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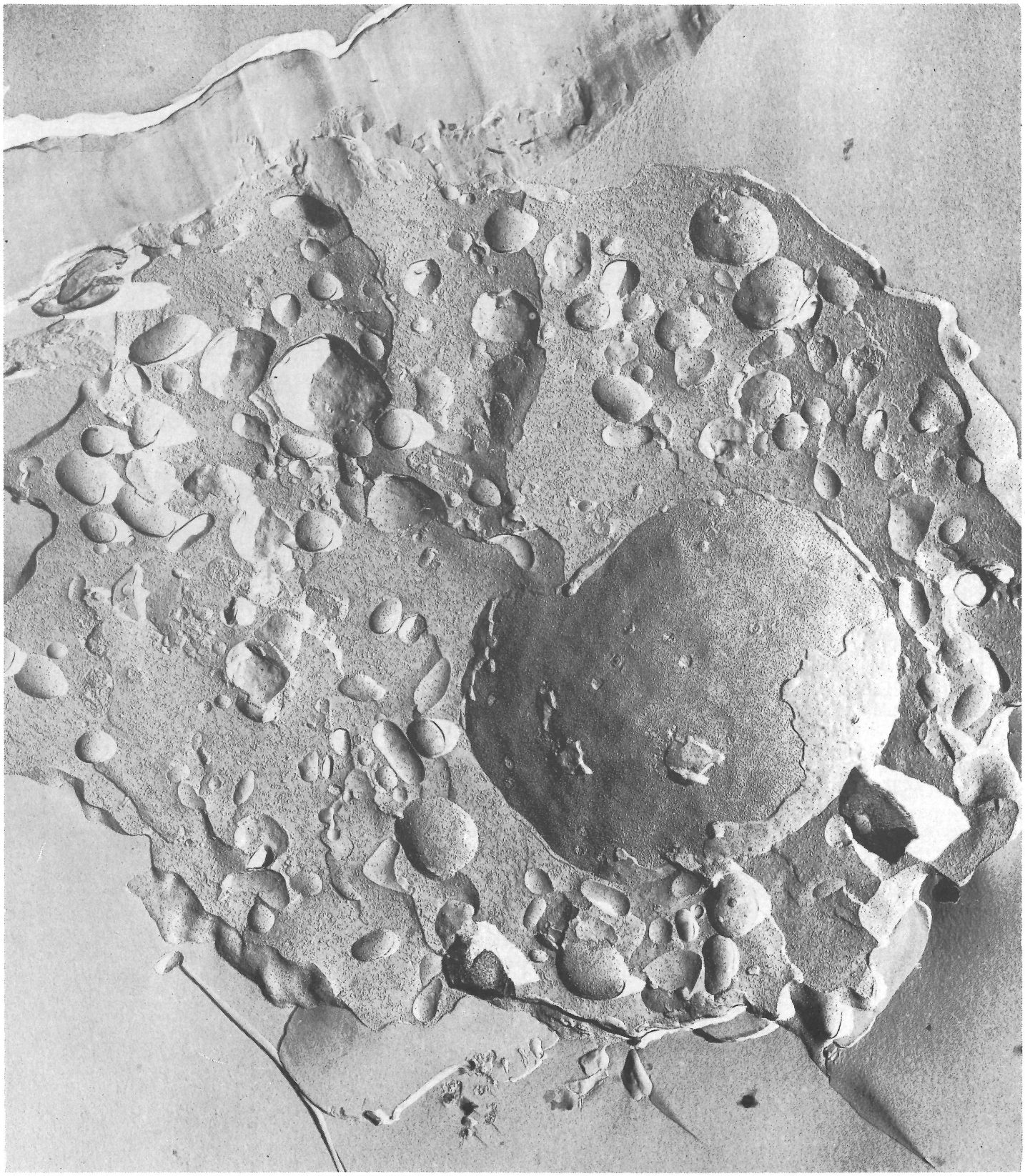

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Contents

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Texas Society for Electron Microscopy
"For the purpose of dissemination of research with the electron microscope"

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 (interesting micrographs) are welcome and should be selected with some attention to aesthetic appeal as well as excellence both in technique and in scientific information content.

(Continued On Page 6)

ON THE COVER

Electron micrograph courtesy of Dr. W. Allen Shannon, Jr., Electron Microscopy Unit, V.A. Medical Center at Dallas and Department of Cell Biology at the University of Texas Health Science Center at Dallas. Freeze-etch electron preparation of polymorphonuclear leukocyte neutrophil isolated from rabbit peritoneal exudate. Demonstrated are prominent nucleus with pores, larger primary cytoplasmic granules and smaller elongate secondary cytoplasmic granules. x22400.

President's Message

This year has passed so quickly, I find it hard to believe the time for a farewell message has come. I have enjoyed working for you as President. I have had such fine officers to work with me: Hilton Mollenhauer, Marilyn Smith, Allen Shannon, Paul Baur, Elaine McCoy and Bruce Mackay. My thanks to each of you.

Like many of you, I am drawn to electron microscopy by the pleasure of seeing a beautiful image that no one else has ever seen. I was reminded of this by a recent visit to the library of the Houston Museum of Fine Arts. There was an exhibit of macrophotographs by Karl Bloussfeldt and Ernst Fuhrmann for a book on the structure of plants, *"Art Forms in Nature."* Each photographic plate was not only of scientific importance but was also a work of art showing the beauty of nature. These works bring to mind the recent collection of scanning electron micrographs brought together as an art book. Here the same concept of beauty in nature was illustrated with a different kind of image.

At the same time at the Houston Museum of Fine Arts and at Rice University, there were collections of drawings by Leonardo da Vinci. As a scientist and engineer, Leonardo was fascinated by movement — muscles pushing and pulling, flowers blowing in the wind, water flowing past a stationary object. As an artist with unsurpassed hand-eye coordination, he rendered faithfully what he observed in nature. For example, his study of the bird in flight has only recently been duplicated with high speed photographic techniques. In his anatomical drawings of the ox heart, he documented that the heart was four-chambered and not two-chambered as dogma of his time dictated. Moreover, he drew the right ventricular wall thinner than the left ventricular wall and showed accurately the relative sizes of the ventricular cavities. Da Vinci's notes suggest that he did not understand the significance of these differences, and that he tried to make them fit

Galen's doctrines. The concept of the circulation of blood was not developed until Harvey in the mid 18th century. Yet, after all the development of the ideas about pressure and flow and muscle hypertrophy, Leonardo's drawings still attest to the accuracy of his initial observations in the 15th century.

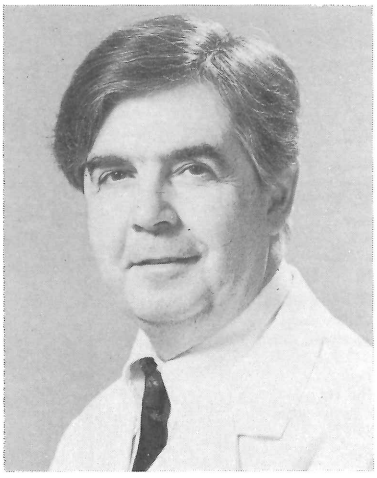
As microscopists, it is very important that we observe carefully, and document accurately what we see. We deal with statis images, but they suggest movement. We may not have an explanation for what we see, and our interpretations may change, but our visual abstractions will remain.

Today the need for basic research is being challenged. Honest scientific inquiry is being discussed heatedly. As scientists who deal with visual images, we are in a unique position to "speak" eloquently for science. There is a Chinese proverb that says, "The eyes believe themselves, the ears believe others."

Fare you well,
Ann Goldstein, Ph.D.
President

The occasion of taking office as President of TSEM is an appropriate time to reflect on one's feelings about the Society and to consider goals that might be set for the coming year. There is a natural temptation to strive for bigger and better, but I expect the number of members will remain more or less the same as it has been over the past few years, with the usual fluctuations as some leave to take up positions outside Texas and others come up to the student ranks. Each of us should, however, feel an obligation to conduct whatever missionary work we can on behalf of the Society, by informing colleagues and students about the activities of TSEM and encouraging them to come along to a meeting to see how good it really is. As far as becoming better is concerned, I personally feel that TSEM is pretty good the way it is. I have had the opportunity to participate in the meetings of a number of the local E.M. societies, and TSEM still sets the standard. The caliber of the scientific content of our meetings, and the opportunity they provide to associate with colleagues from other parts of the state and to learn what is going on in other field of ultrastructural studies, are excellent.

All of this does not mean that we can afford to be complacent. I think one of the most important challenges to the Executive Council is the responsibility of assisting students to attend the meetings, and this is more complex than it might seem since variables such as distance travelled and reimbursement from the parent institution have to be taken into consideration. Nevertheless, we have to devise a system that is equitable and can not be abused. Pooling of transportation and sharing accomodations certainly help, now that costs have escalated to the point where many of us have to budget our travel opportunities very carefully.



The TSEM Journal reflects the Society's excellence, and I am pleased that it is going to appear quarterly, and that there are

President's Message Continued . . .

plans for expansion of the contents, including more original papers, and for wider dissemination to other States, societies and libraries. We are a Texas society but we have a lot to offer and should be willing to share with colleagues outside the state.

From a personal standpoint, the most important thing I can say is that TSEM belongs to each of us, and we have an equal opportunity and responsibility to share in the conduct of its affairs. I believe there has been too little input from individual members in the past regarding the content of the meetings. The Executive Council meets at every meeting, and its members conduct telephone discussions or get together in small groups between meetings to transact business. A question from a member directed to anyone on the Executive Council (names, addresses and telephone numbers are listed on the contents page of the journal) will receive prompt and sympathetic attention, and constructive comments, suggestions, criticisms and even compliments are welcomed. Specifically, if there is anything you would like to see covered at our meetings - a workshop on a technical subject, or a particular topic or person for guest speaker, for example - do let us hear from you. The same invitation is of course extended to our Company representatives. The society must avoid commercial overtones in its meetings, but comments, suggestions, requests and complaints are solicited and will be carefully considered.

Last, but by no means least, I want to record my appreciation to Ann Goldstein, our immediate past president, and Paul Baur, her predecessor in the office, for all they have done on behalf of the society. Their shoes are going to be difficult to fill but I intend to try.

Bruce Mackay

Editor's Message

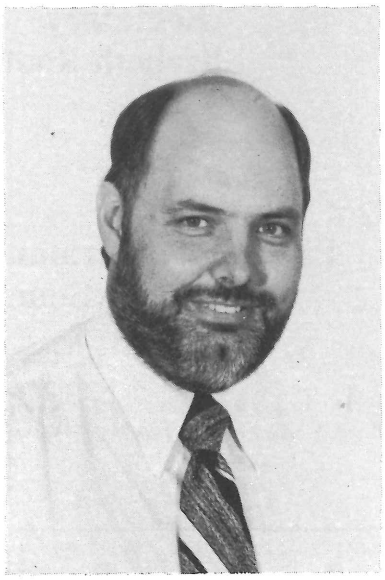
This is the first issue of the "new Journal." Like fine wine, it will continue to improve with age. However, TSEMJ urgently needs your support in the form of proffered papers, regional news, reports, technical notes, helpful hints, etc. This means that the Journal is a reflection of **our** concerted efforts. If we don't hear from you, you won't be able to hear from us. We can't compile blank pages into a plausible Journal issue!

Advertisements are informative, educational, and even sometimes recreational. They are usually of great value to the Journal and its readers. However, I'm going to be very cautious about overselling TSEMJ ad space. There are a few magazines to which I once subscribed that were originally filled with material that was of great interest to me. With time the publishers turned the issues into "advertising rags" with the intellectual text representing far less than half of the total pagination. I got tired of spending my money for 60 pages of ads and 20 pages of topical material. It is my intention to guard against that happening to TSEMJ. It is my hope that our Journal never gets above the 4 to 1 ratio of text to ads. Of course, if we choose to go the other way, to a truly self-sufficient Journal, it will mean that each issue will embody about 20 pages of advertisement and 10 pages of text. With that type of format, our Journal will not flourish!

The meeting in Denton was very nicely arranged and well attended. Dr. Ed Reynolds, Chairman of the Department of Pathology at U.T.M.B. ("Reynolds" lead stain) was our invited speaker. TSEM's immediate past-president also gave an excellent talk!

Bargains are rare! In spite of that, TSEMJ remains a true bargain to its readers. The publication costs average about $3.00 per issue and you get four of them each year. Thus, for your $10 membership fee, you'll receive about $12 worth of publication. Who said miracles don't happen? They do in Texas!

Some student members are occasionally overhead complaining about high registration charges, hotel/motel rents, travel expenses, etc. Well, I'd like to make a comment about this situation. TSEM meetings are exceptional opportunities for students,



to turn their $2 membership dues investment into two or three abstracts during their student years, valuable meeting experiences, easy access to a regional EM Journal, and the opportunity to meet and know those people that can be most helpful, careerwise (laboratory and/or research directors, departmental chairmen, etc.). The bottom line of TSEM's overall investment is that we as a society underwrite the students' registration by approximately $10 per meeting, disburse about $1500 per year in overall student travel, and of course the Journal costs are nearly totally absorbed. Next time you buy a record, swimming suit, or drop a quarter or two into "Pac Man," remember TSEM's impact on your life and how much the society supports you! By the way students, what about a "thanks" now and again. Think about it!

Paul S. Baur, Jr., Ph.D.
Editor, TSEMJ

Editorial Policy Continued . . .

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 these 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; and 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.

1982 ELECTION RESULTS

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Letters to the Editor

I would like to remind the general membership of the supportive role of its corporate members for the Society. These members pay higher dues, keep us informed of the latest in equipment, supplies and methodology, have given us seminars in techniques, supported symposia and special speakers, helped cover meeting costs, directly or indirectly, have "wined and dined" us, and most are regular advertisers in the TSEM Journal which supports its publication.

It will help this relationship to drop by the corporate displays and meet their representatives. It also helps our corporate members, and the Society indirectly, to purchase from them.

Take a look at the corporate displays in Denton and be sure to get their catalogs. They've supported TSEM — let's support them.

