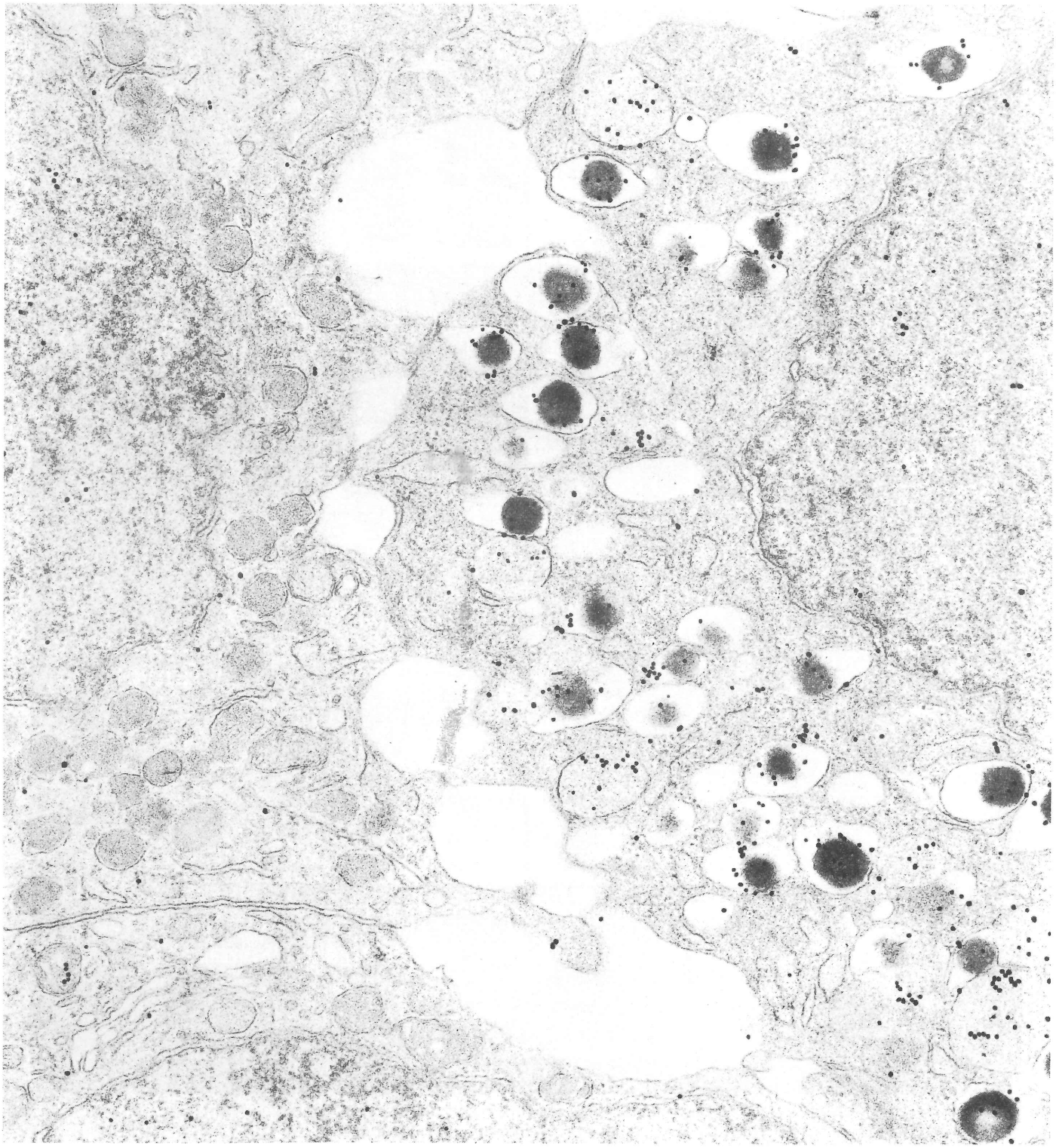
Texas Society for Electron Microscopy

JOURNAL
VOLUME 14, NUMBER 2, 1983
ISSN 0196-5662



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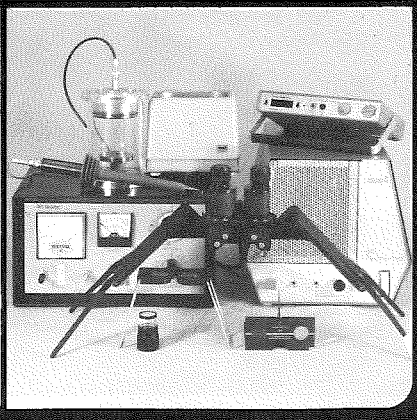

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Contents

JOURNAL
VOLUME 14, NUMBER 2, 1983
ISSN 0196-5662

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 13)

ON THE COVER

Electron Micrograph courtesy of Richard Dey, Ph.D. and Allen Shannon, Ph.D., Department of Cell Biology, The University of Texas Health Science Center at Dallas and the Dallas V.A. Medical Center. Gold Protein-A immunocytochemical reaction for insulin. The reaction occurs over the secretory granules of a B-cell. An adjacent D-cell is non-reactive.

President's Message

It is with a great deal of pleasure that I write my first "presidential message" to the general membership of TSEM. It is a distinct honor for me to serve as your president and I promise to do everything in my power to see that TSEM has an active and productive year.

We have just returned from a most successful and enjoyable meeting in Austin. Many people worked hard to make this meeting a success but, I would especially like to thank Pat Davis and Beth Root for their efforts in our behalf as well as those individuals who opened their labs for us on the University of Texas Campus. John Guyton, Ann Goldstein and H. Fernandez-Moran also deserve a big "thank you" for the special lectures that added so much to the meeting. We also appreciate the participation and support of our faithful corporate members.

It is now time to turn our attention to the future. As you are probably aware, our Fall Meeting is scheduled for Tyler, October 13-15, 1983. The meeting is shaping up well under the guidance of Ernie Couch and Ron Dodson and his eager "helpers". I hope that each of you will plan to attend the meeting and participate in the program. October is a very popular tourist time in the "Rose City" so don't delay in making reservations once you receive the mailout for the meeting.

During my term in office I have three major goals. The first is to increase our membership. As you are probably aware there are many electron microscopists in Texas who are not members of TSEM. Many of these individuals are new to Texas and probably are not familiar with TSEM. I hope that each of you will make a special effort to nominate someone for membership in our organization. In particular I hope that you will encourage student participation in TSEM. Usually major professors can be quite effective in regard to this matter!

My second goal is to work to see that our Journal is a success. Paul Baur has worked long and hard on the Journal the past few years but, he can not do it all himself. If you would like to help in some way please let Paul know. Perhaps even more importantly, why not submit a paper to the Journal? Unless we can attract quality papers it is unlikely that we can be successful on a long term basis.

My third goal is to see that TSEM engages in some long range planning. It is evident that past TSEM officers have done the same or we would not have the successful organization that we have today. We can not, however, confine our plans simply to future meeting sites. We must identify specific goals (special programs or symposia, workshops, joint meetings, etc.) and then take appropriate steps to realize the goals. Before we can do anything, however, we need to know what the membership wants TSEM to do. I hope that each of you will offer your suggestions and/or comments either to me or to any officer of the Society. I think, for instance, that it would be valuable for you to comment on what you see as our strong areas as well as our weak areas. Unless you speak up the officers can only guess as to your needs and wants.

Once again let me say that I am honored to serve as President of TSEM. I look forward to working with you this year. (Remember — nominate a new member prior to the Tyler meeting).

Charles W. Mims
President, TSEM 1983-84

Editor's Message

We failed to proof one tiny part (TECHNICAL NOTE SECTION) of Issue 4, Volume 13 and wouldn't you know it there were errors aplenty. A repeat of the insertion is found herein.

New blue-line abstract forms will be mailed out with each future meeting announcement. Please follow the instructions to the absolute letter when submitting your abstract. Note also that reproduction machines usually enlarge 3 to 6% so don't use a reproduction of the abstract form. If you have to, use a clean white sheet of bond paper and keep the abstract within the boundaries of the original form.

There are no papers awaiting publication. Now is a good time for you to get yours to me for consideration.

Submitted micrographs will be accepted for inclusion in the Journal as "text-fillers' and/or as a cover photo. The contributions should be sharp (finely focused) and embody an excellant contrast range. None will be acknowledged or returned but ALL will be appreciated by the editor. Normally, the cover photos will be derrivations of one of the features articles in that issue.

Every once in a while TSEMJ is going to spotlight a TSEM member with a brief bibliography and a "mug" shot. It will be a way of keeping abreast of our fellow members and their many accomplishments. It ought to be obvious who I'd select for the first insertion. If you have nominations for the spotlight section then let me hear from you.

The next issue of the TSEMJ will include abstracts from the Eleventh Western Regional Meeting of Electron Microscopists. The meeting was held in Asilomar, California in May. I hope you take the time to read and appreciate the efforts of our "western" colleagues.

My best wishes and regards.

Paul S. Baur
Editor, TSEMJ

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Glenn Williams, The University of Texas Health Center at Tyler, P.O. Box 2003, Tyler, TX 75710. (214) 877-3451.

Financial Report

ASSETS ON OCTOBER 4, 1982		
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Certificate of Deposit No. 10-141-345, Houston First Savings	2,580.70	
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Includes Paul Enos Memorial Fund ($50.00) and Merrill Lynch Money Market Cashed ($2,016.36)	9,301.59	9,301.59
RECEIPTS		
Galveston Meeting - Registration	2,281.12	
Donations; AMRAY-$25, EG&G Ortec-$20, Electron Microscopy Sciences-$25, Hitachi-$25, ISI-$30, JEOL-$55, Ladd Res. Industries-$25, LKB-$25, Philips-$375, Princeton Gamma Tech-$25, Schares-$50, Ted Pella, Inc.-$75, Tracor Northern-$50	805.00	
Interest		
CD No. 91099	75.00	
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DISBURSEMENTS:		
Galveston Meeting		
Social	477.06	
Banquet	2,251.95	
Guest Speaker	310.13	
Student Travel	118.00	
Secretarial Expenses	300.00	
Local Arrangements (Austin Meeting)	100.00	
Treasurer Expenses	20.70	
TSEM Journal Printing	1,758.00	
	5,335.84	5,335.84 –
ASSETS ON APRIL 7, 1983		
Certificate of Deposit No. 91099, Univ. Natl. Bank, Galveston	2,000.00	
Certificate of Deposit No. 10-141345, Houston First Savings	2,713.95	
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Respectfully submitted
W. Allen Shannon, Jr., Treasurer

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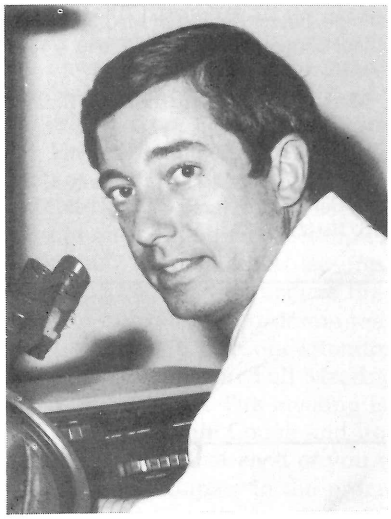

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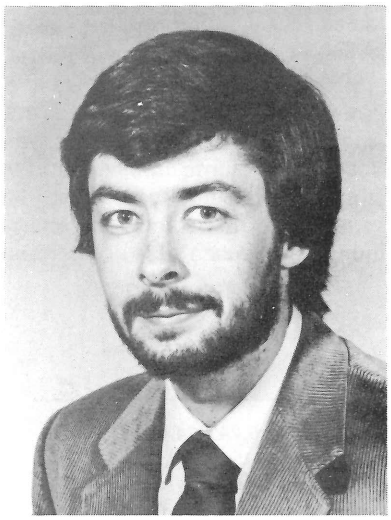

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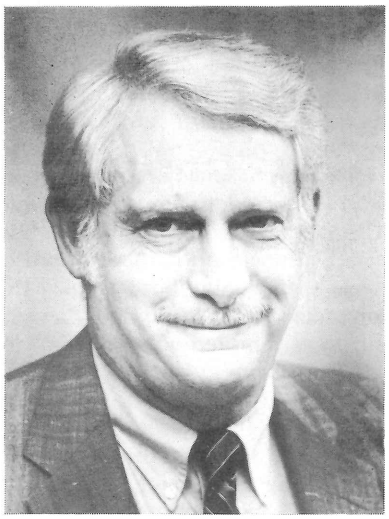

