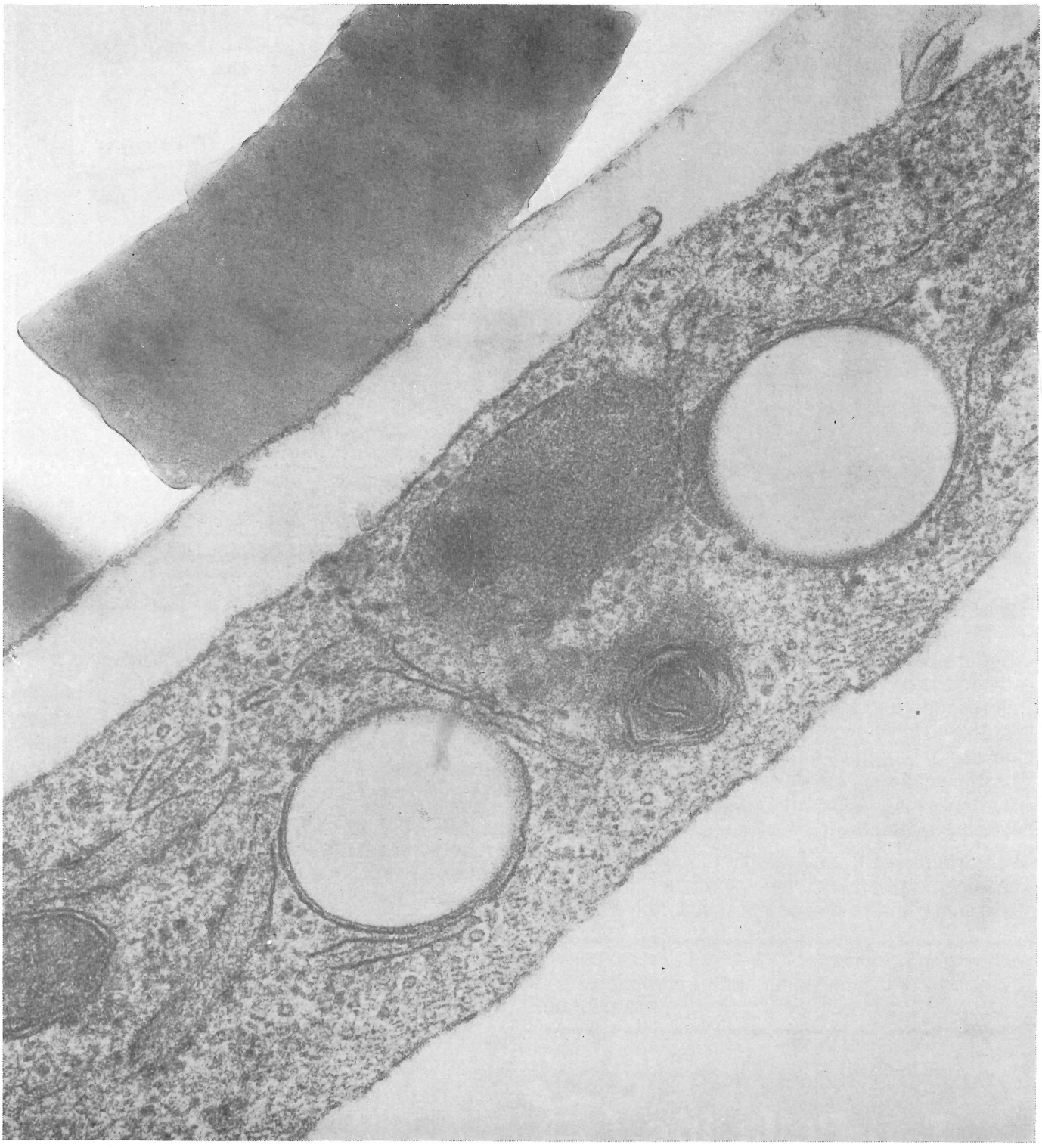