W. Allen Shannon, Jr.

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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 _____

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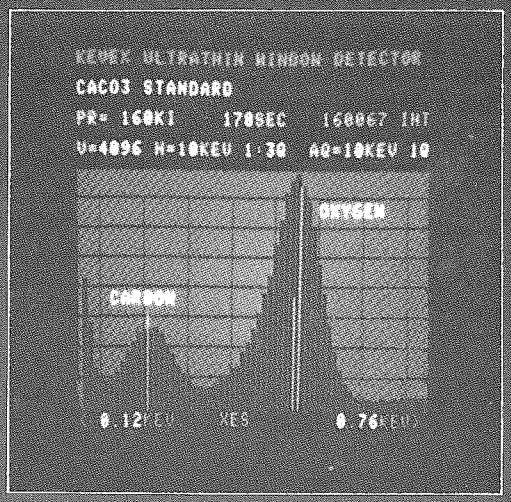

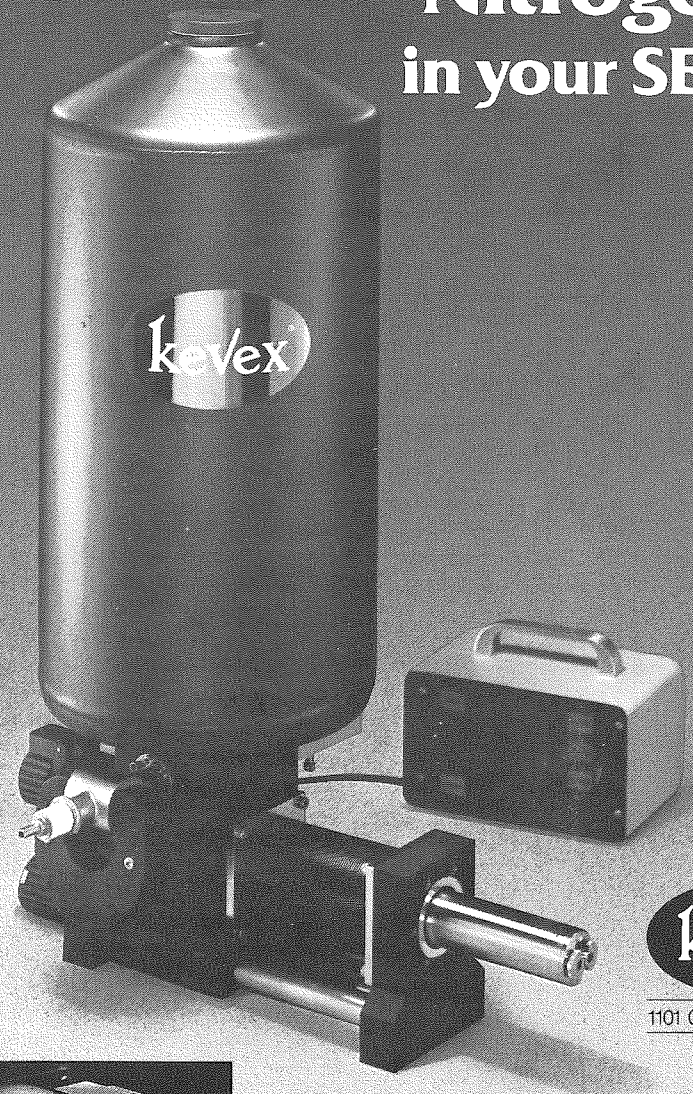

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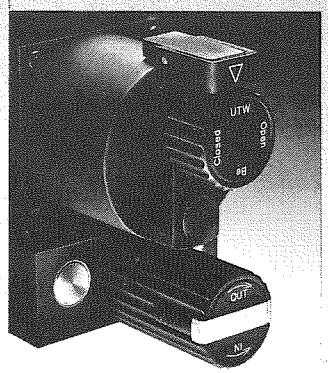

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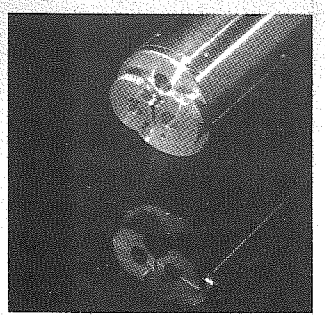

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THE CYTOCHEMICAL LOCALIZATION OF ACID PHOSPHATASE IN PLANT CELLS

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

Phosphatases in plants are a large group of non-specific enzymes that are able to hydrolyse phosphate esters with the concommitant release of inorganic phosphate as a reaction product. These enzymes can be localized cytochemically by two different methods: (1) capture of the "R" group to which the phosphate was originally attached (i.e., the azo dye method), and (2) capture of the liberated phosphate group with lead ions (Fig. 1).

THE AZO DYE METHOD

The most common type of light microscopical licalization of acid phosphatase is the azo dye method (Fig. 2). This method of enzyme localization is based upon the fact that naphthol (which results from the hydrolysis of naphthyl phosphate) can combine with a diazonium salt to give an isoluble colored precipitate (i.e., the azo dye) at the site of phosphatase activity. Unfortunately, however, the azo dye technique for localizing phosphatase activity is not easily modified for electron microscopical cytochemistry. Specifically, the azo dye is (a) partially soluble in post-fixation treatments, (b) not very electron-opaque, and (c) often obscures cytological detail (1). Furthermore, diazonium salts themselves can inhibit phosphatase in plants (2). However, recent improvements in the azo dye technique (3) have led to claims of enzyme localization comparable to those obtained with lead-based techniques (4-6).

LEAD-BASED TECHNIQUES

In spite of the increasing popularity of the azo dye method, lead-based techniques remain the most widely used methods of the electron microscopical localization of phosphatases in plant cells. According to these methods, tissues fixed in aldehyde are placed in an incubation medium containing a phosphorylated substrate and lead ions which immediately trap the phosphate liberated at the site of enzyme activity. Early light microscopists typically converted the insoluble (and difficult to see) lead phosphate reaction product into a black lead sulfide precipitate by treating the tissue with ammonium, sodium, or hydrogen sulfide (Fig. 3). This technique was developed independently by Gomori (7) and Takamatsu (8), and the technique is now known by the former scientist's name (Fig. 4).

Although lead sulfide was shown to be a suitable stain for electron microscopy, investigators soon discovered that lead phosphate is also electron dense and easily detectable with the electron microscope. Thus, the conversion of lead phosphate to lead sulfide was deemed to be unnecessary, and has been eliminated.

Although widely used by cytochemists, the validity of the Gomori-type reaction (depicted in Fig. 4) for the cytochemical localization of phosphatases has been ques-

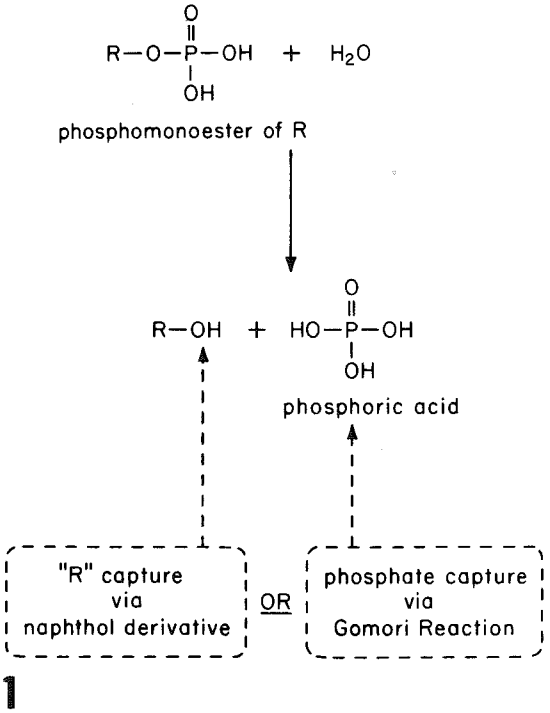


Fig. 1. The two general methods for the cytochemical localization of phosphatases.

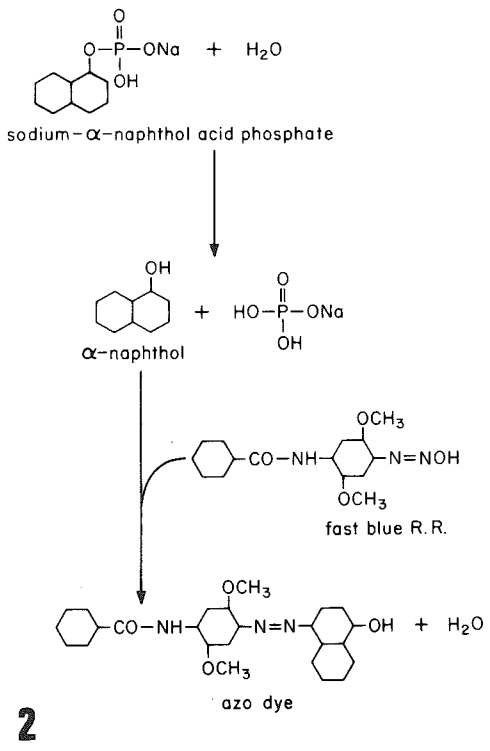


Fig. 2. The azo dye method for localizing acid phosphatase.

tioned by some investigators. The major questions raised by these investigators involve (a) the inconsistent reaction of the nucleus in regard to localization and activity of phosphatase (9, 10), and (b) the diffusion of reaction product from its site of production (11, 12). While disagreements regarding the validity of the technique continue (9, 12, 13), the application of the Gomori-type localization of acid phosphatase has nevertheless produced a great deal of valuable information about the distribution of phosphatases in plant cells (14).

ACID PHOSPHATASE IN PLANT CELLS

One of the most extensively studied phosphatases in plant cells is acid phosphatase (E.C. 3.1.3.2.). Acid phosphatases in plants are quite non-specific (15, 16), a feature that makes them well suited for their participation in the various lytic processes in the cell. Acid phosphatases in plant cells have pH optima that range from 5.0-6.5 and possess broad (but differing) specificities for substrate (17).

Acid phosphatases have been ascribed numerous functions in plant cells, including intercellular transport (18-20), mobilization of food reserves (21, 22), and cellular autolysis (16, 23-25). Although various workers have reported different subcellular locations for the enzyme (20, 26), cytochemists are in general agreement on the absence of acid phosphatase from the cytosol of healthy plant cells (16). Similarly, the loss of this subcellular compartmentation of acid phosphatase (and its subsequent release into the cytosol) has been positively correlated with lethal cellular senescence in plant cells (16, 23-25). Whether this release occurs as the result of, or as the cause of, cellular autolysis remains unresolved (27).

CYTOCHEMICAL PROTOCOL FOR THE LOCALIZATION OF ACID PHOSPHATASE

Although localization procedures for acid phosphatase will doubtless vary with different plants and plant tissues under investigation, we have found the following procedure to be a good "starting point" for acid phosphatase cytochemistry. Following fixation in aldehyde for a minimal length of time, the tissue should be hand-sectioned into slices 40-50 um thick in order to insure penetration of the substrate. The tissue should be washed in 0.05 M Tris-maleate buffer (pH 5.2) for 1 hour, and then placed in a reaction mixture consisting of 40 mM Tris-maleate buffer (pH 5.2), 8 mM sodium-β-glycerophosphate, and 2.4 mM lead nitrate at room temperature. Control sections should be incubated in a reaction mixture either (1) without substrate (sodium-β-glycerophosphate), or (2) with substrate but in the presence of 10 mM sodium fluoride, a phosphatase inhibitor. The sections are then washed with buffer (pH 6.9), post-fixed in osmium tetroxide, and processed for electron microscopical observation by standard procedures. A diagramatical representation of this experimental protocol is given in Fig. 5. Those individuals desiring more specific information on the experimental protocol for acid phosphatase localization are referred to Maier and Maier (28), Moore and Walker (29), and Poux (30).

Although sodium-β-glycerophosphate is the most widely used substrate for the cytochemical localization of acid phosphatase at the electron microscopical level, other substrates have also been utilized. These include p-nitrophenyl phosphate (31, 32) (Fig. 3) and cytidine monophosphate (33).

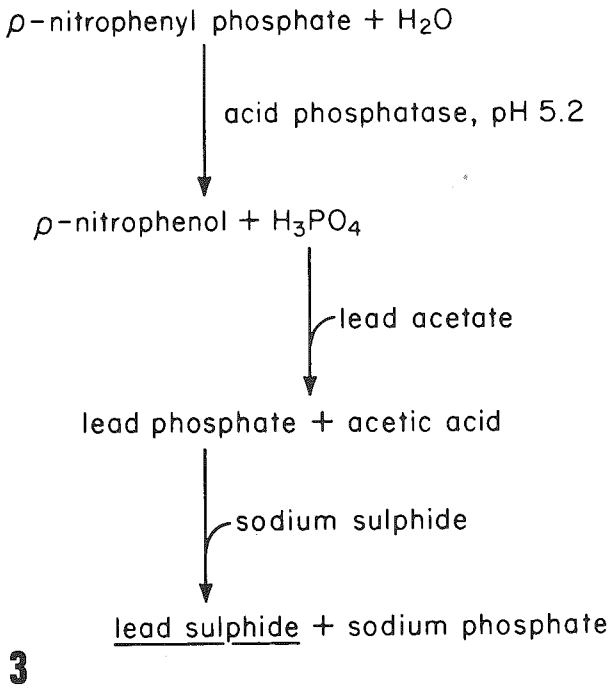


Fig. 3. The lead-based technique for localizing acid phosphatase using p-nitrophenyl phosphate as the substrate.

LOCALIZATION OF ACID PHOSPHATASE IN PLANT CELLS

Acid phosphatase histochemistry as performed according to the above protocol is consistently specific for the designated enzyme and gives precise ultrastructural localization in parenchymal cells of **Sedum telephoides** (Crassulaceae) and **Solanum pennellii** (Solanaceae). Enzymatic precipitate is absent in controls (i.e., those trials with inhibitor and minus substrate). A representative control is shown in Fig. 6.

The precision afforded for the cytochemical localization of acid phosphatase in plant cells by the above protocol is shown in Fig. 7. Although not stained with uranium or lead, the lamellar nature of plastid membranes that are sectioned obliquely is nevertheless discernable (Fig. 7, single arrows). The plastid membranes cut in cross section become especially prominent after cytochemical staining (Fig. 7, double arrows).

The distribution of acid phosphatase in typical internodal cells of **Solanum** and **Sedum** is shown in Figs. 8-9. The majority of enzymatic activity (indicated by the black precipitate) is associated with the plasmalemma and plasmodesmata, with lesser activities associated with the dictyosomes and mitochondria. Similar localizations of acid phosphatase activity have been reported previously in other systems (16, 20). The association of enzymatic activity with the plasmalemma and plasmodesmata has prompted other investigators to suggest a role for acid phosphatase in cellular transport processes (18-20)

Internodal cells of **Sedum** and **Solanum** differ in regard to acid phosphatase activity along the tonoplast (i.e., the vacuolar membrane). The cytochemical localization of acid phosphatase in the vacuole of **Solanum pennellii** (Fig. 8) is consistent with an earlier report in which Pitt and Galpin (34) utilized cell fractionation techniques to demonstrate vacuolar activity of the enzyme in **Solanum tuberosum. Sedum** cells, on the other hand, do not exhibit a vacuolar activity of acid phosphatase (Fig. 9). This absence of a cytochemically-demonstrable activity of acid phosphatase in the vacuole of undisturbed **Sedum** cells is somewhat unexpected, since other investigators have reported the enzyme to be present in the vacuole of other CAM plants using organelle isolation procedures (37, 38). However, organelle isolation procedures involve traumatizing the cells, which doubtless induces wounding and could thus be responsible for the increased activity of acid phosphatase in these studies (34-36). Indeed, wounding does induce a dramatic rise in the vacuolar activity of acid phosphatase in **Sedum** cells (29) (Figs. 10, 11). Furthermore, it is noteworthy that acid phosphatase has been reported to be absent from the vacuole of other plant cells (39).

Thus, low amounts of acid phosphatase activity are associated with cellular membranes and organelles in internodal cells of **Sedum** and **Solanum.** The consistent absence of enzymatic activity from the cytosol of healthy cells is consistent with previously published data on acid phosphatase localization (16). Conversely, cellular necrosis in **Sedum** cells has been correlated with the release of acid phosphatase into the cytosol (29).