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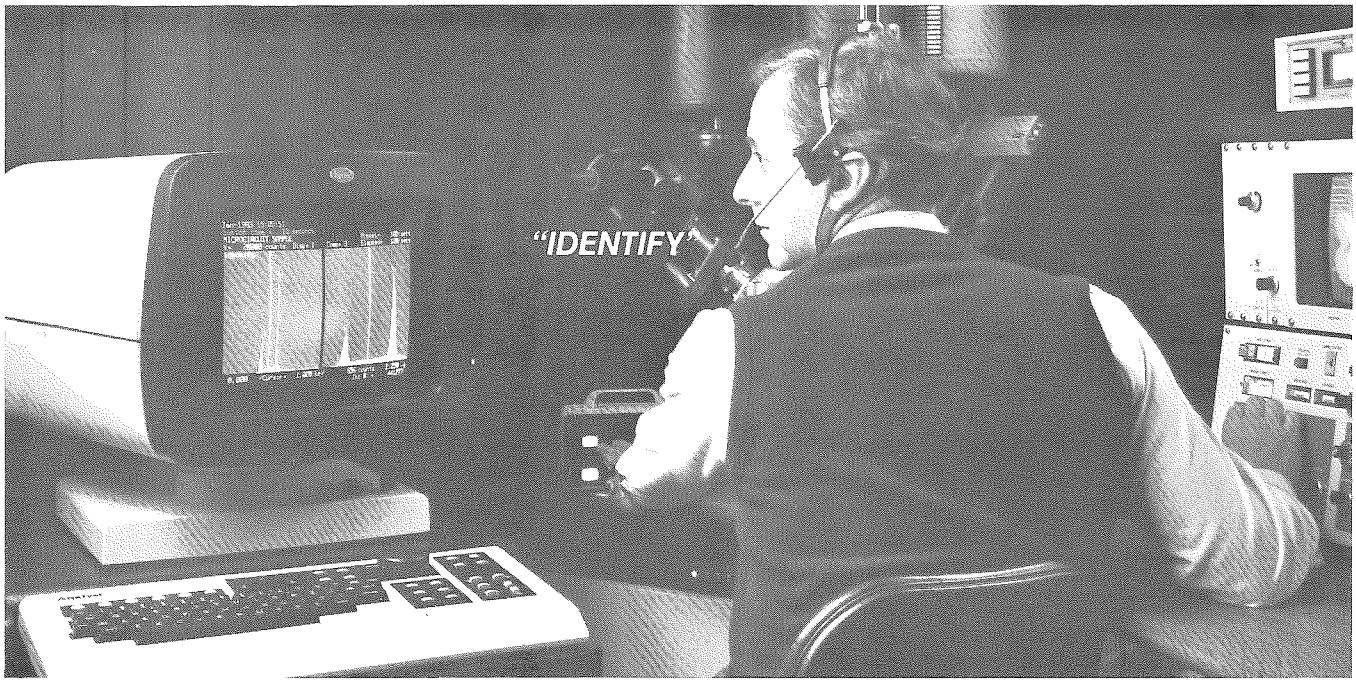

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TSEM/83

A METHOD FOR PREPARING MONOLAYERS OF CELLS GROWING ON COLLAGEN GEL SUBSTRATES FOR SCANNING ELECTRON MICROSCOPY

by

S. K. File[1] and C. P. Davis[2]

Departments of Pathology[1] and Microbiology[2]
University of Texas Medical Branch
Galveston, Texas 77550

INTRODUCTION

Collagen has a variety of effects upon the growth and development of cells in culture (1,2). Studies utilizing primary cultures of liver parenchymal cells have especially benefited from systems using collagen substrate. It prolongs the viability and many cellular functions for days beyond that obtainable from collagen free systems (3). While evaluating cellular injury caused by potential hepato-toxic substances it became important to examine the possibility of hepatocyte membrane alterations (4). The method of choice being scanning electron microscopy (SEM). Other studies have shown that diverse hepato-toxic chemicals cause dramatic changes in the hepatocyte membrane (5,6). Because it was desirable to examine cells after they had been in culture for 1 day or more, the following method was developed. The method is simple and conservative allowing subsequent processing of the tissue for transmission electron microscopy if desired.

MATERIALS AND METHODS

Collagen gels were prepared using a solution of rat-tail collagen, concentrated media and sodium hydroxide (3). Once mixed, the solution was distributed into sterile plastic culture dishes. A depth of 1 mm is optimum. The solution should gel very rapidly and therefore small batches are prepared allowing rapid distribution. Gels were used within 1-12 hours of preparation. They can be stored at 4°C or at 37°C. The collagen gels should not be allowed to dry.

Freshly isolated adult rat hepatocytes were diluted to a concentration of 10^6 cells/ml of media (Williams E supplemented with 10% fetal calf sera, .02 units/ml insulin, .05 mg/ml hydrocortisone and .002 mg gentamicin sulfate). This cell suspension was layered on the collagen gel (1 ml/35mm diameter dish) and cultures incubated in a moist incubator at 37° having a 95:5 air-CO_2 ratio. Unattached cells were rinsed off and fresh media added water 1 hour and again at 24 hours. The cells were fixed for 1 hour (2% gluteraldehyde in .1m PIPES buffer at 20°C) (4), post-fixed for 1 hour in 1% aqueous osmium tetraoxide, rinsed in PIPES buffer then dehydrated in a graded ethanol series. Great care must be taken with all the preceeding steps not to dislodge the collagen gel from the surface of the plastic dish. Once the cultures are in 95 or 100% ethanol, a square piece (approximately 1 cm^2) of the dish and collagen are cut out using a Dremel tool (Emerson Electric Co., Racine, WI.) and a cutting disc. During this step care must be taken not to allow the preparation to become overheated or to dry out. This is accomplished by keeping the plastic dish inverted in ethanol while cutting. The small pieces of collagen-coated culture dish are then dehydrated further in ethanol then critical point dried directly in CO_2. The edges may curl somewhat but the center is flat and suitable for viewing. The preparations were coated with gold in a Technics Hummer VI apparatus. Viewing and photography were done using a ISI III scanning electron microscope using 15 kV and a tilt of 40 degrees.

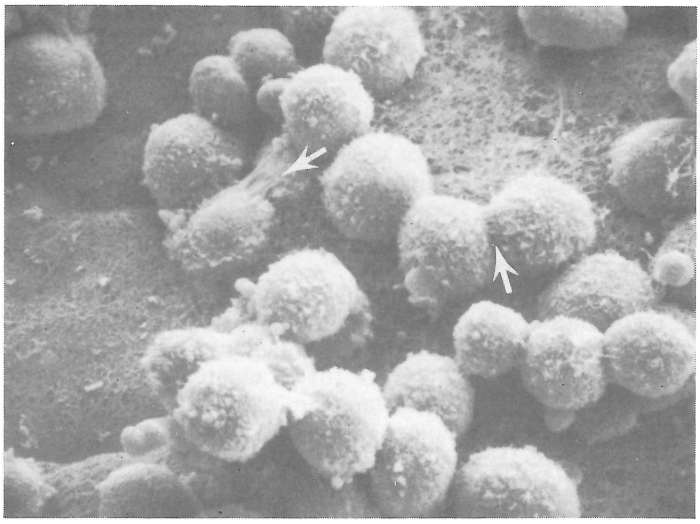

FIGURE 1: Hepatocytes after 1 hour on collagen gel. Cells still have microvillus surfaces, some are beginning to flatten out. Cells attach to collagen and each other via fine filapodia (arrow). X1220.

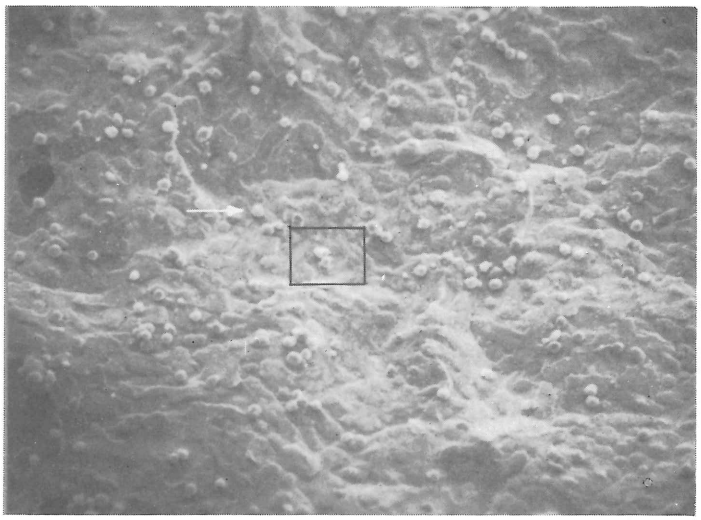

FIGURE 2: Hepatocytes after 24 hours on collagen gel. Living cells have formed a monolayer. Subviable cells and/or large blebs remain spherical (arrows). Surface of gel is slightly uneven producing wavey appearance. Note the absence of cracks and distortions in gel. X122.

RESULTS AND DISCUSSION

This technique allows examination of cells monolayers without producing excessive tearing and cracking at intracellular junctions (8). The well preserved surface features also suggest the advantages of growing cells on a preferred substrate instead of plastic or glass (Figures 1-3). The method also allows SEM examination of monolayers of hepatocytes on collagen several days after isolation. Instead of suspension cultures which generally survive for only a few hours. The portion of the monolayer not removed for SEM can be floated off the plastic and embedded for TEM. Alternatively, the SEM specimen can be removed, embedded and sectioned if close correlation of structures seen with SEM and TEM is desired.

FIGURE 3: Higher magnification of box indicated in Figure 2. Flattened cells have formed trabeculae, some areas on surface of cells have numerous microvilli and there is a definite pattern of overlaping indicating intracellular junctions (double arrows). Numerous small indentations may be bile caniculae (single arrow). Note the absence of distortion in the gel-cell surfaces and intracellular relationships using the technique described. X1220.

REFERENCES

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(2.) S. Hauschka and I. Konigsberg. The influence of collagen on the development of muscle clones. Proc. Natl. Acad. Sci. USA (1966). 55:119.

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(8.) J.C. Wanson, D. Bernaert and C. May. Morphology and functional properties of isolated and cultured hepatocytes. Prog. in Liver Disease (1979) Vol. VI. 1-22.

Job Opportunities

The University of Texas Health Science Center at Houston is looking for a Research Scientist with a materials science/physics/electronic background to aid in operation of a biological x-ray microprobe facility (Cameca MBX, and Tracor Northern EDS). Previous experience with x-ray microprobe analysis is essential. Responsibilities would include maintaining equipment, programming, development of new instrumentation and techniques, as well as aiding biological scientists in appropriate applications of XMP methods. Salary and position commensurate with experience. Contact: Albert J. Saubermann, Director, Microprobe Center, University of Texas Health Science Center at Houston, MSMB 5182, 6431 Fannin, Houston, Texas 77030, 713-792-5570.

The University of Texas Health Science Center at Houston is an Equal Opportunity Employer. Women and minorities are encouraged to apply.

Announcements

(1) Texas Society for Electron Microscopy's Annual Fall Meeting — 1983

October 13-15, 1983
Sheraton Inn-Tyler, Texas

Guest Speaker: Benjamin Trump, M.D.
Department of Pathology
University of Maryland School of Medicine, Baltimore

Agenda: Rose Garden Tour
Social Event(s)
Platform and Poster Sessions
Local Arrangements: Ronald Dodson and Mlynn Davis

Contact: Ernest F. Couch
Program Chairman, TSEM
Dept. Biology
Texas Christian University
Ft. Worth, Texas 76129

(2) EMSA/MAS Annual Meeting — 1983

August 6-12, 1983
Hyatt Regency and Adams Hilton
Phoenix, Arizona

Local Arrangements: C. Ward Kischer
Department of Anatomy
College of Medicine
University of Arizona
Tucson, AZ 85724

Contact: Pat Calarco
Department of Anatomy
University of California
San Francisco, CA 94143

(3) American Society for Cell Biology's Annual Meeting — 1983

November 29-Dec. 3, 1983
Convention Center
San Antonio, Texas

Local Arrangements: Bob Turner
Department of Pathology
Scott and White Clinic
Temple, Texas 76508
(817) 774-3688

Contact: Professional Associates
2012 Big Bend Boulevard
St. Louis, MO 63117

(4) High Voltage Electron Microscopy in Medical Research

A symposium on High Voltage Electron Microscopy in Medical Research will be co-hosted by Bowman Gray School of Medicine of Wake Forest University and the Appalachian Regional Electron Microscopy Society in Winston-Salem, North Carolina on October 14 and 15, 1983. The purpose of this symposium will be to examine present uses of high voltage electron microscopy (HVEM) in the biomedical sciences, with the goal of stimulating interest in the application of this technique to medical research. Symposium topics will illustrate the correlative use of conventional and HVEM along with other techniques such as computer image reconstruction, cytochemistry, x-ray tomography, and immunoelectron microscopy in the areas of cardiovascular disease, hematologic disorders, chromosome defects, neuromuscular pathology, viral diseases, and tumor pathology.