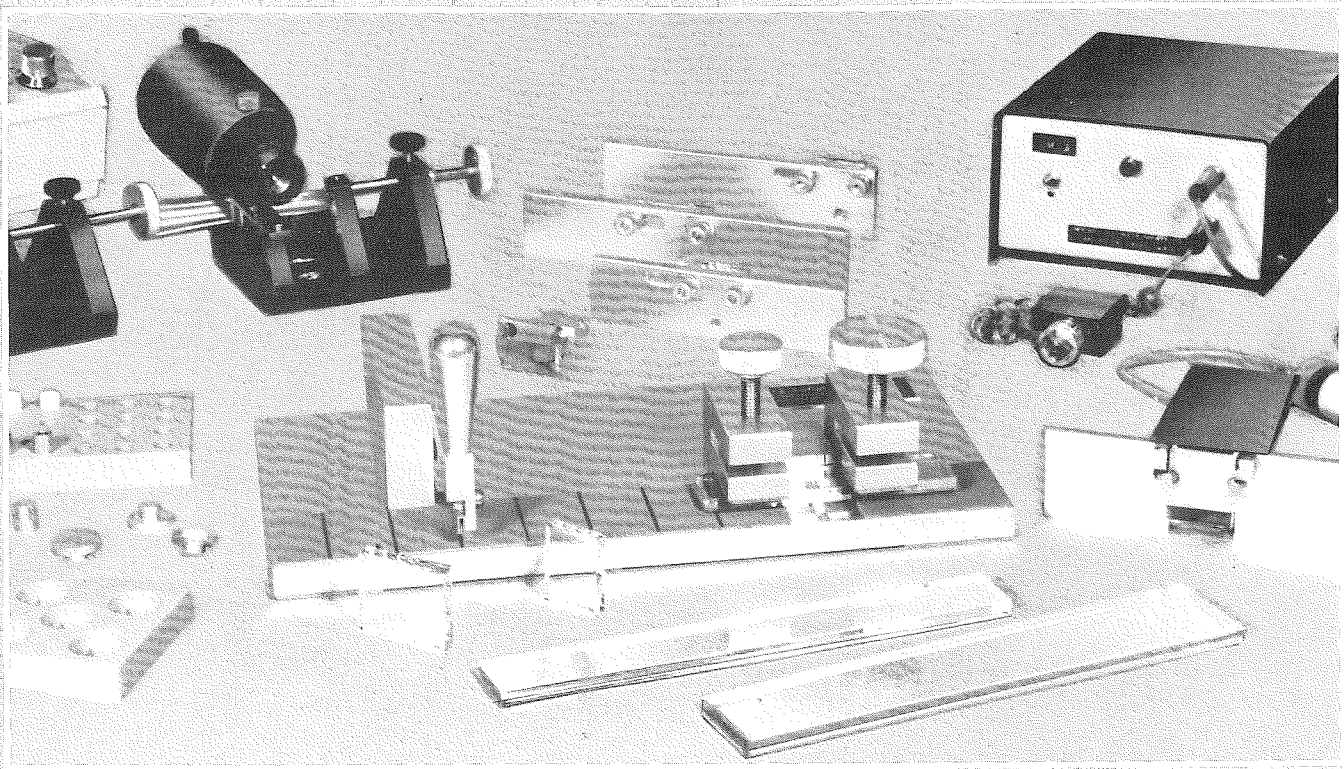
Texas Society for Electron Microscopy

