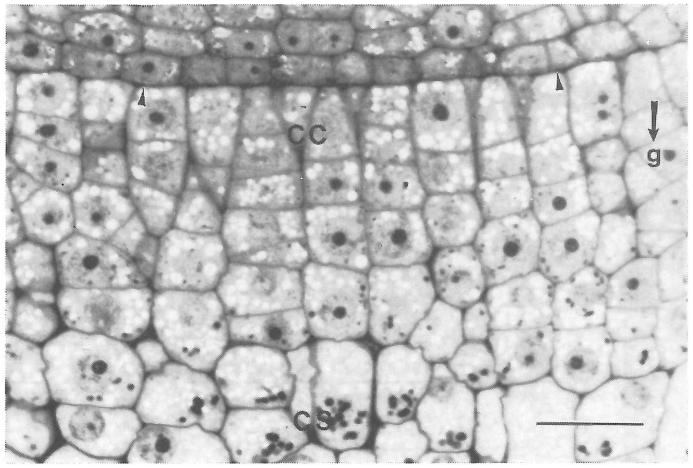

FIGURE 2. Differentiation of columella statocytes (CS) from calyptrogen cells (CC). Arrowheads indicate junction with root proper. Gravity vector (g) is indicated by arrow. Scale bar = 25 μm. (unpublished micrograph of E. McClelen)

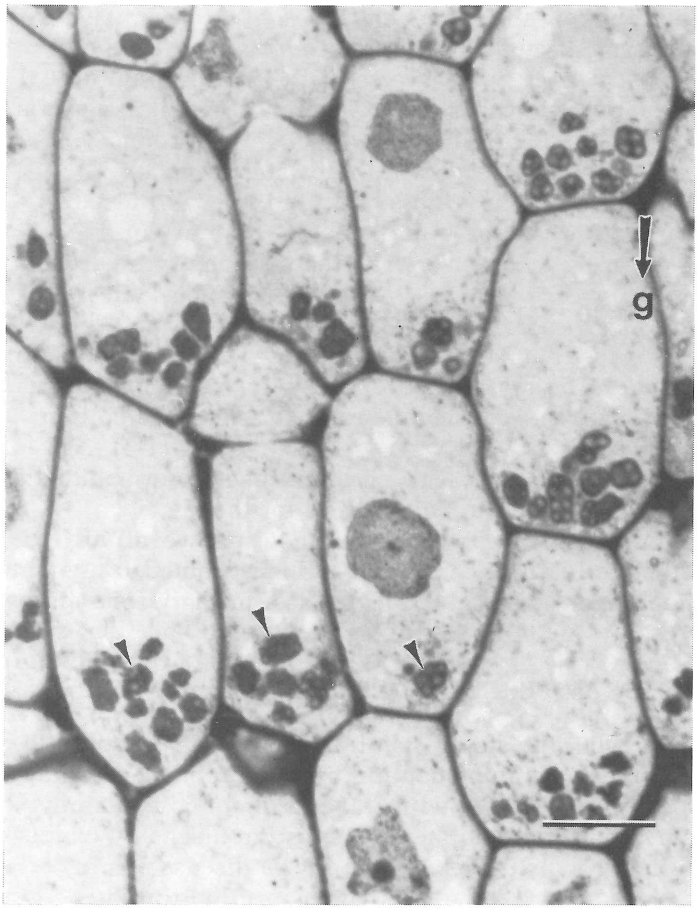

FIGURE 3. Columella tissue of primary root of **Zea mays**. Arrowheads indicate sedimented amyloplasts. Gravity vector (g) is indicated by arrow. Scale bar = 10 μm. (unpublished micrograph of E. McClelen)

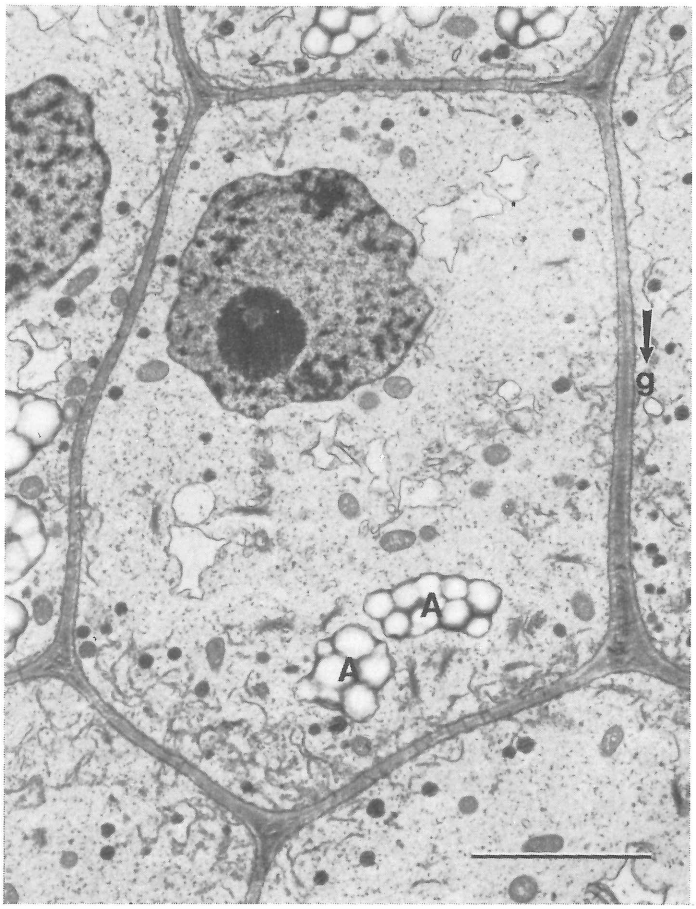

FIGURE 4. Columella cell of primary root of **Zea mays**. Columella cells are characterized by the presence of numerous sedimented amyloplasts (A). Gravity vector (g) is indicated by arrow. Scale bar = 5 μm. (from reference 4)

calyptrogen cells into columella cells are the (i) differentiation of proplastids into amyloplasts, and (ii) sedimentation of these amyloplasts to the bottom of the cells (3). Both of these events occur rapidly, since the entire root cap is regenerated in as little as 24 hours (6). Also, both of these changes in cellular structure are believed to be essential for graviperception by primary roots (3, 4). A more thorough discussion of the differentiation of columella cells from calyptrogen cells is presented elsewhere (3, 4).

STRUCTURE OF COLUMELLA CELLS

The quantitative ultrastructure of columella cells of primary roots of **Zea mays** is shown in Table 1. On a relative volume basis, the ultrastructure of columella cells of **Zea** is similar to that reported for those of pinto bean (**Phaseolus vulgaris**) (7, 8) and other plants (Stoker and Moore, unpublished results; Moore and Pasieniuk, unpublished results), suggesting that this ultrastructure (i.e., as presented in Table 1) may be characteristic of columella cells in plant roots. Columella cells typically have a length:width ratio of approximately 1.5:1, a granular cytoplasm, and thin primary cell walls (Fig. 3, 4).

Table 1. The relative volumes ($\pm$ standard deviation) of cellular components in columella cells of primary roots **Zea mays**. All volumes are expressed as a percentage of the protoplasm. (from reference 7)

Nucleus	9.51 $\pm$ 3.7
Vacuole	11.4 $\pm$ 5.1
Mitochondria	3.42 $\pm$ 1.0
Amyloplasts	7.57 $\pm$ 3.3
Dictyosomes	0.55 $\pm$ 0.3
Hyaloplasm	67.5 $\pm$ 5.4

Amyloplasts — Columella cells of primary roots of **Zea** each contain 25 to 40 amyloplasts (8, 9) that have an average volume of approximately 35 μm^3 per amyloplast (7). Interestingly, this is about the same number of statoliths as observed by Sievers in rhizoids of **Chara**, a green alga (10, 11). The numerous starch grains in each amyloplast occupy approximately 65% of the amyloplast volume (4, 8). Amyloplasts in columella cells are of similar size and divide infrequently (9). Aside from starch, these amyloplasts contain fragmentary thylakoids (9).

Amyloplasts are located in the bottom portion of columella cells (5, 9, 12, 13 — also see Fig. 1-4). This

sedimentation of amyloplasts is believed to be the basis for graviperception by plant roots (5, 13 — see "Graviperception By Columella Cells" below).

Endoplasmic reticulum — The area of endoplasmic reticulum (ER) increases from 400 μm^2 to 10,000 μm^2 as calyptrogen cells differentiate into columella statocytes (14). Most of the ER is rough ER (9), and its distribution in graviperceptive cells is highly variable. Indeed, the ER in columella cells has been described as being (i) located parallel to the longitudinal cell walls (e.g., **Zea** — see reference 9), (ii) located at the distal pole of the cell (e.g., **Lepidium** — see reference 13), and (iii) distributed randomly (e.g., **Phaseolus** — see reference 8).

Ribosomes — The number of ribosomes in columella cells is smaller than that of other cells in the root cap (9). This decreased density of ribosomes has been suggested to be responsible for the decreased staining intensity of columella cells (9) reported by O'Brien and McCully (15) and Barlow (16).

Nuclei — Nuclei of columella cells are typically lobed.

Mitochondria — Mitochondria in columella cells have a normal appearance. The approximately 810 mitochondria in individual columella cells of **Zea** have an average volume of 0.75 μm^3 per mitochondrion (7).

Dictyosomes — Dictyosomes of columella cells also have a normal appearance. The approximately 210 dictyosomes of **Zea** columella cells have an average volume of 0.47 μm^3 per organelle (7).

Vacuome — The vacuome of columella cells is typically comprised of numerous small vacuoles (4, 8), each of which is irregularly shaped.

Microtubules — Microtubules are rare in columella cells. When present, they are located parallel to the longitudinal axis of the cell (9).

Plasmodesmata — Plasmodesmata are 2.8 times more frequent in transverse walls than in longitudinal walls of calyptrogen cells. The longitudinal expansion of calyptrogen cells as they differentiate into columella cells results in the plasmodesmatal frequencies being differentially "diluted". Indeed, plasmodesmata are approximately 10 times more frequent in transverse walls than in longitudinal walls of columella cells (i.e., 772 versus 75 plasmodesmata um^{-2}, respectively) (9).

Intracellular distribution of organelles in columella cells — The most striking feature of columella cells is the presence of numerous amyloplasts at the bottom of the cell (5, 8, 9, 12, 13 — also see Fig. 1-4). As already mentioned, this structural feature of columella cells is believed to be the basis for their graviperceptive function (5, 13, 17). However, all other cellular organelles are also distributed nonrandomly in columella cells of primary roots (Fig. 5).

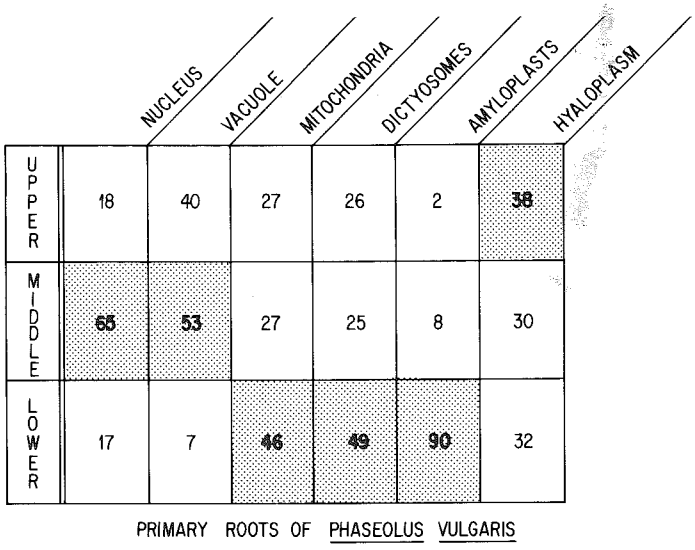

FIGURE 5. Probabilities that organelles will be located in the upper, middle, or lower thirds of columella cells of primary roots of **Phaseolus vulgaris**. Shaded portions indicate zones in which the organelle tends to be located most frequently. (after data in reference 24)

GRAVIPERCEPTION BY COLUMELLA CELLS

In 1900, the starch-statolith theory was proposed to account for graviperception by plant roots. According to this theory, the sedimentation of starch-containing amyloplasts in columella cells is the means by which primary roots perceive gravity (see discussions in references 2, 5, 13, 17). Evidence supporting this theory includes the following observations:

i) Decapped roots are not graviresponsive (2). The recovery of graviresponsiveness by decapped primary roots correlates with the differentiation and sedimentation of amyloplasts in cells of their root tips (18).

ii) Treatments which result in the disappearance of starch from amyloplasts also render roots unresponsive to gravity (19). When amyloplast starch is regenerated, the root again becomes graviresponsive (19).

iii) Sedimentation of amyloplasts occurs fast enough to be temporally correlated with gravicurvature (5). Conversely, the direct involvement of other organelles in graviperception has been discounted due, for example, to their highly dissected shape (e.g., dictyosomes — see reference 17), their low density (e.g., mitochondria — see reference 5), and that they often require longer to fall through the cytoplasm than is compatible with gravitropic presentation times (e.g., spherosomes — see reference 20).

These observations have led some investigators to conclude that the movement of amyloplasts in columella cells somehow initiates the sequence of events that leads to root gravicurvature (5). Furthermore, it appears that the movement of amyloplasts relative to some other cellular component (rather than amyloplast movement **per se**) initiates the graviresponse (21).

The sedimentation of amyloplasts is ultimately transformed into an imbalance of growth inhibitors(s)

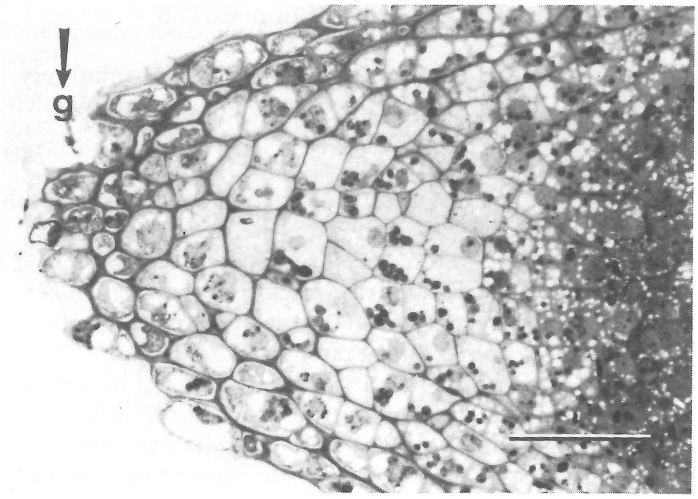

FIGURE 6. Median longitudinal section of the cap of a lateral root of **Helianthus annuus**. Note the columella cells in the center of the cap. Gravity vector (g) is indicated by arrow. Scale bar = 100 μm. (unpublished micrograph of R. Stoker)

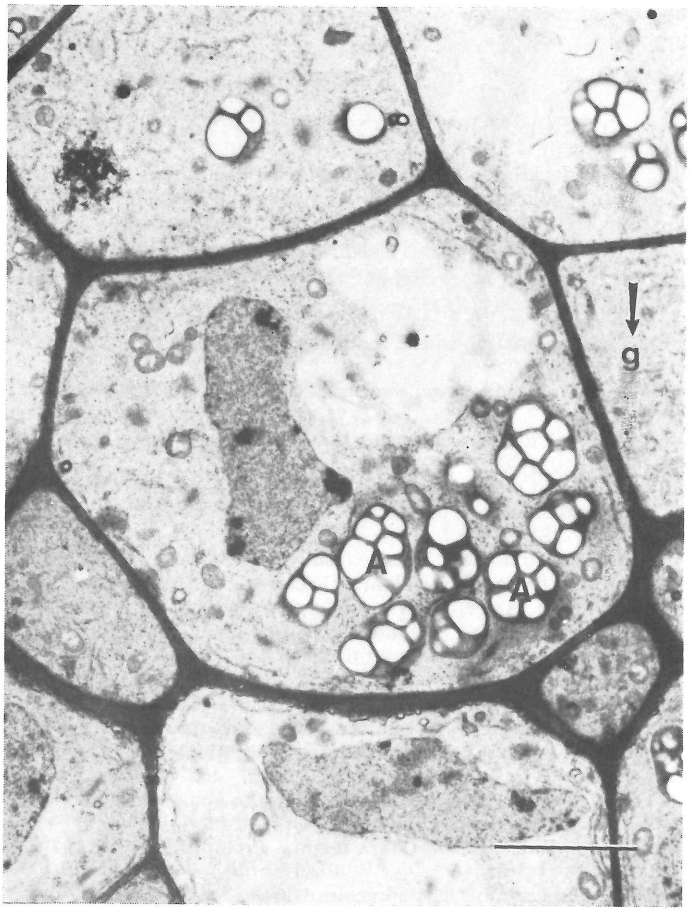

FIGURE 7. Columella cell of a lateral root of **Phaseolus vulgaris**. Like those of primary roots, columella cells of lateral roots are characterized by the presence of numerous sedimented amyloplasts (A). Gravity vector (g) is indicated by arrow. Scale bar = 5 μm. (from reference 8)

in the root cap (see discussion in reference 22), with the largest concentration of the inhibitor being in the lower half of the cap. The inhibitor(s) are subsequently transported basipetally to the elongating zone of the root (i.e., at greater concentrations in the lower versus upper portion of the root), which is located 1 to 3 mm behind the root tip (22). There the imbalance of growth inhibitor(s) results in a more rapid elongation of the upper versus lower halves of the root, thereby accounting for the downward gravicurvatures of horizontally-oriented primary roots (see discussion in reference 22). A discussion of the proposed mechanisms for how the sedimentation of amyloplasts (i.e., a physical stimulus) is transformed into physiological changes in columella cells is is presented elsewhere (23).