In addition to invited speakers, the submission of abstracts for an informal poster session is welcomed. Further information can be obtained by contacting either Dr. Jon Lewis, Department of Pathology, Bowman Gray School of Medicine, Winston-Salem, NC 27103, telephone (919) 748-2668, or Dr. Carole Browne, Department of Biology, Wake Forest University, Winston-Salem, NC 27103, telephone (919) 761-5527.

Regional News

BAYLOR UNIVERSITY
DEPARTMENT OF BIOLOGY

PUBLICATIONS

C.W. Mims and R.W. Roberson. 1983 Ultrastructure of ornamentation development on aeciospores of *Cronartium quercuum* - *Mycologia* 75:401-411.

Moore, Randy. 1982.Studies of vegetative plant tissue compatibility-incompatibility. V.A morphometric analysis of the development of a compatible and an incompatible graft. Can. J. Bot. 60:2780-2787.

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Moore, Randy and C. Edward McClelen. A morphometric analysis of cellular differentiation in the root cap of *Zea mays*. Amer. J. Bot. 70:611-617.

Sampson, H.W. and D.E. Bowers. 1982. Intracellular Calcium Localization in Stimulated and Non-Stimulated Eccrine Sweat Glands. J. Anat. 135:565-575.

Sampson, H.W., D.E. Bowers, M.S. Cannon and I. Piscopo. 1982. Intracellular Calcium Localization in Stimulated and Non-Stimulated Extraorbital Lacrimal Glands of Rats. Tiss. Cell. 14:735-749.

Sampson, H.W. 1982. Increased Mitochondrial Size During Cholinergic Induced Exocrine Gland Secretion. Cell. Boil. Int. Reports. 6:981.

NEW FACULTY AND/OR STAFF

Dr. Thomas Caceci has recently been appointed Assistant Professor of our Anatomy and Director of the EM. Lab.

Pam Neill has accepted a position as an EM Tech II with Dr. Vincent in Entomology.

INVITED LECTURES/SEMINARS

Development of the embryonic chick heart - scanning and transmission electron microscopic study - Don A. Hay, Department of Anatomy, College of Medicine, Univ. of Florida.

TEXAS WOMAN'S UNIVERSITY
DEPARTMENT OF BIOLOGY

NEW FACULTY

Dr. Fritz Schwam is the new chairman of the Biology Department at TWU. Dr. Schwam is a developmental biologist who uses the electron microscope as one of his tools for research.

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PUBLICATIONS

K. Wang and R. Ramirez-Mitchell, A Network of Transverse and Longitudinal Intermediate Filaments is Associated with Sarcomeres of Adult Vertebrate Skeletal Muscle. J. Cell Biol. 95 (1982) Part 2, 234 a.

K. Wang and R. Ramirez-Mitchell, Titin is an Extraordinarily Long and Flexible Myofibrillar protein. J. Cell Biol. 95 (1982) Part 2, 372 a.

DEPARTMENT OF BOTANY

PUBLICATIONS

R. Malcolm Brown, Fr., Ed., Cellulose and Other Natural Polymer Systems. Biogenesis, Structure and Degradation. Plenum Press, New York/London, 1982.

Candace H. Haigler, R. Malcolm Brown, Jr., and M. Benziman, Calcofluor White ST Alters in **In Vivo** Assembly of Cellulose Microfibrils. Science 210 (1980) 903-906.

Candace H. Haigler, Alan R. White, R. Malcolm Brown, Jr. and Kay M. Cooper, Alteration of **In Vivo** Cellulose Ribbon Assembly by Carboxymethylcellulose and Other Cellulose Derivatives. J. Cell Biol. 94 (1982) 64-69.

Karl J. Niklas, R. Malcolm Brown, Jr., Richard Santos and Brigitte Vian, Ultrastructure and Cytochemistry of Miocene Angiosperm Leaf Tissues. Proc. Nat'l. Acad. Sci. USA 75 (1978) 3263-3267.

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Moshe Benziman, Candace H. Haigler, R. Malcolm Brown, Jr., Alan R. White and Kay M. Cooper, Cellulose Biogenesis: Polymerization and Crystallization Are Coupled Processes in **Acetobacter Xylinum**. Proc. Nat'l. Acad. Sci. USA 77 (1980) 6678-6682.

M. Dauwalder and W.G. Whaley, Ethanol Induced Changes in Membrane Systems in Higher Plants. J. Cell Biol. 95 (1982) 264 a.

J.W. LaClaire II, Light and Electron Microscopic Studies of Growth and Reproduction in **Cutleria** (Phaeophyta). Phycologia 21 (1982) 273-287.

NEW EQUIPMENT

A Phillips 420 electron microscope with equipment for image processing is being installed in the laboratory of Dr. Malcolm Brown.

NEW FACULTY AND/OR STAFF

Dr. Tako Itoh of Kyoto, Japan is a visiting faculty member in the Department of Botany. Dr. Sharda Saradambal has a postdoctoral position in Dr. Malcolm Brown's laboratory.

OTHER NEWS OF INTEREST TO MEMBERS OF TSEM

Dr. W. Gordon Whaley, Ashbel Smith Professor of Cellular Biology, died the night of December 14, 1982, at the age of 68. Professor Whaley was a foremost authority on the Golgi Apparatus and one of the first to describe this organelle in plants. He was indeed a father of electron microscopy of plant systems in the United States, and greatly influenced development of electron microscopy in Texas. His tenure at UT included terms as Chairman of the Botany Department, Dean of the Graduate School and Director of the Cell Research Institute. Many of us in TSEM knew him as an excellent teacher, mentor, and friend. Prof. Whaley had planned to retire in 1984.

COLLEGE OF PHARMACY, PHARMACOLOGY

OTHER NEWS OF INTEREST TO MEMBERS OF TSEM

Dr. Daniel Acosta and Dr. Elsie Sorensen have received a grant from the Johns Hopkins Center for Alternatives to Animal Testing (director, Dr. Alan Goldberg), entitled Hepatotoxicity: An **In Vitro** Approach to the Study of Toxicity and Membrane Interactions of Cadmium Using Cultured Rat Hepatocytes.

REFERENCES

Opium poppy laticifers - Ultrastructure and alkaloids - Craig L. Nessler, Department of Biology, Texas A&M University.

Spermiation: a target for male contraception - Curtis Gravis, Department of Anatomy, UTHSC at San Antonio.

Biology and Ultrastructure of **Pleistophora compostomi** n. sp. from stone rollers from Southern Illinois - Robert price, Department of Zoology, Southern Illinois University.

TEXAS A&M UNIVERSITY

NEW FACULTY

Dr. Robert Burghardt is now Director of the EM Center.

NEW EQUIPMENT

A new Zeiss 10C Teaching Microscope is now in operation in the EM Center.

UNIVERSITY OF TEXAS AT AUSTIN

DEPARTMENT OF BOTANY

PUBLICATIONS

G.T. Cole, S.H. Sun and M. Huppert, Isolation and Ultrastructural Examination of Conidial Wall Components of **Coccidioides** and **Aspergillus.** Scanning Electron Microscopy 1982, Vol IV (1983) 1667-1676.

L.M. Pope and G.T. Cole, Comparative Studies of Gastrointestinal Colonization and Systemic Spread by **Candida albicans** and Nonlethal Yeast in the Infant Mouse. Scanning Electron Microscopy 1982, Vol. IV (1983) 1677-1685.

G.T. Cole and R.A. Samson, Airborne Fungi, in: Mold Allergy, Ed. Y. Al-Doory (Lea and Febiger, 1983)

G.T. Cole, **Graphiola phoenicis:** a Taxonomic Enigma. Mycologia 75 (1983) 93-116.

NEW FACULTY AND/OR STAFF

Dr. Cecilio R. Barrera, associate professor at New Mexico State Univ., spent the spring semester, 1983, in Dr. Garry Cole's laboratory working on morphogenesis of **Mucor rouxii.**

OTHER NEWS OF INTEREST TO MEMBERS OF TSEM

Dr. Garry Cole spent two weeks during March, 1983 at the Pasteur Institute in Paris, France working on wall structure and chemistry of **Conidiobolus obscurus,** a pathogen of cereal aphids which is being studied as a possible biological control for these insects.

COLLEGE OF PHARMACY, PHARMACOLOGY

PUBLICATIONS

Elsie M.B. Sorensen, T.L. Bauer, J.S. Bell and C.W. Harlan, Selenium Accumulation and Cytotoxicity in Teleosts Following Chronic, Environmental Exposure. Bull. Environm. Contam. Toxicol. 29 (1982) 688-696.

DEPARTMENT OF ZOOLOGY

PUBLICATIONS

W.R. Jeffery and S. Meier, A Yellow Crescent Cytoskeletal Complex in Ascidian Eggs and Its Role in Early Development. Developmental Biol. 96 (1983) 125-143.

UNIVERSITY OF TEXAS HEALTH CENTER AT TYLER

PUBLICATIONS

Dodson, R.F., R.R. Martin, M.F. O'Sullivan and G.A. Hurst. 1982. **In vitro** response of human pulmonary macrophages with volcanic ash: a morphological study. Expmtl. Molec. Path., 37:406-412.

Lawrence, E.C., H.W. McClung, R.K. Wilson, M.M. Key, R.F. Dodson and G.A. Hurst. 1982. Alteration of **in vitro** immuno-globulin secretion by amosite asbestos. J. Immuno., 129:1931-1935.

Dodson, R.F., S.D. Greenberg, M.G. Williams, Jr., C. Corn, and G.A. Hurst: Ferruginous body content from lung tissue of occupationally and non-occupationally exposed groups. Presented at the International Academy of Pathology, Atlanta, Georgia, February 28, 1983.

NEW APPOINTMENTS

Dr. Ronald F. Dodson, Chief and Research Professor of Cell Biology and Environmental Sciences has been appointed Assistant to the Director for Research.

UNIV. OF TEXAS AT ARLINGTON

PUBLICATIONS

Allen, R.D., D.A. Prier, and L.H. Bragg. 1982. Protein bodies and lipid bodies in the dormant cotyledons of **Prosopis glandulosa** (mesquite). Scanning Electron Microsc. 1982/I: 221-228.

Arnott, H.J. 1982. Calcium oxalate (weddellite) crystals in forest litter. Scanning Electron Microsc. 1982/III: 1141-1149.

Arnott, H.J. and M.A. Webb. 1983. Twin crystals of calcium oxalate in the seed coat of the kidney bean. Protoplasma 114: 23-24.

Bragg, L.H. 1982. An SEM comparison of the seed coats of **Prosopis glandulosa** and **P. pallida.** Scanning Electron Microsc. 1982/I: 213-219.

Grimson, M.J., H.J. Arnott, and M.A. Webb. 1982. An SEM study of winged twin crystals in the bean legume. Scanning Electron Microsc. 1982/III: 1133-1140.

Webb, M.A. and H.J. Arnott, 1982. Cell wall conformation in dry seeds in relation to the preservation of structural integrity during desiccation. Am. J. Bot. 69:1657-1668.

Webb, M.A. and H.J. Arnott. 1982. A survey of calcium oxalate crystals and other mineral inclusions in seeds. Scanning Electron Microsc. 1982/III: 1109-1131.

NEW EQUIPMENT

The University has acquired a Tracor Northern TN-2000 energy dispersive x-ray analysis apparatus that is attached to the JEOL JSM-35C scanning electron microscope.

EM EDUCATION OPPORTUNITIES

Dr. H.J. Arnott will offer a course in Scanning Electron Microscopy in the fall semester, 1983.

TEXAS TECH

NEW FACULTY

Drs. Reid Norman and Peter Doris have accepted faculty positions in the department of Anatomy at Texas Tech Health Sciences Center. Dr. Norman, an Endocrinologist, is currently at the Oregon Regional Primate Research Center in Portland. Dr. Doris comes to us from the Department of Physiology at the University of Reading, England. His research interests are hypothalamic hypophyseal interactions.

JOB AND EDUCATIONAL OPPORTUNITIES

The department is in the process of interviewing for two more faculty positions and also anticipates having a number of graduate assistantships in the amount of $7,000 each available to qualified applicants to our graduate program.

PUBLICATIONS AND ABSTRACTS

Runyan, R.B., G.T. Kitten and R.R. Markwald. 1982. Proteins of the embryonic extracellular matrix: regional and temporal correlation with tissue interactions in the heart. In "The Extracellular Matrix". (S. Hawkes and J. Wang eds.) Academic Press, N.Y. pp. 153-158.