JOURNAL
VOLUME 14, NUMBER 3, 1983
ISSN 0196-5662



The MI Longknife Maker

- *Ralph-Bennett Glass Knives*
- *Lowest Priced*
- *Sharper*
- *Disposable*
- *Simple Operation*



Production, evaluation, mounting of Ralph-Bennett glass knives for conventional microtomy specimen collection and mounting — microtome specimen retraction

U.S. Patent Numbers 4,175,684 and 4,231,503

GLASS knives routinely can produce very-thin (1—2um) sections of plastic or paraffin embedded specimens. Very-thin plastic sections take advantage of the full resolving power of the light microscope and show morphological detail comparable to low power electron micrographs. Very-thin (2um) paraffin sections, while not as good as plastic sections, are superior to thicker (5um) paraffin sections cut with steel knives. They will work in cryostats.

MI Longknives are adaptable to any microtome. The Longknife Maker rapidly produces 25—36mm long, ultra-sharp, disposable glass knives.

ordering information

8000 MI Longknife Maker, with Instructions **$1195.00**

Salient Features

- **Lowest priced** glass knife-breaking instrument for histology microtomy
- A **System** for glass knife production, including inspection, retraction, mounting, section collection and accessories to be announced, such as Specimen Stubs and Molds for block mounting
- Longknives are **disposable** and save the time and expense associated with steel knives
- Longknives are **inherently sharper** than steel knives and thus allow production of **superior specimen sections**
- Incredibly, despite the material being glass, cutting edges are **durable** and will section hundreds of slices
- **Simple operation** requiring neither special skill nor previous experience

Ted Pella, Inc.

P.O. Box 510 • Tustin, CA. 92681
714-557-9434 • Calif: 800-432-3059
U.S.A.: 800-854-7553 (exc. HI & AK)

Contents

JOURNAL
VOLUME 14, NUMBER 3, 1983
ISSN 0196-5662

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

ON THE COVER

RECRUITING FIBROCLASTS IN VITRO

Fibroclasts (sic) in vivo appear to mediate soft tissue remodeling by phagocytizing and then enzymatically degrading selected collagen fasicles. These functionally specialized cells appear to be derived from the tissue's fibroblast population.

When a suspension of 0.5um diameter latex particles is added to a human foreskin fibroblast culture some of the spheres are phagocytized by the in vitro cells. This micrograph shows a cross-sectional view of a fibroclast from a culture challenged with the particles for 24 hours prior to collection for electron microscopic study. Two latex spheres are incorporated in separate membrane-bound vesicles. One particle appears to be in a phagosome while the other is either within a secondary or tertiary lysosome. A non-inclusion containing lysosome can also be observed in the cytoplasm. X 105 K.

Submitted by Paul S. Baur, Robert Cox, and Darrell Hudson, Graduate School of Biomedical Sciences, UTMB, Galveston, Texas.

President's Message

I am writing this letter approximately one week prior to our Fall Meeting to be held in Tyler. At this time all indications are that we will have an outstanding meeting. Over thirty contributed papers will be presented along with special invited lectures by Dr. Benjamin Trump of the University of Maryland School of Medicine and Dr. Richard Trelease of Arizona State University.

In regard to the Fall Meeting, I would like to sincerely thank the University of Texas Health Center at Tyler for its support. In particular our thanks go to Ronald Dodson for his hard work and efficiency in planning this meeting. He was capably assisted by Lynn Davis, Glenn Williams and Carolyn Tuley. I hope that each of you will take time to personally thank them for their work.

While on the topic of meetings I should note that the Spring Meeting of TSEM is scheduled for College Station. The tentative dates are April 26-28, 1984. Ernest Couch informs me that Wayne Sampson and Hilton Mollenhauer have agreed to handle local arrangements for us.

As many of you already know, TSEM will celebrate its twentieth anniversary in 1985. I have appointed an **ad hoc** committee consisting of Randy Moore, Howard Arnott and Jerry Berlin to begin making special plans for this event. Please feel free to offer suggestions regarding the anniversary meeting either to me or to any member of the **ad hoc** committee. We want this anniversary to be very special and something that all members of TSEM can take pride in.

I have some important news regarding the TSEM Journal. Paul Baur has resigned as Editor and will be replaced by Randy Moore. I would like to express our sincere thanks to Paul for his dedication and hard work during his term as Editor. We will certainly miss Paul but, I feel confident that the editorship will be in good hands with Randy in charge. As has been the case in the past, however, one person alone can not make the Journal a success. Randy will need the help and support of all TSEM members in his new job.

I look forward to seeing all of you both at Tyler and at College Station. Please do your best to bring at least one new member with you when you attend one of our meetings.