WHAT ABOUT LATERAL ROOTS?

A longitudinal section of a cap of a lateral root of sunflower (**Helianthus annuus**) is shown in Fig. 6. In the center of caps of lateral roots are columella cells, which are characterized by the presence of numerous sedimented amyloplasts (Fig. 6, 7). Indeed, the qualitative (cf. Fig. 4, 7) and quantitative (cf. Fig. 5, 8) ultrastructures of columella cells of lateral roots of **P. vulgaris** (8) are not significantly different from those of primary roots. These results have prompted Ransom and Moore to conclude that the differing gravibehaviors of primary and lateral roots of **P. vulgaris** must be due to factors other than the structures of their individual columella cells (24). If it is assumed that columella cells are responsible for graviperception by plant roots (as is indicated by data

	NUCLEUS	VACUOLE	MITOCHONDRIA	DICTYOSOMES	AMYLOPLASTS	HYALOPLASM
UPPER	34	37	24	20	2	37
MIDDLE	50	50	28	23	8	31
LOWER	16	13	48	57	90	32

LATERAL ROOTS OF PHASEOLUS VULGARIS

FIGURE 8. Probabilities that organelles will be located in the upper, middle, or lower thirds of columella cells of lateral roots of **Phaseolus vulgaris**. Shaded portions indicate zones in which the organelle tends to be located most frequently. Note similarities with distribution of organelles in columella cells of primary roots (Fig. 5). (after data in reference 24)

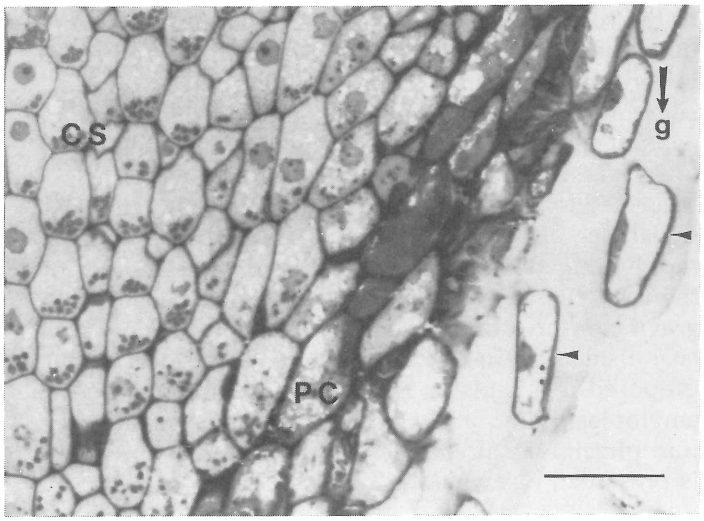

FIGURE 9. Differentiation of columella statocytes (CS) into peripheral cells (PC). Note peripheral cells in process of being sloughed from the cap (arrowheads). Scale bar = 50 μm. (unpublished micrograph of E. McClelen)

indicated by data — see discussion in reference 5), then lateral (i.e., minimally graviresponsive) roots perceive gravity as do primary (i.e., graviresponsive) roots. What, then, uncouples gravipeception from gravitropism in lateral roots?

Moore and Pasieniuk have recently suggested that the minimal graviresponsiveness of lateral roots is due to the inability of their caps to establish a concentration gradient of the inhibitor(s) which affect gravicurvature (25). These same authors have also suggested that the basis for a lateral root being unable to establish a concentration gradient of the inhibitor(s) results from the smaller amounts of columella tissue in their root caps (25). Supporting this suggestion are the following observations:

i) The columella tissue of lateral roots is much less extensive than that of primary roots (8, 25).

ii) The onset of graviresponsiveness by lateral roots of some plants (e.g., **Ricinus communis**) is positively correlated with a pronounced increase in the volume of the columella tissue (25).

iii) Primary roots with larger caps (and, presumably, more extensive columellas) are more graviresponsive than roots with smaller caps (and, presumably, less extensive columellas) (26).

iv) The graviresponsiveness of serially detipped primary roots is proportional to the amount of cap (and, presumably, the amount of columella tissue) remaining on the root (26).

v) The graviresponsiveness of hypocotyls is proportional to the amount of graviperceptive tissue present (27).

Finally, it is interesting to note that according to the model of Moore and Pasieniuk (25), graviperception is a **cellular** event, while gravitropism is dependent on the presence of an extensive columella **tissue.**

FATE OF COLUMELLA CELLS

The unique structure of columella cells is relatively short lived. Within hours, continued meristematic activity in the calyptrogen results in cells being pushed out of the columella tissue toward the periphery of the root cap. Concurrent with this change in cellular position is (i) the disappearance of most amyloplast starch, (ii) a redistribution of cellular organelles, and (iii) hypertrophication of dictyosomes as columella cells differentiate into peripheral cells (3, 4). Correspondingly, these changes in cellular structure are correlated with the assumption of a new cellular function, that being the production and secretion of mucilage by peripheral cells (see Fig. 9). Eventually, peripheral cells are sloughed from the cap as the root tip moves through the soil. A more thorough discussion of the differentiation of peripheral cells from columella cells is presented elsewhere (3, 4).

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SHORT COURSES AND WORKSHOPS

WORKSHOP IN BIOLOGICAL HVEM
May 29-June 2, 1984
University of Colorado, Boulder, CO
For further information contact Mircea Fotino, Laboratory for High-Voltage Electron Microscopy, MCD Biology, Campus Box 347, University of Colorado, Boulder, CO 80309, phone (303) 492-8593.

SCANNING AND TRANSMISSION ELECTRON MICROSCOPY
TEM - June 4-15
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The George Washington University, Washington, D.C.
For further information contact Fred Lightfoot, The George Washington University, Department of Anatomy, 2300 Eye Street, N.W., Washington, D.C. 20037, phone (202) 676-2882 or 676-3511.

TRANSMISSION ELECTRON MICROSCOPY OF MATERIALS
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Massachusetts Institute of Technology, Cambridge, MA
For further information contact L.W. Hobbs, Room 13-4066, MIT, 77 Massachusetts Ave., Cambridge, MA 02139.

SCANNING ELECTRON MICROSCOPY
June 24-29, 1984
University of California, Davis, CA
For further information contact D.G. Howitt, Department of Mechanical Engineering, University of California, Davis, CA 95616, phone (916) 752-1318.

ANALYTICAL ELECTRON MICROSCOPY WORKSHOP
July 16-20, 1984
Lehigh University, Bethlehem, PA
For further information contact David C. Joy, Bell Laboratories, Room 15-211, 600 Mountain Ave., Murray Hill, NJ 07974 phone (201) 582-7216.

THIRD ASIA PACIFIC ELECTRON MICROSCOPY WORKSHOP
August 24-28, 1984
Singapore
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SCANNING ELECTRON MICROSCOPY AND X-RAY MICROANALYSIS: THEORY AND PRACTICE - MATERIALS SCIENCE
October 15-19, 1984
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For further information contact Dr. Angelos V. Patsis, Department of Chemistry, CSB 209, State University of New York, New Paltz, NY 12561, phone (914) 257-2175.

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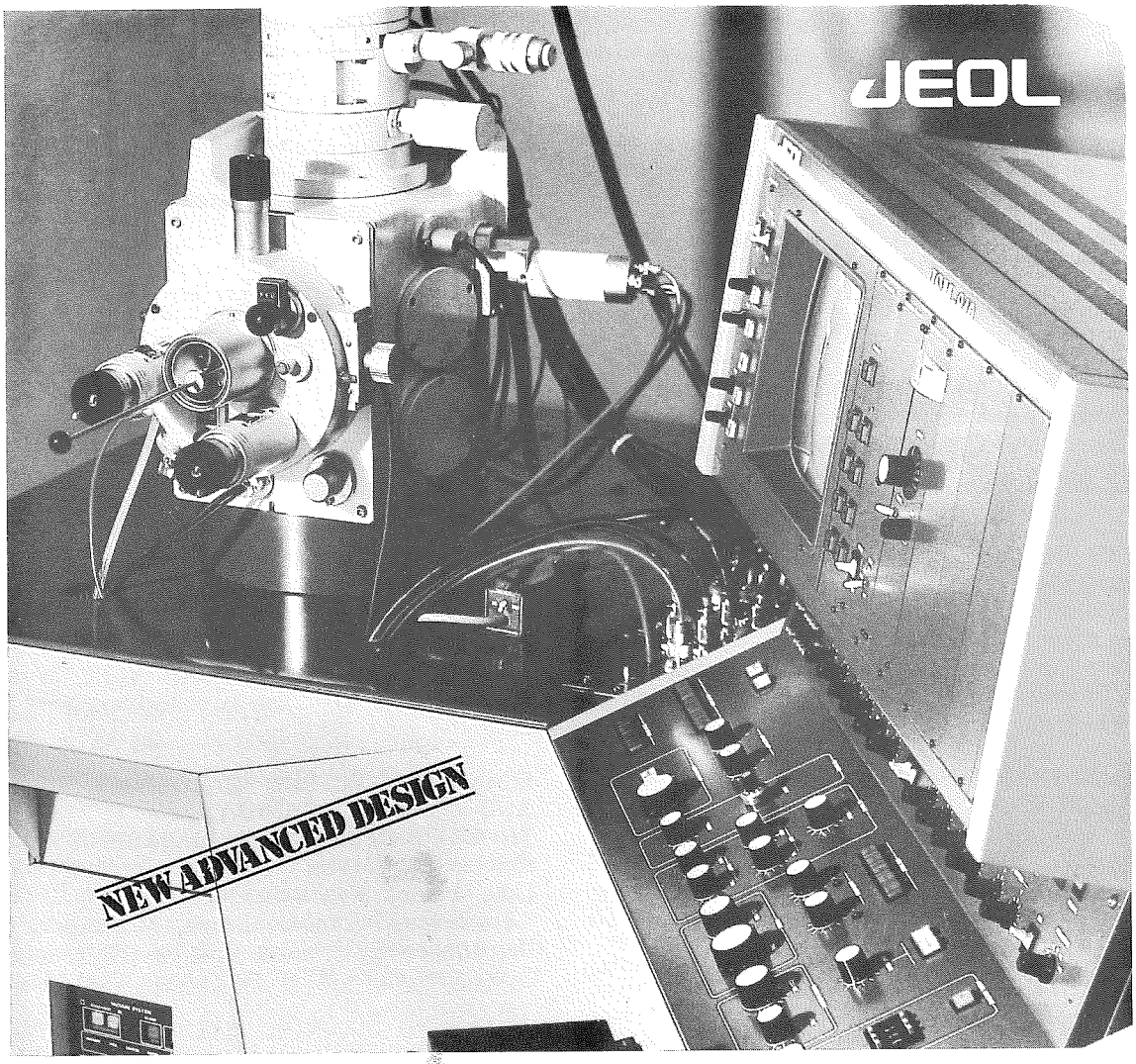

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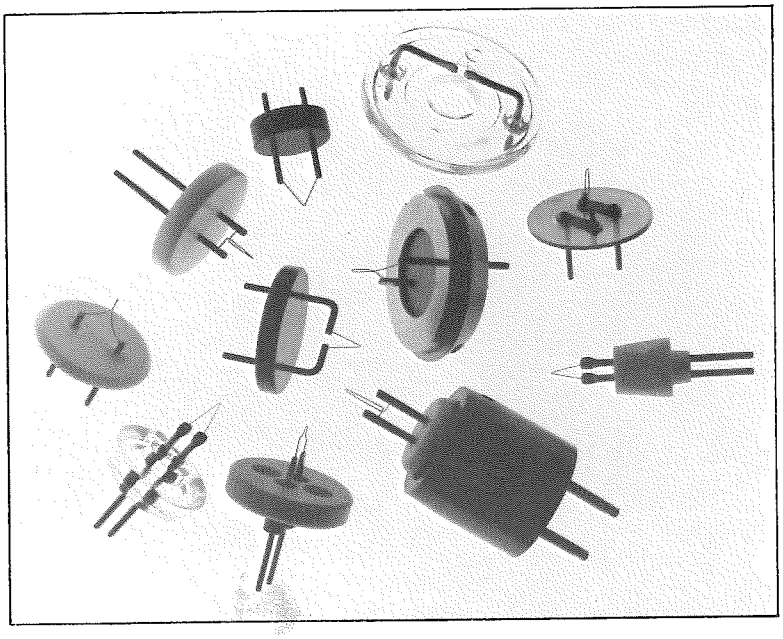

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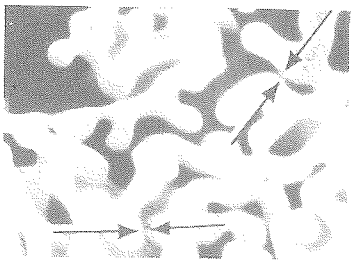

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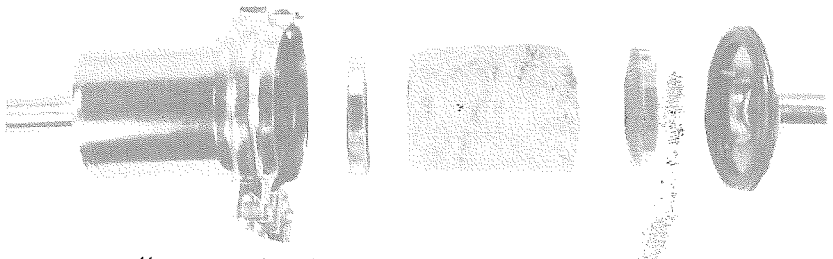

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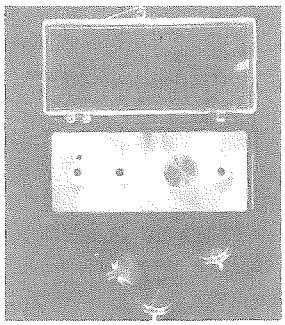

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A GRAVIMETRIC FORMULA FOR ERLANDSON'S MARAGLAS, D.E.R. 732 EMBEDDING MEDIUM

By

Thomas Caceci
Assistant Professor

Department of Veterinary Anatomy
Texas A&M University
College Station, Texas 77843

Introduction:

Several different epoxy embedding media are used to prepare ultrathin sections of biological material for transmission electron microscopy. Each of these has its own advantages and disadvantages, and no doubt most microscopists have a "favorite" medium that is used for routine work. For years the "favorite" was usually Epon 812, the most widely used of the epoxy embedments. Epon 812 was introduced in 1961 by Luft (1), and soon was accepted as a versatile embedment with excellent sectioning characteristics, whether used alone or in combination with other resins, such as Araldite (2).

About 1979, the manufacture of Epon 812 resin was terminated, and the remaining supplies on distributor's shelves dwindled rapidly as laboratory directors who had adopted it as a standard medium stockpiled as much as possible. Production never resumed, and subsequently most of the EM supply houses began marketing what they termed "Epon equivalents." These are often sold under proprietary names which imply that the product is actually identical to Epon. However, there is no real assurance that this is the case, and some if not all of these "Epon equivalents" are in fact different resins. Their duplication of Epon's performance varies considerably, especially when substituted for real Epon in mixtures with other resins. In these mixtures the resins may or may not interact to produce the desired results.

Other problems may arise in those labratories which still have stocks of genuine Epon 812 on hand. First is the undeniable fact that the freshest of these stocks are by now almost five years old, and may be coming to the limit of their shelf life. When this limit is reached, it is to be expected that the characteristics of blocks produced with this stale material will be affected. Second, sooner or later the day will arrive when the stockpiled reserves are exhausted and a substitute **must** be found.

When the Great Epon Famine began, our laboratory tested several other embedding media as replacements. We rejected several popular media as unsuited to routine use: Spurr's medium (3), introduced for use on plant material, is not ideal for softer animal tissues due to excessive shrinkage in curing, and in our hands Araldite (4) proved to be excessively sensitive to the duration of the cure. We found the Maraglas, D.E.R. 732 mixture of Erlandson (5) to be a versatile and extremely useful general-purpose embedding medium. It has ideal cutting and handling characteristics, shrinks very little in curing, pentrates tissue easily, and is not sensitive to moderate variations in curing time or temperature, thus yielding a good block over a wide range.