Kitten, G.T., R.R. Markwald and R.B. Runyan. 1982. Parameters influencing the formation of cardiac mesenchymal cells in 3-dimensional collagen gel culture. In "The Extracellular Matrix". (S. Hawkes, J. Wang eds.) Academic Press, N.Y. pp. 159-164.

Markwald, R.R., F.M. Funderburg, R.B. Runyan and G.T. Kitten. 1982. Extracellular glycoprotein mediates the binding of hyaluronate to **in situ** migrating chick cardiac mesenchyme. In "The Extracellular Matrix" (S. Hawkes and J. Wang eds.) Academic Press, N.Y. pp. 239-246.

CORPORATE MEMBERS

AMRay, Inc., Thomas Levesque, 5209 Kisor Dr., Box 83416, Lewisville, TX 75056.

Bausch & Lomb., Bill Burton, 4885 Alpha Road, Suite 105, Dallas, Texas 75234.

Cambridge Scientific, Mike Webber, 3945 Fairington Dr., Marietta, Georgia 30066.

E.I. DuPont de Nemours, Inc., Biomedical products Div., Harry Vacek, Concord Plaza-Quillen Bldg., Wilmington, DE 19898.

EBTEC Corp., Margrit Barry, 120 Shoemaker Lane. Agawam, Mass. 01001.

EDAX International, Inc., Jim Moore, P.O. Box 2253, Boulder, CO 80306.

Electron Microscopy Sciences, Dr. Richard Rebert, Box 251, Ft. Washington, PA 19034.

Ernest F. Fullman, Inc., Richard Kemmer, 900 Albany Shaker Rd., Latham, NY 12110.

Hitachi Scientific Instruments, Jonni Fischer, 2407 W. Settlers' Way, Woodlawns, TX 77380.

Int'l Scientific Instr. Inc., John Fitzpatrick, 3255-6C Scott Blvd., Santa Clara, CA 95051.

JEOL, Richard Lois, 1 Kingwood Place Suite 122-B, 600 Rockmead Dr., Kingwood, TX 77339.

Kevex Corp., Dick Cushing, 1101 Chess Dr., Foster City. CA 94401

Ladd Research Industries, Margaret Ladd, P.O. Box 901, Burlington, Vermont 05402.

Link Systems, Bill Stewart, P.O. Box 2160, Sunnyvale, CA 94087

LKB Instruments, Inc., Charles Weinert, 8319 Sharpcrest, Houston, TX 77036.

EG&G Ortec Instruments, Richard Neiman, 21718 Rotherham Dr., Spring, TX 77379.

Philips Electronics Instruments, Robert L. Peterson, 7302 Harwin, Suite 106, Houston, TX 77036.

Ted Pella, Inc., T.P. Turnbull, 16812 Milliken Ave., Irvine, CA 92714.

Polaron Instruments, Inc., Dermot O. Dinan, 2293 Amber Drive, Line Lexington Industrial Pk, Hatfield, PA 19440.

B. David Halpern, Polyscience, Paul Valley Industrial Park, Warrington, PA 18976.

Princeton Gamma Tech, Dick Stancher, 17756 Kings-Park lane, Houston, TX 77058.

Rockwell International, R.W. Max, Mail Station 406-146, Richardson, TX 75081.

SPI Supplies, Charles A. Garber, President, 535 East Gay Street, P.O. Box 342, Westchester, PA 19380.

Technics EM Systems, Inc., Diane A. Hurd, 7653 Fullerton Rd., Springfield, VA 22153.

Carl Zeiss, Inc., Dietrich Voss, 3233 Weslayan 191, Houston, TX 77027.

EDITORIAL POLICY

(Continued from Page 3)

REGIONAL NEWS

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

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

TECHNICAL SECTION

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

PUBLICATION PRIVILEGES

The right to publish in the TSEMJ is restricted to TSEM members or to those whose membership is pending. A membership application form can usually be found in each issue of the TSEMJ. Membership dues are as follows: students $2.00; regular members $10.00; Corporate members $75.00. Individuals who belong to TSEM by virtue of a corporate membership are invited to participate in Journal submissions as are our regular or student members. However, papers of a commercial nature, either stated or implied, will not be accepted for publication as a Research Report or Techniques Paper. Such papers may be acceptable as advertising copy.

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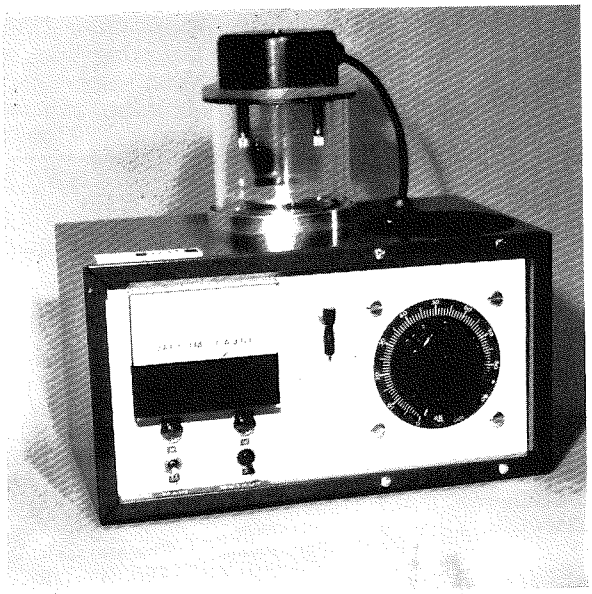

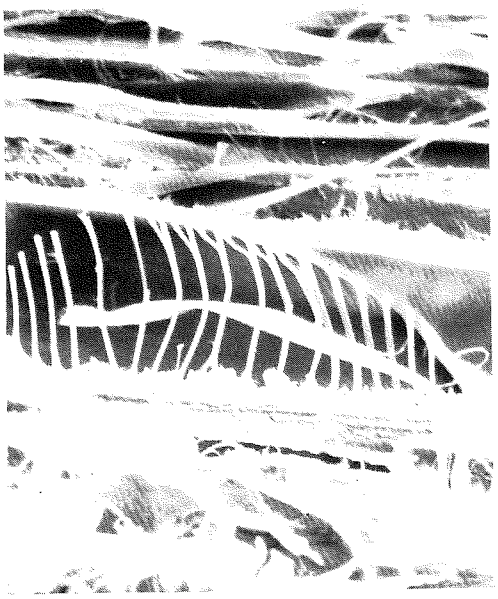

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TSEM SPOTLIGHT

Humberto Fernandez-Moran

BORN

Maracaibo, Venexuela
Feb. 18, 1927

EDUCATION

A.B. Schulgemeinde Wichersdorf, Saalfeld (Germany) 1940
M.D. University of Munich, 1944
M.D. University of Caracas, 1945
M.S. Cell Biology, University of Stockholm, 1951
Ph.D. Biophysics, University of Stockholm, 1952

POST-DOCTORAL STUDIES

George Washington University, 1945-1946, Serafimerlasarettet, Stockholm
1946-1948, Nobel Institute of Biophysics, Stockholm
1947-1949, Karolinska Institutet, Stockholm
1948-1951.

PROFESSIONAL APPOINTMENT

A.N. Pritzker Divisional Professor of Biophysics in the Division of Biological Sciences and the Pritzker School of Medicine The Research Institutes - University of Chicago.

MEMBER OF

More than 20 professional societies, boards, and advisory or editorial committees.

AWARDED

Claude Bernard Medal (University of Montreal),
John Scott Award (City of Philadelphia),
Special EMSA Citation for contributions to EM,
And honorary membershipe TSEM 1980.

Call Home Memo #1

To: All TSEMJ Readers
From: T.E.*

Message: Why don't you send us one of your manuscripts (Research Reports, Technical Notes, or Historical Reviews) for publication? We really need your input! Without them, T.E. really will have to "Go Home".

*The Editor (TSEMJ)

Call Home Memo #2

To: TSEM Members (Present, Past, Potential)
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Message: We need your contributions NOW! When I must wait; the Journal is late!!!

*The Editor (TSEMJ)

Abstracts

AMYL NITRITE INDUCED ULTRASTRUCTURAL ABERRATIONS IN IN VITRO LEUCOCYTES. A.M. Andrews*, T.R.Hoage*, Y.White* and R.F.Jacobs. *National Center for Toxicological Research, Jefferson, AR. 72079 Dept. Pediatrics, University of Arkansas for Medical Science, Little Rock, AR. 72205.

Amyl nitrite (AN) ($_5H_{11}NO_2$, MW 111.15) is a common drug used in medicine as a vasodilator in the treatment of angina pectoris. AN use by homosexual males and the outbreak of Kaposi sarcoma-opportunistic infection (KSOI) syndrome in males that use AN, suggests that an interaction exists. The acquired immunodeficiency syndrome (AIDS) associated with this male population suggests related lymphocyte immunity reaction suppression. AN vapors effect on cultured peripheral blood sampled leucocytes were studied by ultrastructural analysis. Human mononuclear cells (MC) were isolated from normal human peripheral whole blood and adjusted to 2 x 10^6cells/ml in RPMI 1640 with 20% serum. MC cell suspensions in Warburg Flasks at 3-4ml volumes had AN ampules (USP) emptied into the middle chamber. The sealed flask was incubated at room temperature for 5 or 10 mins. MC suspensions for electron microscopy were fixed in phosphate buffered 4% glutaraldehyde (2 hrs), washed, the pellet resuspended and fixed in phosphate buffered 1% osmium (1 hr), washed and dehydrated. One half of each suspension was prepared for SEM and one half was centrifuged in preparation for TEM. SEM mounts were examined in a JEOL/JSM-35 SEM at 25kV and thin sections were examined using a Philips 201 at 60kV. All cells treated had the same response to AN, as seen by plasma and cytoplasmic membrane profile alterations. The outer nuclear membrane exhibited vesicular blebbing as did the Golgi apparatus. It was concluded that AN affects plasma membrane integrity closely allied with the immunological binding sites. Alteration of the membrane immune system could contribute to the increased AIDS and KSOI seen in amyl nitrite users.

CELLULAR STRUCTURE AND LIGHT ABSORPTION IN LEAVES OF FRITHIA PULCHRA (MESEMBRYANTHEMACEAE). Lois Simpson and Randy Moore, Department of Biology, Baylor University, Waco, Texas 76798

In order to quantify the structural differences between cell types constituting leaves of a "window" plant, we have performed a morphometric analysis of the ultrastructures of the epidermal, upper and lower window, and upper and lower chlorenchyma tissues of Frithia pulchra. Epidermal cells are the largest cells found in Frithia leaves, and are characterized by the presence of a thick cell wall, a thick cuticle, and numerous vacuolar inclusions. Epidermal tissue has an optical density of 0.3. Transparent window cells have a uniform ultrastructure throughout the length of the leaf, are characterized by a vacuolar volume of approximately 97% of the protoplasm, and have an optical density of 0.08. Chlorenchyma cells possess thin cell walls surrounded by numerous intercellular spaces. In cells of the upper chlorenchyma tissue there is an average of 30 chloroplasts per cell, each having a volume of 220 um^3. Cells of the lower chlorenchyma tissue compensate for lower light intensity by producing chloroplasts that are 5.4 times smaller and 5.7 times more numerous than chloroplasts in cells of the upper chlorenchyma tissue. The increased relative volume of chloroplasts in the protoplasm of the upper versus lower chlorenchyma cells (i.e., 31.4% and 20.2%, respectively) is positively correlated with the facts that the optical density of the upper chlorenchyma is 1.46, while that of the lower chlorenchyma is 0.97.

CYTOCHEMICAL LOCALIZATION OF GLYCOGEN IN FRACTIONS OF CANINE CARDIAC SARCOPLASMIC RETICULUM. D.L. Murphy, M.A. Goldstein, W.V. VanWinkle and M.L. Entman, Dept. Med., Baylor College of Medicine.