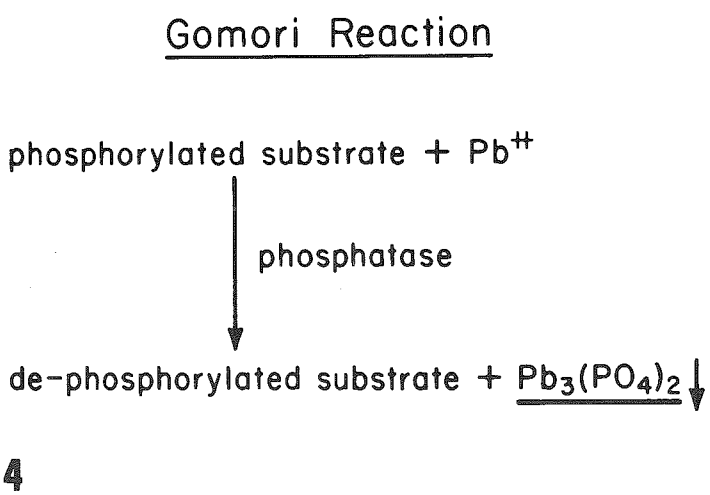


Fig. 4. The Gomori reaction for acid phosphatase cytochemistry.

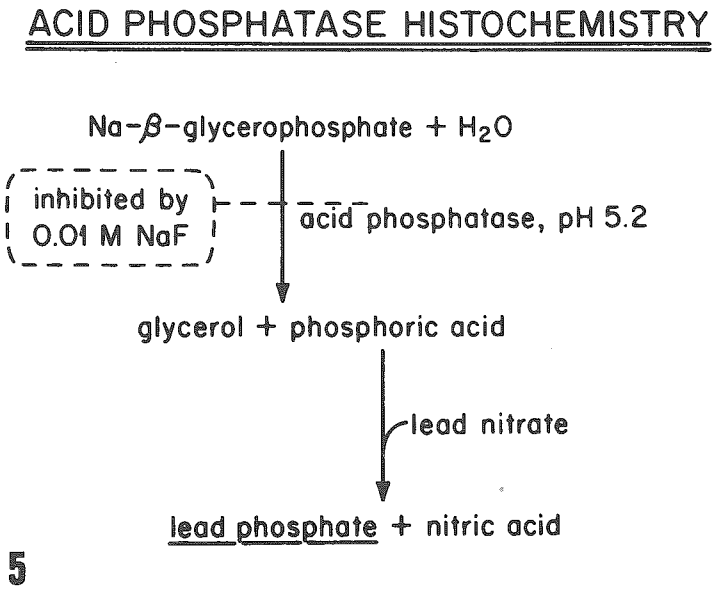


Fig. 5. The lead-based technique for localizing acid phosphatase using sodium-β-glycerophosphate as the substrate. A positive control includes the addition of sodium fluoride, a phosphatase inhibitor.

ACKNOWLEDGEMENTS

This work was supported in part by research grants from Baylor University, Sigma Xi, and the American Philosophical Society. The author is grateful to Steve Ransom for technical assistance.

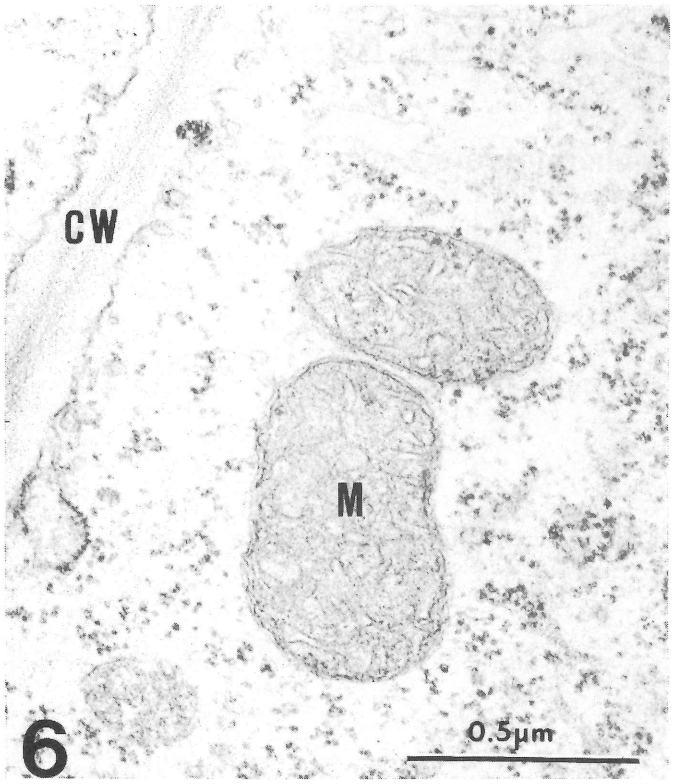


Fig. 6. Representative control section of an internodal cell of **Sedum** incubated without substrate but in the presence of lead. Sections incubated in the presence of substrate and 0.01 NaF showed a similar absence of reaction product throughout the cell. CW=cell wall; M=mitochondria. 63,000 X.

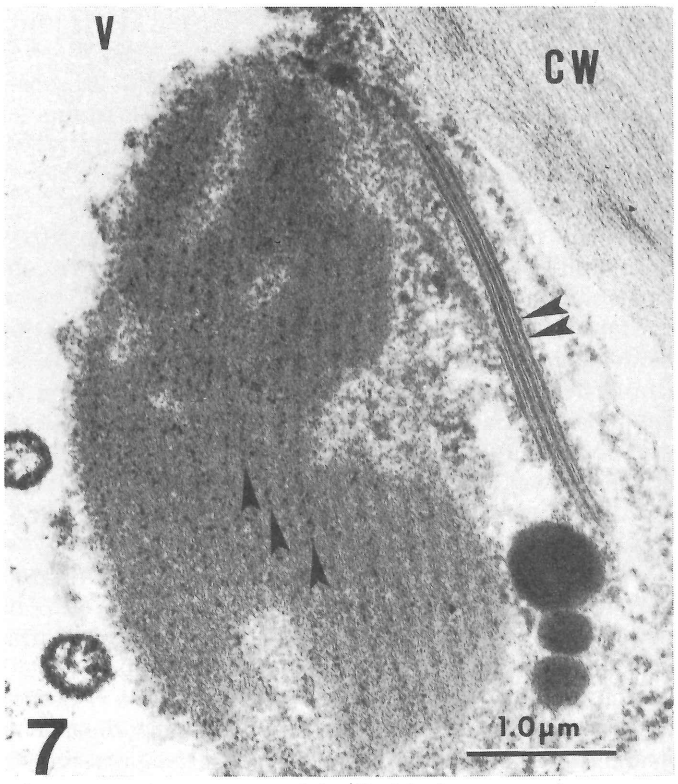


Fig. 7. Plastid in an internodal cell of **Sedum** stained cytochemically to demonstrate the localization of acid phosphatase. Single arrows indicate reaction product on obliquely sectioned thylakoids. Double arrows indicate thylakoids sectioned in cross-section. CW=cell wall; V=vacuole. 20,000 X.

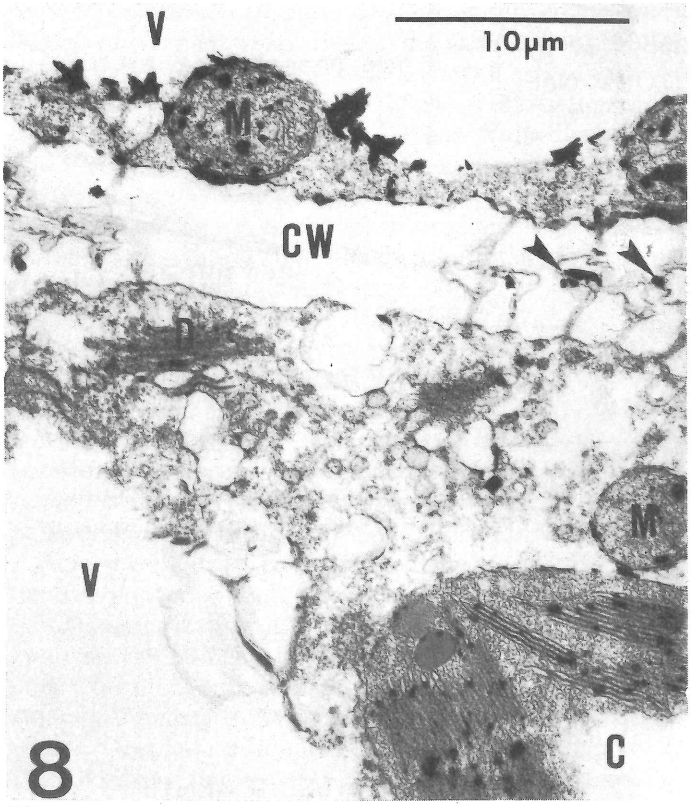


Fig. 8. The distribution of acid phosphatase in a typical internodal cell of **Solanum.** Arrows indicate reaction product at plasmodesmata. C=chloroplast; CW=cell wall; D=dictyosome; M=mitochondrion; V=vacuole. 31,500 X.

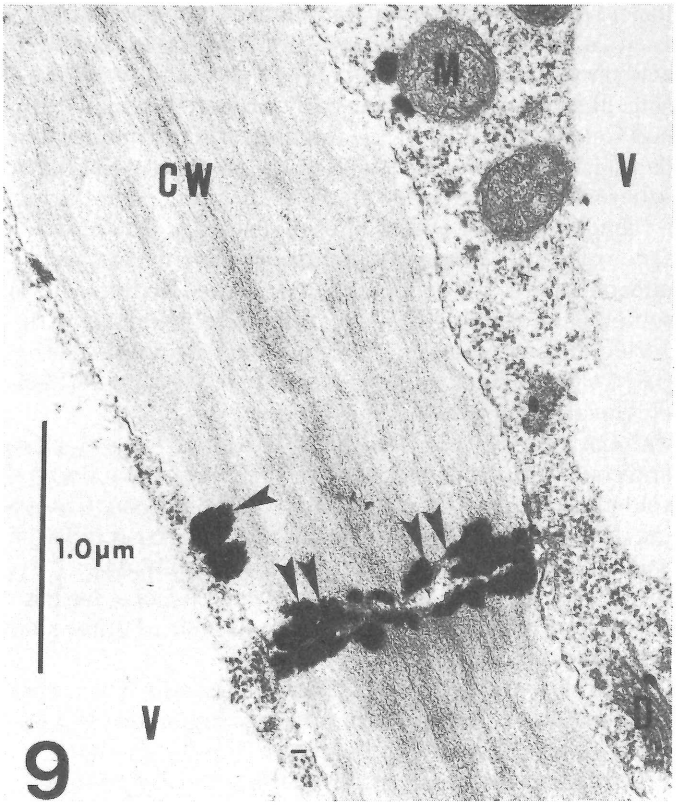
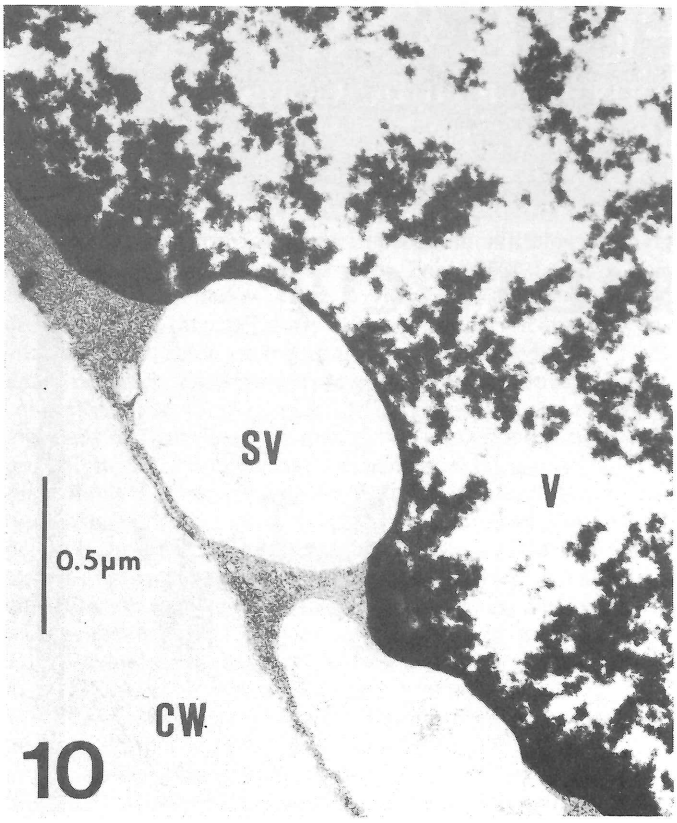
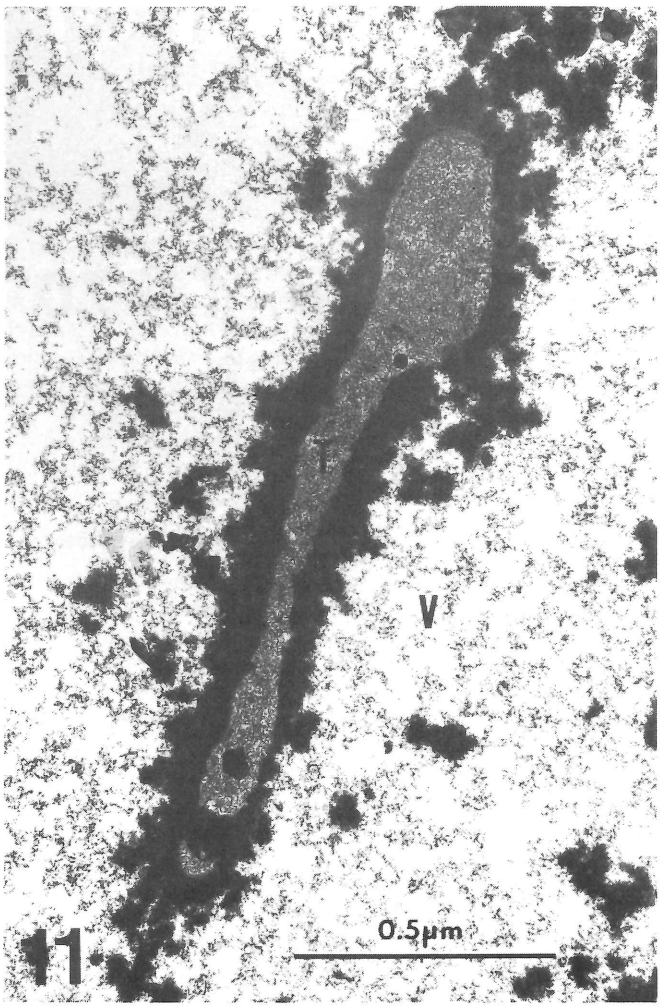


Fig. 9. The distribution of acid phosphatase in a typical internodal cell of **Sedum.** Double arrows indicate reaction product at plasmodesmata; single arrows indicate reaction product at plasmalemma. CW=cell wall; D=dictyosome; V=vacuole. 32,800 X.





Figs. 10-11. The distribution of acid phosphate in **Sedum** cells 7 days after wounding. The majority of the enzymatic activity is associated with the vacuolar membrane. CW=cell wall; SV=small vacuole; T=transvacuolar strand; V=vacuole. Fig. 10: 21,800 X; Fig. 11: 31,800 X.