A significant drawback to the formula as originally given by Erlandson, however, is that it is volumetric. The measurement of viscous liquids by volume is almost always imprecise, especially when only small quantities are involved. The slow flow of such materials out of pipets, and their tendency to adhere to the sides, cause a good deal of variation in the amount actually delivered versus the amount measured. This imprecision of measurement causes blocks to be inconsistent.

The measurement of embedding chemicals by weight, however, presents no such difficulty. Gravimetric measurements are reproducible to any degree of accuracy that may be desired because the variability of the method is limited to the inherent error of the balance, and this usually very small. The blocks produced are uniform in composition, which results in uniformity of curing and cutting characteristics. A gravimetric formula for the

preparation of Maraglas, D.E.R. 732 embedding medium is given here.

This formula has been worked out in our laboratory and has been in use for the past four years with excellent results. It is an adaptation of Erlandson's 1964 formula, and it is not applicable to the earlier Maraglas formulas of Freeman and Spurlock (6) or that of Spurlock **et al**. (7).

Method of Procedure:

The complete Maraglas mixture has four components: Maraglas 655 resin, D.E.R. 732 resin, dibutyl phthalate (DBT), and the catalyst, which may be either benzyldimethylamine (BDMA) or dimethylaminoethanol (DMAE). The first three components are measured by weight, and the last by volume. The Maraglas resin is the most viscous component, and the catalyst the least.

Using a toploading laboratory balance, tare out a 50-milliliter polypropylene or polyethylene beaker. Weight out the first three components in the following order:

Maraglas 655 ..... 16.1 grams
D.E.R. 732 ..... 3.3 grams
DBT ..... 2.0 grams

This produces a total volume of about 20.0 ml. Do not stir at this point. To the first three components add 0.4 milliliters of the catalyst. This material is quite fluid, and can be measured precisely using a disposable pipet.

Mix the four components thoroughly with a pair of wooden sticks. The mixture will be almost colorless and its viscosity will decrease slightly as mixing occurs. During curing of the blocks, the mixture will turn slightly yellow. This is normal and does not affect the quality of the blocks.

The following embedding schedule has been found to work well with a wide variety of tissues. We have used it in our laboratory on cell suspensions, as well as pieces of intestine, kidney, liver, heart and skeletal muscle, gill, lung, and teeth:

- After fixation and washing of the tissue are completed, dehydrate it in a series of ethanol solutions of increasing concentration from 50% to 100% (two changes), for 2-10 minutes per change, depending on the size of the tissue pieces.
- "Clear" in propylene oxide (PO), two changes of five minutes each.
- Incubate the tissue blocks overnight in a 1:1 mixture of Maraglas medium:PO at room temperature.
- On the following day, incubate the tissue in a 3:1 mixture of Maraglas medium:PO for six hours at room temperature.
- Incubate one hour in pure Maraglas mixture, then transfer to a curing oven at 53-55° C.
- Cure for 24-48 hours.

Blocks prepared in this way can be cut and will give satisfactory ultrathin sections after as little as 18 hours of curing time, but they will be rather soft. Maximum hardness is reached after 48 hours, and further incubation will not harden them beyond this point, In fact, on occasion we have left blocks in the oven for as long as a week with no deterioration in cutting characteristics.

On occasion it may be necessary to avoid the use of propylene oxide as a dehydrant or "clearing" agent. In these cases the propylene oxide can be replaced with 100% ethanol, usually with satisfactory results.

It is advisable to make a fresh batch of Maraglas mixture for each stage of the embedding schedule, especially when a partially-full vessel of catalysed mixture has been allowed to sit overnight. It is also better not to attempt the common practice of storing catalysed mixtures in a freezer. It has been our experience that this latter practice causes inconsistent blocks, possibly due to condensation of water in the resin.

So far, we have not found it necessary to modify the mixture for any of our specimens. The moderately hard blocks that are produced section well with glass or diamond knives, and are dimensionally stable. Sections can be expanded after cutting by exposure to chloroform vapor. By varying the proportions of D.E.R. 732 and Maraglas, the hardness of the block can be varied. A softer block will be produced by adding more D.E.R. 732; the amount of Maraglas should be adjusted to give the same total weight of 21.4 grams for the first three components.

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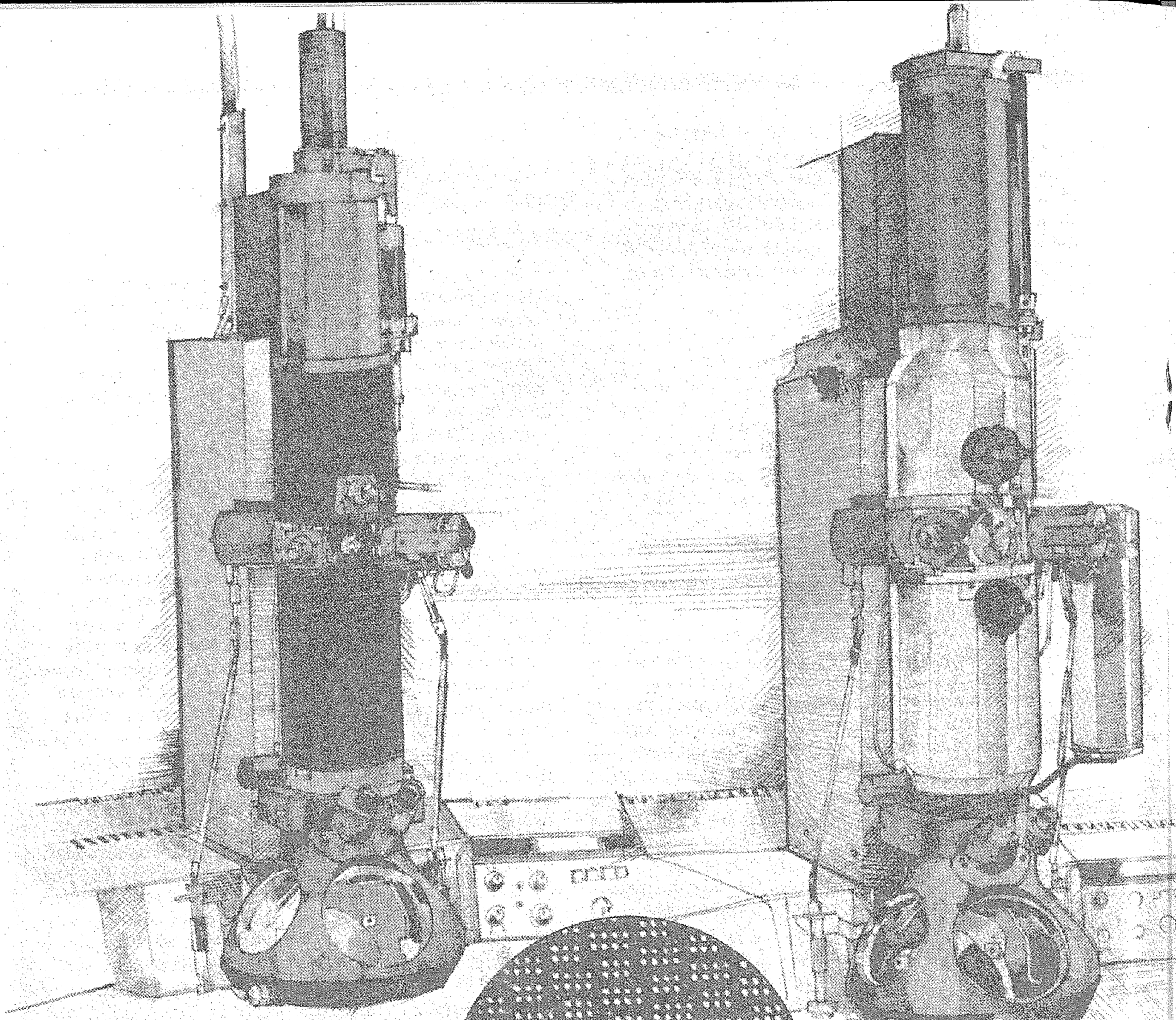

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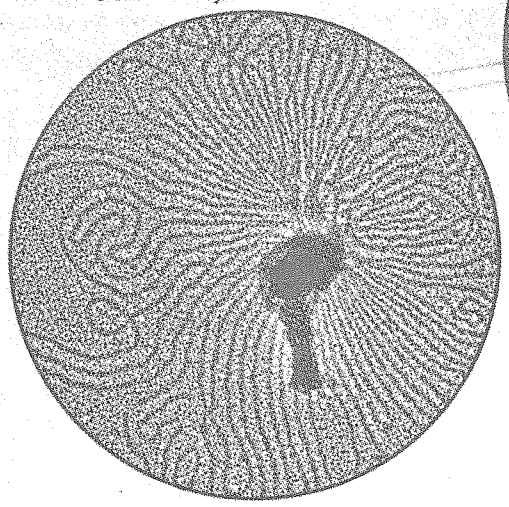

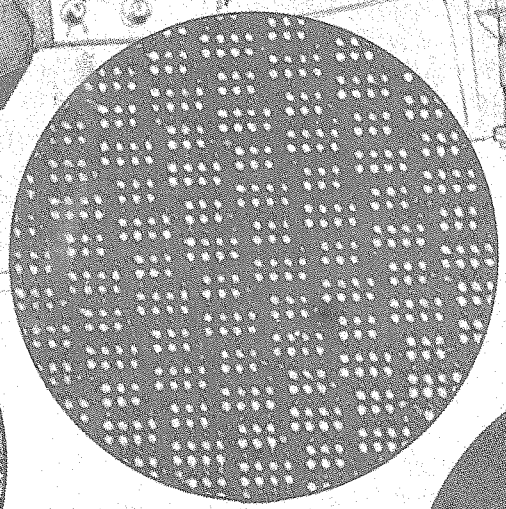

Block lattice atom imaging in $H\text{-}Nb_2O_5$ crystal.

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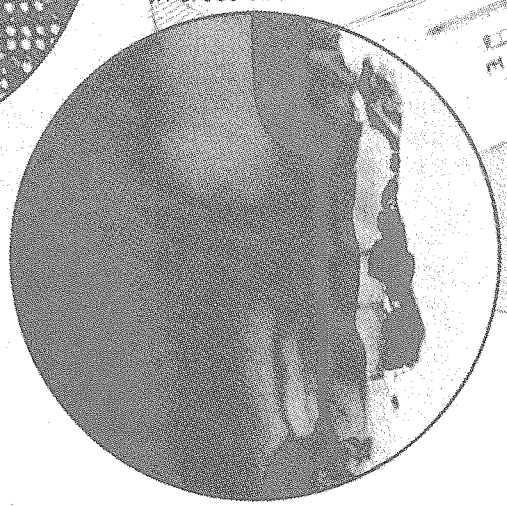

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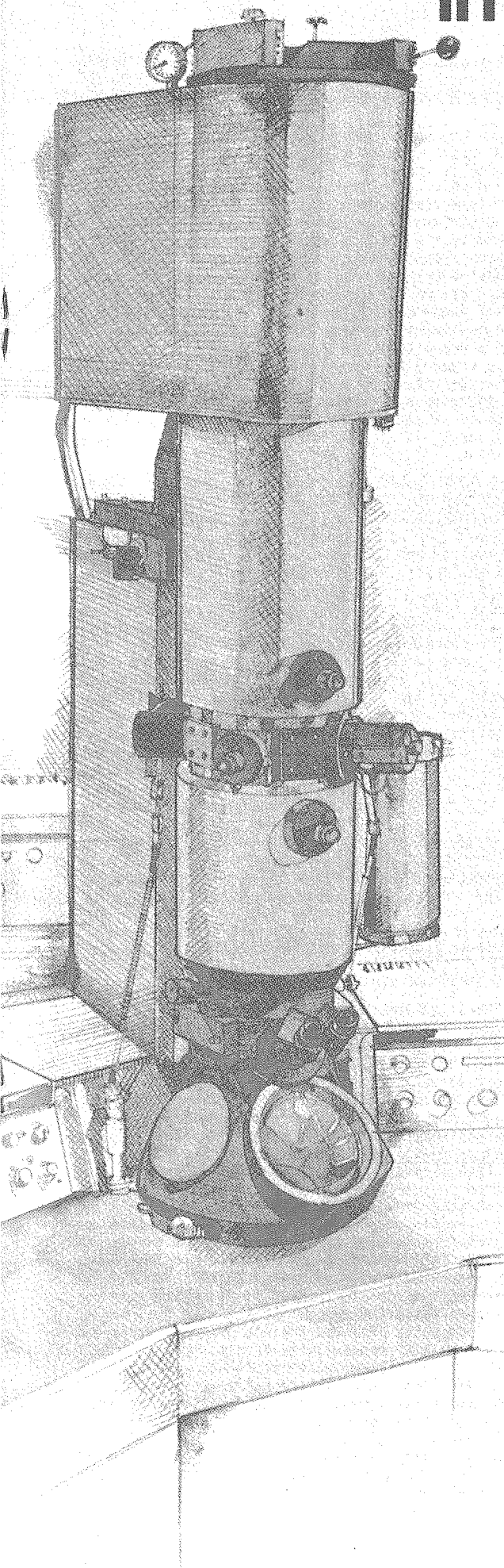

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Abstracts

Abstracts from the Spring 1984 Meeting in College Station, Texas

WOUND CONTRACTION: EFFECTED BOTH BY MYOFIBROBLASTS AND COLLAGEN FASICLES. Paul S. Baur, Galveston, TX.

The contractile nature of repairing soft connective tissue (SCT) accounts for wound contraction. SCT myofibroblasts are the cell mediators that effect the physical closure of the wound. Studies of *in vivo* wound models show the apparent insensitivity of these cells to the drug Cytochalasin B an agent commonly used to disrupt microfilaments. Colcemid, a microtubule inhibitor which also blocks dividing cells in metaphase, was able to delay closure for several days before normal healing and/or contraction rates resumed. Both Colcemid and Cytochalasin B were able to surpress the contractility of the SCT in an *in vitro* study but their contributions were not additive. Measurable contractility was also observed in SCT whose cells had been dissociated. This data implies that the microtubules and actin microfilaments concert a cell contraction event and that a similar contractile capacity is built into the newly fabricated collagen fasicles. The absence of mitotic fibroblast configurations in the treated tissues suggests that cell proliferation in and to the wound was accomplished by migration and not by division. This gives rise to the "QUEUE SYSTEM HYPOTHESIS" which teaches that SCT is repaired by cells that divide elsewhere and migrate to the wound space through the SCT fabrics of the body in the manner of a queue. THE QUEUE SYSTEM allows that cell migration distance is minimal (one cell space), fibroblast division need not take place in a more or less hostile milleau (wound space), and insures that an optimal number of cells is provided for repair.

SEM COMPARISON OF FRUITS, SEEDS, AND EMBRYOS OF *SYRINGODIUM ISOETIFOLIUM* AND *S. FILIFORME*. Louis H. Bragg, Biology Dept., University of Texas, Arlington, TX 76019 and Calvin McMillan, Dept. of Botany, University of Texas, Austin, TX 78712.

One seeded fruits of the seagrasses, *Syringodium isoetifolium* from the Phillipines and *S. filiforme* from the U.S. Virgin Islands were compared with scanning electron microscopy for differences that may aid in establishing their taxonomic status. The thick-walled cells of the fruit in *S. isoetifolium* has sculptured pitting which is half the size of that in *S. filiforme*. The endocarp if 7-10 cells in cross-section, each cell with multi-layered concentric walls. The smaller fruits of *S. isoetifolium* has a different morphology than the larger fruits of *S. filiforme*. The seed coats of both species are composed of elongated cells but become polygonal near the stylar end of the fruit. The embryos of both seeds are composed of an oblong hypocotyl with an attached plumule-cotyledon folded into an invagination which lies adjacent to the operculum flap of the fruit wall. Between the plumule-cotyledon and the radicle is a dumbell-shaped area which produces trichomes at the time of germination. The above features of this genus will be compared with those of *Halodule*, another genus in the same family.

PAROTOID GLAND OF BUFONIDAE. M.S. Cannon, Dept. Anatomy, College of Medicine, Texas A&M University, College Station, TX 77843.

The gross and microscopic anatomy of the venom producing parotoid glands of *Bufo marinus* has been studied by light and electron microscopy and reactions for the presence of glycoprotein and mucopolysacchardies, the catecholamines, 5-hydroxytryptamine or dopamine, glucose-6-phosphatase, adenosine triphosphatase and the steroid nucleus and cholesterol and its esters have been performed. The gland is composed of numerous individual lobules, each lobule surrounded by a double cell layer. The interior surface of the outer layer is thrown into small cytoplasmic projections which traverse an intercellular space and interdigitate with microvilli formed by the outer plasmalemma of the inner layer. The outer layer resembles smooth muscle-like cells, is rich in adenosine triphosphatase, contains many pinocytotic vesicles and various organelles and may function in some aspect of venom synthesis, active cellular transport and contraction in the discharge of the secretory product. The inner layer shows a positive chromaffin reaction, contains various organelles, appears devoid of a plasmalemma on its inner surface and is involved in venom formation and release via an apocrine type of secretion. The intercellular space is rich in PAS positive materials, while the secretory product, itself, demonstrates a positive chromaffin reaction.