Isolated fractions of cardiac sarcoplasmic reticulum (SR) contain glocogen as shown biochemically and by electron Microscopy (Entman et al., Life Sciences 19:1623, 1976). Entman and co-workers have shown that extensive amylase digestion of these SR fractions removes phosphorylase and debrancher activity-enzyme activity involved in glycogenolysis-and that primary functions of cardiac SR membranes, namely calcium uptake and release, can be modulated by factors which modulate these enzymes. The present study provides cytochemical evidence for association of glycogen with enzymatically active SR. Fractions of cardiac SR were prepared and aliquots were digested by 0.5% α-amylase for 2 hrs. at 37°C, then fixed 20 min in 1.5% gluteraldehyde alone (no OsO_4 postfixation), pelleted, and processed for EM. Sections were oxidized by 0.8% buffered ethanolic periodic acid, rinsed, and stained 30-60 min. with alkaline bismuth subnitrate. Some grids were treated with sodium borohydride to block the Shiff aldehyde reaction prior to or following periodic acid treatment. Granules of a fine-grained reaction product, presumably metallic bismuth, averaging 25 nm in diameter were localized ultrastructurally. These granules were associated with vesicles of SR in control samples incubated in buffer only, but were largely absent from α-amylase-treated sections. Granules remaining were those most closely associated with SR membranes as shown by comparisons with α-amylase treated samples osmicated and stained with uranyl acetate and lead nitrate. The morphological and cytochemical demonstration of glycogen in enzymatically active cardiac SR preparations provides additional support for the biochemical concept of a structured enzyme complex involving the SR and glycogenolytic enzymes.

DEVELOPMENT AND ANATOMY OF THE PARASITE TRISTERIX APHYLLUS (LORANTHACEAE) INFECTING TRICHOCEREUS CHILENSIS (CACTACEAE). J. D. Mauseth, Dept. of Botany, University of Texas, Austin, TX 78712.

Many of the large columnar cacti Trichocereus chilensis near Santiago are infected by Tristerix (=Phrygilanthus) aphyllus. This is one of the most highly reduced plants known: it is an endoparasite, the flowers being the only parts of the plant ever to emerge from the host, all the rest existing as an endophytic haustorial system; roots, stems and leaves are not produced. After infection, the parasite spreads in all directions through the thick cortex of the host, eventually reaching the vascular cambium and conducting tissues. The parasite in this invasive stage occurs as a "mycelium" of uniseriate filaments that grow between host cells, deforming them, but only rarely entering them. Later growth is by apparently random cell division that produces irregular parenchymatous strands. Ultimately xylem and phloem are produced in these strands; the phloem is normal but the xylem is almost pure parenchyma, with only occasional idioblastic tracheary elements. Strands close to the epidermis of the host are able to produce adventitious flower buds that emerge through either soft regions in the epidermis (the areoles) or through accidental breaks in it. The flower stalk may persist, forming a small perennial inflorescence that has normal wood, phloem and bark but is without leaves or chlorophyll. The portions of the endophyte that produce these exophytic inflorescences do not develop normal anatomy, but persist as irregular parenchymatous strands with small amounts of xylem and phloem. Host cells appear healthy and normal, with no sign of damage caused by the presence of the parasite.

EFFECTS OF SURGICAL TRAUMA AND SYMPATHETIC INTERRUPTION ON DUODENAL COLUMNAR CELLS: STEREOLOGICAL ANALYSIS. J. Leon McGraw, Jr., Dept. Biology, Lamar Univ., Beaumont, Tx. 77710

Three groups of three male rabbits constituted three study populations: Group I-normal; Group II-mock abdominal surgery exposed sympathetic ganglia; Group III-amdominal surgery removed coeliac and mesenteric ganglia and a portion of sympathetic trunk. Groups II and III were kept four days post-surgery before biopsy. All groups were given water adlibidum but no food two days prior to biopsy. Nembutol was used during surgery and biopsies. The duodenum of all groups was biopsied one inch below stomach and prepared for TEM with a 3% glutaraldehyde-0.1M sodium cacadylate-ethanol-Spurr method and sectioned. Stereological analysis was performed on columnar epithelial cells. Group II had significant decreases ($p<0.05$) in real and relative volumes of mitochondria, lysosomes and E.R. and significant increases in real and relative volumes of the nucleus and cytoplasm ($p<0.05$) compared to Group I but had little change in total cell volume. The assumption is that surgical trauma decreases the oxidative metabolism and secretory activity of this cell. In contrast, there were significant increases ($p<0.05$) in real and relative volumes of mitochondria and lysosomes of columnar epithelial cells of Group

III compared to Group II. There was no sognificant change in relative volume of E.R. but there was a slight increase in real volume of E.R. of Group III compared to Group II. The real and relative volumes of the nucleus decreased significantly ($p<0.05$) while real and relative volumes of the cytoplasm increased significantly ($p<0.05$) in Group III compared to Group II. Removal of sympathetic inhibition to this cell resulted in an apparent increase in oxidative metabolism and secretory activity, a decrease in nuclear size and cytoplasmic hypertrophy. Stereological analysis appears to monitor ultrastructural changes associated with sympathetic and parasympathetic stimuli.

THE EFFECTS OF WATER STRESS ON CHLOROPLAST LIPID BODIES IN COTTON LEAVES. Jerry D. Berlin, Susan L. Middleton, Jerry Quisenberry, Department of Biological Sciences, Texas Tech University, Lubbock, Texas 79409 and USDA, Lubbock, Texas 79401.

Long-term water stress causes a number of morphological and physiological changes in plants. We previously reported a number of water stress induced subcellular changes in palisade cells of a commercial cultivar, Paymaster 266 (Berlin et al. 1982 Plant Physiol. 70: 238-243), including an increase in the volume of chloroplast lipid bodies. Such an increase might afford a stress mechanism in that less energy would be required to store lipids compared to carbohydrates. Also, the continued reduction of carbon dioxide may be beneficial to stressed plants. Preliminary stereological analysis of water stressed leaves of T-25, a stress resistant geontype, revealed a 77% reduction in the number of lipid bodies per chloroplast, but the volume per lipid body increased 74%. We are investigating the effect of water stress on chloroplast lipid bodies in other genotypes in an attempt to evaluate the role of these bodies in the plants' response to water stress. Supported by Cotton Incorporated and by the Institute for Research in Plant Stress, Texas Tech University.

THE FINE STRUCTURE OF DEVELOPING LATEX DUCTS IN TUBERCLES OF MAMMILLARIA HEYDERI (CACTACEAE). G. H. Wittler and J. D. Mauseth, Dept. of Botany, University of Texas, Austin, TX 78712.

Electron microscopy was used to investigate early development of latex ducts in tubercles (persistent leaf bases) of Mammillaria heyderi (Cactaceae). In this species, latex ducts consist of vesicle-filled lumens (formed by partial cell autolysis) surrounded by epithelia. During the formation of the lumen, parietal vesicles form from invaginations of the plasmalemma near sites of wall thinning. The endoplasmic reticulum (ER) and older plastids may contribute to the formation of secondary vacuoles. Dictyosomes, though they occur in young duct cells, do not seem to be responsible for the formation of vesicles. Vesicles may contain fibrillar, globular, or crystalline matter, and they may be responsible for the production and storage of numerous laticiferous components. Lysosomal materials could be stored in some vesicles and contribute to the degradation of the protoplast. Some nuclei contain condensed chromatin and are subject to deformation and collapse. Mitochondria and spherosomes are common in young duct cells, but ER is rare. When ducts form in young tissues, plastids in the lumen do not produce starch grains or extensive membranous networks. The plastids eventually degenerate to become a part of the latex. If ducts form in older, established tissues having mature plastids, the plastids undergo extreme modification and ultimately degenerate.

IMPROVED METHODS FOR MOLDING, FACING, AND SECTIONING GLYCOL METHACRYLATE TISSUE BLOCKS FOR HIGH RESOLUTION LIGHT MICROSCOPE HISTOLOGY. J. K. Butler, Dept. Biology, University of Texas, Arlington, TX 76019.

Improvements are described in glycol methacrylate embedding, block facing and trimming, and sectioning involving the use of a novel molding system, an instrument for rapid block facing and trimming, and a device that rapidly removes unwanted sections from the microtome knife during sectioning. Together, these methods substantially facilitate specimen preparation resulting in significant reduction in the amount of time required to prepare high resolution very-thin sections for light microscopy.

THE INTERNAL STRUCTURE OF DRUSES AND GLOBOIDS IN VITIS VINIFERA ENDOSPERM. M. A. Webb and H. J. Arnott, Department of Biology, The University of Texas at Arlington, Arlington, TX 76019.

In the endosperm of Vitis vinifera each cell contains a large protein body which has a mineral inclusion that is either a druse of calcium oxalate or a globoid composed of phytin. The druses are composed of many individual crystals which radiate out from a central noncrystalline core. In an effort to understand the structure and composition of these cores and their relationship to the crystals in the druse, endosperm tissue was sectioned to expose the cores, treated in various ways, and observed with scanning electron microscopy (SEM) following treatment. The core was found to have two distinct regions, a central core region composed of protein and a peripheral core region that is a complex of organic and mineral substances. The peripheral core is closely associated with the base of the crystals and may serve to nucleate their growth. The globoids, the other type of mineral inclusions found in the protein bodies, have a variety of internal structures. From light microscopic observations we have categorized them as having 1) no internal structure, 2) a pattern of concentric rings, 3) a central granular area, or 4) a central crystalline body. Energy dispersive x-ray analysis of isolated globoids shows variation in elemental composition from one globoid to another in the relative amounts of Mg, P, K, and Ca present. In sectioned tissue the internal structure of the globoids can be observed with SEM and correlated with light microscopic observations.

THE IN VITRO OBSERVATION OF THE PROPOSED SEXUAL STAGES OF BABESIA BOVIS. Robert Droleskey[1], Patricia J. Holman[2], Thomas M. Craig[2], Hilton H. Mollenhauer[1], and Gerald G. Wagner[2]. [1]United States Department of Agriculture, Agricultural Research Service, Veterinary Toxicology Research Laboratory, P.O. Drawer GE, College Station, TX 77841 and [2]Center for Tropical Animal Health, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843

A Boophilus microplus continuous cell line of embryonic origin was inoculated with a Babesia bovis enriched fraction of parasitized bovine erythrocytes. After 24 hr incubation in 3% CO_2 at 32°C, electron microscopic examination of the cultures revealed B. bovis merozoites in the medium as well as within B. microplus cells. Also observed were forms of B. bovis which closely resembled the reported sexual forms of other Babesia species normally found within tick intestine. The proposed sexual stages observed in this study possessed a spike-like projection and a vacuolated area containing numerous microtubules. Other characteristics of the ultrastructure of these proposed sexual forms will be described.

A MODIFIED, SHORT PROTOCOL FOR PREPARATION OF BRYOPHYTES FOR SCANNING ELECTRON MICROSCOPY. A. J. Neumann, A. E. Rushing and D. M. J. Mueller, Department of Biology, Texas A&M University, College Station, TX 77843

The relatively thin cell walls of most bryophyte tissues have made critical point drying (CPD) a necessity in the preparation of bryophyte specimens for scanning electron microscopy (SEM) in order to overcome the problems of cell wall and tissue collapse inherent in air dried specimens. In an effort to simplify and shorten preparation protocols, several procedures using 2,2-dimethoxypropane (DMP) were tried before attaining acceptable results for SEM. Our results show that a standard protocol of immersing unfixed specimens in DMP for five minutes followed by a five minute wash in absolute acetone prior to CPD geve excellent preparations for SEM. This protocol bypasses problems of cell collapse and surface artifacts due to fixative reagents and utilizes the instantaneous chemical conversion of water to methanol and acetone by DMP, instead of the tedious and often damaging physical dilution and gradual replacement method of dehydration.

MONENSIN AT LOW CONCENTRATIONS INHIBITS ROOT GROWTH IN SEVERAL GRASSES. Hilton H. Mollenhauer and Robert E. Droleskey, USDA, ARS, VTERL, P.O. Drawer GE, College Station, TX 77841.

Monensin is a common agricultural compound used extensively as a coccidiostat in the poultry industry and as a feed additive to promote weight gain in cattle. In cattle, some monensin is metabolized by the liver and the metabolites, along with the remaining monensin, are excreted in dung. A recent report (Harold et. al., J. Animal Sci. 54:1128, 1982) indicates that monensin fed to cattle acts as a pesticide in that it partially inhibits the growth of fly larvae developing within the dung. The minimum effective amount of monensin within the dung has not been determined, but related studies indicate a possible level of 1-10 ppm. Thus, monensin could have a further beneficial affect on meat productivity by reducing the biting and face fly population. We have found, however, that monensin at concentrations of less than 10 ppm also inhibits root tip growth in corn and several other grasses, presumably by inhibiting cellular secretion, cell plate formation, and cell elongation. Thus, monensin accumulating in dung could inhibit the growth rate of grass adjacent to the dung. This could be significant near feedlots where dung, or its residues, might accumulate or where dung is used as fertilizer.