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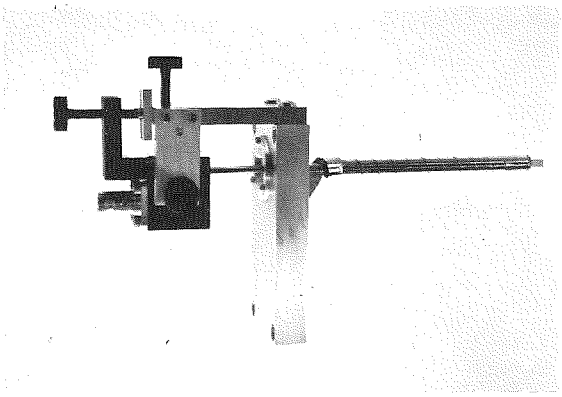

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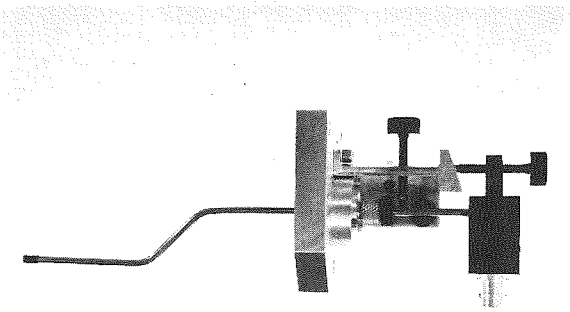

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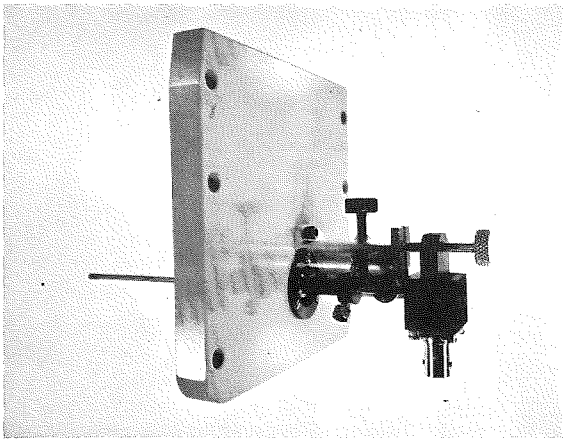


JSM U-3

Abstracts

HORMONAL CONTROL OF STORAGE PROTEIN MOBILIZATION IN SUNFLOWER COTYLENDONS. Randy D. Allen and Howard T. Arnott, Department of Biology, University of Texas at Arlington, Arlington, Texas 76019.

Protein storage bodies which occur in a variety of seed tissues are hydrolyzed intracellularly during germination and the constituent amino acids are transported to the developing embryonic axis. Observations of germinated sunflower cotyledons with scanning electron microscopy suggest that protein body hydrolysis occurs during two successive periods of proteolytic activity. The first period occurs uniformly throughout the cotyledon on about day 2 post imbibition (PI) and results in fluidization and fusion of protein bodies. The second period begins during day 3 PI in cells adjacent to the embryonic axis and spreads progressively to more distal cells on succeeding days. Protein is transported from cotyledon cells during the second period and all cells appear devoid of storage proteins by day 5 or 6 PI. Additional observation of excised cotyledons germinated either in the presence or absence of isolated embryonic axes suggest that the first wave of protease activity is axis independent. However, the second proteolytic wave does not occur in the absence of germinated axis tissue. Early, low level storage protein hydrolysis may be mediated by enzymes endogenous to protein bodies. Later, complete protein hydrolysis is facilitated by enzymes produced **de novo** within the cotyledon in response to hormonal signals from the embryonic axis. Gibberellic acid appears to trigger storage hydrolysis in many monocot seeds. However, cytokinins have been implicated in a similar role for some dicot seeds. Preliminary experiments involving externally supplied hormones indicate that kinetin is capable of replacing the axis effect on storage protein hydrolysis in sunflower.

FINE STRUCTURE OF THE TEGUMENT AND ASSOCIATED STRUCTURES IN THE TAPEWORM CITTOTAENIA. Kemi I. Adewusi, Department of Biology, Stephen F. Austin State University, Nacogdoches, Texas 75962.

The primary objective of this study was to examine using both TEM and SEM the external covering or tegument of the tapeworm **Cittotaenia**. The specimens used in this study was obtained from the intestine of the cottontail rabbit **Sylvilagus floridanus**. Freshly collected specimens were quickly rinsed in isotonic saline to remove any adhering intestional debris and then prepared for examination with both TEM and SEM.

Tapeworms lack both digestive and respiratory systems and the absorption of nutrients and gas exchange take place through the tegument. As a result, the fine structure of the tegument is of considerable interest. In **Cittotaenia** the tegument has two distinct regions of cytoplasmic modifications, the distal cytoplasm and the perinuclear cytoplasm that lies in the parenchyma. A fibrous zone which is continuous with the parenchyma separates the two regions. Cytoplasmic processes connect the distal and perinuclear cytoplasm. The free surface of the tegument is covered by projections called microtriches. These microtriches are covered by a plasma membrane that is continuous with the limiting membrane of the distal cytoplasm. Each microtrix has two distinct regions, the shaft and the base.

FIBROCLAST RECRUITMENT IN VITRO, P.S. Baur[1,2], and R.A. Cox[2], [1]Shriners Burns Institute, 610 Texas Avenue, Galveston, Texas 77550; [2]Graduate School of Medical Sciences, University of Texas Medical Branch, Galveston, Texas 77550.

Fibroclasts (sic) mediate soft connective tissue degradation in normal skin, granulations, wound healing tissues, and scars. This cell-mediated degradation of interstitial collagen is most pronounced during the "remodeling phase" of wound repair with fibroclasts accounting for as many as 35% of the dermal cells observed in a mature scar. Our evidence suggests that fibroclasts first phagocytize interstitial collagen fasicles and then enzymatically digest the collagenous fibril or filament fragments vis a vis lysosomal activity. Fibroclasts appear to be derived from fibroblasts and/or myofibroblasts.

In a previous **in vivo** study, we reported that the fibroclast population could be significantly increased if the soft connective tissues were perfused **in situ** with a particulate suspension of collagen fragments or latex spheres. To determine if fibroclasts could be recruited **in vitro,** we added a suspension of 0.5 μ diameter latex particles to a human foreskin fibroblast culture being maintained in a minimum essential media (MEM) at 37°C. Samples of treated and control culture cells were collected 2, 4, 8 and 24 hours later and were processed for transmission and scanning electron microscopy (TEM-SEM) using routine preparative procedures. Stages of phagocytosis, mediated by the fibroblasts, were observed by SEM while the TEM surveys documented the formation of phagosomes, phagolysosomes and tertiary lysosomes in those same cells. Both lines of microscopic evidence strongly suggest that fibroclasts can be recruited **in vitro** and further imply that a population of predominantly synthetic cells may be transformed to one that is primarily comprised of degradative cells.

CALCIUM OXALATE CRYSTALS IN FOREST LITTER. H.J. Arnott, Department of Biology, University of Texas at Arlington, Arlington, Texas 76019.

Forest litter from the Wasatch National Forest, Utah County, Utah, consisted of a mixture of angiosperm and gymnosperm leaves. Aspen, fir, Douglas fir and spruce are native in the local area. The litter was composed of compressed leaves which often were white in color. The white color comes from calcium oxalate crystals associated with hyphae that grow on the surface and in the spaces between leaves. The crystals have been identified by x-ray diffraction as weddellite (calcium oxalate dihydrate) and the litter studied by SEM. The crystals are needle-shaped and may reach a length of 22 μm with a thickness of about 1μm. In some cases the hyphal strands may be completely covered with these acicular crystals, and in other cases they are somewhat separated along the hyphae that bear them. The hyphal strands bearing the crystal may cover the surface completely in some areas while in other parts of the litter they are sparce. Observation of the hyphae in the sparce areas revealed many hyphae in the process of crystal formation. This process appears to occur inside the hyphae, and as the crystals grow, they are exteriorized. It is probable that these hyphae and the crystals they produce are important in soil evolution (Graustein, W.C., et al. 1977. Science 198:1252).

CALCIUM OXALATE CRYSTALS IN THE LEAVES OF OXALIS. H.J. Arnott, Mary Lou Kelly and Mary Alice Webb. University of Texas at Arlington, Arlington, Texas 76019.

In a continuing effort to examine crystal systems in the leaves and other organs of plants, crystals of the leaf of **Oxalis violacea** L. were examined using SEM and LM. The plants used in this study were grown in our greenouse. Crystals were isolated

from the leaves using techniques described in Arnott and Workman (Scanning Electron Microsc. 1981; III: 293-298, 250) and are of a completely unique character. Careful attention to the nature of the crystals in the living leaf was made because of this unique character (appearance of errosion). The crystals have the same general shape and dimensions inside the cells that produce them as they have when isolated. Under crossed nicols, the crystals are strongly birefringent and show two and three orders of diffraction colors. This and acid solubilities point strongly to the crystals being whewellite (calcium oxalate monohydrate). With SEM, the crystals appear to be very bizarre twins which often have irregular shapes, unlike those reported in any other plant or in the seeds of Oxalis. The most prominent characteristic of these crystals is two flat faces which parallel the twin plane. Extending from these faces are many ridges which run toward the twin plan at an angle. The surface faces sometimes appear to be geometric in shape, but others are completely irregular. Occasionally, irregular but recognizible druses and prismatic crystals are seen.

THE MYOFIBROBLAST ANCHORING STRAND-A CELL TETHER WHERE COLLAGEN ARE PUT TOGETHER. P.S. Baur[1,2], J.D. Hudson[2] and R.A. Cox[2], [1]Shriners Burns Institute, 610 Texas Avenue, Galveston, Texas 77550; [2]Graduate School of Medical Sciences, University of Texas Medical Branch, Galveston, Texas 77550.

Myofibroblast anchoring strands (MAS) are extracellular fibrous structures that connect the fibronexus of myofibroblasts to collagen fasicles in the extracellular spaces. MAS are 40-500 nm in diameter and up to 60 Gm in length. The strands appear to be comprised of 20-50 A diameter microfibrils of fibronectin, 100-110 A diameter intermediate sized filaments (ISF), and an amorphous matrix composed of collagen. ISF can also be observed scattered throughout the cytoplasm and incorporated in parallel arrays within the matrix of the contractile bundles. The constituent ISF of each strand often is observed in a continuum with what appears to be forming collagen fibrils suggesting that the MAS are the loci of collagen assembly. Within the matrix of the MAS the linear force required for cell migration (produced by bundle contraction) is translated into a force which aligns and compresses the ISF together into the fibrils. The migration events also deform the collagenous structures to which the cells are attached. At the site of fibril assembly the fibronectin component of the strand is then utilized to cement the newly formed fibrils to the collagen filament being reinforced.

ASSAY OF RADIATION AND DRUG INDUCED CHROMOSOME DAMAGE THROUGH THE CELL CYCLE. Arthur Cole, Ruthann Langley and Margaret Hall; The University of Texas M.D. Anderson Hospital, Houston, Texas 77030.

We have previously reported on the structure of CHO cell chromosomes through the cell cycle (J. of Texas Soc. for Electron Microscopy 11 (3); 33 (1980) and 12: (3); 18 (1981)). The evidence supports the proposal that eight circular DNA subunit molecules, arranged side-by-side, extend the full length of the chromatid and loop outward between attachment sites spaced every 0.1 Gm along a backbone structure. The backbone appears to be closely associated with the nuclear membrane during cell interphase. **In-vivo** and **in-vitro** treatment of cells or chromosomes with radiation or drugs induced damage which could be assayed as aberrations in backbone and DNA loop structures. In general the damage is expressed to a greatest extent in metaphase chromosomes, with later stages of mitosis or cell interphase exhibiting less damage. Reduced sensitivity is believed to be related to a progressive organization of the chromosome backbones to form a stable nuclear cage in interphase cells. The sensitivity of the assay is increased by exposing chromatin to very low concentrations of proteases or nucleases that do not affect untreated samples. The results will be illustrated with stereo electron microscope projections. Supported in part by DOE contract DE-AS05-76EV02832.

BACTERIAL ATTACHMENT TO ACID-MODIFIED BLADDER MUCOSA. C.P. Davis and A.E. Avots-Avotins. Department of Microbiology, University of Texas Medical Branch, Galveston, Texas.

Short acid treatment of bladder epithelium has been reported to remove bladder mucin and trigger increased bacterial adherence to bladder mucosa. The technique of short time (60 seconds) acid modification of bladder mucin as a means to evaluate bacterial interactions with bladder mucin and mucosal cells was examined. Using a rat model system, the experiments indicated that short term acid treatment removed the mucin layer but it also modified the bladder epithelial cell surface. The modification consisted of removal of transitional epithelial cells from the mucosal surface. Some areas showed that large numbers of cells were removed. In addition, cell borders were no longer continuous. Although the treatment which removed mucin allowed an increase in non-localized bacterial attachment, the increase could also be attributable to acid modification of the mucosa. Examination of rabbit bladders also showed that mucin removal and bladder epithelium modification occurred simultaneously. The changes observed in rabbit bladders were essentially the same as those described for rat bladders. Consequently, we conclude that short term acid treatment removes mucin and modifies the epithelial layer. The data also suggests that acid treatment nonspecifically increases bacterial attachment on the mucosa.

DEMONSTRATION OF RNA WITHIN ANAPLASMA MARGINALE BY DIFFERENTIAL STAINING. Robert E. Droleskey[a], Gerald G. Wagner[b] and Hilton H. Mollenhauer[a] [a]USDA, ARS, VTERL, P.O. Drawer G.E., College Station, Texas 77841 and [b]Center for Tropical Animal Health, Dept. of Vet. Micr. and Parsit., College of Vet. Med., Texas A&M University, College Station, Texas 77843.

Anaplasma marginale is a rickettsia that infects bovine erythrocytes causing anemia and, in severe cases, death. Although the ultrastructure of **A. marginale** has been well documented, its nucleic acid content remains unclear. The presence of DNA within **A. marginale** has been demonstrated, but reports concerning the presence of RNA have been inconclusive. In this study, **A. marganale** infected blood was processed and stained according to the Bernhard method for differentially staining RNA from DNA (1). Differentially staining **A. marginale** revealed RNA which appeared as distinct granules within the initial body. Filamentous material, possibly DNA, sometimes radiated in a spoke-like fashion from these granules. Ribosomes contained within blood cells present in the preparation served as controls in that they stained positive for RNA. The presence of RNA within **A. marginale** is important in the further elucidation of its **in vivo** life cycle and to the prospects of genetically altering the organism.

[1]W. Bernhard, J. Ultrastruct. Res. 27:250 (1969).

DIURNAL VARIATION IN THE SIZE OF SEVERAL CELLULAR COMPARTMENTS WITH EMPHASIS ON CHLOROPLAST/MITOCHONDRIAL MEMBRANE SURFACE AREA IN LEAF PALISADE CELLS OF SUNFLOWER. W.R. Fagerberg, Dept. of Biology, Southern Methodist Univ., Dallas, Texas 75275.