HAMSTER AND RAT PINEAL GLAND β-ADRENOCEPTOR CHARACTERIZATION WITH IODOCYANOPINDOLOL AND THE EFFECT OF DECREASED CATECHOLAMINE SYNTHESIS ON THE RECEPTOR. C.M. Craft, W.W. Morgan, *D.J. Jones, and R.J. Reiter, Department of Cellular and Structural Biology and *Department of Anesthesiology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284.

Rat and hamster pineal glands were used in binding studies to characterize their β-adrenoceptors with the new specific antagonist, iodocyanopindolol. The ligand was saturable, selective, and stereospecific for both species but the rat pineal had a 15-fold greater density and 10-fold lower affinity compared to the hamster pineal. Utilizing this radioligand binding technique, we examined the supersensitivity of pineal β-adrenergic receptors in both species. Decreased sympathetic input to the pineal was accomplished either by superior cervical ganglionectomy or exposing the animals to continuous light for 36 hrs. Parallel studies were conducted in hamster pineal gland in which catecholamine synthesis was measured. Catecholamine production was estimated by measuring dihydroxyphenylalanine accumulation after NSD-1015 administration. The results indicate that a selective decrease in catecholamine synthesis in the hamster pineal does not change the β-adrenoceptor density or affinity. In contrast, a concomitant increase in β-adrenoceptor density but not affinity in the rat pineal gland occurs after similar decreased sympathetic input. (Supported by NSF grant no. PCM 8304706 to RJR, NIH grant nos. DA 00755 and DA 00083 to WWM and NIH-NINCDS grant no 14546 to DJJ.)

A PRELIMINARY INVESTIGATION OF THE STRUCTURE AND ELEMENTAL ANALYSIS OF CYSTOLITHS IN WEEPING FIG (*FICUS BENJAMINA*). Ronald W. Davis, Dept. Anatomy, Texas A&M University College of Medicine, College Station, TX 77843

Cystoliths are large mineral deposits that occur in specialized cells called lithocysts. They occur in many plant families and are particularly prominent in species of the genus *Ficus* which includes the popular ornamentals, rubber plant (*Ficus elastica*) and weeping fig (*Ficus benjamina*). Although cystoliths have been periodically studied for a hundred years, little is known concerning their detailed structure, development, or function. The current study was initiated as a preliminary investigation of the ultrastructure and development of cystoliths. Mature *F. benjamina* leaves were used to examine the fine structure and elemental composition of cystoliths and lithocysts. Leaves from container-grown *Ficus benjamina* were collected and processed for TEM, SEM and STEM by conventional methods which included the use of Karnovsky's fixative, OsO_4 and plastic embedding or critical point drying where applicable. Elemental analysis was conducted using a Phillips 420 STEM equipped with a Tracor 2000 X-ray fluorescence detector. Portions of the cystolith contained calcium, silicon, zinc, sulfur and phosphorus. Two areas of the cystolith had particularly high concentrations of silicon. A band of silicon was also present in the cell wall of the lithocyst and other epidermal and hypodermal cells. The deeper-lying chlorenchyma cell walls contained

AN UNUSUAL STRUCTURED GRANULE IN LEAF CELLS OF THE MISTLETOE PHORADENDRON. W.R. Fagerberg, Dept. of Biology, Southern Methodist University, Dallas, TX 75275.

A granule of varying size appears to be a common constituent of leaf cells in the mistletoe Phoradendron serotinum. This granule has been observed in all cell types of the leaf with the exception of vascular tissue and guard cells. The granule has been observed in a number of "states" of structural "development" varying from an amorphous body to an object maximally packed with tubular inclusions. These granules stain, variously, for the presence of lipid, proteins and carbohydrates i.e. they are "stainophilic". At the ultrastructural level these granules may be very osmiophilic and at other times stain only slightly. These granules have been intimately associated with membrane systems, crystals and apparently isolated. Rampant speculation will be made as to their possible function or role in the cell.

CARDIAC JELLY GLYCOPROTEIN DISTRIBUTION IN RELATION TO ENDOCARDIAL CYTODIFFERENTIATION. D.A. Hay, Dept. Biology, Stephen F. Austin State University, Nacogdoches, TX 75962.

Prior to H-H stage 17, all endothelium is attenuated and separated from the myocardium by cardiac jelly (extracellular matrix). After stage 17, the atrioventricular (AV) endothelium is induced to undergo endothelial/mesenchymal transformation. It is suggested that myocardially secreted jelly glycoproteins (GP) induce AV endothelial activation. This was tested by determining 1) whether matrical glycoproteins extend across the cardiac jelly (CJ) space and directly contact the endothelium, and 2) if EDTA extracts of CJ would bind to isolated endothelium maintained in collagen gel cultures. TEM examination of cryopreserved tissues revealed extracellular electron dense materials that were distributed as a gradient from the myocardium to the endothelium. This material is thought to be glycoprotein on the basis of 1) incorporation of ^{3}H fucose and ^{3}H amino acids given to whole embryo cultures and 2) fucose binding lectins. Examination by two dimensional gel fluorography of tissue extracted with EDTA and hyaluronidase revealed the presence of 35 protein moieties. EDTA-extracted proteins were bound to the basal surfaces of unactivated endothelium maintained in collagen gel cultures; pretreatment with albumin did not block binding. These results indicate that the CJ contains glycoproteins whose distribution and binding is consistent with the hypothesis that glycoprotein moieties influence endothelial cytodifferentiation.

EFFECT OF GROWTH RATE AND OF MORPHOGENIC ACTIVITY ON SHOOT APICAL ULTRASTRUCTURE. J.D. Mauseth, Dept. Botany, University of Texas, Austin, TX 78712.

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); 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. The ultrastructure varied according to each of these conditions: 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 are performing different morphogenic activities. Hyaloplasm is an excellent indicator of metabolic rate; mitochondria, nuclei, and vacuoles are not.

ULTRASTRUCTURE OF THE HOST-PATHOGEN RELATIONSHIP IN RED LEAF DISEASE OF BLUEBERRY. C.W. Mims, Dept. of Biology, S.F. Austin State University, Nacogdoches, TX. 75962 and N.L. Nickerson, Research Station, Agriculture Canada, Kentville, Nova Scotia, B4N 1J5.

The causal organism of red leaf disease of blueberry is the basidiomycetous fungus Exobasidium vaccinii. This fungus produces a perennial mycelium that invades the rhizomes of the low bush blueberry Vaccinium angustifolium. Symptoms are seen on infected shoots as buds break in the spring and the reddish leaves for which the disease is named soon become apparent. In this study TEM was used to examine the host-pathogen relationship in these infected leaves.

Exobasidium vaccinii produces a system of slender, branched, septate hyphae within the leaves of V. angustifolium. Although a few intracellular hyphae were observed in cells of the lower epidermis, elsewhere in the leaf the hyphae of this fungus grow almost exclusively in an intercellular fashion. The hyphae typically fill the intercellular spaces immediately adjacent to the lower epidermis, but are rather sparse elsewhere in the leaf. The haustorial apparatus of E. vaccinii is a branched or lobed structure that arises from an intercellular hypha in close association with a host cell. Each haustorial lobe is initially ensheathed by host cell wall material, but eventually the wall is penetrated by the lobe. Membranous inclusions were observed within the haustorial apparatus. Each lobe also possesses an electron dense haustorial cap that is intimately associated with the host cell plasma membrane.

AN ULTRASTRUCTURAL STUDY COMPARING TWO POTENTIAL TREATMENTS FOR LEAD POISONING IN CALVES. H.H. MOLLENHAUER, G.R. BRATTON*, J. ZMUDZKI*, R.E. DROLESKEY, AND L.D. ROWE, U.S.D.A., Vet. Toxicol. Entomol. Res. Lab., College Station, TX 77841, and *Dept. Vet. Anat., College of Vet. Med., Texas A&M Univ., College Station, TX 77843.

Lead (Pb) is one of the most ubiquitous natural toxins, and Pb poisoning constitutes the second most common toxicosis in livestock. This study was undertaken to evaluate the therapeutic effects of two potential treatments for Pb poisoning. Calves 3-4 weeks old were dosed orally with Pb acetate (5 mg/kg b.w./day) for 7 days. Following dosing, calves were either left untreated, treated with EDTA (75 mg/kg b.w./day sub Q), or treated with thiamin (30 mg/kg b.w./day sub Q) for an additional 7 days. Following treatment, the calves were killed and the kidneys sampled for electron microscopy. In Pb-dosed-untreated and Pd-dosed-thiamin treated calves, Pb containing substances were found in the nuclei and mitochondria of proximal tubules. Chemical analyses demonstrated that both thiamin and EDTA reduced the Pb in kidneys from Pb-dosed-treated calves as compared with kidneys from Pb-dosed-untreated calves, but thiamin effects were not markedly visible via electron microscopy. On the other hand, Pb deposits were absent in the kidneys of calves treated with EDTA as compared with the kidneys from untreated and thiamin treated calves, but the EDTA treatment was accompanied by a generalized disorganization of cell ultrastructure and mitochondrial damage. Similar disorganization and damage were not observed in kidneys from thiamin treated calves. Some calves in the Pb-dosed-untreated group died, but all calves in both treatment groups remained in good general health. Thus, both thiamin and EDTA have value in treatment of Pb poisoning, but EDTA appears to have severe side effects as judged by changes in kidney ultrastructure.

TOXICITY OF LASALOCID: AN ULTRASTRUCTURAL STUDY USING RATS AS A MODEL SYSTEM. H. H. MOLLENHAUER, L. D. ROWE, AND R. E. DROLESKEY. U. S. Department of Agriculture, Agricultural Research Service, Veterinary Toxicology and Entomology Research Laboratory, College Station, TX 77841.

Lasalocid, like monensin, is an important agricultural chemical used as a coccidiostat for poultry and as a feed additive for cattle. It is a carboxylic acid ionophore which functions by forming complexes with cations (principally Ca^{2+}) to catalize electroneutral exchanges of cations or H^{+} across biological membranes. When given in excess of recommended levels, lasalocid is toxic and incidents of poisoning have been reported. However, there does

not appear to be any ultrastructural data from animals exposed to lasalocid. Therefore, this study was undertaken to evaluate the ultrastructural effects associated with lasalocid poisoning. Rats were given toxic levels of lasalocid and then sacrificed at intervals of 1-14 days. Heart, diaphragm, skeletal muscle, and liver were processed for electron microscopy. A consistent observation of toxicity was vacuolization of cardiac mitochondria. Only some cardiac mitochondria became vacuolated, and these mitochondria were dispersed throughout the cell. Additionally, excessive numbers of dense granules were present in some partially vacuolated mitochondria. The nonvacuolated mitochondria, as well as the other constituents of the muscle cells, usually appeared normal. Other striated muscle such as diaphragm and skeletal muscle sometimes exhibited similar aberrations. Ultrastructural aberrations to the liver were observed also, but mitochondrial vacuolization did not occur in this tissue. At the ultrastructural level and in these tissues, the effects of lasalocid were similar to those of monensin even though monensin is a sodium-selective ionophore rather than a calcium-selective ionophore like lasalocid.

HAUSTORIUM FORMATION BY CUSCUTA SALINA, A HOLOPARASITIC ANGIOSPERM. Randy Moore, Department of Biology, Baylor University, Waco, Texas 76798.

In order to investigate development of the haustorial apparatus of a parasitic plant, I have studied haustorial connections between the holoparasite Cuscuta salina (dodder) and its host Cardiospermum halicacabum (balloonvine). The endophyte often grew completely through leaves of the host, thus exposing portions of the endophyte and search hyphae. Search hyphae were (1) comprised of 4 to 5 cells each measuring approximately 45 x 15 μm (i.e., length x width), and (2) covered at their tips by numerous projections of varying shapes and sizes. These results suggest that search hyphae of Cuscuta haustoria encounter vascular tissues of the host randomly (i.e., they do not sense the location of and "purposefully" grow toward vascular tissue).

CALCIUM AND PHOSPHORUS DEPOSITION IN THE SKIN OF BUFO MARINUS. H. Wayne Sampson, R.W. Davis and M.S. Cannon. Dept. Medical Anatomy, Texas A&M University Medical School, College Station, TX 77843

The integument of Bufo marinus is similar to that of other anurans in that it has a stratum corneum, an epithelium, a stratum spongiosum and a stratum compactum. The stratum spongiosum contains the three chromatophores common to anurians, the melanophore, iridophore and xanthopore. The xanthophores contain both pterinosomes and carotenoid granules. The skin of these toads contains more than 28% mineral deposited in an amorphous layer in the form of small crystals between the stratum compactum and the stratum spongiosum. These crystals reveal a high content of calcium and phosphorus aggregated in membrane-bound vesicles with an appearance very similar to matrix vesicles. Electron microscopy gives the appearance that these vesicles are formed from fibroblasts located between alternating bundles of collagen.

NON-SPECIFIC NEGRI BODY-LIKE INCLUSION BODY FORMATION IN THE BRAIN OF THE DOG. R.W. Storts and M.S. Frey, Dept. of Veterinary Pathology, Texas A&M University, College Station, Texas 77843.

Non-specific intracytoplasmic neuronal inclusion bodies closely resembling Negri bodies of rabies were detected and examined in 24 dogs. Breeds most frequently affected were the Poodle(13) and Pointer(5). The average age was 5.93 years with the range being 0.33 to 14 years. Only 1 dog was under 2 years. Definite neurologic signs occurred in 13 dogs. Sixteen dogs had non-neurologic signs of different types. Both neurologic and non-neurologic signs occurred in 5 dogs. Microscopically, only 9 dogs had lesions of the CNS in addition to inclusions. Inclusions, primarily occurred in Purkinje cells of the cerebellum and neurons of thalamic nuclei. Inclusions which varied in size, shape and intracytoplasmic position and had a definite internal structure that was often indistinguishable from inner body formation of the Negri body, did have some features that differentiated them from Negri bodies. Histochemical characteristics of inclusions were comparable to those of the Negri body. The brains of 3 dogs examined were negative for rabies viral antigen. Ultrastructurally, inclusions were relatively well defined and consisted of laminated membranous structures that were accompanied by short linear deposits of electron dense material generally oriented perpendicular to, or parallel with, the membranous lamina. No rabies virions were detected in association with inclusions. The cause of the inclusion body formation could not be determined.

TRANSISTION FROM LIGHT TO ELECTRON MICROSCOPIC EXAMINATION, Donna S. Turner and Irene Piscopo*, Dept. Cell Biology, Baylor College of Medicine, Houston, Texas 77030, and *Applications Laboratory, Philips Electronic Instruments, Mahwah, N.J. 07430.

A 1u thick section of normal rat testis was stained with toluidine blue and photographed on a light microscope. The section was removed from the glass slide and placed on a formvar coated slotted grid and stained with uranyl acetate and lead citrate. The section was sandwiched with an additional grid and viewed in a Philips EM420 transmission electron microscope. Comparable magnifications of the same area with light and electron optics were compared. An additional sterio pair of the same thick (1u) section were made.

This technique may be employed to guide the individual whose experience has been with light micrographs into the study of electron micrographs.

THE FORMATION OF INTERCELLULAR SPACES IN THE DEVELOPMENT OF LATEX DUCTS IN MAMMILLARIA GUERRERONIS (CACTACEAE). G.H. Wittler and J.D. Mauseth, Dept. Botany, University of Texas, Austin, TX 78712.

Latex ducts in Mammillaria guerreronis, sect. Subhydrochylus are complex, having a distinct outer epithelium without intercellular spaces and an inner epithelium in which schizogenous spaces arise. Schizogeny begins with formation of bulbous wall thickenings and/or production of dark streaks in certain walls. Spaces formed by these processes ultimately contain a combination of electron dense materials, vesicles, and numerous thin, convoluted wall layers. Schizogeny may be responsible for initial formation of lumen and latex and may also separate some inner epithelial cells from the surrounding layers. This peculiar type of schizogeny may occur even after a well defined lumen is produced. Lysigeny of the inner epithelial cells contributes materials to the latex and allows enlargement of the duct. We consider the schizo-lysigeny in the members of sect. Subhydrochylus to be ancestral to the lysigeny of the members of sect. Mammillaria. The inner and outer epithelia of M. guerreronis are thought to be homologous to the lumen and epithelium respectively of M. heyderi.