A MORPHOMETRIC ANALYSIS OF CYTOLOGICAL CHANGE DURING SPORE MATURATION IN DIDYMIUM IRIDIS. W.R. Fagerberg and C.W. Mims. Dept. of Biology, Southern Methodist Univ., Dallas, TX 75275 and Dept. of Biology, Stephen F. Austin State Univ., Nacogdoches, TX 75962

Young and mature spores of the slime mold Didymium iridis were analyzed using stereological analytical techniques. The V_V ratios of the nuclear, autophagic vacuole, mitochondrial, microbody, lipid and cell wall compartments were determined to evaluate changes in these compartments during maturation. During the 24 hr. maturation period the nuclear and mitochondrial compartments showed a significant decrease in the relative proportion (V_V) of the cell occupied while the autophagic vacuole and cell wall compartments showed significant proportional increases. The microbody and lipid compartments did not change. During spore maturation there was a 44% decrease in cell volume between young and mature spores. In actual volumes the nuclear, mitochondrial, microbody and lipid compartments decreased by varying amounts during maturation. The cell wall increased by over 1100% in actual volume during this period denoting considerable synthetic activity. The autophagic vacuole compartment did not change in real size during the maturation process. These results indicate considerable activity in the organelle compartments during maturation. Changes in compartment volume, except for the lipids, appeared to be independent of changes in cell volume during this period.

A MORPHOMETRIC ANALYSIS OF THE EGG SHELL STRUCTURE OF THE LIZARD CNEMIDOPHORNS SEXLINEATUS. W. R. Fagerberg and S. E. Trauth, Dept. of Biology, Southern Methodist University, Dallas, Texas 75275.

The egg shells of the lizard C. sexlineatus provides a unique opportunity to study a system where groups of cells secrete and construct a complex extracellular structure. Stereological parameters were used to describe the relationships between egg shell fibers and interfibrillar space as well as space found within the fibers. Three distinct zones of the shell were recognized with the innermost (Z1) being the first to be laid down and outermost (Z3) the last. As the egg shell is laid down a number of structural changes occur. In the first zone (Z1), which occupied 13% of the shell volume, fibers were significantly more dense (less intrafibrillar space) than fibers in the other zones. The ratio of interfibrillar space to fibrillar volume was equal in Z1. The fibers in this zone were equal in size to those of Z2. Zone 2 fibers are less dense than those of Z1 (more intrafibrillar space) but they do not change in size. There was significantly more fiber volume relative to space volume in this zone. Zone 2 occupied 61% of the total shell volume. Fibers of Z3 were significantly smaller than those of Z1 or Z2 but did not change in fiber density (intrafibrillar space). There was a significant decrease in the proportional volume of the fiber-interfibrillar space in Z3 compared to Z1, both (space-fiber) occupied equal volume in Z3. Zone 3 occupied 26% of the total volume of the shell. Based on actual volume measurements the Z1 fibers were 1.3 times more dense than Z2 fibers and 4.5 times more dense than Z3 fibers. The Z2 fibers contributed 5 times as much fiber to the total shell as Z1 and 3 times the fiber contributed by Z3. These results suggest that the construction of this extracellular structure is a cell controlled phenomenon.

A MORPHOMETRIC ANALYSIS OF THE ULTRASTRUCTURE OF COLUMELLA STATOCYTES IN PRIMARY ROOTS OF ZEA MAYS. Randy Moore, Biology Department, Baylor Unversity, Waco, Texas 76798

A morphometric analysis of the ultrastructure of columella statocytes in primary roots of Zea mays was performed in order to determine the precise locations of cellular organelles in graviperceptive cells. Vacuoles occupy the largest volume of the cell (11.4%). The nucleus (9.51%), amyloplasts (7.57%), mitochondria (3.42%), spherosomes (2.13%), and dictyosomes (0.55%) occupy progressively smaller volumes of the statocytes. All organelles are distributed asymmetrically within the cells. Amyloplasts, spherosomes, and dictyosomes are found in greatest numbers (and relative volumes) in the lower (i.e., "bottom") third of the cells. The largest numbers and relative volumes of mitochondria are in the lower and middle thirds of the cells. Nuclei tend to be found in the middle third of the statocytes. Only the hyaloplasm is concentrated in the upper (i.e., "top") third of Zea statocytes. The sedimentation of amyloplasts (and the resulting exclusion of other cellular organelles from the lower third of the cells) alone is not responsible for the differential distribution of other cellular organelles in Zea statocytes. The quantitative ultrastructure of Zea statocytes will be discussed relative to the graviperceptive function of these cells.

A MORPHOMETRIC STUDY OF EPIDERMAL DIFFERENTIATION IN ROOTS OF ZEA MAYS. Houston S. Smith and Randy Moore, Department of Biology, Baylor University, Waco, Texas 76798

The differentiation of epidermal cells in roots of Zea mays is characterized by six changes in the length:width ratio over a distance of approximately 2.2 cm. The first three stages of differentiation (i.e., apical protoderm (AP), cuboidal protoderm (CP), and tabular epidermis (TE)) will be reviewed from an earlier presentation. As cells of the TE differentiate, their shape changes from anticlinally flattened to cuboidal. This stage is arbitrarily labeled A1. A1 cells differentiate into a second group of cuboidally shaped cells (labeled A2) with a 120% increase in the relative volume of the vacuole, but without an increase in cellular volume. In the A1 to A2 transition there are areas in the hyaloplasm that are devoid of organelles and that are surrounded by expanded ends of endoplasmic reticulum. These areas may be localized transformations of the hyaloplasm to vacuole, which would explain the increase in vacuolar volume without a corresponding increase in cellular volume. The last cells studied, the columnar epidermal cells (CE), are characterized by cellular elongation and continued vacuolation. The ultrastructural changes observed during the differentiation of epidermal cells will be discussed relative to the functions of these cells.

MURINE GRAFT-VERSUS-HOST SKIN REACTION (GVHSR) J.H. Dees, M.R. Charley, R.D. Sontheimer & J.N. Gilliam, Dept. of Dermatology, U.T.H.S.C. at Dallas, Dallas, Texas 75235.

Graft-versus-host reaction is an important and potentially fatal clinical entity following bone marrow transplantation and other instances of the inoculation of immunologically competent donor cells into an immunologically incompetent host of disparate histocompatibility. The histopathology of human GVHSR resembles a number of autoimmune diseases of unknown etiology, such as lupus erythematosus (LE) and scleroderma. Studies on an animal model of GVHSR would provide insight into the pathogenesis of this disease as well as insight into the development of the lesions of LE and scleroderma. Therefore we have studied a murine model of GVHSR by sequential ultrastructural analysis of abdominal skin biopsies at 5,7,9,12 and 15 days post-transplantation.

Both epidermal and dermal alterations can be classified into early and late phases. Early epidermal changes include spongiosis, isolation of desmosomes, infiltration of the epidermis by lymphocytes, and the appearance of lipid-like inclusions in mitochondria of basal keratinocytes. Later, Keratinocytes exhibit several patterns of cell injury, including condensation and formation of colloid bodies, and liquifaction necrosis, while others show evidence of proliferation and dedifferentiation. Mitochondrial swelling and the persistence

of the mitochondrial lipid-like inclusions occurs in some keratinocytes. A striking feature of the dermal alterations is the presence of numerous mast cells at early time intervals (5,7 days), degranulating mast cells at 9 days, and the disappearance of mast cells from the dermis at 12 and 15 days. These and other dermal and epidermal cell reactions in GVHSR will be discussed in relationship to the known pathogenesis of this disease.

A NEW PROCEDURE FOR THE CYTOCHEMICAL LOCALIZATION OF PECTINASE ACTIVITY AT THE ULTRASTRUCTURAL LEVEL. R. D. Allen and C. L. Nessler, Department of Biology, Texas A&M University, College Station, TX 77843.

A technique that localizes pectinase activity in vivo has been developed for transmission electron microscopy. The method can be outlined as follows - Fix specimens in ice cold Karnovsky's fixative for 2 hr, rinse with 20 changes of 0.05 M cold phosphate buffer, pH 7.2 and store overnight in the same buffer at 0 C. Incubate tissues for 20 min in a substrate solution composed of 0.5% pectin dissolved in 0.1 M acetate buffer at pH 5.0, then transfer to hot Benedict's reagent and boil for 10 min. Following Benedict's treatment tissues are post-fixed for 2 hr in 1% osmium tetroxide, dehydrated through ethanol-acetone and embedded in Spurr's low-viscosity resin. Controls include: 1. specimens that are boiled prior to substrate incubation, 2. specimens that are incubated in buffer without pectin, and 3. specimens that are not treated with Benedict's reagent. Electron-dense crystals are deposited in areas where the Benedict's reagent reacts with galacturonic acid residues that have been liberated from the substrate by pectinase activity. This method has been used to localize pecinase activity in the nonarticulated, branched laticifers of Nerium oleander.

PERINUCLEAR MICROTUBULES IN THE DEVELOPING RAT HEART MUSCLE. R. S. Hawley, M. F. Legier, J. Cartwright, Jr. and M. A. Goldstein, Dept. Med., Baylor College of Medicine, Houston, TX 77030.

The numbers of microtubules per μm^2 in cross sectional area of developing rat heart and skeletal muscle increase in the postnatal rat to a maximum at 5-9 days and decline to the steady value of the adult muscle (J. Ultrastr. Res. 79:74, 1982, TSEM J. 13 (4):18, 1982). These numbers include two subpopulations of cytoplasmic microtubules 1) those randomly distributed between myofilament bundles and 2) others adjacent to the nucleus (perinuclear). In this study, we have determined the numbers of perinuclear microtubules. The perinuclear region was defined as that area around the nucleus which extends no further than .273 μ from the nuclear envelope. Perinuclear microtubules per μm^2 decrease after birth to a minimum at 5-9 days, then increase to a steady level characteristic of the adult. This trend is opposite to that of the whole population previously described.

These studies further indicate that microtubules have a role in orienting the intracellular structures of the developing muscle cells. In 1 day old rats, the myofilament bundles are arranged randomly with respect to each other. As the cells develop, the bundles increase in size and become oriented parallel to the muscle cell axis. During this time, the nuclei decrease in cross sectional area. Our study suggests a dynamic role of microtubules accomodating the needs of the developing cells.

PERMEABILITY OF RETINAL CAPILLARIES TO INTRAVITREOUS HORSERADISH PEROXIDASE IN DIABETIC RATS. W. Allen Shannon, Jr., Sally B. Bates and Michael L. Chandler, VA Medical Center Dallas, Department of Cell Biology, The University of Texas Health Science Center at Dallas and Alcon Laboratories, Inc., Fort Worth, TX.

Microvascular "basement membrane," i.e., basal lamina (BL) thickening has been demonstrated in the retina of diabetics. There is also an increase in vascular permeability. These phenomena have clinical importance in the pathogenesis of diabetic retinopathy. The thickening may result from overcompensation to increased permeability by an increase in endothelial cell synthesis of BL.

Horseradish peroxidase (HRP) in sterile saline (10% in 0.9% saline) was injected intravitreously (1.0 or 2.0 μl) in anesthetized streptozotocin diabetic and age-matched normal white rats. Following 2 to 60 min. the animals were sacrificed and the posterior half of each eye was fixed in glutaraldehyde for 3 hr. Following several rinses overnight the specimens were sliced thin, incubated in diaminobenzidine-H_2O_2 medium for visualization of the HRP, osmicated, and processed for TEM.

Normally there exists a blood-retinal barrier to small proteins, e.g., HRP ($\sim$40 kd mol. wt., $\sim$5 nm mol. dia.). Light and TEM sections revealed HRP penetration into the retinal inner cell layers. TEM further demonstrated intercellular deposition of HRP in normal and diabetic conditions. The greatest difference was in an apparent resistance to HRP diffusion through normal capillary BL relative to the diabetic at the same time. Many of the normal BL had a peripheral buildup of HRP. In addition, HRP was seen in endothelial cell junctions in the diabetic capillaries.

The increase in vascular permeability reported in diabetes is probably due to a defect in the BL of capillary endothelial cells as well as in the endothelial cell junctions.

THE ROLE OF ELECTRON MICROSCOPY IN THE DIAGNOSIS OF SOFT TISSUE TUMORS. B. Mackay, Dept. Pathology, Univ. of Texas M.D. Anderson Hospital and Tumor Institute, Houston, Tx.77030

Tumors arising from mesenchymal cells are remarkably varied in their histopathology, and frequently they fail to display any resemblance to the normal soft tissues of the body. Consequently they are often misdiagnosed, or can not be classified, when studied by light microscopy alone. The ultrastructural features of the tumor cells may be sufficiently distinctive to indicate the cell type and allow classification, but the spectrum of fine structure of a number of the malignant soft tissue tumors is broad and at the present time poorly defined. For example, the range of morphology that fibroblasts are capable of assuming appears to be extensive. In identifying soft tissue tumors, specific ultrastructural features of the cells such as the surface configuration, number and type of organelles, and presence and type of cytoplasmic filaments provide the principal diagnostic criteria, but in some instances the differences between cells of one sarcoma and another of a different type are of degree only. It is probable that electron microscopy will assume an increasing role in the identification of problem sarcomas as more data on the spectrum of the ultrastructure of the various types are obtained through the study of larger series of cases.