Sincerely,

Charles W. Mims
President, TSEM 1983-84

Editor's Message

This will be my last issue as TSEMJ editor. A multitude of unforseen dilemmas have made it nearly impossible for me to continue in this office. For the most part, I have enjoyed this task immensely. Randy Moore of Baylor University has agreed to assume the responsibilities of editorship and I know he will do a good job.

During my tenure as editor I did not receive a single letter of complaint, suggestions, or an offer to help. However I did get one letter of praise from Bruce Mackay which gladdened my heart for weeks on end. If you have had an opinion about the Journal then you have either kept it to yourself, lacked courage to contact me, or you have complained to other TSEM officials. Remember, I have pleaded with you for your input. In the future, I'd suggest that all TSEM members consider "putting their money, time, or effort where their mouths are" and contribute actively and directly to the TSEMJ and its editor. My submissions to the printer have always awaited the arrival of text (manuscripts, technical notes, historical reviews, letters, etc) to my desk. Such contributions have been rare indeed. In fact, the Journal may have to be abbreviated to two issues per year because of "material shortage". If this happens, its your fault!

Some of the material we receive (Regional News in particular) has consisted of hand scribbled notes. These have been difficult to read and translate. Next time you send the editor something, type it as you want it to appear in print. Give the editor a little respect and a lot of help.

The TSEMJ has made a lot of progress over the last year or two. The Editorial Policy has solidified into a fair and prudent doctrine, the Instructions for Authors appears to work well, and the Journals issue scheduling has worked into a workable routine. Likewise the TSEMJ's style has settled down into a sophisticated format. The manuscripts published have all been reviewed by an editorial review committee and a mechanism for galley proof review for the contributing authors has been used for the last several issues. However, there is still room for improvement which we will undoubtedly see as the TSEMJ matures. Hostorically each issue of the Journal has always been better than its predecessor.

My thanks to Bob Blystone and Randy Moore for their help and to the general membership for their patience and understanding.

Best wishes to everyone in their future endeavors...

Paul S. Baur Jr., Ph.D.
Editor, TSEM Journal

Letters to the Editor

Meeting Report:

Sixth Veterans Administration National Electron Microscopy Conference

The Veterans Administration (V.A.) has been one of the major motivating factors in the development of diagnostic electron microscopy in this country. Some years ago, under the aegis of Dr. Marjorie Williams, microscopes were installed in V.A. hospitals across the country, and the National Conference, which is held every two years, brings together workers in the field from the V.A. and many academic institutions. The meetings provide a forum for didactic presentations and for less formal gatherings at which exchange of experiences, discussion and informal questions are possible. The Sixth Conference was held this year at the University of Michigan in Ann Arbor.

At some national meetings, there is sensible use of the available time by the addition of evening sessions. At Ann Arbor, 4 evening workshops on selected topics were held on each of the two nights of the conference. Each workshop was organized and chaired by a moderator, and the basic format was to show slides of interesting or problem cases, many of them brought by the participants. Sessions of this type are always interesting and useful to pathologists.

The lecture sessions were held in an impressive auditorium on the campus of the University of Michigan, and they included talks of up to 1 hour by invited speakers, some from overseas, and shorter presentations (usually 15 minutes) by participants from the V.A. hospitals.

At Michigan, Wolverine fans are justifiably proud of their football stadium which seats over 100,000, but there are other impressive edifices including the bell tower from which a carillon peals forth each noon. An added pleasure to participants at the meeting was the profusion of flowering trees along many of the roads around the campus.

Bruce Mackay
M.D. Anderson Hospital and Tumor Institute

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.

REGIONAL NEWS

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

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

TECHNICAL SECTION

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

PUBLICATION PRIVILEGES

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

Regional News

BAYLOR COLLEGE OF MEDICINE, HOUSTON

DEPARTMENT OF OTORHINOLARYNGOLOGY AND COMMUNICATIVE SCIENCES

PUBLICATIONS

Fermin, C.D. and Igarashi, M., 1982. The vestibular ganglia of the squirrel monkey (**Saimiri sciureus**). Ann. Otol. Rhinol. Laryngol., 91, 44-52.