Diurnal variation in biochemical, physiological and

behavioral activities is well documented. Such variation in cell structure has not been thoroughly examined. In this case palisade cell structure was described using stereological parameters (percent volume, V_V; surface to volume S_V ratios). ANOVA-LSD and Mann-Whitney statistics showed that significant differences occurred in V_V values for the chloroplast, vacuole, oil and starch compartments over a 24 hour period. Significant changes also occurred in the S_V ratio of chloroplast and mitochondrial membranes between light and dark sampled cells. In addition to restructuring the relative size of organelle compartments (V_V) the "mean" palisade cell showed significant changes in actual volume over 24 hours. Since the expression of the ratio values in absolute terms (Gm^3, Gm^2) is related to "mean" cell vol., changes in cell vol. affected the actual volume of each organelle compartment. The most spectacular change occurred in the chloroplast membrane surface area which more than doubled during the 24 hour period with maximum values 6 hours into the dark period. The possible role of multivesicular bodies in rapid membrane turnover will be discussed.

ELECTRON MICROGRAPH STUDY OF MORPHOLOGICAL CHANGES IN MICROCOCCUS LUTEUS INFLUENCED BY THE PHYSICAL STATE OF THE MEDIUM. Shellye Gathings, M. Louise Higgins and Robert Fuerst, Microbiology Research Laboratory, Department of Biology, Texas Woman's University, Denton, Texas 76204.

Bacteria are often prepared for transmission electron microscopy by growth in broth culture and subsequent suspension of the pelleted cells in agar (Noble). The agar suspension is then treated as a tissue in preparation for sectioning. Growth in a broth culture provides the bacteria with the proper environment as the cells are bathed in nutrients and waste products are constantly being removed and diluted. If agitation is not supplied, a layering of available oxygen confines the growth to optimum areas.

Micrococcus luteus colonies were grown using the same nutrient and temperature parameters but with the addition of the solidifying agent, two per cent agar. A surface grown colony would have different spheres of gas, moisture, nutrients, and concentrations of waste than would cells grown in broth. Individual colonies were fixed and embedded for sectioning and the micrographs were examined. Cell wall thickness and deviations in planes of division were compared for indications of environmental influences.

APPEARANCE OF GRANULAR CELLS NEAR LUNG ARTERIOLES IN CHICK EMBRYO TREATED WITH DEXAMETHASONE. Eric Gilchrist and R.V. Blystone, Dept. of Biology, Trinity University, San Antonio, Texas 78284.

The synthetic glucocorticoid, dexamethasone, is known to enhance lung surfactant appearance in mammalian feti and avian embryos. Preliminary observations indicate that dexamethasone may also affect the vasculature and connective tissue of the embryonic bird lung as well.

White leghorn chick embryos were given a single lung injection of dexamethasone phosphate intravenously at one of five different incubation ages (15 through 19 days incubation). Embryos were sacrificed at 20 days incubation and lung tissue prepared for and examined by light and transmission electron microscopy.

Changes in the lamellar inclusions were observed and these results have been reported elsewhere. Arterioles in the connective tissue of treated avian lung frequently displayed "granular cells" around their perimeter. Data subjected to one way analysis of variance confirmed that treated tissue had a significantly larger number of these "granular cells" around lung arterioles than control. Positive identification and function of these arteriole connected "granular cells" has yet to be determined.

AN SEM STUDY OF TWINNED CRYSTALS IN THE BEAN FRUIT. Mark J. Grimson and H.J. Arnott, Department of Biology, University of Texas at Arlington, Arlington, Texas 76019.

Kinked and straight crystals of calcium oxalate monohydrate were found in the fruit of the common bean **(Phaseolus vulgaris)**. A winged condition is characteristic for these crystals which have ridges running along both sides of the crystal. A ridge extends unevenly along the length of the crystal and is present in both the straight and kinked form. The crystals are found within a sheath which appears continuous with the cell wall and are generally termed Rosanoffian crystals. Scanning electron microscopy and light microscopy were used to show twinning of the crystals as well as to determine crystallographic and extinction angles of the twinned counterparts. The contact twinning in the crystals occurs by two basic crystallographic laws, a mirror image along a twin plane (kinked) and rotation about a twin axis (straight). The crystals appear to be a model monohydrate twin, with a standard twinning plane and twinning angles indicative of the monohydrate. The apparent random distribution of kinked and straight crystals throughout the tissue suggest that the morphology at the twin plane (i.e. straight vs. kinked) is due more to random thermal influences than to direct genetic control. Due to the relative simplicity of the twinned bean crystals, they could represent an excellent model system for the study of twinning in more complex crystals such as druses.

ULTRASTRUCTURE ANALYSIS OF NEURITE TRAUMA AND LESIONS PRODUCED IN CELL CULTURE WITH PULSED UV LASER MICROBEAMS. Guenter W. Gross and M. Louise Higgins, Department of Biology, Texas Woman's University, Denton, Texas 76204.

The simplication of neuronal monolayers in culture is a vital step in the **in vitro** analysis of circuit development and behavior. We have developed methods for the selective deletion of cells and the precise transection of neurites in closed chambers during high magnification light microscopy. A UV laser emitting 12 ns pulses at 337 nm, coupled to a microscope, is used to carry out this surgery with a precision of %0.5 Gm.* Two immediate and one delayed transection techniques have been established: 1) direct single shot transection due to cytoplasmic cavitation; 2) indirect single shot transection due to minute shock waves from vaporizing substrate and 3) multiple shot, low energy density transection due to unknown slower cytoplasmic reactions at the laser focus. We are analyzing these effects with TEM and SEM. Damage is greatest with technique 2 which causes membrane disruptions, swelling of mitochondria, vacuolization and formation of electron dense areas in regions as far as 4 Gm diameter impact site. This represents primarily physical damage from the fluid shock wave. Small cytoplasmic cavitations produced with method 1 show such damage only in the laser focus. However elastic readjustments in the cytoskeleton obscure local effects, especially in cell processes. Method 3 causes cytoplasmic pinching, and slow transection. Cytoskeletal changes responsible for this phenomenon have not yet been identified.

*Higgings, Smith and Gross, J. Neurosci. Meth. **3** (1980) 83-99.

AN SEM AND X-RAY DIFFRACTION STUDY OF THE CALCIUM OXALATE DIHYDRATE (WEDDELLITE) CRYSTALS IN THE LEAVES OF BEGONIA. Mary Lou Kelly and H.J. Arnott. Department of Biology, University of Texas at

Arlington, Arlington, Texas 76019.

The leaves of **Begonia maculata** Raddi contain massive numbers of birefringent crystals throughout the lamina and petiole. Leaf clearings showed the crystals evenly dispersed throughout the leaf. Using techniques reported previously, pure samples of clean crystals were extracted and isolated. For the most part, the crystals were isolated using 95 to 100% ETOH in order to obviate the chemical change from weddellite to whewellite (calcium oxalate monohydrate) which occurs in aqueous solutions. The isolated crystals were identified by powder x-ray diffraction and confirmed as weddellite. Their relatively low birefringence, acid solubilities, and basic tetragonal shape are in accord with this identification. The basic crystal morphologies are bipyramidal octahedrons (8-sided crystal) and dodecahedrons (12-sided crystal), characteristic of weddellite. The druses appear to originate with a large single crystal as the basis for the development of interpenetrant crystals which occurs on the surface. Additional crystals then develop on the surface of these. In simple cases the twinning can be clearly analyzed. Many multiple interpenetrant twins become druse-like and may be composed of tens of crystals in which the plane of twinning is not readily obvious. The crystals can be observed **in situ** by cryofracturing glutaraldehyde-osmium-fixed materials.

ULTRASTRUCTURE OF THE WOUNDED TREE BEETLE, NOSODENDRON CALIFORNICUM. David L. Kulhavy[1], Robert W. Roberson[2], and Mark N. White[1], [1]School of Forestry and [2]Department of Biology, Stephen F. Austin State University, Nacogdoches, Texas 75962.

The wounded tree beetle, **Nosodendron californicum** Horn, inhabits slime fluxes of true firs in the Pacific Northwest and the California live oak and true firs in California. Scanning electron microscopic examination of the beetle revealed vestiture on the elytra (protective wings) that may be mechanical structures to move exudate from the surface of the beetle. These setae are arranged in tufts of ten spaced at two eye-diameters apart. The distal portion of these setae are sculpted. Elytral punctures interspersed between the setal tufts each have a peg-like structure arising from a pit. Each pit has a minute pore into the eleytron. This pattern is repeated on the scutellum and the head. Intraommatidial structures may provide lubrication for the eye. The ventral surface of the beetle has short peg-like setae located by shallow depressions. These observed structures may assist the beetle in moving through the slime flux exudate.

THE EFFECT OF INTERMEDIATE FILAMENT INHIBITORS ON STEROIDOGENESIS AND CYTOSKELETON IN Y-1 MOUSE ADRENAL TUMOR CELLS. Hyun S. Lee* and James J. Mrotek. Dept. of Biol. Sci., North Texas State University, Denton, Texas 76203.

The cytoskeleton of the Y-1 mouse adrenal tumor cells consists of microfilaments, intermediate filaments (IF), and microtubules. By using cytochalasins and electron microscopy, Mrotek demonstrated that microfilaments were involved in mediating the steroidogenic response of Y-1 cells to ACTH (Biochemistry, 16:3177). Anti-microtubule agents do not inhibit the acute (30 min) response to ACTH by Y-1 cells (Gen. Pharmacol., 9:269). In an attempt to elucidate the influence of IF on steroidogenesis in Y-1 cells, we have investigated the effects of anti-intermediate filament agents on steroid production, and on the cytoskeleton. When cells were treated with sodium orthovanadate, which is known as IF inhibitor in BHK21-F cells, there was no change in the amount of steroid produced. Another IF inhibitor, 3,3'-iminodipropionitrile which affects neurons, was found to affect the ability of Y-1 cells to produce steroid in response to ACTH. Whole cytoskeletal changes as well as the changes in the arrangement of IF, due to these anti-intermediate filament agents, were examined by transmission and scanning electron microscopy. (Supported by NIH Grant AG 01055).

ISLET CELL TUMORS. Bruce Mackay and Nelson G. Ordonez, University of Texas, M.D. Anderson Hospital and Tumor Institute, Houston.

Several cell types with differing functional activity have been identified in islets of Langerhans of the animal and human pancreas, and it is not surprising that a variety of islet cell tumors, benign and malignant, occur, differing in their histology and hormone production. Transmission electron microscopy is often helpful in the diagnosis of these neoplasms, particularly when the tumor is malignant and presents as a metastasis in the liver or some other extrapancreatic location. The light microscopic histology may reveal or suggest that the tumor is of endocrine origin, but some islet cell tumors have unusual histologic features, and demonstration of dense-core granules by TEM may be necessary to prove that the cells are indeed endocrine. The contribution of TEM is limited to establishing that granules are present and showing their number, size and shape, but in some instances the morphology of the secretory granules does suggest the primary site of a metastatic tumor. Usually immunocytochemical studies are required to reveal the functional nature of the tumor cells, and the immunocytochemical findings must be interpreted with the awareness that a tumor cell may form more than a single hormone, or may produce a hormone that differs from the one normally produced by that particular cell type.

EFFECTS OF EXOGENOUS STEROIDS ON THE ADRENAL PLASMA MEMBRANE: ALTERATION OF STEROIDOGENESIS AND CELL MORPHOLOGY. Mark P. Mattson and J.J. Mrotek. Dept. of Biol., Sciences, North Texas State University, Denton, Texas 76203. (Supported by NIH grant AG 01055).

Experiments were conducted to determine and localize the effects of exogenous steroids on steroidogenesis in adrenocortical cells. Using cultured Y-1 mouse adrenal tumor cells which produce the steroid 20x-hydroxypregn-4-en-3-one (20-DHP) it was found that corticosterone and deoxycorticosterone at 10-5 M significantly increased 20-DHP production in a dose-dependent manner in 30 min and 120 min incubations. This stimulation was inhibited by the microfilament inhibitor cytochalasin D, indicating a mode of steroid stimulation similar to that of the hormone ACTH. Exogenous steroid significantly inhibited ACTH-stimulated 20-DHP production in a dose-dependent and reversible manner in 30 and 120 min incubations, preventing the normal rise in intracellular cAMP associated with ACTH stimulation. Exogenous steroids did not significantly inhibit $(Bu)_2$cAMP-stimulated or cholera toxin-stimulated steroidogenesis, indicating a site of steroid inhibition of ACTH-stimulated steroidogenesis occuring between ACTH binding to the cell surface receptor and adenyl cyclase activation. Other steroids tested (cortisol, cortisone, dehydroepiandrosterone, testosterone, aldosterone, dexametnasone) did not stimulate 20-DHP production, and varied in their ability to inhibit ACTH-stimulated 20-DHP production. Scanning electron microscopy indicated that exogenous steroids altered cell surface morphology, also suggesting that steroids affect the plasma membrane.

HOMOGENEITY OF ZONES IN THE SHOOT APICAL MERISTEM OF ECHINOCEREUS ENGELMANNII (CACTACEAE). James D. Mauseth, Department of Botany, University of Texas, Austin, Texas 78712.

Median longitudinal sections of shoot apical meristems of **Echinocereus engelmannii** were photographed such that the location of each cell was mapped. The maps of the peripheral zone and the pith-rib meristem were divided into upper, middle, and lower thirds, and then the upper and lower regions vs. the lower regions within each zone indicate that there are no significant differences between them. The cells do not change detectably as they "flow" from the top of the meristem to the bottom.

AN ULTRASTRUCTURAL STUDY OF CELLULAR DIFFERENTIATION IN THE ROOTCAP OF ZEA MAYS. C. Edward McClelen and Randy Moore, Department of Biology, Baylor University, Waco, Texas 76798.

The rootcap of **Zea mays** is composed of three tissues: (1) the calyptrogen, (2) the columella, and (3) the peripheral zone. The calyptrogen is the meristem that gives rise to the rootcap. Cells of the calyptrogen are densely cytoplasmic and contain the usual compliment of cellular organelles, the most prominent of which are the nucleus and the numerous small vacuoles. The columella, which is located in the center of the rootcap, is the tissue responsible for detecting gravity in roots. The most distinguishing feature of columella cells is the presence of numerous amyloplasts which have sedimented to the "bottom" of the cells. Peripheral cells are located at the periphery of the rootcap. Their differentiation from columella is characterized by a dramatic increase in dictyosomes, the production and secretion of mucilage, and an increase in the size of the primary vacuole. The results of this study will be discussed relative to other studies of rootcap structure and function.

THE CINGULA PATTERNS OF TWO THALASSIROSIRA SPECIES. Mischelle McMillan, Department of Oceanography, Texas A&M University.

The cingula patterns of two diatom species belonging to the genus **Thalassirasira** were examined using scanning electron microscopy and compared to those normally characteristic of the genus. The arrangement of the ligulas and antiligules were also examined. The morphological features of the cingulum could prone to be concernative taxonomic characters which would allow differentiation of **Thalossirosira** species and designation species groups within the genus.

STEREOLOGICAL ANALYSIS OF EPIDERMAL CELLS FROM WATER STRESSED COTTON LEAVES. Susan Middleton, Jerry Berlin and Jerry Quisenberry, Department of Biological Sciences, Texas Tech University, Lubbock, Texas and USDA, Lubbock, Texas 79401.