SEED SURFACES, HILA, AND TRACHEID BARS OF SELECTED MEMBERS OF THE SUBFAMILY PAPILIONOIDEAE (LEGUMINOSEAE). Terry L. Bridges and Louis H. Bragg, Biology Dept. University of Texas, Arlington, TX 76019.

Three species of Sophora and one species each of Sesbania and Strophostyles occurring in Texas were compared for features that may be useful in their taxonomies. The seed testae of the three Sophora species are distinct from each other as well as the testae of the three genera being distinct. The hila of the five examined species are also distinctively different. The tracheid bar and tracheoids of Sophora, Sesbania, and Strophostyles show the common patterns for the papilionaceous members but variations in the shape of the bar and tracheoid pitting differ slightly from that previously reported for the three tribes represented by these three genera. This is the first report of the testae, hila, and tracheid bars of Sophora. Although this preliminary study has been useful in separating the three genera as well as the three species using these criteria, additional representatives should be examined to ascertain the validity of these characters for further taxonomic usage.

SEM COMPARISON OF FRUITS, SEEDS, AND EMBRYOS OF HALODULE WRIGHTII AND H. UNINERVIS. Louis H. Bragg, Biology Dept., University of Texas, Arlington, TX 76019 and Calvin McMillan, Dept. of Botany, University of Texas, Austin, TX 78712.

One seeded fruits of the seagrasses, Halodule wrightii Aschers. from Texas and H. uninervis (Forsk.) Aschers. from Australia were compared for differences that may aid in establishing their taxonomic status. Cells of the fruit wall, cells of the seed coat, and a separation layer in the fruit wall are similar in both species, as are the polygonal cells of the hypocotyl which contain starch grains and protein bodies. The cotyledon-plumule folded into an invagination of the hypocotyl are also similar in both species. The surface furrows of the fruit wall are deeper in H. uninervis and the fruit has an inconspicuous rostrum whereas the H. wrightii fruit has a prominent beak. It is suggested that additional collections be examined to fully resolve the status of the two species.

SMALL CELL CARCINOMAS OF THE PAROTID GLAND: A CLINICO-PATHOLOGIC STUDY OF 3 CASES. B.B. Kraemer, B. Mackay and J.C. Batsakis, Dept. Pathology, Univ. of Texas M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030.

Three small cell tumors arising in the parotid gland have been studied by light and electron microscopy. By light microscopy, two of the tumors were thought to show neuroendocrine differentiation, and in both instances ultrastructural study confirmed this impression. The cells possessed slender dendritic processes containing accumulations of small (approximately 120 nm) dense-core, membrane-limited granules. The third tumor did not show these features and instead was composed of uniform, round to ovoid cells forming irregular groups within which adjacent cells were united by frequent long desmosomes with sparse tonofilaments, features suggesting intercalated duct cell differentiation. These observations confirm the occurrence of two different types of small cell carcinomas arising from salivary gland tissue, and the possibility that both types arise from the same primitive cell is suggested. A distinction between duct cell and neuroendocrine small cell carcinoma may not be possible by routine light microscopy, but the ultrastructural features will serve to distinguish between the two types of differentiation.

A SOFT PLASTIC SUITABLE FOR USE AS A CELLOIDIN SUBSTITUTE FOR THICK SECTIONS OF BIOLOGICAL MATERIALS. E.J. Root, Graduate Nutrition Division, The University of Texas at Austin, Austin, TX. 78712-1097.

Prior to electron microscopy of biological materials it is usually desirable to obtain an overview of the tissue from large sections examined in the light microscope. When such sections need also to be thick, as for Golgi impregnated specimens of brain, celloidin has been the embedding medium of choice; however, the preparation involves considerable time and expense. The following plastic can be used for specimens approximately one cm. square and can be cut 50 microns thick with a steel knife on an ordinary rotary microtome without a retraction device.

Tissue is stained in the block. After an optinonal 1 to 2 hours in 60% ethanol, tissue is dehydrated for 2 hours each in 80% ethanol and 3 changes of 95% ethanol, and left in 100% ethanol overnight. Dehydration continues with 1 hour each in 2 changes of 100% ethanol and 2 changes of 100% acetone. Times can be shortened for smaller samples. Plastic is composed of 10 ml Embed 812 (Epon 812 substitute Electron Microscopy Sciences), 12 ml DDSA and 0.8 ml DBP, well mixed, for each specimen. For infiltration, tissue is left overnight in 33% plastic, 66% acetone plus 1/2 to 1 drop DMP-30 per ml of plastic, then all day and overnight in 66% plastic, 33% acetone plus DMP-30. Tissue is embedded in 100% plastic plus 1/2 to 1 drop DMP-30 per ml and polymerized at 60° C for 1 to 2 days. Blocks are trimmed with a razor blade and affixed to microtome holders with ordinary household cement. Sections are coverslipped using Permount and kept flat with weights upon the coverslips.

SPORES OF THE LIVERWORT FOSSOMBRONIA Raddi. Steven E. Ehlers and Dale M. J. Mueller, Department of Biology, Texas A&M University, College Station, TX 77843.

Spore ornamentation has long been considered important in the identification of species within the liverwort genus Fossombronia Raddi. Two basic patterns were observed in species collected from Texas, reticulate and ridged. There are three broad categories found in the ridged pattern: parallel around the circumference, anastomosing and papery lamellae. Some problem species were encountered and their possible misidentification is discussed.

TAXOL INHIBITS STEROID PRODUCTION IN MOUSE Y-1 ADRENAL TUMOR CELLS W.E. Rainey, R.E. Kramer, J.I. Mason, and J.W. Shay. Depts. of Cell Biology, Biochemistry & Obstetrics and Gynecology, Univ. of Texas Southwestern Medical School, Dallas, Texas 75235.

The effects of taxol, a drug known to promote microtubule assembly on steroidogenesis and microtubular polymerization in cultured mouse Y-1 adrenal tumor cells have been examined. The Δ^4-3-ketosteroid released from the tumor cells were quantified by use of high pressure liquid chromatography. ACTH (0.4 μm) addition produced a six-fold increase in total steroid release over basal secretion in a six hour assay period. The addition of taxol (1 μm) produced a 50% inhibition of both basal and ACTH-stimulated Δ -3 ketosteroid synthesis. Pregnenolone formation, the initial step in cholesterol metabolism also decreased to 50% of basal and ACTH-stimulated rates in the presence of taxol (1μm). Pregnenolone was quantified by use of a radioimmunoassay procedure. When the tumor cells were studied by thin section electron microscopy, ACTH and taxol treatments were observed separately to increase slightly microtubules within the cytoplasm. The combination of ACTH and taxol treatment greatly increased the number of cytoplasmic microtubules above controls. Similar results were also obtained when microtubules were observed using an antibody to tubulin and indirect immunofluorescence microscopy. We and others have demonstrated previously that cochicine, a microtubule disrupting agent, stimulated steroid production in Y-1 adrenal cells. Other investigators have observed that when microtubules were stabilized with deuterium oxide, an inhibition of steroid production was obtained. The present study is further evidence that stabilization of microtubules may block cholesterol mobilization to the adrenal mitochondria and thus inhibit steroid biosynthesis.

TWIN RAPHIDE CRYSTALS IN THE MUSTANG GRAPE (VITIS MUSTANGENSIS). By Howard J. Arnott and Mary Alice Webb. Department of Biology, The University of Texas at Arlington, Arlington, TX 76019

Raphide crystal cells have been found in the leaves, tendrils, and seeds of Vitis mustangensis where they are sometimes intermixed with druse-producing cells. Using isolated raphides and druses, we were able to identify only one species of calcium oxalate, whewellite (calcium oxalate monohydrate), using powder x-ray diffraction. When raphides and druses are isolated in ethanol by a blending-filtering technique, there is still part of a sheath surrounding the packet of raphides, whereas druses are apparently free of any adhering material. When subjected to energy dispersive x-ray analysis, the druses show only $K\alpha$ and $K\beta$ peaks for calcium, however, the raphide packets show an additional strong peak for potassium. When the alcohol-isolated crystals are washed in distilled water, the sheath material is solubilized, and clear raphide crystal packets can be isolated. The latter produce exactly the same spectrum as druses with the potassium peak being absent. Potassium is apparently present in the sheath surrounding the raphide crystal packet. In both SEM and TEM observations of raphide crystal cells, we are able to demonstrate a complicated array of structure surrounding and interpenetrating the crystal packet. Dense membranous bodies, small paracrystalline bodies, membranes and fibrils are among the structures associated with the crystal chambers in which the raphides are formed. The raphides are twins with a sharp pointed end and a strong reentrant angle at the opposite end. An apparent twin plane can be seen in SEM observations. Early stages in the development of raphide crystals have been seen and their reentrant angle termination is always present.

ULTRASTRUCTURE OF PROSOPIS GLANDULOSA COTYLEDON CELLS DURING STORAGE MOBILIZATION. R. D. Allen, D. A. Prier and L. H. Bragg, Department of Biology, Texas A&M University, College Station, TX 77843 and Department of Biology, University of Texas at Arlington, Arlington, TX 76019. Cotyledon cells of Prosopis contain numerous large protein bodies which are closely surrounded by a single layer of minute lipid bodies. Lipid bodies also line the inner face of the plasmalemma. After germination, the protein body matrix first develops a granular appearance. Later, electron transparent regions form, usually at the protein body periphery. These areas are membrane bound and have been termed "protein body vacuoles." These areas swell and may become much larger than the original protein bodies. Electron dense protein body fragments remain within the protein body vacuoles. Fusion of protein body vacuole areas eventually results in the formation of a large main cell vacuole which still contains spherical fragments of undigested storage protein. Lipid bodies enlarge slightly but retain their position around protein bodies until enlargement of the protein body vacuole. Some evidence of lipid body fusion is seen and fewer, larger lipid bodies are observed in cells at later stages of development.

ULTRASTRUCTURE OF SPORE MATURATION IN THE SLIME MOLD DIDYMIUM IRIDIS. C. W. Mims, Department of Biology, Stephen F. Austin State University, Nacogdoches, Texas 75962.

During the reproductive process in D. iridis the naked,

multinucleate, acellular somatic phase (the plasmodium) gives rise to numerous sporangia containing thick walled spores. When first formed these spores are surrounded only by the cell membrane, but eventually each spore deposits a wall around itself. At maturity this wall appears to consist of at least three layers and is about 1 um thick. Mature spores are 7-9 um in diameter. Small wart-like projections are present on the spore surface.

Most young spores are uninucleate and contain mitochondria, microbodies, ribosomes, glycogen particles, lipid droplets and bands of microtubules. As a spore matures cellular components are phagocytized presumably to provide energy for the spore. Autophagic vacuoles are at first small, but enlarge and coalesce to form a large vacuole that occupies much of the volume of the mature spore. Most mature spores contain a single nucleus although two, three and even four nuclei were observed in some spores.

ULTRASTRUCTURAL IMMUNOCYTOCHEMICAL LOCALIZATION OF 5-HT IN RABBIT ENTEROCROMAFFIN CELLS. J. Hoffpauir & R.D. Dey, Dept. of Cell Biology, U.T.H.S.C.D., Dallas, Texas 75235.

Enterochromaffin cells of the rabbit pyloric mucosa are known to contain 5-hydroxytryptamine (5-HT). The ultrastructure of EC cells is characterized by the presence of pleomorphic vesicles with osmiophillic cores. This investigation provides evidence that 5-HT is stored in the cores of these vesicles. Pieces of rabbit pyloric mucosa were fixed with a combinationof 0.5% glutaraldehyde and picric acid-formaldehyde, dehydrated, and embedded in LX-112. In order to locate EC cells, 1 μM thick plastic sections were mounted on glass slides, incubated with a 1:1400 dilution of anti-5-HT antiserum for 30 min. at 37° C, rinsed, and incubated in a second labelled antibody consisting of goat anti-rabbit IgG-FITC-gold complexes. Adjacent regions of the epithelium containing positive cells were trimmed and thin sectioned. Thin sections were exposed to the anti-5-HT antiserum (diluted 1:1400), washed and then incubated in the IgG-FITC-gold complexes. Controls consisted of adsorption of the primary antiserum with an excess of 5-HTor with the 5-HT percursor 5-hydroxytryptophan (5-HTP). The number of gold particles/unit area were counted over the cell cytoplasm, the nucleus and the dense core vesicles. Gold particles were located predominantly over the dense core vesicles of EC cells. Labelling over the nucleus of EC cells was higher than background labelling, but lower than labelling over the granules. The cytoplasmic labelling was equal to the background levels. Labelling over the vesicles was abolished after the primary antiserum had been absorbed with 5-HT. Interestingly absorption with 5-HTP only slightly reduced the labelling over the dense core vesicles, but reduced labelling over the nucleus to background levels. Our results demonstrate that serotonin is present in the dense core vesicles of EC cells. The significance of the apparent 5-HTP labelling over the nucleus is not clear.