Abstracts from the Fall 1983 Meeting in Tyler, Texas

SPERMATOGENESIS IN THE HEPATICAE: PETALOPHYLLUM AND FOSSOMBRONIA (CODONIACEAE). Steven Ehlers and Dale M. J. Mueller, Texas A&M University, College Station, Texas 77843.

The general course of development of the male gamete in bryophytes has long been known. Early light microscopists described a series of anatomical and cytological changes which occur during spermatogenesis. With the increased resolution of the electron microscope the spermatid is revealed as the most structurally complex cell produced by any bryophyte. The spermatid develops from a simple cell and undergoes remarkable cytological transformation with (1) the de novo development of the locomotive apparatus (two flagella and the underlying multilayered structure), (2) the morphogenesis and condensation of the nucleus and (3) the loss of extraneous cytoplasm. Ultrastructural aspects of spermatogenesis have not previously been described in the liverworts Petalophyllum and Fossombronia.

SEED COATS OF THREE CAESALPINIOIDEAE (LEGUMINOSAE) GENERA. Louis H. Bragg and Terry S. Bridges, Department of Biology, The University of Texas at Arlington, Arlington, Texas 76019.

Seed coats of three of nine Caesalpinioideae genera occurring in Texas were examined in this initial phase of a survey to determine the usefulness of the surface and internal features in separating and characterizing the genera of this subfamily. The testae of Gleditsia, Cassia, and Cercis each have distinctive surface ornamentation. Gleditsia and Cassia have surface cracks with Cassia also having pits in rows. Cercis lacks both cracks and pits. A double palisade layer occurs in Cassia but a single layer is present in Gleditsia and Cercis with Gleditsia having palisade cells almost twice the length of those in Cercis. There are also distinctive differences in the hypodermal layer between these three genera. The observed differences in the surface and internal features are useful diagnostic characters for separating these genera and warrants further investigation of the six remaining genera.

A PRELIMINARY STUDY OF CRYSTAL SAND PRODUCTION IN THE STEM OF POTATO (SOLANUM TUBEROSOM). M. J. Grimson and H. J. Arnott, Department of Biology, The University of Texas at Arlington, Arlington, TX 76019

The common potato (Solanum tuberosom) produces abundant quantities of calcium oxalate crystals in the form of crystal sand in all major organs of the plant (stem, leaves, root, tuber). In the stem, crystal formation begins very early, being observed just below the apex. Crystal cells become more numerous in older parts of the stem, leading to the conclusion that idioblast development is not restricted to the apical area alone. It is possible to distinguish between immature and mature crystal cells with the light microscope and TEM. Developing idioblasts produce a complex membrane system in the center of the vacuole. The membrane complex is highly convoluted and appresses closely to the crystal surfaces and appears responsible for crystal development. As crystals are produced, they move to opposite ends of the cell, where the membrane complex does not occur. As the cell matures, crystals eventually fill the vacuole and the membranes disappear. At maturation, the crystal cell is still living. The tonoplast still delimits the vacuole which contains the crystal sand, the plasmalemma is still intact and the nucleus and other organelles are present in the cytoplasm. In older parts of the stem, lysogeny occurs, as parenchyma cells breakdown in the pith, producing a central lacuna. As the lacuna enlarges, the crystal cells also lyse and in the process deposits crystal sand on the walls of the air space. Without studying the development, it would appear as though the crystals have an extracellular origin. However, development clearly indicates that they are produced intercellularly.

STRUCTURE AND FUNCTION OF THE PERISTOME IN THE MOSS FORSSTROEMIA TRICHOMITRIA. Alan J. Neumann and Dale M. J. Mueller, Department of Biology, Texas A&M University, College Station, Texas 77843.

Forsstroemia trichomitria (Hedw.) Lindb. is a corticolous moss commonly found on hardwood trees in mesic habitats throughout eastern North America, including east Texas. The sporophyte of this moss has an arthrodontous peristome consisting of a highly reduced endostome and an exostome of sixteen stout teeth. The endostome exists only as a basal membrane composed of the thickened, adjacent periclinal cell walls of the inner (IPL) and primary (PPL) peristome tissue layers. The exostome is composed of undissolved portions of thickened, adjacent periclinal cell walls of the primary (PPL) and outer (OPL) peristome layers cemented together by the middle lamellae. The primary walls of the PPL and OPL consist of densely packed cellulosic microfibrils oriented parallel to the longitudinal axis of the tooth. The lamellae formed from the PPL exhibit thicker wall deposition than the lamellae formed by the OPL, especially in the basal regions of the teeth. Wall thickenings are composed of loose arrays of microfibrils embedded in a matrix of polysaccharides and lipoidal compounds. Matrix materials of the teeth are hydrophilic and increase in volume as water is imbibed. Swelling of the PPL wall thickenings upon hydration cause the teeth to reflex away from the mouth of the urn, thus allowing unhindered release of spores from the capsule. Dehydration causes shrinkage of the matrix and the bending of the teeth over the mouth of the urn. Hence the peristome functions as a regulator of spore release and is classified as hygrocastique because spore release is favored during periods of high humidity.

ULTRASTRUCTURE OF BASIDIOCARP DEVELOPMENT IN THE FUNGUS GANODERMA LUCIDUM. C. W. Mims, Department of Biology, S.F. Austin State Univ., Nacogdoches, TX 75962.

Ganoderma lucidum is a wood rotting fungus belonging to the order Aphyllophorales. Members of this order are commonly called "polypores" because their spore bearing layer or hymenium lines well defined pores or tube-like depressions in the basidiocarp. This study uses both TEM and SEM to examine various aspects of basidiocarp development in G. lucidum.

The pores of G. lucidum develop on the undersurface of the cap-like portion of the typically stalked basidiocarp. The layer in which the pores develop is composed of thin-walled, dikaryotic hyphae possessing clamp connections. These hyphae are characteristically coated with an extracellular material. Pores first appear as small, shallow depressions near the margin of the cap but, eventually increase in diameter and form deep, tubular structures that extent into the layer of hyphae comprising the under surface of the cap. As the pores develop many of the surrounding hyphae disintegrate. Although most of the surviving hyphae remain thin-walled, some differentiate into thick-wall structures that add to the strength of the basidiocarp.

Eventually basidia arise from the hymenial layer that lines the surface of each pore. Four basidiospores develop from each basidium. Basidiospores of G. lucidum are ellipsoid in shape and measure 10-12 um x 6-9 um.

THE INTRACELLULAR DISTRIBUTION OF ORGANELLES IN COLUMELLA CELLS OF PHASEOLUS VULGARIS (FABACEAE). Steve Ransom and Randy Moore, Biology Department, Baylor University, Waco, TX 76798

A morphometric analysis of the ultrastructures of columella cells in primary and lateral roots of Phaseolus vulgaris was performed in order to determine the precise location of cellular components in these cells. Roots were fixed in situ in order to preserve the in vivo ultrastructures of the cells. All cellular components in columella cells of both types of roots were distributed asymmetrically. The nucleus and vacuome were located primarily in the middle third of both types of columella cells. Dictyosomes, mitochondria, and amyloplasts were most abundant in the lower third of the columella cells in both types of roots. The distribution of amyloplasts was the most asymmetrical of all cellular components examined, with the lower third of the columella cells containing approximately 90 % of the relative volume of amyloplasts in both types of roots. The distribution of cellular components in columella cells of primary roots was not significantly different from that of columella cells of lateral roots. These results indicate that differences in graviresponsiveness of primary and lateral roots of P. vulgaris are probably due to factors other than the ultrastructures of their individual columella cells.

THE STRUCTURE OF COLUMELLA CELLS IN PRIMARY AND LATERAL ROOTS OF HELIANTHUS ANNUUS. Robert Stoker and Randy Moore, Biology Department, Baylor University, Waco, Texas 76798

Primary and lateral roots of sunflower (Helianthus annuus) were fixed in situ, and the ultrastructures of their columella cells determined using stereology. Organelles were distributed asymmetrically in columella cells of both types of roots. The nucleus tended to be located in the upper thirds of the columella cells, while the vacuoles tended to be located in the middle thirds of the cells. Amyloplasts, dictyosomes, and mitochondria were most frequent in the lower thirds of the cells in both types of roots. The distributions of organelles in columella cells of primary versus lateral roots were not significantly different, suggesting that the differential graviresponsiveness of these roots is probably due to factors other than the ultrastructures of their individual columella cells.

THE SURFACE MORPHOLOGY OF SEVERAL SOUTH ATLANTIC DINOFLAGELLATES, GENUS PROTOPERIDINIUM. Cynthia H. McKenzie, Department of Biology, Texas A&M University, College Station, Texas 77843.

The speciation of the genus Protoperidinium Bergh. has been almost exclusively determined using light microscopy. Observations with the light microscope describe size and shape only. However, information now available from scanning electron microscope studies provides descriptions of the surface details of the organisms.

About 25 species of Protoperidinium were observed from net hauls taken in the southern Atlantic Ocean. Details of surface morphology not revealed by light microscopy are elucidated in this study. These additional morphological 'traits' include trichocysts pores, apical pores, thecal plate ornamentation, and growth margins or sutures. An estimate of the age of the cells can also be inferred by changes observable in the surface morphology.

The surface morphology of Protoperidinium revealed by this scanning electron microscope study is very distinctive. The surface details should now be included as taxonomical characteristics.

THE CYTOCHEMICAL LOCALIZATION OF GLUCOSE-6-PHOSPHATASE AND CATALASE IN CELLS COMPRISING THE ROOT CAP OF ZEA MAYS. C. Edward McClelen and Randy Moore, Dept. of Biology, Baylor University, Waco, Texas 76798

In order to determine the sites of enzyme activity in cells of root caps, we have cytochemically localized glucose-6-phosphatase and catalase in root cap cells of corn (Zea mays). Glucose-6-phosphatase was localized using a modified Gomori procedure. The enzyme is associated with the plasmalemma and plasmodesmata of columella and peripheral cells of the cap. Catalase was localized using 3'-diaminobenzidine. The enzyme was localized in lysosome-like bodies in the inner peripheral cells. Catalase was also localized in the cytosol of the outer peripheral cells. This association is evidence that the outer peripheral cells are in the process of autolysis as they are sloughed from the cap.

CYTOCHEMICAL LOCALIZATION OF CELLULASE ACTIVITY IN CELL WALLS OF SYRRHOPODON TEXANUS HYALOCYSTS. R. D. Allen, C. L. Nessler, S. Galewsky, and A. J. Neumann, Department of Biology, Texas A&M University, College Station, TX 77843.

Sheathing leaf bases of Syrrhopodon texanus are composed primarily of large hyalocysts which may function in water storage and transport. Hyalocysts have thin walls which, at maturity, are marked by large pores. These pores develop by gradual thinning of the protoplasmic side of the cell wall followed by eventual rupture. Cellulase activity was detected in extracts of Syrrhopodon gametophores. This enzyme exhibited a broad active pH range with an optima near 4.5. Cytochemical localization indicated cellulase activity which was associated with the thinning cell walls of immature hyalocysts. Cellulose digesting enzymes are apparently involved in removal of cell wall materials from hyalocysts during the formation of pores.

SCANNING ELECTRON MICROSCOPY OF THE THECAL PLATE TABULATION OF PERIDINIUM BALTICUM (LEV.) LEMMERMAN (PYRRHOPHYTA, PERIDINIALES). Joby M. Chesnick, Department of Biology, Texas A&M University, College Station, Texas 77843.

Motile forms of the marine binucleate dinoflagellate, Peridinium balticum were examined using SEM to determine thecal plate tabulation and variation. The cells observed were unornamented. Plate formulae consisted of an apical pore plate, a preapical (canal) plate, six precingular plates, five cingular plates, four sulcal plates, five postcingular plates, and two antapical plates (PP, PR, 4', 2a, 6'', 5c, 4s, 5''', 2''''). Six variations to the major plate tabulation were observed. Note is made of a closing plate, adjunct to the apical pore plate, with 180 degree placement with respect to the preapical plate, previously undescribed for Peridinium.

Questions concerning the phylogeny of P. balticum occur because this alga has five cingular plates and the organism, from which this clone was established, was isolated from a saline habitat. Most marine species with plate tabulation similar to Peridinium are placed in the genus Protoperidinium, characterized by four cingular plates. Current studies are underway to determine salinity range tolerances for this organism. It is suggested that P. balticum may be a salt-tolerant alga rather than a truly marine one.

INTRACELLULAR CALCIUM COMPARTMENTALIZATION. H. Wayne Sampson, Dept. Human Anatomy, Texas A&M University School of Medicine, College Station TX 77843.

The significance of calcium involvement in the cell physiology of specialized tissue is becoming increasingly apparent. In some instances, the required calcium enters the cell from the exterior, but many cell types depend upon a reservoir of intracellular calcium. The organelles functioning as reservoirs for calcium vary from cell to cell. The mitochondria is quite prominent in calcium compartmentalization, but other structures active in this manner include the endoplasmic reticulum, smooth membrane vesicles, lysosomes, subsurface cisternae, and the plasma membrane.

Specific instances of these compartments and methods of study will be discussed.

ULTRASTRUCTURAL AND IMMUNOCYTOCHEMICAL CHARACTERISTICS OF PULMONARY ENDOCRINE CELLS IN ORGAN CULTURE. R.D. Dey and J.M. Snyder, Dept. Cell Biology and Obstetrics-Gynecology, University of Texas Health Science Center at Dallas, Dallas, TX 75235.

Pulmonary endocrine cells are known to contain the peptide bombesin (BOM) and the amine 5-hydroxytryptamine (5HT). We have investigated the ultrastructural and immunocytochemical features of pulmonary endocrine cells from explants of mid-trimester human fetal lung airways that have been maintained in organ culture. Fragments of the bronchial tree were dissected from the lung then incubated for 2 or 5 days in serum-free medium in an atmosphere of 95% air - 5% CO_2 at 37°C. Frozen sections of airway explants from the two incubation periods as well as the starting tissue were used for immunocytochemical localization of 5HT and BOM. Explants were also examined using electron microscopy.

Endocrine cells from the starting tissue contained 5HT and BOM. Ultrastructurally, the airway endocrine cells were characterized by the presence of cytoplasmic dense core vesicles. In cultured airway explants, numerous 5HT- and BOM-containing cells were observed in the airway epithelium. There was no evidence of necrosis in the explants. Ultrastructurally, the endocrine cells in the cultured airway explants contained abundant dense core vesicles. Our results are suggestive that human fetal lung endocrine cells retain their ability to store BOM and 5HT during 5 days of culture and are ultrastructurally similar to the endocrine cells in the starting tissue. Fetal lung airway explants maintained in organ culture may be a useful model to study pulmonary endocrine cell function.

THE SYNTHESIS OF POLYANIONIC MATERIALS BY EMBRYONIC ATRIOVENTRICULAR (AV) CANALS EXPLANTED ONTO COLLAGEN GELS. D.A. Hay, Dept. of Biology, S.F. Austir State Univ., Nacogdoches, TX 75962.

The purpose of the study was to compare the macromolecular cytology of polyanions (PA) produced in vivo with those produced in a 3-dimensional collagen gel culture system. PAs are examined using ruthenium red (RR) as regulated by specific glycosidases, i.e., Streptomyces hyaluronidase (S-HAase), testicular hyaluronidase (T-HAase) as well as trypsin. The following RR positive (+) constituents were observed: 5-10 nm filaments (HAase-sensitive), 50 nm granules (resistant to S-HAase but T-HAase- and trypsin-labile) that coated collagen fibers and CT cell surfaces, and a flocculent, fuzzy layer on CT cell surfaces (resistant to both HAases, but trypsin-sensitive). The desposition of filaments and granules was greatest near the myocardium. Based on the foregoing enzyme sensitivities, the 5-10 nm filaments were identified as hyaluronate (HA), the 50 nm granules as chondroitin sulfate and protein, whereas the flocculant cell surface layer (T-HAase resistant) appears to be a different protein-polysaccharide complex (perhaps heparan sulfate). The following conclusions may be made from preliminary observations:

1) The chondroitin sulfate-protein granules (50 nm) are synthesized primarily by the myocardium, but also by the cardiac mesenchymal cells.

2) The similarity of RR desposition in this model system to that found in vivo suggests that the cellular constituents of AV canal explants retain the same synthetic capabilities as their in situ counterparts.

EFFECT OF METHYL MERCURIC CHLORIDE ON HEPATIC PEROXISOMES AND LYSOSOMES. 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 after treatment with methyl mercuric chloride. Optimum cytochemical localization of acid phosphatase activity in lysosomes was obtained by fixing in 4% glutaraldehyde in 0.09 M cacodylate buffer, PH 7.4 for 3 hours at 0°C. followed by incubation with 5' cytidine monophosphate, PH 6.2 for 25 minutes at 25°C. Liver tissue for peroxisome localization was fixed in 3% glutaraldehyde in 0.1 M Millonig's phosphate buffer PH 7.4 for 3 hours at 0°C. 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). Tissues were preincubated in DAB medium without H_2O_2 for 25 minutes at 45°C, then incubation was continued in complete DAB medium, PH 9.0 for 1 hour at 45°C. Normal structures as illustrated by these histochemical techniques will be discussed. Changes in morphology and number of lysosomes and peroxisomes at 2, 7 and 21 days after treatment with methyl mercuric chloride will be presented.