The subcellular mechanisms whereby plants exhibit resistance or tolerance to water stress remain enigmatic. Previous water stress studies have concentrated on yield losses or whole plant parameters and have virtually ignored specific cell types within the plants. We have previously described water stress effects in palisade cells (TSEM J. 11:35-36, 1981; Plant Physiol. **in press**). We report here the effect of water stress on different cell types, namely, the epidermal cells. Water stress generally ellicited a similar response in both the upper and lower epidermal cells. For example, the water stressed cells were always smaller, had reduced cell walls and fewer and smaller chloroplasts. In contrast, water stress resulted in more and larger lipid bodies in the lower epidermis, but fewer and smaller lipid bodies in the upper epidermis.

ULTRASTRUCTURE OF THE TELIOSPORE AND TELIOSPORE GERMINATION IN THE RUST FUNGUS KUNKELIA NITENS. Charles W. Mims, Department of Biology, Stephen F. Austin, State University, Nacogdoches, Texas 75962.

The rust fungus **Kunkelia nitens** parasitizes various species of **Rubus** producing bright orange sori on infected leaves. These sori are filled with spores that morphologically resemble aeciospores. When germinated, however, these spores function as teliospores and each gives rise to a promycelium bearing basidiospores. The object of this study is to examine these spores ultrastructurally and follow the sequence of events leading to the production of basidiospores.

The teliospores of **K. nitens** are globoid to ellipsoid and measure 16-24 by 19-30 Gm thick and contains numerous germ pore regions. A mature spore appears to contain a single nucleus surrounded by a dense cytoplasm filled with many lipid droplets. When placed in water the spore germinates to form a short, hyphal-like promycelium that emerges from one of the germ pore regions. During germination structures thought to represent synaptonemal complexes appear in the nucleus. The nucleus eventually enters the promycelium and presumably divides meiotically to produce four haploid nuclei that are separated from one another by the development of septa in the promycelium. A slender sterigma then develops from each uninucleate compartment of the promycelium and eventually gives rise to a small basiodiospore initial at its tip. The nucleus then migrates through the sterigma into the spore initial thus completing the process of basidiospore formation.

MONENSIN TOXICITY IN EUGLENA. Hilton H. Mollenhauer and Robert Droleskey, Veterinary Toxicology and Entomology Research Laboratory, ARS, USDA, P.O. Drawer GE, College Station, Texas 77841.

Monensin is a sodium selective ionophore synthesized by **Streptomyces cinnamonensis.** It is used extensively as a coccidiostat in poultry production and as a feed additive to promote weight gain in cattle. However, monensin is toxic when used in excess and has caused death in poultry, horses, and cattle. Cardiomyopathies are the outstanding lesions in mammals; whereas, changes in secretory activity are most prominent in cultured cells and in the root tips of maize. To further evaluate these effects, we exposed cultures of **Euglena gracilis** to monensin and then examined the cells by electron microscopy for subcellular aberrations. We found that monensin selectively altered the cisternae of the mature (trans) half of each dictyosome of the Golgi apparatus. All of these cisternae were altered equally by the monensin, and the same alterations were present after either 1 or 4 hours of monensin exposure. No other cell constituents were noticeably altered by the monensin. Thus, our results confirm that at least one intracellular site of monensin activity is the Golgi apparatus and that there is a differential effect of monensin within the dictyosomes of the Golgi apparatus. In regard to the latter, monensin appears to be more active toward the mature (trans) poles of the dictyosomes than toward the forming (cis) poles of the dictyosomes.

FURTHER EVIDENCE FOR CELL WALL DEPOSITION DURING GRAFT FORMATION IN HIGHER PLANTS. Randy Moore, Department of Biology, Baylor University, Waco, Texas 76798.

Cells of **Sedum telephoides** (Crassulaceae) undergoing lethal cellular senescence in response to grafting with **Solanum pennellii** (Solanaceae) often possess plasmalemmal tubules (PT). These PT are unbranched, 27 to 31 nm in diameter, and are bordered by a wall measuring 4.8 to 5.2 nm in thickness. No regular substructure is discernible in the lumen of the PT. At 36 hours after grafting, PT extend into the cytoplasm of **Sedum** cells at the graft interface between **Sedum** and **Solanum.** By 10 days after grafting, however, PT are typically found embedded in

newly deposited cell wall. These results indicate that cell wall deposition occurs during the early stages of graft formation.

ELECTRON AND LIGHT MICROSCOPE EXAMINATION OF ACTH-INDUCED CELL SURFACE AND CYTOSKELETON CHANGES IN CULTURED Y-1 ADRENAL CELLS. J. Mrotek, W. Rainey and T. Sawada, Department of Biology Sciences, North Texas State University, Denton, Texas 76203. Supported by NIH grant AG01055.

Using fluorescent actin antibodies, SEM and TEM, cytoskeletal changes in whole and Triton X-100 solubilized mouse adrenal tumor cells were examined; cells were examined after one hour incubation with saline, ACTH, cytochalas in D or ACTH/cytochalasin D. Steroid production was also measured in medium from similarly treated cells. The cytoskeleton of control cells was typical of that associated with flattened, polymorphic cultured cells. Cable-like bundles of intermediate filaments and microtubules radiated from the nuclear area; in addition, an extensive, random lattice-work of actin-containing filaments connected the radiating bundles. Following treatment with ACTH, the cells became "rounded-up", exhibiting numerous surface blebs and thread-like microvilli. The rounded cell resulted from the lattice-work actin filaments being drawn close to the nucleus; the intermediate filament-and microtubule-cables still radiated away from the nucleus, giving the rounded cell a stellate appearance. Time-lapse photomicrography revealed that filopodia actually elongate outward from the periphery of the rounded cell. The morphological changes observed in cells incubated with either cytochalasin-containing treatment were similar. The Triton digests of cytochalasin-treated cells rounded-up and appeared to have a stellate shape, the radiating cables were thicker, and few actin-containing thin filaments were observed in the perinuclear region. Microvilli and blebs capped in the center of the cell surface.

IDENTIFICATION OF CRYSTALLINE INCLUSIONS IN ACER SACCHARUM MINERAL STAIN BY ANALYTICAL ELECTRON MICROSCOPY. R.F. Pinizzotto, Texas Instruments Incorporated, P.O. Box 225936, MS-147, Dallas, Texas 75265 and A.J. Mia, Department of Life Sciences, Bishop College, Dallas, Texas 75241.

The heartwood of many hardwood tree species of Eastern North America is commonly discolored. This is due to the presence of mineral deposits in the xylem cells. Analytical electron microscopy has been used to identify the crystalline inclusions in Acer Saccharum. X-ray energy dispersive spectroscopy, electron energy loss spectroscopy and electron diffraction were performed on ultathin sections of heartwood material. The elements Ca, C, O, Mg and Mn were identified. The electron diffraction patterns are consistent with the crystalline inclusions being Kutnahorite, a form of $CaCO_3$ that may contain various amounts of both Mg and Mn. These results are in agreement with results obtained from bulk chemical analyses of sugar maple wood.

PROTEIN BODIES AND LIPID BODIES IN THE DORMANT COTYLEDONS OF PROSOPIS GLANDULOSA (MESQUITE). D.A. Prier, R.D. Allen, and L.H. Bragg, Department of Biology, University of Texas at Arlington, Arlington, Texas 76019.

Storage protein in mesquite cotyledons is organized into distinct protein bodies. Scanning electron microscopy (SEM) was used to observe the compact arrangement of the protein bodies within a cytoplasmic matrix of the fractured cotyledon cell. Upon closer observation the surface of the protein bodies exhibits a uniform hexagonal pitting arrangement. In fractured protein bodies spherical globoid inclusions are found in a granular protein matrix. Transmission electron microscopy indicates the protein bodies are composed of a homogenous protein matrix and no protein crystalloids can be seen. Globoids are removed or shattered during thin sectioning which leaves holes that sometime contain electron dense fragments which are the remnants of the globoids. Small spheres surround the protein bodies and also line the inner surface of the plasma membrane. Histochemical data as well as the location and electron density of these spheres suggest that they contain lipid. In grazing sections tangential to protein body surfaces, the lipid bodies are seen in hexagonal arrays and are responsible for the organized surface detail observed with SEM. Light microscopy reveals two distinct populations of protein bodies and a gradation between the two types with respect to the presence of globoids. Globoids are numerous in parenchymal cells, but absent in palisade cells.

MORPHOMETRIC ANALYSIS OF ROOTCAP STATOCYTES OF PHASEOLUS VULGARIS by Steve Ransom and Randy Moore. Department of Biology, Baylor University, Waco, Texas 76798.

Work by early investigators into the problem of geotropism in the roots of plants proved that geoperception occurs in the rootcap of the plant. Cells containing amyloplasts in the columella of the rootcap are the site of gravity perception. In our study we compared the statocytes of lateral and primary rootcaps of **Phaseolus vulgaris.** Primary roots grow downward under the influence of gravity while lateral root growth is unaffected by gravity. We wanted to find out if this difference in geotropic behavior was relfected in the anatomy of the rootcap statocytes. Plants were grown in soil and fixed in situ with glutaraldehyde. The roots were then post-fixed with osmium tetroxide, embedded in Spurr's plastic, and sections cut for light and electron microscopical study. These investigations revealed that both lateral and primary rootcaps contained statocytes with amyloplasts that sedimented under the influence of gravity. Qualitative analysis of the data suggested that there were more amyloplasts in the statocytes of primary roots than those of lateral roots. A stereological study was undertaken to determine if this was the case. Results indicated that statocytes of primary roots did have more amyloplasts than statocytes of lateral roots. This difference could be a contributing factor in the contrasting geotropic behavior of lateral and primary roots.

ULTRASTRUCTURE OF AECIOSPORE ornamentation development in the rust fungus cronartium fusiforme. Robert W. Roberson and Charles W. Mims, Department of Biology, Stephen F. Austin State University, Nacogdoches, Texas 75962.

The plant parasitic fungus **Cronartium fusiforme** attacks various species of **Pinus** producing large, fusiform galls on the infected trunk or branches. In the spring these galls bear masses of bright yellow aeciospores. These spores are ovoid to pyriform or elongated pyriform in shape and measure 10-21 x 18-32 Gm. The surface of the mature spore wall is coarsely verrucose except for a smooth area at the base of the spore that also extends up one side. The purpose of this study is to use both transmission and scanning electron microscopy to examine the formation of the surface ornamentations present on the mature spore. Previous ultrastructural studies on both this species as well as other members of the genus **Cronartium** have failed to elucidate the mechanism by which ornamentations develop over most of the spore surface yet not in the smooth area. Other details of aeciospore development in **C fusiforme** will also be compared to those in other rust fungi.

OBSERVATIONS OF THE GEMMAE OF THE MOSS

OEDIPODIELLA AUSTRALIS. Ann E. Rushing and Dale M.J. Mueller, Department of Biology, Texas A&M University, College, Station, Texas 77843.

Multicellular gemmae produced at the apex of the moss **Oedipodiella australis** (Wag. & Dix.) Dix. are discoid in shape and average 250 Gm in diameter. The gemmae are uniseriate at the margins and four to six cells in thickness at the center. Light and electron microscope studies show formation of apical cells from apical initials on both sides of the central regions of gemmae. Both apices of a gemma are capable of developing if sufficient light is present. The thin walled cells of the apex are smaller than the marginal and central cells. Marginal and central cells are highly vacuolate. The central cells apparently function in storage. Functional plastids containing starch are present.

ENZYMATIC AND ULTRASTRUCTURAL CHANGES IN LIVERS OF RATS FED POOR QUALITY PROTEIN DIET DURING PRE- AND POST-NATAL PERIODS. Takako I. Sawada, M. Louise Higgins and Andie M. Hsueh, Department of Nutrition and Food Science and Department of Biology, Texas Woman's University, Denton, Texas 76204.

Pregnant McCollum rats were fed a diet of either 20% casein (C) or 20% wheat gluten (WG) during gestation and lactation. After weaning, all progeny were maintained on the same diet as their mothers had received, for 12 weeks. At the end of the 12-wk feeding period, activity of hepatic aniline hydroxylase was determined and ultrastructure of the liver was examined. Compared to the C rats, the WG rats had lower birth weight, weaning weight, and final weight at the end of experimental period. In the liver of WG rats, microsomal protein per g of liver was decreased slightly, while the activity of aniline hydroxylase expressed as per g of microsomal protein was significantly depressed. Ultrastructural changes in the rough endoplasmic reticulum, lipid accumulation, and Golgi vesicles with lipid inclusions were observed in the WG rat livers. These results suggest that feeding wheat gluten diet to rats during pre- and postnatal periods can lead to ultrastructural changes in the liver and reduced microsomal aniline hydroxylase activity concomitantly.

FUNCTIONAL ALTERATIONS IN THE SUBSTRUCTURE OF POLYMORPHONUCLEAR LEUKOCYTE (PMN) MEMBRANES REVEALED BY TANNIC ACID-GLUTARALDEHYDE (TAG) FIXATION. W.A. Shannon, Jr., and D.M. Zellmer. VA Medical Center and Department of Cell Biology, The University of Texas Health Science Center at Dallas, Texas 75216.

TAG-fixation (Shannon and Zellmer, J. Supramolec. Struct. Cell. Biochem. 16: 155, 1981) has been used in a study of membrane-associated proteins and cytoplasmic granules in PMNs collected from rabbit peritoneal exudate. TAG-fixation of the cells revealed plasma membrane substructure and symmetry which are not evident with other fixatives. TAG-fixed cells had a 3 nm electron-dense coat not apparent with glutaraldehyde fixation. Both azurophil and specific granules exhibited distinct classes of granules based on the thickness of these membranes which were found to be 11.4-15.7 nm and 7.7-11.7 nm, respectively. Calcium ionophore A23187-treated, non-degranulating cells had a 4 nm coat while degranulating cells had a 6.5 nm coat. Similarly, a dense coat was observed on the inner side of the membrane of some azurophil-type granules. Subsequently, we have used other membrane perturbants and have found that each exhibits a specific response. Cytochalasin B promoted extensive thickening of the protein coat. Archidonic acid, whether in the presence of absence of cytochalasin B, depleted the protein coat. The alteration of the protein coat appeared to be stimulus-specific and indicated recruitment, rearrangement, and/or addition of proteins to the membrane. It is suspected that the induced protein flux is indicative of a functional response of the membrane, and that this response regulates initial and subsequent mechanistic events which determine the response of the PMN.

AN ATTEMPT AT THE IMMUNOCYTOCHEMICAL LOCALIZATION OF CITRATE CLEAVAGE ENZYME IN RAT HEART AND LIVER. W.A. Shannon, Jr., C.S. Chang, and S.B. Bates. VA Medical Center and Department of Cell Biology, The University of Texas Health Science Center at Dallas, Texas 75216.

The enzyme citrate cleavage enzyme (CCE) ATP-citrate lyase) catalyzes the formation of acetyl-CoA from citrate, ATP and CoA. Although this reaction has been shown to be extramitochondrial, it has been suggested that it might take place in the vicinity of mitochondria.

Rats were fasted 48 hours and then fed 48 hours from a special diet to elevate CCE levels. Specimens of heart and liver were fixed in a mixture of 4% paraformaldehyde-1% glutaraldehyde-0.2% picric acid in 0.1 M sodium acetate buffer. Sections chopped at 30 Gm on a Sorvall TC-2 tissue sectioner were incubated in CCE antiserum (supplied by Dr. Paul Srere). Following rinsing in PBS, they were incubated in peroxidase (kindly supplied by Dr. Ludwig Sternberger). The peroxidase was visualized by incubation in diaminobenzidine medium with H_2O_2, followed by postfixation in O_8O_4.