Fermin, C.D. and Igarashi, M. 1982. The vestibular nerve and ganglia of the squirrel monkey after labyrinthectomy. 40th Ann. Proc. Electron Microscopy Soc. Amer. G.W. Bailey, ed., Claitor's Publ. Div., LA. Pages 196-197.

NEW FACULTY AND/OR STAFF

Hideo Yamane, M.D. from the Department of otorhinolaryngology Osaka City University Medical School, Japan is visiting with Dr. M. Igarashi in the Department of Otorhinolaryngology and Communicative Sciences at Baylor, where he will stay until the end of 1983 as a Research Associate. Dr. Yamane's research in Japan has concentrated on the relationships between nephrotic syndrome and sensorineural defness, and aminoglycoside ototoxicity. He will also work with Dr. C. Fermin in the departmental EM facilities.

BAYLOR UNIVERSITY

DEPARTMENT OF BIOLOGY

PUBLICATIONS

Moore, Randy. 1982. A SEM study of the early events in graft formation in plants. Scanning Electron Microscopy/1982 3: 1103-1107.

Moore, Randy and C. Edward McClelen. 1983. Ultrastructural aspects of cellular differentiation in the root cap of **Zea mays**. Can. J. Bot./ In Press.

Moore, Randy and C. Edward McClelen. 1983. A morphometric analysis of cellular differentiation in the root cap of **Zea mays**. Amer. J. Bot. 70: 611-617.

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.

Moore, Randy and Dan B. Walker. 1983. Studies of vegetative plant tissue compatibility-incompatibility. VI. Grafting of **Sedum** and **Solanum** callus tissue in vitro. Protoplasma 115: 114-121.

Ransom, J. Steve and Randy Moore. 1983. Geoperception in primary and latara1 roots of **Phaseolus vulgaris** (Fabaceae). I. Structure of columella cells. Amer. J. Bot. 70: 1048-1056.

Moore, Randy. 1983. Studies of vegetative plant tissue compatibility-incompatibility. IV. The development of tensile strength in a compatible and an incompatible graft. Amer. J. Bot. 70: 226-231.

THE UNIVERSITY OF TEXAS AT AUSTIN

DEPARTMENT OF BOTANY

NEW EQUIPMENT AND/OR FACILITIES

Renovation of Dr. Malcom Brown's first floor laboratory in the Biological Laboratories building has been completed, and the Ibas is being connected to the Philips 420.

NEW OR VISITING FACULTY AND/OR STAFF

Debra Brown is working in Dr. Malcolm Brown's laboratory, on gravitational response in plants.

Dr. Robert Canon from the University of North Carolina at Greensboro visited Dr. M. Brown's laboratory during May, 1983, to work on **Acetobacter** cellulose synthesis. Jean Francois Revol, an expert in electron diffraction, was also a visitor in May.

In 1985, Dr. Henri Chanzy of Grenoble plans to spend a sabbatical as a visitor in Dr. M. Brown's laboratory.

EM EDUCATIONAL OPPORTUNITIES

A Plant Cell Biology meeting will be held in Austin on November 28, 1983, the day preceding registration for the American Cell Biology meeting in San Antonio. Six eminent speakers have been invited. Meetings will be held in the Thompson Conference Center on the University of Texas at Austin campus, and 155 rooms are reserved at the nearby Villa Capri Motel. The Chevy Chase Travel Agency is arranging travel to the Cell Biology meetings with no extra charge for stopover in Austin. Persons wishing to attend the Austin meeting should plan to spend nights of November 27th and 28th in Austin and for details should contact Dr. R.M. Brown, Jr., Department of Botany, The University of Texas at Austin, Austin, TX 78712 Telephone (512) 471-3364

OTHER NEWS OF INTEREST TO MEMBERS OF TSEM

At the 3rd International Mycological Congress in Tokyo, Japan, August 28 to Sept. 3, 1983, Dr. Garry T. Cole served as chairman for two of the symposia, one on the "Biology of the Black Yeasts" and the other on "Fungal Ultrastructure and Morphogenesis."

In Japan Dr. Cole also presented a paper on "Morphogenesis in **Coccidioides immitis**" at the First International Symposium on Filamentous Microorganisms — Current Topics of Infection, Toxicosis and Control, held at Chiba University, September 5th-6th, 1983.