Use of Modified Timm's Stain to Follow Movement of Metals in Secretory Tissue Using Oviduct of the Japanese Quail. Phyllis Butzen, Elizabeth Root and Barry Starcher, Graduate Nutrition Division, The University of Texas at Austin, Austin, Texas 78712.

The modification of Timm's sulphide silver method for electron microscopy by G. Danscher (Histochemistry 71 (1981) 1-16) was used to monitor location of zinc in oviduct of the Japanese quail. The method of fixation was altered to employ concurrent, rather than alternate, treatment of the tissue with sulphide and glutaraldehyde. Fixative consisted of 0.1% sodium sulphide, $Na_2S \cdot 9H_2O$, and 3% glutaraldehyde in 0.15 M sodium phosphate buffer, pH 7.4. Slices of the magnum portion of the oviduct were immersed in the freshly-made solution for 4 to 5 hours.

Reaction product was confined to the epithelial cells of the secretory face of this albumin-producing region of the oviduct in untreated tissue. When inverted sacs made from the magnum were filled with zinc solution and incubated, metal was seen to be taken up in the smooth muscle layer, to move through the lamina propria via connective tissue channels without entering the glandular cells in most areas, and to be highly concentrated in the epithelial cells and subjacent interstitial areas. Electron microscopy of untreated tissue verifies absence of reaction product in glandular cells, and shows it in epithelial cells in aggregates of discrete particles sequestered with material apparently destined for secretion.

A LIGHT AND ELECTRON MICROSCOPE STUDY OF EPITHELIOCYSTIS IN JUVENILE STEELHEAD TROUT. Ronald W. Davis,* A.W. Rourke** and T.M. Bradley,** *Dept. Human Anatomy, Texas A&M University Medical School, College Station TX 77843 and **University of Idaho, Moscow, Idaho 83843.

A light and electron microscope investigation was conducted using juvenile steelhead trout, Salmo gairdneri, showing symptoms of the disease epitheliocystis in gill tissue. Epitheliocystis is a benign or proliferative disease occurring in the epithelium of marine and freshwater fish. It can be very pathogenic and cause severe mortalities in fish grown in culture.

Three distinct forms of the apparent causal organism were found. Two were chlamydia-like and similar to epitheliocystis organisms previously described. A third form was found having a distinct head and tail region and was unlike any organism reported from previous investigations. The large membrane bound cysts containing all three prokaryotic forms were surrounded by host cell cytoplasm except at the top where they were contiguous with the outside environment. Cysts were observed that had apparently ruptured in this area. Numerous small vesicles were present within the cysts and adjacent to the membrane separating the cyst from the host cell cytoplasm. These vesicles were not present in the area of the cyst that ruptured.

HORMONAL REGULATION OF GAP JUNCTIONS IN UTERINE SEROSA. D. Gaddy and R.C. Burghardt, Dept. of Biology, Texas A&M University, College Station, TX 77843

Gap junction modulation was studied in one of the two uterine cell types that respond to exogenous estradiol in hypophysectomized (HX) rats by amplifying junctional membrane. Although dose-dependent increases in uterine serosal cell macular and annular junction membrane were observed following 5 daily injections of estradiol benzoate (E_2B) or diethylstilbestrol (DES) (0.5 to 500 µg range), indomethacin antagonized this estrogenic effect. The possibility of E_2B directed prostaglandin (PG) synthesis leading to junction modulation was therefore tested. Exogenous PGE_1 (1mg/day) increased while $PGF_{2\alpha}$ decreased the number of macular junctions compared to HX controls, although PGE_1 did not cause the induction of annular junctions. Isoproterenol (50 µg/day) and colchicine (50 µg/day) were also found to cause gap junction amplification in rats primed for 2 days with 5 µg E_2B over that seen with E_2B priming alone. Since estrogens are associated with changes in PG levels, and PGs are known to affect intracellular cAMP metabolism, these studies suggest that the action of estrogen on serosal cell gap junction membrane is indirect and may be mediated by PGs. Further, the involvement of cAMP and cytoskeletal perturbations in junction amplification is suggested. (Supported by NIH grant HD-14781).

TEMPORAL ANALYSIS OF GAP JUNCTION DEVELOPMENT DURING FOLLICULOGENESIS IN MICE . Philip Mitchell, Dept. Biology, Texas A&M University, College Station, TX 77843.

The appearance and relative quantities of gap junctions present during ovarian histogenesis were examined in mice from fetal day 13 through 19. Thin sections rather than freeze fracture methodology were used to improve the precision of identifying cell types. Length measurements of plasma and junctional membrane were recorded from micrographs using a digitizer, and both lengths and numbers of junctions were analyzed statistically. A significant increase in both gap junction numbers ($p<0.01$)

and lengths ($p<0.05$) was observed with increasing fetal age. While the day of development is a major factor for the observed increase in the number and size of junctions, it appears that other factors contribute to this increase as well. These studies indicate that the presumptive granulosa cells derived from the intraovarian rete cells are morphologically coupled prior to and during folliculogenesis, suggesting that ovarian follicles begin acting as functional sycitia at the earliest stages of their development. (Supported by NIH grant HD-14781)

MODULATION OF GAP JUNCTION CONTACTS IN CULTURED MOUSE GRANULOSA CELLS BY cAMP AND ESTRADIOL. R. Kurten and R.C. Burghardt, Dept. Biology, Texas A&M University, College Station, TX 77843

The role of gonadotrophic and steroid hormones in regulating gap junction membrane in ovarian granulosa cells was examined in cultures derived as monodisperse cells from immature mice. The length of junctional membrane, normalized to the length of apposed cell membrane was analyzed in situ by quantitative electron microscopy following incubation for 48 hr in serum-free medium in the presence or absence of exogenous hormones. Evaluation of untreated control and E_2 (10^{-7}M) treated cultures revealed the reestablishment of basal levels of both macular and annular gap junctions with no significant differences between the two treatments. Inclusion of FSH (50ng/ml) in the culture medium resulted in a significant increase in macular gap junction levels while annular levels did not differ significantly from untreated controls. Addition of 0.1mM 8-Br-cAMP also induced a significant increase in the level of macular junctions and, in contrast to FSH, proved capable of significantly increasing levels of annular junctions as compared to controls. Combination of E_2 and FSH resulted in slight elevations of both macular and annular profiles as compared to FSH treatment alone. Addition of E_2 and 8-Br-cAMP resulted in decreased macular and increased annular junction levels as compared to 8-Br-cAMP treatment alone. The total junctional membrane did not differ significantly between these two treatments. We conclude that: (a) cAMP may mediate FSH-induced increases in macular domains, (b) E_2 augments the FSH-induced increases in junctional domains and (c) E_2 exerts a synergistic effect on both FSH- and cAMP-induced increases in annular junctional membrane. (Supported by NIH grant HD-14781)

AN ULTRASTRUCTURAL STUDY OF THE BLOOD/AIR BARRIER IN THE GUINEA PIG. Joan O. Ford, Ronald F. Dodson, and M. Glenn Williams, The University of Texas Health Center at Tyler, Tyler, Texas.

A small molecular weight protein, horseradish peroxidase (HRP; Sigma Type II, 40,000 MW), was intravenously injected into guinea pigs to ultrastructurally examine the permeability of the blood/air barrier in the lung. Adult animals were given 300 mg/kg of the tracer in a small volume of saline (4% of total blood volume), anesthetized and sacrificed at varying intervals by either intratracheal filling or right-ventricular perfusion with 3% glutaraldehyde buffered with 0.1M phosphate. Representative sections of lung parenchyma were incubated for peroxidase using a method modified from that of Graham and Karnovsky.[1] The reaction product in the form of a black granular material had penetrated the endothelial layer and accumulated in the interstitium as early as 1.5 minutes after injection. Junctions between pneumocytes prevented passage of the reaction product into the alveoli. Pinocytotic vesicles within both endothelial and epithelial cells exhibited uptake of the tracer, but did not appear to significantly contribute to HRP transport.

REORGANIZATION OF HYDRATED COLLAGEN LATTICES BY HUMAN SKIN FIBROBLASTS• C.R.Lamke, F.Grinnell, Dept. Cell Biology, University of Texas Health Science Center, Dallas TX 75235

Fibroblasts were cultured on top of or at the bottom of hydrated collagen lattices. Shortly after initially interacting with collagen lattices, fibroblasts appeared to attach to individual collagen fibrils and in many cases cell processes were found wrapped around clusters of collagen fibrils. Tension generated by cells during spreading resulted in proximal collagen fibrils becoming aligned in the plane of spreading and more densely packed. During subsequent culture, the collagen fibrils distal to the cells underwent a similar reorganization and the lattice thinned to 1/10th of its original thickness. The rate of thinning was similar regardless of whether the cells were originally above or at the bottom of the lattices. The presence of cells distributed throughout the lattice was unnecessary for lattice reorganization to occur. When the lattices were allowed to come off of the underlying substratum, compaction of the collagen gels was observed, and the resulting matrix had the typical appearance of dermis as observed by both light and electron microscopy. Collagen fibrils associated with the cell surface often appeared to be under tension, and in regions of close fibril binding, there was a prominent reorganization of submembranous microfilaments. It is suggested that reorganization of the collagen lattice by fibroblasts may depend upon secreted cell factors as well as physical forces generated by cells.
Supported by a grant from the NIH, GM 31321.

ELEMENTAL CONTENT OF CARDIOCYTES IN THE SNELL DWARF MOUSE. A. Lewiński[1], N.K.R. Smith[1], A. Bartke[2] and S. Stabler-Morris[1], Departments of Anatomy[1] and Obstetrics and Gynecology[2], The University of Texas Health Science Center, San Antonio, Texas 78284.

X-ray microanlaysis of ultrathin (0.2 μm), freeze-dried cryosections prepared at -100°C has been used to determine elemental content in nucleus, myofibrillar cytoplasm and mitochondrially enriched cytoplasm of cardiocytes in Snell dwarf mice in comparison with phenotypically normal mice from the same strain. It was found that there was significantly lower chlorine concentration in all three subcellular locations and significantly lower sodium concentration in the nucleus of dwarf mouse cardiocytes. These findings are possibly connected with the absence of prolactin (PRL) and growth hormone (GH) and with the extremely low serum thyroxine and triiodothyronine levels in Snell dwarfs. Since PRL, GH and also thyroid hormones are known to reduce substantially the renal excretion of sodium, chlorine and water, their deficiency presumably results in salt wasting and leads to extracellular hypochloremia and hyponatremia. In turn, extracellular hypochloremia in hypopituitary mice can result in the loss of intracellular chlorine and is probably responsible for the significantly lower chlorine concentration in the three probed subcellular compartments of the cardiocytes of dwarf mice as compared to nondwarf controls. The present results confirm the existence of a positive correlation between the concentration of sodium and chlorine in various animal tissues. Additionally, in both normal and dwarf mice, statistically significant subcellular compartmentalization was found in the present study for phosphorus, sulfur and potassium.

ASSAY OF RADIATION AND CHEMICAL DAMAGE TO MAMMALIAN CHROMOSOMES AND CHROMATIN BY STEREO ELECTRON MICROSCOPY. Arthur Cole, Physics Dept., University of Texas System Cancer Center, Houston, Texas 77030.

Compact, swollen, histone-depleted, and extensively dissociated chromosomal structures from synchronized mammalian cells were studied using stereo electron microscopy and a variety of biophysical and biochemical techniques. Histone-depleted mitotic chromosomes contained three prominant components: (1) dense (lipo) protein particles which overlay a (2) multistranded central backbone from which radiated (3) many chromosomal DNA loops. Histone-depleted interphase nuclei appeared as cages (shells) with similar chromosomal DNA loops radiating outward from the surface of the cage. Histone-depleted chromosomes from irradiated cells expressed damage, in order of decreasing sensitivity, as a loss of (lipo) protein overlay, disruption of backbone and degradation of the DNA loops. Expression of damage was attenuated as cells progressed through mitosis into interphase. Further dissociation of histone-depleted structures by protease and detergent yielded separated chromosomal "unit" structures which contained DNA, residue protein, and cholesterol lipids. Damage was assayed in unit structures as changes in sedimentation and buoyant density properties, which probably related to configurational changes. We are currently developing protocols to image "unit"

structures by stereo electron microscopy. The studies will be shown as a sequence of stereo pair micrographs using a new viewing system developed for poster presentation. Micrographs are mounted as a continuous roll and viewed with a stereoscope adjustable for the individual's height, eye separation and focal level.

SCANNING ELECTRON MICROSCOPIC STUDY OF IMPULSE NOISE-INDUCED MECHANICAL DAMAGE IN THE COCHLEA. R.P. Hamernik, G. Turrentine, R. Salvi, Callier Center for Communication Disorders, University of Texas, 1966 Inwood Road, Dallas TX 75235

Binaural chinchillas were exposed at normal incidence to 160 dB peak SPL impulse noise at the rate of 2 impulses per minute for 50 minutes. Animals were sacrificed at post-exposure times varying from t = 0 through 30 days. The cochleas were prepared for SEM observation using a standard protocol. Immediately following exposure, a large (6 mm) area of the organ of Corti was separated from its attachments to the basilar membrane along a fracture line that follows the outer pillar cells. The separated portion of the organ of Corti is left floating in the scala media with both ends attached to viable portions of the remaining sensory epithelia. Surprisingly, in the denuded areas of the basilar membrane, the inner hair cell surface structure remains comparatively normal during the early post-exposure times while outer hair cells in the region bordering the main lesion show considerable changes in cilia structure and in the appearance of the reticular lamina - cuticular plate complex. Scar formation and the absorption/phagocytosis of the free-floating portions of the organ of Corti will be described, as well as the differing susceptibilities of inner and outer hair cell cilia to morphological changes.

ULTRASTRUCTUAL CHANGES IN EPITHELIAL CELLS OF FROG URINARY BLADDER STIMULATED BY ADH, CALCIUM IONOPHORE AND VERAPAMIL. A.J. Mia, L.X. Oakford, J. Aschenbrenner, J. Carnes, N. Tarapoom and T. Yorio, Dept. of Life Sciences, Bishop College, Dallas, Tx and Depts. of Anatomy and Pharmacology, TCOM, Ft. Worth, Tx 76107.

Epithelial cells, of frog urinary bladder, contain smooth apical membranes covered with numerous microridges and short microvilli covered with glycocalyx. The cytoplasm contains rough ER thin Golgi profiles, numerous mitochondria, a few dense granules, minute vacuoles and some lysosomes. There is also a sporadic distribution of microtubules but numerous microfilaments and intermediate filaments are present both singularly and in bundles. Cells stimulated by ADH, calcium ionophore and verapamil undergo a series of cellular changes. The apical membrane becomes highly contoured while the basal-lateral membranes of adjacent cells appear to separate from each other. The cytoplasm reveals a general distortion with a proliferation of rough ER, degenerated mitochondria with a loss of cristae and an accumulation of numerous membrane bound heterogenuous secretory granules. Cells challenged by ionophore contain a large number of highly elongated microvilli. Microvilli are often absent or reduced to slight elevations in verapamil treated cells. The apical membranes of the treated cells contain vast arrays of filamentous glycocalyx which appear aligned with many microfilaments occurring within the microvilli and microridges, indicating possible transmission of hormonal and chemical signals across the plasma membrane to the cell interior. The microfilaments within the cytoplasm may play a role in transmural water and ion transport, or the discharge of secretory products across the cell membrane.

QUANTITATIVE ENERGY DISPERSIVE ELECTRON PROBE X-RAY MICRO-ANALYSIS OF ELECTROLYTES IN THIN CRYOSECTIONS. I. L. Cameron, K.E. Hunter and N.K.R. Smith, Department of Anatomy, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284.

For microprobe analysis, biological specimens are subjected to rapid freezing and then thin frozen sections of the specimen are cut and freeze-dried at low temperatures to prevent diffusion of ions. Mammalian erythrocytes, whose ionic composition is accurately known, provide us with an excellent system for validating the accuracy of specimen preparation and microprobe procedures. What temperature prevents the diffusion down the known ion gradients during the preparative procedures? In this regard cryosectioning and cryosorption were done at -40°C in one case and at -100°C in the other case. From the erythrocyte data on Na, K and Cl we conclude that at -100°C we have validated a procedure for the accurate quantification of intracellular electrolytes using x-ray microanalysis in thin cryosections of mouse erythrocytes. The processing of erythrocytes suspend in blood plasma at a temperature of -40°C lead to spuriously high erythrocyte Na and Cl concentration values. The most reasonable explanation for this finding is that Na and Cl have diffused from their higher concentration in the extracellular environment into the erythrocyte. Because of the small size of the erythrocytes, the area rastered for analysis is never positioned more than one micron from the extracellular space. Recent studies on large frog oocytes show that if the distances of Na and Cl diffusion are great enough, the use of -40°C for the preparation of thin biological sections for microprobe analysis can give accurate quantitative analysis.