Reaction product in both tissues appeared only at the periphery of the tissue sections. It was especially concentrated in the cytoplasmic areas adjacent to mitochondria. In some mitochondria, the outer compartment also appeared reactive.

The location of CCE reaction product suggests an intimate relationship between the mitochondria and cytoplasmic loci of CCE activity. It also appears that some of the enzyme is present in the mitochondrial outer compartment and/or diffuses into the compartment. The cytochemically-indicated location for CCE is functionally appropriate since citrate, ATP and CoA are functionally associated with mitochondria.

AN ULTRASTRUCTURAL STUDY OF THE DIFFERENTIATION OF THE EPIDERMIS IN ROOTS OF ZEA MAYS. Houston S. Smith and Randy Moore, Department of Biology, Baylor University, Waco, Texas 76798.

Protodermal cells of 10 day old roots of **Zea mays** are characterized by the presence of occasional small vacuoles, small proplastids, and numerous plasmodesmatal connections with adjacent cells of the quiescent center and calyptrogen. The nucleus is relatively inconspicuous in the densely staining hyaloplasm. The youngest cells of the protoderm (i.e., those cells at the root apex) measure 17.3 % 2.8 Gm x 9.5 % 1.8 Gm when viewed in a radial section (i.e., these cells are characterized by a length: width ratio Z 1.82). Protodermal cells begin maturation at the periphery of the root apex, and maturation is complete at approximately 7.5 Gm behind the root apex. Mature epidermal cells measure 15.5 % 4.4 Gm x 30.0 % 3.7 Gm (i.e., length: width ratio Z 0.52). These mature cells are characterized by the presence of (1) a densely staining hyaloplasm, (2) increased numbers of proplastids, (3) a large, centrally located nucleus with a prominent nucleolus, (4) numerous small vacuoles, and (5) prominent dictyosomes that appear to be active in the secretion of mucous. A morphometric analysis of these ultrastructural changes will be discussed relative to other studies of cellular differentiation in the root epidermis.

THIN FILAMENTS IN RAT SKELETAL MUSCLE. Laurel Traeger and Margaret A. Goldstein, Cardiovascular Sciences,

Baylor College of Medicine, Houston, Texas 77030.

Structural data on thin filaments have come primarily from adult frog sartorius and rabbit psoas muscles. We have studied thin filament length in slow and fast muscle from adult and neonatal rats. Soleus (SOL; slow) muscle from adult and 3, 7, and 9 day old rats, and extensor digitorum longus (EDL; fast) muscle from adult rats were serially cross sectioned (60nm sections). The number of thin filaments/0.06Gm^2 (TF') was counted for individual myofibrils followed from the H zone of one sarcomere, through the I-Z-I region, to the H zone of an adjacent sarcomere. TF' was pooled by distance from the Z band (Z+1, 2, 3 . . . sections) or AI junction (AI+1, 2, 3 . . . sections). In adult 7 and 9 day SOL, I band TF' decreased linearly with distance from the Z band, such that TF' was 25% lower at the AI junction than at Z+1 (pY .01). In 3 day SOL, TF' remained constant throughout the I band. In adult EDL, I band TF' decreased 30% from Z+1 to the AI junction (pY .01). Although the slopes of the regression lines of TF' vs distance from the Z band were similar for adult, 7 and 9 SOL and adult EDL, in 7 and 9 SOL, TF' was 15% lower at all distances compared to adult SOL and EDL (pY .01). In 3 day SOL, TF' was 25% lower at Z+1 and 7% lower at the AI junction compared to adult SOL (pY .01). TF' was similar at all distances for adult SOL and EDL. In all muscles studied, A band TF' decreased from the AI junction to the H zone. We conclude: 1) In rat skeletal muscle, thin filaments are not of uniform length, ranging in length from 0.18 to 1.1Gm. 2) There may be two stages of thin filament assembly in neonatal muscle, between 3 and 7 days when short thin filaments may be preferentially synthesized or inserted near the Z band, and between 9 days and adult when thin filaments of all lengths may be synthesized or inserted into the myofibril. Supp. by MDA and NIH Grants HL17269, HL17376, HL07282 and HL05925.

A STEREOLOGICAL STUDY OF DEVELOPING PALISADE CELLS IN WATER STRESSED AND NONSTRESSED COTTON LEAVES. Usha Vishnoi, Jerry Berlin and Jerry Quisenberry, Department of Biological Sciences, Texas Tech University, Lubbock, Texas 79409 and USDA, Lubbock, Texas 79401.

We previously reported a sterological analysis of palisade cells from water stressed and nonstressed cotton leaves (TSEM J. 11:35-36, 1981; Plant Physiology in press). We report here a comparative study of developing palisade cells from leaves of water stressed and nonstressed cotton (Gossypium hirsutum L. var. Paymaster 266). The palisade cells from young leaves of nonstressed plants had a greater fractional volume of cell wall compared to palisade cells in fully expanded leaves. The young cells from nonstressed plants had reduced fractional volumes for the central vacuole, chloroplasts, starch granules, intrachloroplast bodies and peroxisomes. There was no significant difference in the mitochondrial fractional volumes in cells of young or fully expanded leaves. The imposition of water stress on developing palisade cells resulted in greater fractional volumes for chloroplasts, starch granules, intrachloroplast bodies and peroxisomes. Mitochondrial fractional volumes were significantly increased by water stress.

MINERALIZED LAYERS IN SEED COATS. Mary Alice Webb and Howard J. Arnott, Department of Biology, University of Texas at Arlington, Arlington, Texas 76019.

In the seeds of many angiosperms and some gymnosperms a cell layer of the testa (seed coat) is the site where large quantities of minerals are deposited. In many cases each cell of a particular layer contains one to several crystals, which have been tentatively identified as calcium oxalate on the basis of acid solubilities and structural evidence. Examples of such crystal layers have been observed with SEM in a variety of species, and the crystal forms present vary greatly. In **Phaseolus vulgaris** twinned prismatic crystals are present in sclerified cells. In **Carica papaya** crystals are rounded on one side and angular on the other and are surrounded by a sheath. **Sesamum indicum** has an epidermal crystal layer consisting of druses, which are attached on one side to the cell wall. Masses of prismatic crystal and fill cells of the crystal layer in **Papaver somniferum.** In **Juniperus virginiana,** a gymnosperm, twinned prismatic crystals occur in sclereids of the testa. Deposits of silica have been observed in similar layers in **Elettaria cardamomum,** and deposits of carbonate occur in a testal layer in **Ricinus communis.** These mineralized layers may play an important role in protection of the embryo during dormancy by forming both a chemical and physical barrier to penetration of the seed.

AMINOTRIAZOLE-INSENSITIVE CATALASE IN RABBIT POLYMORPHONUCLEAR LEUKOCYTES. D.M. Zellmer and W.A. Shannon, Jr. VA Medical Center and Department of Cell Biology, University of Texas Health Science Center at Dallas, Texas 75216.

Catalase has been demonstrated to exhibit maximal cytochemical reactivity at pH 9.7-10.5. This range allows a definitive differentiation of catalase activity from myeloperoxidase (MPO) activity, both of which use H_2O_2 as substrate in identical incubation media, since the peroxidase activity was reported not to be present at pH 10.5. The catalase reactivity in rabbit polymorphonuclear leukocytes (PMN) was more intense than myeloperoxidase reactivity, and the reactivity was more intense at pH 10.5 than at pH 9.7. At pH 9.7 or 10.5, mitochondria was strongly reactive. It was also apparent that a darker appearing cell in the PMN population had reactive mitochondria which were longer or more tubular than in the majority of the PMN population. We further attempted to differentiate between the catalase and MPO activity of rabbit PMN by using 2.5 mM 3-amino-1,2,4-triazole (AT) which is a potent inhibitor of catalase, but only infrequently inhibits peroxidase activity, to establish the identity of the catalase. We found that the catalase activity was not inhibited by AT, even after preincubation in AT for 1 hour. This probably indicates a specialized nature of the catalase in PMN granules in that it is used as an anti-bacterial agent as opposed to the more generalized catalase functions of other cells.

Regional News

AUSTIN
THE UNIVERSITY OF TEXAS AT AUSTIN
THE CELL RESEARCH INSTITUTE

PUBLICATIONS

D.T. Brown, Assembly of an Alphavirus Membrane. International Congress of Virology, Strasbourg, France (1981) (Abstract).

H. Scheefers, U. Scheefers-Borchel and D.T. Brown, Nearest Neighbor Analysis of Sindbis Virus Proteins. International Congress of Virology, Strasbourg, France (1981) (Abstract).

E.M. Sorensen, L.L. Wenz, B.C. Windsor and R. Ramirez-Mitchell, Sterological Analysis of Arsenic-Induced Cytological Structures. J. Tennessee Acad. Sci. 56(1981) 131-134.

K. Wang and R. Ramirez-Mitchell, Titin: a Candidate as a Protein Component of a New Type of Longitudinal Filament in the Sarcomere of Striated Muscles. Presented at the annual meeting of the American Biophysical Society, Boston, Mass., February, 1982.

INVITED LECTURES AND SEMINARS

Dr. Dennis T. Brown has recently given invited lectures or seminars on "Development of a Virus Membrane" at the following institutions: Rice University; The University of Texas at San Antonio; St. Judes Hospital, Memphis, Tennessee; the University of Massachusetts Medical Center; the Institute for Genetics, Cologne, West Germany; the Division of Biology, University of Konstanz, West Germany.

OTHER NEWS OF INTEREST TO MEMBERS OF TSEM

Dr. Dennis Brown has received a grant from the Robert A. Welch Foundation entitled "Fatty Acids and Phospholipids Containing Photosensitive (R-N=N=N-) Groups as Tools for Study of the Structure of Biological Membranes."

DEPARTMENT OF BOTANY
NEW FACULTY

Dr. R. Malcolm Brown, Jr. is joining the faculty to occupy an endowed position. Dr. Brown did pioneering work in electron microscopy of algae. His current research concerns synthesis of cellulose, with grant support from Johnson and Johnson.

OTHER NEWS OF INTEREST TO MEMBERS OF TSEM

Dr. John LaClaire has received a National Science Foundation, Cell Biology Program, grant for an ultrastructural and experimental study of wound healing in giant algal cells.

Dr. Garry T. Cole has received a Public Health Service grant entitled "Study of Gastrointestinal and Systemic Candidiasis".

DEPARTMENT OF ZOOLOGY

PUBLICATIONS

S. Meier and A.G. Jacobson, Experimental Studies of the Origin and Expression of Metameric Pattern in the Chick Embryo. J. of Experimental Zoology 219 (1982) 217-232.

R.H. Richardson, J.R. Ellison and W.W. Averhoff, Autocidal Control of Screwworms in North America. Science 215 (1982) 361-370.

INVITED LECTURES AND SEMINARS

Dr. Stephen Meier spoke on "Experimental Studies of the Origin and Expression of Metameric Pattern in the Chick Embryo" at the University of Iowa Zoology Department.

Dr. Meier gave an invited review at the 1982 SEM meeting in Anaheim, California on "Development of Segmentation in the Cranial Region of Vertebrate Embryos".

EM EDUCATION OPPORTUNITIES

In the fall semester of 1982 Dr. Stephen Meier will teach Principles and Techniques of Electron Microscopy (Zoo 390K), a graduate level course stressing EM applications to biological tissues.

NACOGDOCHES
DEPARTMENT OF BIOLOGY, STEPHEN F. AUSTIN STATE UNIVERSITY

NEW EQUIPMENT

With the installation of a Hitachi 405-A scanning electron microscope and supporting equipment, The Department of Biology now offers graduate level instruction in both TEM and SEM.

LECTURES

Charles W. Mims presented a seminar entitled "Ultrastructure of the host-pathogen relationship in selected fungal diseases of plants" in the Department of Biology at The University of Texas at Arlington.

Bruce Mackay of M.D. Anderson Hospital and Tumor Institute, Houston, Texas, presented a seminar at Stephen F. Austin State University entitled "Diagnostic Electron Microscopy of Tumors."

Ronald F. Dodson of the Department of all Biology and Environmental Sciences at the U.T. Health Center at Tyler, presented a seminar at Stephen F. Austin State University entitled "Asbestos - understanding the problem."

PUBLICATIONS

Mims, C.W. 1981. SEM of aeciospore formation in **Puccinia bolleyana.** Scanning Electron Microscopy. 1981/111: 299-303.

Mims, C.W. 1981. Ultrastructure of teliospore gerimation and basidiospore formation in the rust fungus **Gymnosporangium clavipes.** Canadian Journal of Botany 59: 1041-1055.

WACO
DEPARTMENT OF BIOLOGY, BAYLOR UNIVERSITY

LECTURES

Dr. Robert Baldridge presented a seminar at Southern Methodist University entitled "Behavior of Army Ants".

Dr. Randy Moore presented a seminar at Trinity University entitled "Graft Compatibility-Incompatibility In Higher Plants."

EM EDUCATION OPPORTUNITIES

"Biology 4402 — Electron Microscopy". A formal course in electron microscopy, offered Fall and Spring Semesters in the Biology Department.

DALLAS

UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL SCHOOL

PUBLICATIONS

Shannon, W.A., Jr. 1981. Interrelationships of cytoplasmic tubular structures in circulating lymphocytes. Tx. Soc. Electron Micros. J. 12(2):7-9.

Shannon, W.A., Jr., and D.M. Zellmer. 1981. Tannic acid-glutaraldehyde fixation reveals calcium ionophore-induced changes in rabbit polymorphonuclear leukocyte membranes. J. Supramolec. Struct. Cell. Biochem. 16:155-165.

Dey, R.D., W.A. Shannon, Jr., and S.I. Said. 1981. Localization of VIP-immunoreactive nerves in airways and pulmonary vessels of dogs, cats, and human subjects. Cell Tissue Res. 220:231-238.

Shannon, W.A., Jr. 1981. Light and electron microscopy cytochemistry of monoamine oxidase and other amine oxidative enzymes, pp. 193-243. In J.E. Johnson, Jr. (ed.) Current Trends in Morphological Techniques, Vol. III. CRC Press, Inc., West Palm Beach, Fl.

OTHER NEWS OF INTEREST TO MEMBERS OF TSEM:

Jay Yaquinto was a recent competitor in the annual UTHSCD Southwestern Medical School Medical Student Research Forum with "Fine structural study of human laryngeal epithelium: normal and pathologic." This study was performed last summer with Dr. Allen Shannon and Dr. Werner Schulz.

Dr. Steffen Rogers, Nick Martin and Ellen Cookson from the Department of Life Sciences at the University of Tulsa recently spent a week on a "crash" course in TEM in Dr. Shannon's lab. They return to Tulsa to start up their new EM lab.

NEW FACULTY AND/OR STAFF

Dr. Fred Shipkey has recently joined the VA Laboratory Service Staff and will serve as Director of the Lab Svc. EM program. He is a new member of TSEM. Sally Bates has rejoined Dr. Allen Shannon's group to work on diabetes and heart research.

SCHOOLS, TRAINING PROGRAMS, COURSES

SCANNING AND TRANSMISSION ELECTRON MICROSCOPY

A series of practical courses will be offered during June 1982.
Transmission Electron Microscopy — June 7-18.
Scanning Electron Microscopy — June 21-25.
Combined TEM & SEM — June 7-25.