ULTRASTRUCTURAL SIMULATION OF STEROID-PRODUCING TUMORS BY NON-ENDOCRINE NEOPLASMS. B.B. Kraemer, E.G. Silva & B. Mackay, Dept. Pathology, Univ. of Texas M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030.

Many steroid-forming cells possess distinctive ultrastructural features, notably mitochondria with tubular cristae and prominent smooth endoplasmic reticulum. The same fine structural characteristics are observed to varying degrees in tumors derived from steroid-producing cells. Our earlier studies of adrenal cortical carcinomas have shown that these features may not be present, and that their occurrence cannot be correlated with the functional behavior of the tumors. Nevertheless, they are widely viewed as useful diagnostic criteria, and their presence in a particular neoplasm is usually taken to indicate derivation from steroid-forming cells. We have observed similar ultrastructural features in cells of five unrelated types of nonendocrine neoplasms, indicating that it is necessary to take the clinical setting into consideration when evaluating the diagnostic significance of these electron microscopic findings. Tumor tissue from nine patients with diagnoses of metastatic melanoma, neuroblastoma, liver cell carcinoma, primary anaplastic carcinoma of small intestine, and alveolar soft part sarcoma has been studied. Preservation was satisfactory in every instance, and therefore the changes observed could not be attributed to inadequate fixation. Clinical data, including radiologic and endocrinologic studies, failed to demonstrate evidence of a tumor of steroid-forming cells in any of the patients, and specific ultrastructural features that established the diagnosis were present in several of the tumors.

AN ULTRASTRUCTURAL STUDY OF CRYSTAL IDIOBLASTS IN ASSOCIATION WITH THE AIR SPACES OF MYRIOPHYLLUM. M. J. Grimson Department of Biology, The University of Texas at Arlington, Arlington, TX 76019

Crystals of calcium oxalate have generally been described as developing intracellularly, with the exception of a few species (e.g. Tsuga, Myriophyllum) where crystals were observed to form outside of a crystal cell. A developmental study of crystal formation in Myriophyllum sp. (Haloragaceae) using scanning electron microscopy, transmission electron microscopy, and light microscopy, however, revealed that crystals in Myriophyllum actually develop within idioblasts rather than extracellularly as was previously assumed. Myriophyllum is an aquatic, emergent angiosperm which produces air spaces parallel to the longitudinal axis of the stem. The air spaces are radially arranged and separated by septa giving the plant a "wagon-wheel" appearance in transverse section. Druse crystals are formed in large numbers on the axial and septal walls of the lacunae in older portions of the stem. Developmentally, a cell adjacent to an air space will differentiate into an idioblast. This cell begins to bulge into the air space as a druse begins to form within a vacuole. As the crystal matures, the idioblast becomes more spherical, bulging further into the space. At maturity, the cell senesces and the wall collapses, conforming to the shape of the druse, giving it an "externalized" appearance.

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Message: Send me something, even a postcard would do. Remember; when the pickins are slim, the Journal is thin.

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(3) D. Gabor. Information Theory in Electron Microscopy. in: Quantitative Electron Microscopy. Eds. G.F. Bahr and E. Zeitler (Williams and Wilkins, Baltimore, 1956) 63-68

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

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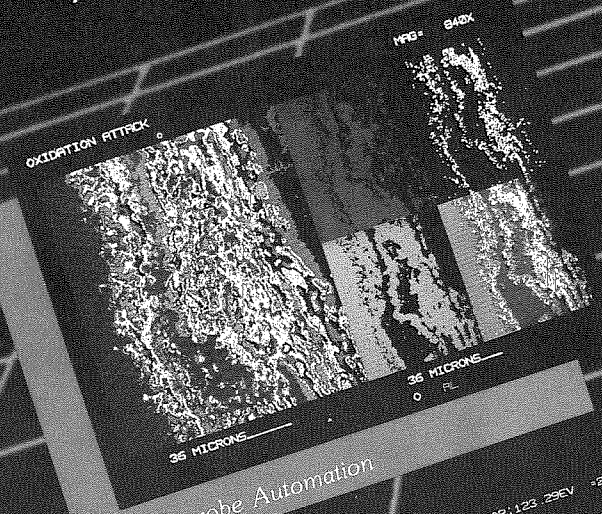

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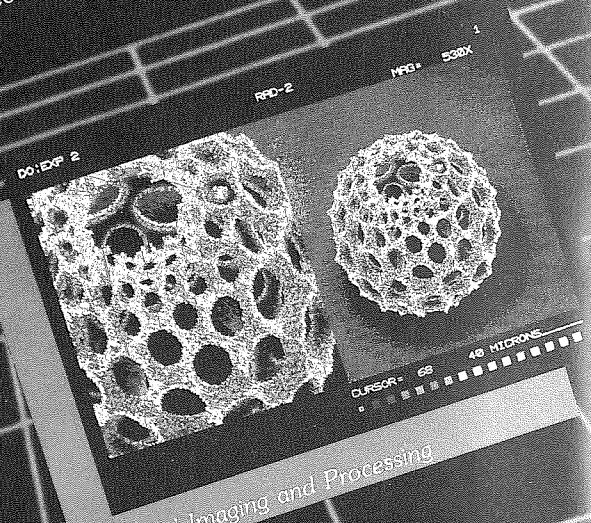

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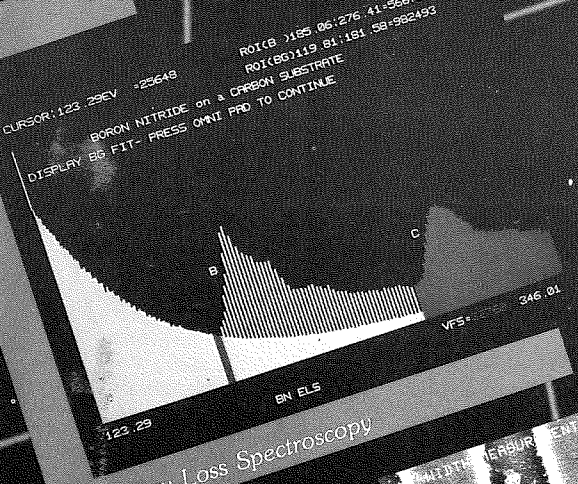

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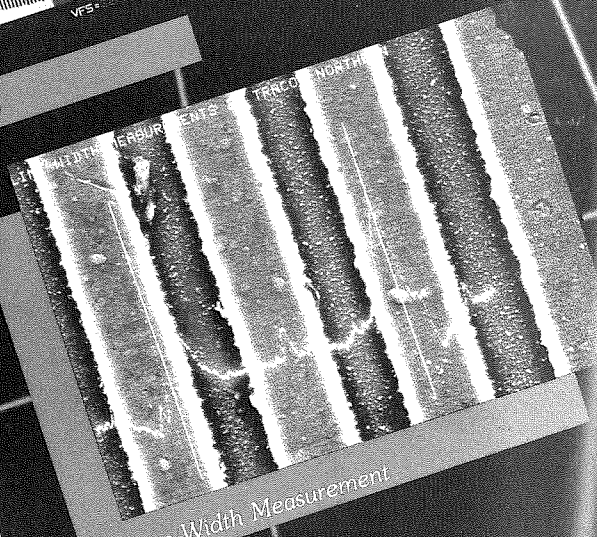

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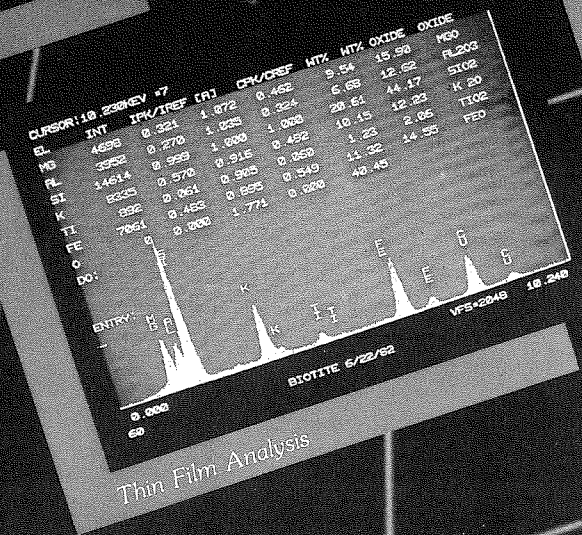

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Presented to the Council at _____ meeting. Date _____

Action _____

Send Application to:

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*The Editor

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Message: TSEMJ is going to start publishing an occasional photograph (s) of TSEM activities both current and from the past. If you have a sharp photograph of such a TSEM function, you know the names of the participants pictured, the date, location, etc.; then send it to the editors office. You will receive acknowledgement of your contribution and the original photo will be returned.

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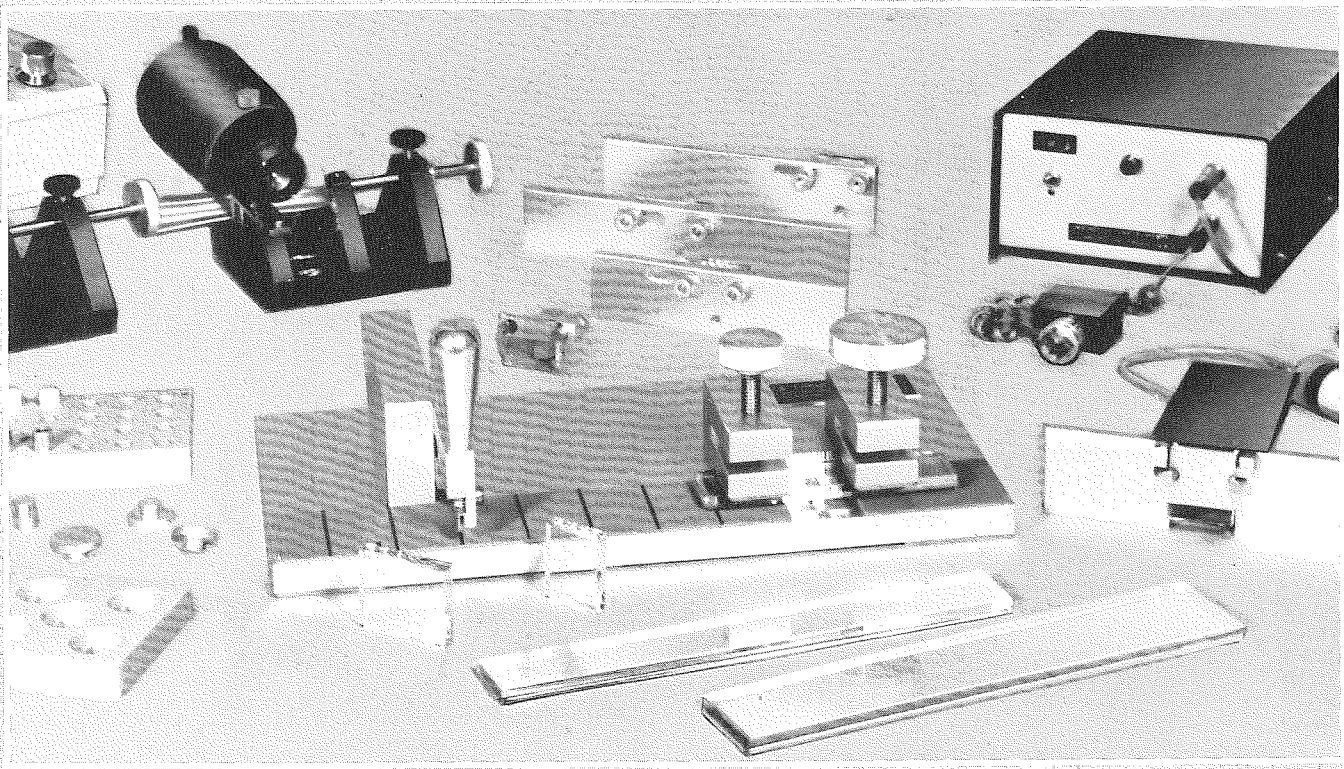

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