DEPARTMENT OF ZOOLOGY

PUBLICATIONS

S.P. Meier, The Development of Segmentation in the Cranial Region of Vertebrate Embryos. Scanning Electron Microscopy 1982 III (SEM, Inc., AMF O'Hare, Chicago, 1982) 1269-1282.

D.S. Packard, Jr. and S. Meier, An Experimental Study of the Somitomeric Organization of the Avian Segmental Plate. Developmental Biology 97 (1983) 191-202.

DEPARTMENT OF HOME ECONOMICS -NUTRITION DIVISION

PUBLICATIONS

E.J. Root and J.B. Longenecker, Brain Cell Alterations Sugesting Premature Aging Induced by Dietary Deficiency of Vitamin B6 and/or Copper. American J. of Clinical Nutrition 37 (1983) 540-552.

CLAYTON FOUNDATION BIOCHEMICAL INSTITUTE

PUBLICATIONS

Robert M. Oliver and L.J. Reed. Multienzyme Complexes. in: Electron Microscopy of Proteins, Vol.2. Ed. J.R. Harris (Academic Press, New York, 1982) 1-48.

UNIVERSITY OF TEXAS

HEALTH SCIENCE CENTER AT SAN ANTONIO

INVITED LECTURES/SEMINARS

Professor Ivan Cameron was an invited speaker at the Scandinavian Society for Electron Microscopy in Trandheim, Norway June 5-8. He talked analytical EM in Biology

S.W. FOUNDATION

SAN ANTONIO

PUBLICATIONS

G.C. Smith, R.L. Heberling, and S.S. Kalter, Viruses in Nonhuman Primate stools, in Viral and Immunologic Diseases in Nonhuman Primates Pages 243-248.

STEPHEN F. AUSTIN UNIV.

NACOGDOCHES, TEXAS

PUBLICATIONS

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

NEW FACULTY AND/OR STAFF

Dr. Don A. Hay joined the faculty of the Department of Biology for the Fall Semester of 1983 as an Associate professor. Dr. Hay holds an M.S. degree in zoology from Michigan State University and a Ph.D. in anatomy from the University of North Dakota. He has taught previously at the University of Wisconsin — Stevens Point and the University of Florida School of Medicine.

TEXAS A&M UNIVERSITY

PUBLICATIONS

Mollenhauer, H.H. 1982. Some observations on mounting media vs. fading of epoxy resin sections used for light microscopy. Tex. Soc. Elect. Mic. 13:13-16.

Mollenhauer, H.H., Morre, D.J. 1982. The Golgi Apparatus. In McGraw-Hill Encyclopedia of Science and Technology. 5th Ed. Lapedes, D.N. (Ed). pp. 328-330, McGraw-Hill Pub. Co., N.Y. 1982.

Mollenhauer, H.H., Morre, D.J., Droleskey, R. 1983. Monensin affects the trans half of Euglena Dictyosome. Protoplasma 114:119-124.

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. Biol. Int. Reports 6:981.

Leuschen, M.P., C.M. Moriarty and H.W. Sampson. 1983. Calcium movements and intracellular calcium distribution in neoplastic GH_3 cells. Histochem. 77:85-97.

INVITED LECTURES/SEMINARS

Mollenhauer, H.H. and Droleskey, R. The use of maize roots for evaluating the effect of toxicants on cellular secretion and plant growth. Annual Staff Conference, Texas Agricultural Experiment Station, College Station January 10-13, 1983.

UNIVERSITY OF TEXAS

HEALTH SCIENCE CENTER AT DALLAS

PUBLICATIONS

Shannon, W.A., Jr. 1982. Use of BSPT in dehydrogenase histochemistry. Histochem. J. 14:166-167.

Shannon, W.A., Jr., D. Rockholt, and S.B. Bates. 1982. Computer-assisted measurement of the thickness of biological structures. Comput. Biol. Med. 12:149-155.