TWO SIMPLE TECHNIQUES TO MAKE LAB LIFE A LITTLE EASIER. Ronald W. Davis, Dept. Human Anatomy, Texas A&M University Medical School, College Station TX 77843.

1) A method for converting between total magnification values and micron bars.

   A simple method, using only a millimeter ruler, will be described for computing the length of scale bars of any value when total magnification is known and for computing total magnification when only a scale bar is given.

2) A ten second procedure for making coated or uncoated grids hydrophilic.

   Grids whether coated with a Formvar-type film or new and uncoated are often hydrophobic. This can make the precise orientation of sections on a grid difficult. Grid surfaces can be made hydrophilic by exposing them in a Technics Hummer II to a current of 10 mAmps for 5 seconds. This technique works when the gas in the bell jar is air but not with a nonreactive gas such as argon. Sputter coaters that have a magnet associated with the target, such as the Technics Hummer III, will work after the magnet has been removed. The hydrophilic surface aids in picking up sections, replicas and negative staining. This technique has worked with both gold and gold-palladium targets. No disagreeable background has been observed with sections. A slight background may be observable when negative stained preparations are viewed at high magnifications.

SOME SUGGESTIONS FOR MICROTOME DIAMOND KNIFE CLEANING AND USE. Bernard E. Mesa, Micro Engineering Inc. Huntsville, Tx. 77340.

The edge of a microtome diamond knife, estimated to be only about thirty carbon atoms across, is very strong in the cutting direction but very fragile to forces applied from any other angle. For this reason the conventional method of cleaning diamond knives using a wood stick to manually wipe the edge, even though recommended by most textbooks and manufacturers, must be considered suspect of producing at least some of the nicks that appear on the edge after a short period of use. Two years ago, this problem was plaguing our manufacturing process which requires the knives to be thoroughly cleaned after various steps. In many cases a knife tested to have a flawless edge would show nicks after cleaning with a wood stick. To eliminate the possibility that the nicks were originated by vibration or shaking of the hand, a small machine was designed and built using a precision linear bearing which performed the wiping motion in a uniform manner and without any vibration. The results were not significantly different from the manual operation; still on occasions this cleaning procedure would generate nicks on the edge. Other materials, besides wood, were tried with similar results.It was our conclusion that, in order to safely clean and handle the knife, it should never be allowed to touch any solid object no matter how innocuous it may appear. (The exception being the microtome cutting operation where the forces are perpendicular to the edge). The most effective way we have found to clean the microtome diamond knives is using a pressurized jet of distilled water and then drying with pressurized clean air or other gas.

FADING OF ELECTRON MICROSCOPE NEGATIVES DURING STORAGE -- A CASE REPORT. Hilton H. Mollenhauer and Robert E. Droleskey, Veterinary Toxicology and Entomology Research Laboratory, College Station, Texas 77841.

Deterioration of electron microscope negatives was first observed after a period of relatively high humidity that prevailed for several months in the laboratory during repair and upgrading of the air conditioning and dehumidifying systems. The negatives were electron image film stored in paper envelopes (either kraft or white) stacked upright in file cabinets designed for film storage. The file cabinets were kept in the laboratory and no special temperature or humidity conditions were maintained. Deterioration was manifest as a partial fading of the negative and/or fading of areas that coincided with the seams of the negative envelopes. The cause of fading was not due to improper processing since recommended development and fixation procedures have always been followed and since the faded negatives could not be correlated with individual film packs. In controlled tests, we have found that negative fading may occur in periods of only 2-3 weeks in both paper and glassine envelopes even under our present "normal" laboratory conditions. We believe that negative fading is caused by substances present in the paper of the negative envelopes or leached out from the glue used to fabricate the envelopes. We now feel that it is important to avoid the use of paper envelopes for negative storage or the use of any envelopes with seams that fall in the center of the film. Several plastic-based envelope types are currently available that meet these requirements.

ASSAY OF RADIATION AND CHEMICAL DAMAGE TO MAMMALIAN CHROMOSOMES AND CHROMATIN BY STEREO ELECTRON MICROSCOPY. Arthur Cole, Physics Dept., University of Texas System Cancer Center, Houston, Texas 77030.

Compact, swollen, histone-depleted, and extensively dissociated chromosomal structures from synchronized mammalian cells were studied using stereo electron microscopy and a variety of biophysical and biochemical techniques. Histone-depleted mitotic chromosomes contained three prominant components: (1) dense (lipo) protein particles which overlay a (2) multistranded central backbone from which radiated (3) many chromosomal DNA loops. Histone-depleted interphase nuclei appeared as cages (shells) with similar chromosomal DNA loops radiating outward from the surface of the cage. Histone-depleted chromosomes from irradiated cells expressed damage, in order of decreasing sensitivity, as a loss of (lipo) protein overlay, disruption of backbone and degradation of the DNA loops. Expression of damage was attenuated as cells progressed through mitosis into interphase. Further dissociation of histone-depleted structures by protease and detergent yielded separated chromosomal "unit" structures which contained DNA, residue protein, and cholesterol lipids. Damage was assayed in unit structures as changes in sedimentation and buoyant density properties, which probably related to configurational changes. We are currently developing protocols to image "unit" structures by stereo electron microscopy. The studies will be shown as a sequence of stereo pair micrographs using a new viewing system developed for poster presentation. Micrographs are mounted as a continuous roll and viewed with a stereoscope adjustable for the individual's height, eye separation and focal level.

STEREOLOGICAL ANALYSIS OF THE EFFECTS OF HOKU POINT ELECTRO-ACUPUNCTURE ON THE HERRING BODIES OF RAT NEUROHYPOPHYSIS
J. Leon McGraw, Jr. and Steve Tsui, Dept. Biology, Lamar University, Beaumont TX 77711

Three groups of rats were electrically stimulated at points corresponding to Chinese Hoku acupuncture points every other day for a period of nine minutes with a stimulator operated at 0.5 volt, at a duration of 2.5 milliseconds and at 120 cycles per second. Experimental groups were terminated at seven, sixteen and thirty days. Three additional groups of rats served as controls and were handled and treated exactly like the experimental groups except that no electrical stimulation was applied. The pituitary gland from each animal was fixed and prepared for transmission electron microscopy. Semithin sections were taken and stereological methods were utilized to determine the relative volumes of Herring bodies within the neurohypophysis. Data was statistically analyzed for differences ($p \leq 0.05$) between the relative volumes of Herring bodies from control and electro-acupunctured groups of animals after seven days. However, by sixteen days there was a significant increase ($p < 0.05$) of 57% in the Herring body mean relative volume of the experimental group. By 30 days, the mean relative volume of the experimental group. By 30 days, the mean relative volume of Herring bodies had significantly increased ($p < 0.05$) by 94% in the experimental group. Such large changes in stored hormones within the neurohypophysis indicate that electro-acupuncture may have significant effects on the secretion of neurohormones by the hypothalamus or on their storage within the neurohypophysis and raise questions concerning the potential of electroacupuncture in controlling hormone output and physiological parameters.

STEREOLOGICAL ANALYSIS OF THE EFFECTS OF EAR LUNG-POINT ELECTRO-ACUPUNCTURE ON THE HERRING BODIES OF RAT NEUROHYPOPHYSIS
J. Leon McGraw, Jr., and Steve Tsui, Dept. Biology, Lamar University, Beaumont TX 77710

Three groups of rats were electrically stimulated at points corresponding to the Chinese ear lung-point acupuncture site every other day for a period of nine minutes with a stimulator operated at 0.5 volt, at a duration of 2.5 milliseconds and at 120 cycles per second. Experimental groups were terminated at seven, sixteen and thirty days. Three additional groups of rats served as controls and were handled and treated exactly like the experimental groups except that no electrical stimulation was applied. The pituitary gland from each animal was fixed and prepared for transmission electron microscopy. Semithin sections were taken and stereological methods were utilized to determine the relative volumes of Herring bodies within the neurohypophysis. Data was statistically analyzed for differences between control and experimental groups. There was a significant increase (p 0.05 of 25% in the mean relative volumes of Herring bodies from the electro-acupunctured group of animals after seven days. By sixteen days there was a significant increase ($p \leq 0.05$) of 74% in the Herring body mean relative volume of the experimental group. By 30 days, the mean relative volume of Herring bodies had significantly increased ($p < 0.05$) by 239% in the experimental group. Such large changes in stored hormones within the neurohypophysis indicate that electro-acupuncture may have significant effects on the secretion of neurohormones by the hypothalamus or on their storage within the neurohypophysis and raise questions concerning the potential of electroacupuncture in controlling hormone output and physiological parameters.

OPTIMAL FIXATION OF CHICK SYNAPTOSOMES USING DIFFERENT OSMOTIC PRESSURES. F.A. Anthony, E.F. Couch, and J.A. Babitch, Depts. of Chemistry and Biology, Texas Christian University, Ft. Worth, TX, 76129.

Synaptosomes are an in situ artifact of brain homogenization used in helping understand the complex event of neurotransmitter release. Their ultrastructure has been well studied with the majority of electron microscopic analyses on tissue isolated from rat brain. Research on chick synaptosomes, however, has been minimal, despite recent evidence of important species differences (Sorensen and Babitch (1983) Neuroscience, in press). Because these two species may differ in synaptic membrane structure, it is likely that synaptosomal structure and conditions required for fixation of synaptosomes may also differ. Therefore, in the present study, synaptosomes were isolated from chick brain and an analysis of the effects of osmotic pressure on chick synaptosomal ultrastructure during fixation was performed. Chick synaptosomes were found to remain intact following fixation in 2.6% glutaraldehyde, 0.1 M sodium cacodylate, 2% paraformaldehyde, pH 7.3, plus 3 drops of 1% $CaCl_2$, either in the presence of 0.2 M sucrose or 0.3 M sucrose. Increasing the sucrose concentration up to 0.4 M sucrose significantly decreased the number of intact synaptosomes; however, typical intrasynaptosomal organelles such as synaptic vesicles and mitochondria were visible in all preparations. In addition, the presence of invaginations in several membranes was observed and may represent "frozen" exocytotic events.

SOME SEM OBSERVATIONS ON THE EARLY PULMONARY RESPONSE TO "AMOSITE" EXPOSURE IN THE GUINEA PIG.
M. Lynn Davis and Ronald F. Dodson, The University of Texas Health Center at Tyler, Tyler, Texas.

The pathogenesity of asbestiform minerals has been demonstrated for both humans and animals. The present study involves an amphibole form of asbestos known commercially as "amosite" to which workers at a Tyler asbestos plant were exposed. Relatively little information is available in the literature concerning the health effects of this type of asbestos, particularly regarding ultrastructural pathology. The present investigation utilizes SEM to illustrate some of the early pulmonary effects of a single-dose exposure to amosite on the lower airways and alveoli. Healthy adult guinea pigs were anesthetized and intratracheally injected with 17.5 mg commercial grade amosite in 0.35 ml of normal saline. The animals were sacrificed after 2 hr or 4 hr by right ventricular perfusion with phosphate buffered glutaraldehyde. Tissue was removed from upper and lower lobes of the lung and prepared for TEM and SEM.

After 2 hr, heavily exposed regions of the lung were visible at low magnification appearing as solid loci in the parenchyma. Accumulations of asbestos fibers and mucus were apparent in many of the smaller airways. Phagocytes were evident in blood vessels adjacent to affected areas and in some of the affected areas themselves. By 4 hr, most of the asbestos had been phagocytized as reflected by a relative lack of free fibers. Numerous phagocytes were present in the airways and alveoli. Areas which were free of asbestos deposition appeared to be normal.

ENDOCYTOSIS OF AMOSITE ASBESTOS BY PARAMECIUM MULTIMICRONUCLEATUM. Joan O. Ford and Ronald F. Dodson, The University of Texas Health Center at Tyler, Tyler, Texas.

Paramecium, a ciliated protozoa that ingests nonnutritive particulate matter, was exposed to amosite asbestos and examined ultrastructurally for possible uptake of this material. A small fiber preparation of respirable size (90% < 0.4μ in width, 5.6μ in length) was introduced to nonstarved cells in culture media at a concentration of 50 μg/ml. At varying time intervals, the cells were fixed by the addition of 4% glutaraldehyde in 0.1M PIPES buffer, pH=7.2. Examination by transmission electron microscopy revealed a significant uptake of the asbestos fibers as early as five minutes post-exposure. Endocytosed particles were located in membrane-bound vacuoles within the endoplasm and ranged from 0.08μ to 3.83μ in length. These vacuoles progressed to the posterior of the cells, and the particulate contents were voided through the cytopyge. Ultrastructure of the treated cells appeared consistent with that of control Paramecium and so at this level of determination, amosite asbestos gave no evidence of cytotoxicity. These eukaryotic unicellular protozoans represent a relatively untapped model which may provide the basis for future cytotoxicity experiments.

ULTRASTRUCTURAL EVIDENCE FOR MEIOSIS IN A SUPPOSED APOGAMIC ISOLATE OF THE MYXOMYCETE DIDYMIUM IRIDIS. J.D. Sherman and C.W. Mims, Department of Biology, S.F. Austin State Univ., Nacogdoches, TX 75962.

Traditionally two patterns of reproduction have been recognized in the Myxomycetes or true plasmodial slime molds. In some isolates any two gametes appear to be capable of fusing to form a zygote while in others definite mating types exist and fusion occurs only between compatible gametes. Isolates in the former category have been referred to as homothallic, while those in the latter are known as heterothallic. Recently, however, it has been suggested that some isolates are apogamic and complete their entire life cycle in the same ploidy level. The purpose of this was to examine a supposed apogamic strain of D. iridis in an attempt to determine if meiosis occurs. In most Myxomycetes examined thus far meiotic divisions have been reported in maturing spores less than 24 hours old.

The results of this study suggest that meiosis occurs in 12-16 hour old spores of the MOI-1 isolate of D. iridis. Structures resembling synaptonemal complexes were observed in the nuclei of these spores and metaphase, anaphase and telophase division figures were noted. Although some of the resulting daughter nuclei were observed within autophagic vacuoles, the exact fate of the products of meiosis is uncertain. Most mature spores appear to be uninucleate.

CALENDAR OF MEETINGS

MICROBEAM ANALYSIS SOCIETY ANNUAL MEETING
July 16-20, 1984
Lehigh University, Bethlehem, PA
For further information contact: J. I. Goldstein, Dept. of Metallurgy and Materials Engineering, Lehigh University, Bldg. #5, Bethlehem, PA 18015, phone (215) 861-4221.

35th ANNUAL MEETING OF THE AMERICAN INSTITUTE OF BIOLOGICAL SCIENCES (AIBS)
August 5-9, 1984
Colorado State University, Ft. Collins, CO
For further information contact: Dr. Ralph R. Baker, Dept. of Botany and Plant Pathology, Colorado State University, Ft. Collins, CO 80523 Phone (303) 491-6944.

EMSA/MSC JOINT ANNUAL MEETING
August 12-17, 1984
Detroit, MI
For further information contact: Linda Sicko Goad, Great Lakes Research Division, The University of Michigan, 2200 Bonlsteel Blvd., Ann Arbor, MI 48109, phone (313) 763-5393.

3rd ASIA PACIFIC ELECTRON MICROSCOPY CONFERENCE
August 29-September 1, 1984
Singapore
For further information contact: Dr. Ny Cheng Siong, ARC, 303 Tanglin Road, Singapore 1024 Telex UN1ARC RS 38806.

CENTENNIAL SYMPOSIUM ON HIGH RESOLUTION ELECTRON MICROSCOPY
April, 1985
Arizona State University, Tempe, AZ
For further information contact: Dr. P. R. Buseck, Department of Geology, Arizona State University, Temple, AZ 85287.

JOB OPPORTUNITY

ELECTRON MICROSCOPY RESEARCH ASSOCIATE — Requires minimum of M.A. or M.S. in biological sciences, with proficiency in all aspects of TEM including tissue preparation, ultrathin sectioning and microscopy. Experience in ultracytochemistry desired, and independent research encouraged. Position available May 14, 1984. Salary is competitive and consistent with university degree and experience. Contact Paul J. Boor or Nancy Kretschmer, University of Texas Medical Branch, Department of Pathology, 230 Keiller, Galveston, TX 77550. Phone 409-761-3656 (P.J.B.) or 409-761-3653 (N.K.). UTMB is an affirmative action/equal opportunity employer.