These classes are designed to introduce the participants to the theory and practical aspects of electron microscopy. Primary emphasis will be on specimen preparation, operation of electron microscopes, X-ray microanalysis, and photographic and darkroom techniques.

TUITION

$650 for TEM, $595 for SEM, $1,150 for the combined program.

INFORMATION

Write or call: Fred Lightfoot, George Washington University, Department of Anatomy, 2300 "I" Street, N.W., Washington, D.C. 20037, (202) 676-2881 or 676-3511.

JOB ANNOUNCEMENTS

Electron Microscopy: Full time laboratory manager to operate and maintain central EM facility. Duties include instruction in EM courses and operation of an Hitachi H-600 TEM and an Hitachi S-450 SEM with Kevex 7067 EDS. Opportunity to participate in research. Requires B.S. Salary negotiable depending upon qualifications. Applications accepted until March 25 or the position is filled. Resume to: Dr. Roy C. Brown, Biology Dept., University of Southwestern Louisiana, Lafayette, LA 70504.

HISTOLOGY TECHNICIAN

Preparation of tissue specimens (cutting, staining, mounting). Requires 1 year's training in histological techniques and ASCP certification in histotechnology.

ELECTRON MICROSCOPY TECHNICIAN

Duties will include preparation & screening of tissue specimens for SEM evaluation. Will cross-train in TEM, requiring techniques of fixation, resin embeddment, thin sectioning, & grid staining. Requires several years experience in electron microscopy.

For more information contact: Personnel Office, U.T. Health Science Center at San Antonio, 7703 Floyd Curl, 512-691-6116.

WE NEED YOU

TO SUBMIT A PAPER FOR PUBLICATION IN THE TSEM JOURNAL

TSEM Minutes

TSEM BUSINESS MEETING MINUTES

The TSEM Business Meeting was called to order by President Goldstein at 6:00 p.m., October 10, 1981, at the Holiday Inn-Emerald Beach in Corpus Christi, Texas.

1. The minutes were read and approved.
2. The Treasurer's report was presented and approved. The society's assets remain unchanged.
3. Forty-three new members were presented and accepted into membership of the society.
4. Future meetings were announced as follows: April 1-3, 1982, at TWU in Denton. The Oklahoma Society will be invited. — October 7-9, 1982, at the Holiday Inn in Galveston. The Louisiana Society will be invited. — Spring, 1983, San Marcus or Austin.
5. Elaine McCoy reported on the TSEM Journal. Advertisers have been dependable but may not be able to cover future costs for expanded number of issues. Paul Baur will succeed Elaine McCoy as the next editor and proposes to establish editorial review and associate editors.
6. Concern was expressed about the percentage of TSEM members in EMSA because of support from the parent society. Now have 25 local societies. Ben Spurlock in Atlanta is new director of local societies.
7. Dr. Goldstein announced that to encourage student participation, meetings will be held near the center of the state at least every other meeting. Each member must encourage students to attend and present at TSEM meetings.
8. The student representative to the Executive Committee will be Danna Zimmer of Baylor College of Medicine.
9. The nominating committee was announced.
10. President Goldstein expressed appreciation to the Biological Photographic Association for the excellent joint meeting with TSEM and to Gordan Stanley for the local arrangements.
11. The meeting was adjourned at 6:45 p.m.

Respectfully submitted,

Marilyn N. Smith
Secretary, TSEM

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).

_____
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 _____

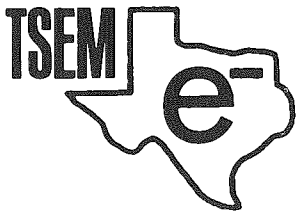
Send Application to:

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

Financial Report

ASSETS ON SEPTEMBER 7, 1981		
Certificate of Deposit No. 1099, Univ. Natl. Bank, Galveston	2,000.00	
Certificate of Deposit No. 10-141345, Houston 1st Savings	2,335.44	
Merrill Lynch Money Market, Dallas	2,000.00	
Checking Account, Forestwood Natl. Bank, Dallas	3,935.53	
Includes Paul Enos Memorial Fund — $50.00	10,270.97	10,270.97
RECEIPTS		
Dues:		
Regular Membership	2,110.00	
Student Membership	142.00	
Corporate Membership	975.00	
Interest on CD No. 1099	75.00	
Interest on CD No. 10-141345	120.43	
(Interest on Merrill Lynch MM Due 3/18/82-approximately $170.)		
Interest on Checking Account	51.43	
Corpus Christi Meeting:		
Registration	2,230.00	
Corporate Donations:		
Hitachi	125.00	
DuPont	75.00	
Commercial Exhibitors	150.00	
	6,054.01	6,054.01+
DISBURSEMENTS:		
Presidential Travel (EMSA-Atlanta)	782.03	
Poster Fabrication	189.44	
TSEM Journal:		
Editor Travel to Temple	99.36	
Open New Bank Account	300.00	
Denton Womens Club (Reservation-Denton Meeting)	50.00	
Secretarial Expenses	402.37	
Misc. Expenses	27.52	
Corpus Christi Meeting:		
Holiday Inn	2,675.30	
Security	66.00	
Student Travel	1,096.00	
	5,688.02	5,688.02-
ASSETS ON MARCH 15, 1982		
Certificate of Deposit No. 1099, Univ. Natl. Bank, Galveston	2,000.00	
Certificate of Deposit No. 10-141345, Houston 1st Savings	2,456.02	
Merrill Lynch Money Market, Dallas	2,000.00	
Checking Account, Forestwood Natl. Bank, Dallas	4,180.94	
Includes Paul Enos Memorial Fund — $50.00	10,636.96	10,636.96

Respectfully submitted: W. Allen Shannon, Jr., Treasurer



CORPORATE MEMBERS

AMRay, Inc. Thomas Levesque, Box 83416, Lewisville, TX 75056, (214) 247-3542.

Cambridge Scientific Instruments, Mike Webber, 3945 Fairington Dr., Marietta, Georgia 30066, (404) 926-9636.

E.I. DuPont de Nemours and Co., Inc., Biomedical Products Division, Harry Vacek, Concord Plaza-Quillen Bldg., Wilmington, Delaware 19898, (800) 441-7493, (302) 772-6024.

EBTEC Corp., Margarit Barry, 120 Shoemaker Lane, Agawam, Mass. 01001, (413) 786-0393.

EDAX International, Jim Moore, P.O. Box 2253, Boulder, Co. 80306, (303) 443-3610.

Electron Microscopy Sciences, Richard Rebert, Box 251, Ft. Washington, PA 19034, (215) 646-1566.

Ernest Fullam, Inc., Richard Kemmer, 900 Albany Shaker Rd., Latham, NY 12110, (518) 785-5533.

Hitachi Scientific Instruments, Jonni Fischer, 2407 W. Settler Way, Woodlands, TX 77380, (713) 643-8339.

International Scientific Instruments, Robert Ruscica, 3255-6C Scott Blvd., Santa Clara, CA 95051, (408) 727-9840.

JEOL, USA, Inc., Dick Lois, 1 Kingwiid Place, Suite 122B, 600 Rockmead Dr., Kingwood, TX 77339, (713) 358-2121.

KEVEX Corp., Dick Cushing, 1101 Chess Dr., Foster City, CA 94404, (415) 573-5866.

Ladd Research Industries, Margaret Ladd, P.O. Box 901, Burlington, Vermont 05402, (802) 658-4961.

Link Systems, Bill Stewart, P.O. Box 2160, Sunnyvale, CA 94097, (408) 749-1656.

LKB Instruments, Inc., Charles Weinert, 8319 Sharpcrest, Houston, TX 77036, (713) 228-4082.

E.G. and G. Ortec, Dick Nieman, 21718 Rotherham, Spring, TX 77379, (713) 353-0078.

Ted Pella, Inc., T.P. Turnbull, 16812 Milliken Ave., Irvine, CA 92714, (800) 854-7553, (714) 557-9434.

Polaron Instruments, Inc., Dermot O. Dinan, 2293 Amber Drive, Line Lexington Industrial Park, Hatfield, PA 19440, (215) 345-1782.

Princeton Gamma Tech, Dick Stancher, 17756 Kings-Park Lane, Houston, TX 77058, (713) 280-8766.

Rockwell International, R.W. Max, Mail Station 406-146, Richardson, TX 75081, (214) 996-6973.

Technics EM Systems, Inc., Diane A. Hurd, 7653 Fullerton Road, Springfield, VA 22153, (703) 569-7100.

Carl Zeiss, Inc., Dietrich Voss, 3233 Weslayan, Suite 191, Houston, TX 77027, (713) 629-0730.

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).

_____
Signature of TSEM Regular Member making the Nomination

_____
Date 19

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Presented to the Council at _____ meeting. Date _____

Action _____

Send Application to:

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

Information for Author's

PURPOSE: The goal of the TSEM Journal is to inform members of the society and the Journal's readers of significant advances in electron microscopy, research, education, and technology. Original articles on any aspect of electron microscopy are invited for publication. However, the TSEM Journal is biologically oriented and articles along those lines will be preferred. Guidelines for submission of articles are given below. The views expressed in the articles, editorials and letters represent the opinions of the author(s) and do not reflect the OFFICIAL POLICY OF THE INSTITUTION with which the author is affiliated or the Texas Society for Electron Microscopy. Acceptance by this Journal of advertisements for products or services does not imply endorsement. Manuscripts and related correspondence should be addressed to Paul S. Baur, Jr., Ph.D., Editor, TEXAS SOCIETY FOR ELECTRON MICROSCOPY JOURNAL, Division of Cell Biology, Shriners Burns Institute, 610 Texas Avenue, Galveston, Texas 77550.

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

PAGE PROOFS & REPRINTS: The author(s) will receive a page proof for review and will be responsible for the content of the article, including copy-editing changes. Page proofs should be carefully read, corrected, and returned to the Editor within 48 hours of receipt. The author(s) should sign the page proofs indicating approval. Reprints may be ordered when page proofs are received, and a table showing the cost of reprints will be enclosed with the proofs. REPRINTS MAY ALSO BE ORDERED FROM THE PRINTER.

MANUSCRIPT PREPARATION. Manuscripts should be submitted in conformance with the following guidelines:

FORMAT: Submit an original and two copies of the entire manuscript, typed, double-spaced, on 8-1/2 x 11 white paper, leaving ample margins. Number each page and identify the article by placing, at the top left of the page, a shortened form of the title, followed by the last name of the first author.

TITLE PAGE. Include:

a. Full title of the article

b. Initials and last names of all authors

c. Current positions of each author (title, department, institution, city)

d. Full name, telephone number and address of the author to whom reprint requests are to be sent.

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

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

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

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

(1) A. Glauert, Practical Methods in Electron Microscopy, Vol. 2 (North-Holland, Amsterdam, 1974) 82-88.

(2) P.S. Baur, Jr., G.F. Barratt, G.M. Brown and D.H. Parks, Ultrastructural Evidence for the Presence of "Fibroclasts" and "Myofibroclasts" in Wound Healing Tissues, J. of Trauma. 19 (1979) 744-756.

(3) D. Gabor, Information Theory in Electron Microscopy, in: Quantitative Electron Microscopy, Eds. G.F. Bahr and E. Zeitler (Williams and Wilkins, Baltimore, 1965) 63-68.

NOTE: Authors are responsible for the accuracy of references.

TABLES

a. Type, double-spaced each table on a separate sheet.

b. Number in order in which they are referred to in the text.

ILLUSTRATIONS

a. Submit three complete sets of illustrations. Copy machine reproductions of photographs will not be accepted. Indicate which set is the original photograph or illustration.

b. Number the figures in the order in which they are referred to in the text.

c. For black and white illustrations, submit sharply focused, glossy prints, or line drawings, 1.5 times larger than they are to appear in print (1/4 or 1/2 page). Scale should be drawn on the photograph itself, not below.

d. For color illustrations, if needed, submit positive 35-mm color transparencies, (not prints) for the original (prints may be used for the two copies). Authors will bear the entire cost of color reproductions.

e. Identify all illustrations (author, title of paper, and number) by a gummed label on the back of each. Do not mount the illustrations, write on the back of them, clip them, or staple them.

f. Illustrations taken from other publications require reprint permission and must be submitted in the form described above.

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

ACKNOWLEDGEMENTS and research funding should appear as a footnote which will appear at the foot of the first page of the article.

REMINDERS FROM THE SECRETARY

— Don't forget to contact the secretary if your address is incorrect or you have an address change.

— Corporate members need to contact the secretary and notify us about local representative changes.

— Corporate members can get a membership list and mailing labels from the secretary.

— Please let your secretary know if you are willing to work in the society or run for an office (the secretary is chairman of the nominating committee). The secretary will keep a file of letters from people volunteering to work. Don't forget! Your secretary changes every two years so let your new secretary know.

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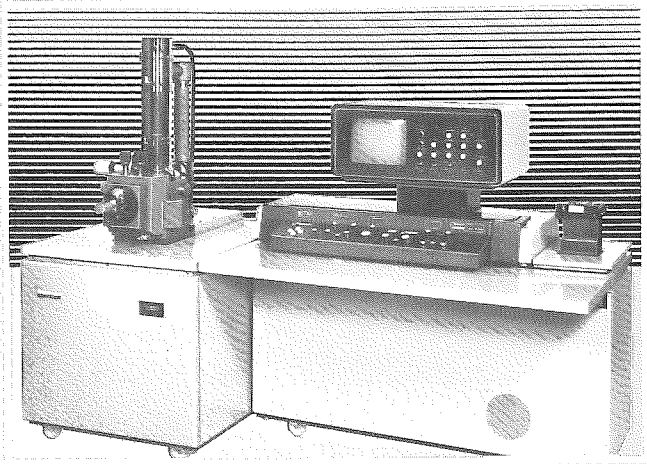

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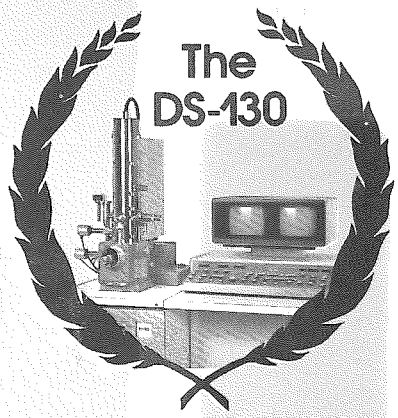

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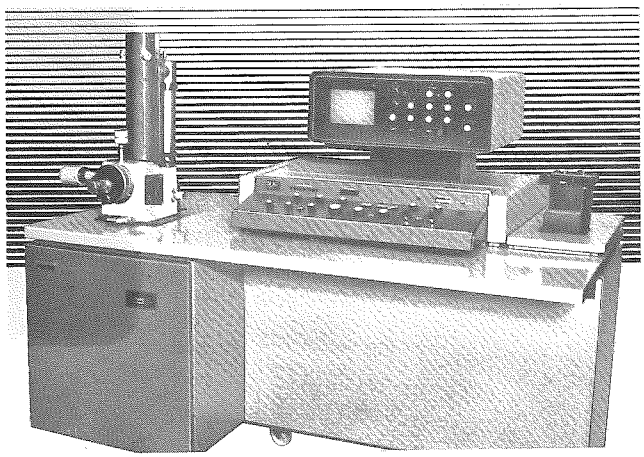

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