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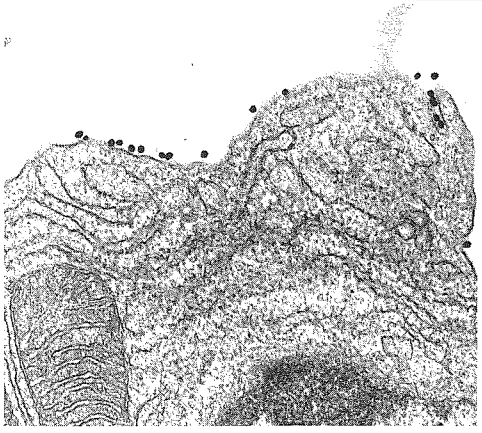

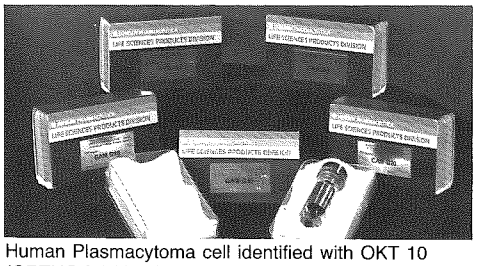

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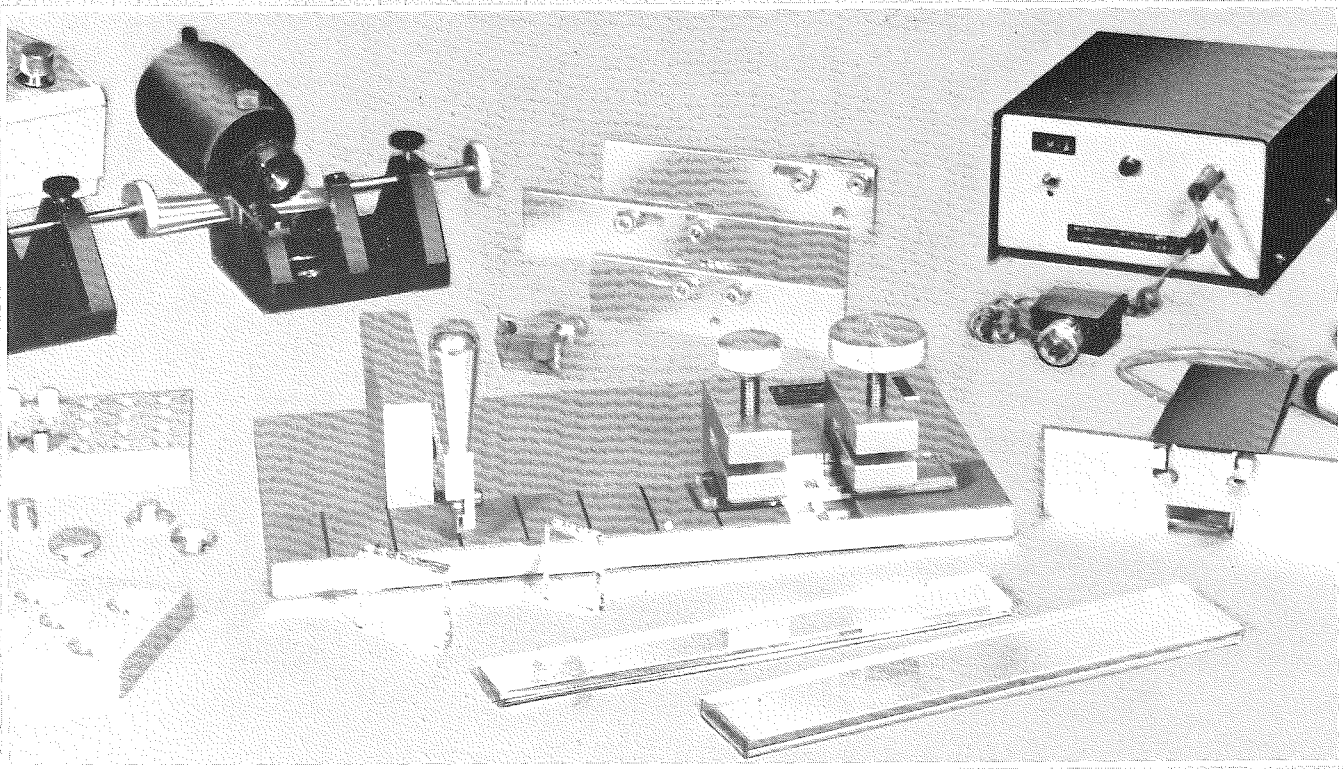

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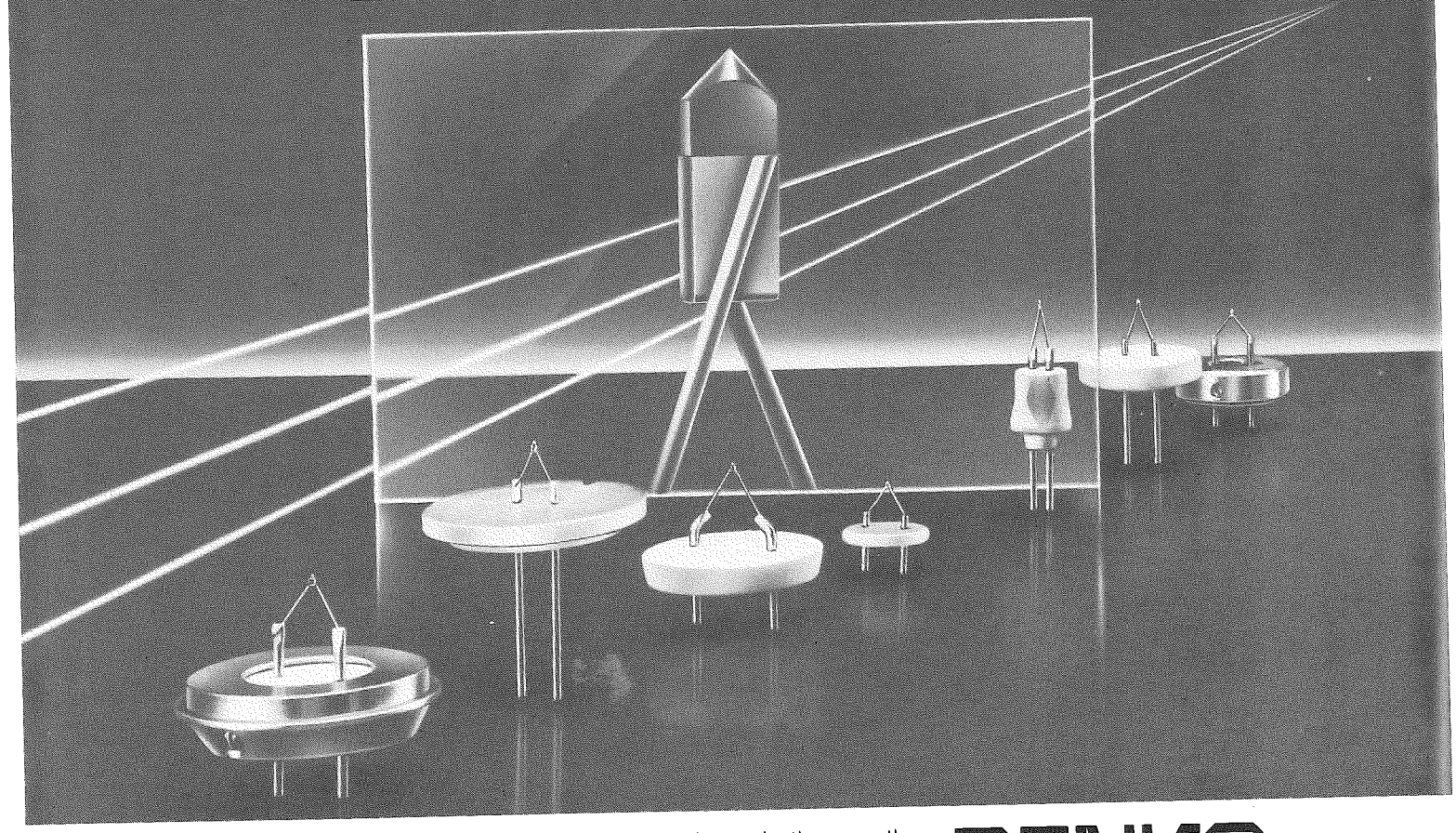

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APPLICATION FORM FOR TSEM MEMBERSHIP

I hereby apply/nominate for Regular / Student / Corporate ☐ membership in the Texas Society for Electron Microscopy.

Name of nominee _____

P.O. Address _____

One year's dues in the form of a check or money order should be sent with the application for Membership form. (Regular $10.00. Student $2.00. Corporate $75.00).

Randy Moore

Signature of TSEM Regular Member making the Nomination

Date 19

This application for Membership in the Society or this application for transfer from the grade of Student to Regular or Regular to Student Member should be sent to the TSEM Secretary. The form will be presented at the next meeting of the Executive Council for their approval (majority vote). The nominees will then be presented by the council to the membership at the next general business meeting for their approval (majority vote). Nominees will be added to the membership rolls at that time.

Presented to the Council at _____ meeting. Date _____

Action _____

Send Application to:

Elizabeth Root
GEA 115
The University of Texas at Austin
Austin, Texas 78712-1097

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ELECTRON MICROSCOPY SOCIETY OF AMERICA
NOMINATION FOR MEMBERSHIP

We hereby nominate for Member ☐, Student Associate ☐, Sustaining Member ☐.
institution

Name of corporation / person nominated _____

P.O. Address _____

Information as to position, degrees, and qualifications for Membership: _____

_____

This nomination is accompanied by a statement of interest in and contributions to Electron Microscopy and associated fields of science. One year's dues in the form of a check or money order should be sent with the Nomination for Membership form. (Member $20.00. Student Associate $2.00. Sustaining Member $50.00).

_____
Signature of EMSA Member making nomination

This Nomination to membership in the Society, or this application for transfer from the grade of Student Associate to Member, signed by one Member should be sent to the Executive Secretary to be presented at the next meeting of the Council for approval by a majority vote of the Council. Notice of approval will be mailed by the Executive Secretary.

Presented to the Council at _____ meeting. Date _____

Action _____

Remarks _____

Send Nominations to:

Blair Bowers, Treasurer
Bldg. 3, Room B1-22
NIH
Bethesda, MD 20205

Regional Editors

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Cheryl Craft, University of Texas Health Science Center, Department of Anatomy, 7703 Floyd Curl Drive, San Antonio, Texas 78284.

Joanne T. Ellzey, Biological Sciences, University of Texas, El Paso, Texas 79968.

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Peter Moller, Department of Human Biology, Chemistry, and Genetics, University of Texas Medical Branch, Division of Cell Biology, Galveston, Texas 77550.

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Anna Siler, Department of Pathology, University of Texas Southwestern Medical School, Dallas, Texas 75235.

Glenn Williams, University of Texas Health Center at Tyler, P.O. Box 2003, Tyler, Texas 75710.

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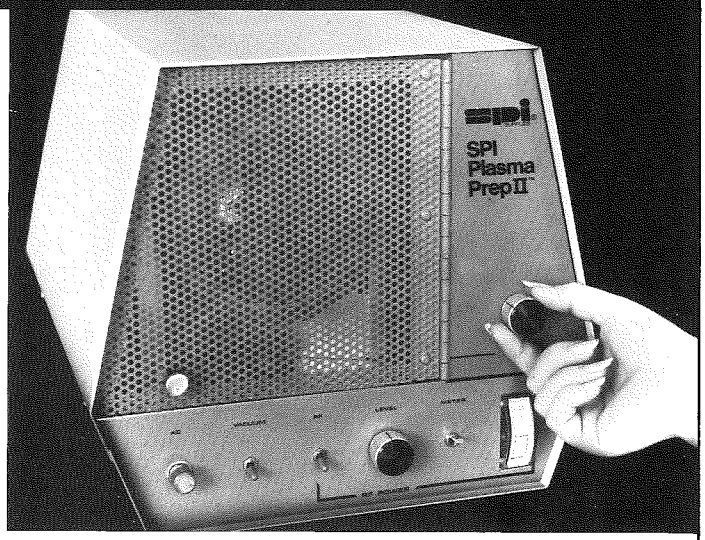

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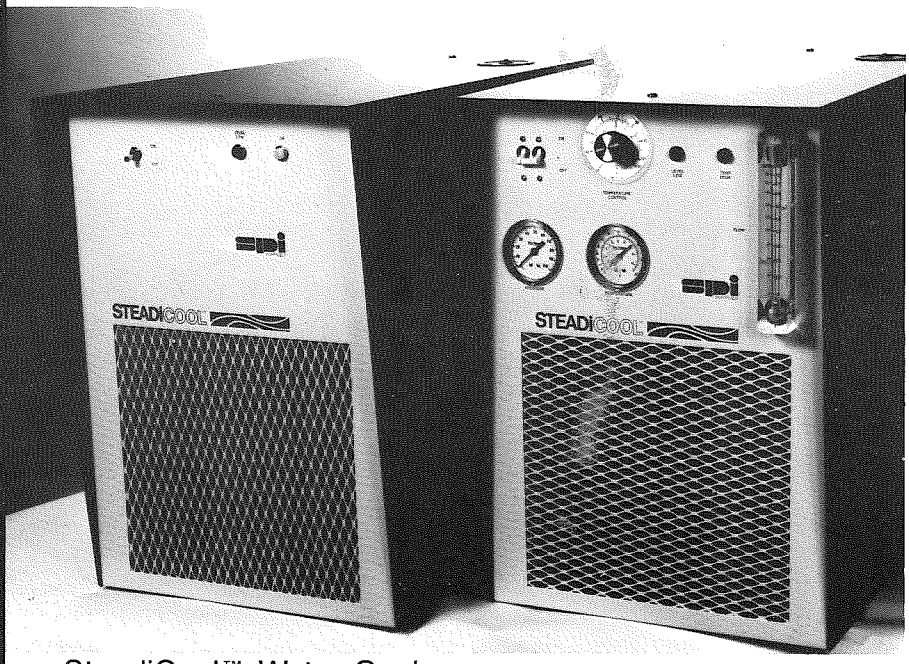

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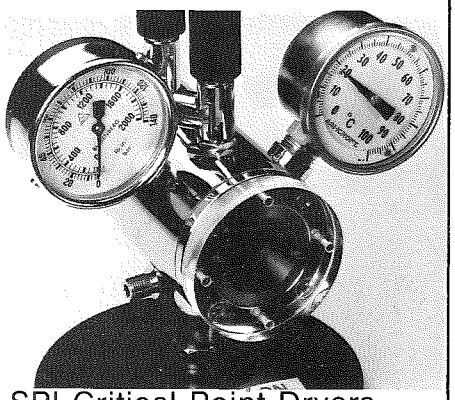

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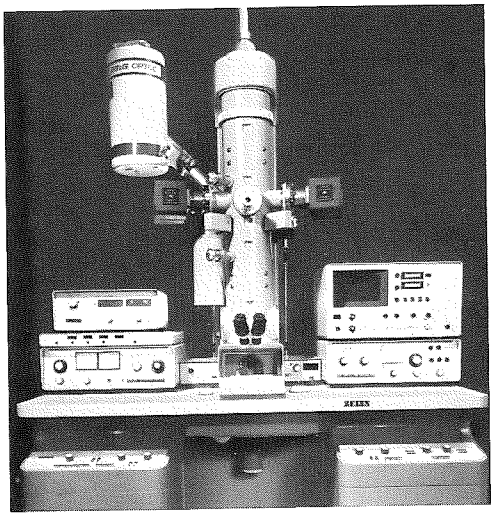

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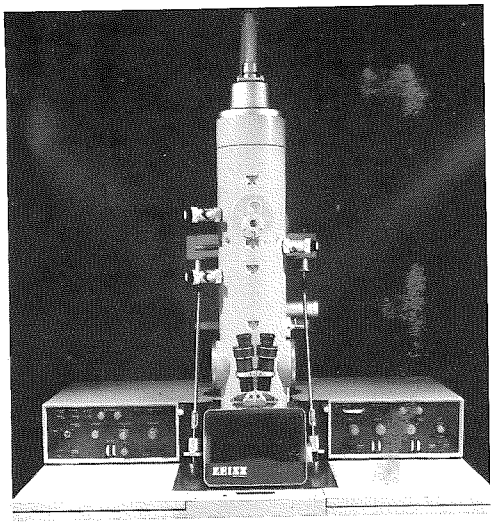

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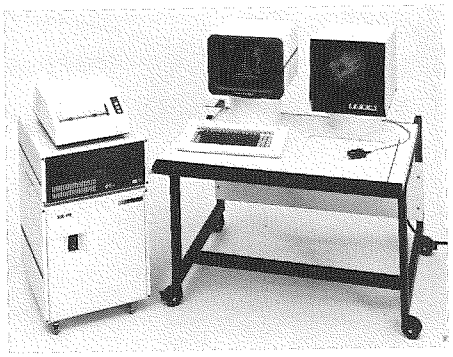

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