Shannon, W.A., Jr., C.S. Chang, and S.B. Bates. 1982. ATP-Citrate lyase localized with the peroxidase-antiperoxidase technique in rat heart and liver. Proceedings, 40th Meeting Electron Microscopy Siciety of America, G.W. Bailey, ed. Claitor's Publishing Division. pp. 40-41.

Zellmer, D.M., and W.A. Shannon, Jr. 1982. Aminotriazole-insensitive catalase activity in rabbit peritoneal exudate monocytes. Proceedings, 40th Meeting Electron Microscopy Society of America, G.W. Bailey, ed. Claitor's Publishing Division. pp. 42-43.

Shannon, W.A., Jr. 1982. Improved preparation of embedding plastics for electron microscopy. J. Microscopy 127:161-164.

Shannon, W.A., Jr., and D.M. Zellmer. 1982. Tannic acid-glutaraldehyde fixation reveals calcium ionophore-induced changes in rabbit polymorphonuclear leukocyte membranes. UCLA Symposia on Molecular and Cellular Biology (Vol. 3): Cellular Recognition, W.A. Frazier, L. Glaser, and D.I. Gottlieb, eds. Alan R. Liss, Inc., pp. 177-187.

Cronin, R.E., E.R. Ferguson, W.A. Shannon, Jr., and J.P. Knochel. 1982. Skeletal muscle injury after magnesium depletion in the dog. Am. J. Physiol. 243:F113-F120.

Shannon, W.A., Jr., and D.M. Zellmer. 1982. Heterogeneity in polymorphonuclear leukocyte neutrophil granules. Histochem. J. 14:847-850.

Dey, R.D., W.A. Shannon, Jr., H.K. Hagler, and S.I. Said. 1983. Histochemical and ultrastructural characterization of serotonin-containing cells in rabbit tracheal epithelium. J. Histochem. Cytochem. 31:501-508.

Shannon, W.A., Jr., and D.M. Zellmer. 1983. Arylsulfatase-containing granules in rabbit polymorphonuclear leukocytes. Histochem. J. 15:201-209.

Zellmer, D.M., and W.A. Shannon, Jr., 1983. Morphometric cytochemistry of catalase and myeloperoxidase-containing granules in the rabbit polymorphonuclear leukocyte. Hostochem. J. 15:211-230.

Brown, W.J., W.A. Shannon, Jr., and W.J. Snell. 1983. Specific and azurophilic granules from rabbit polymorphonuclear leukocytes. I. Isolation and characterization of membrane and content subfractions. J. Cell Biol. 96:1030-1039.

Brown, W.J., W.A. Shannon, Jr., and W.J. Snell. 1983. Specific and azurophilic granules from rabbit polymorphonuclear leukocytes. II. Use of surface labeling methods on intact granules and degranulating cells to determine the spatial orientation of granule membrane proteins. J. Cell Biol. 96:1040-1046.

Zellmer, D.M., and W.A. Shannon, Jr., 1982. Unique catalase activity in rabbit PMN granules. J. Histochem. Cytochem 30:609.

Shannon, W.A., Jr. 1982. Patterns of membrane internalization in phagocytizing and nonphagocytizing polymorphonuclear leukocytes (PMN). J. Cell Biol. 95:429a.

Raskin, P., A.O. Pietri, R.H. Unger, and W.A. Shannon, Jr. 1982. The effect of meticulous diabetes control on quadriceps capillary basement membrane thickness (BMT) in Type I diabetes mellitus. Clin. Res. 30:878A.

Shannon, W.A., Jr., S.B. Bates, D.B. Bellotto, R.H. Unger, and P. Raskin. 1983. Improved methodology for the measurement of capillary "basement membrane" in determining diabetic microangiopathy. Diabetes. 32(Suppl. 1):97A.

INVITED LECTURES/SEMINARS

Al Shannon - "The five structural localization of Krebs Cycle inzymes in Mitochondria" to the Richardson, TX Amer. Heart Assoc.; "Heterogeneity in the rabbit neutrophil" to the Division of Natural Sciences, University of Tulsa; "ATP-Citrate lyase localized with the peroxidase-antiperonidase technique in rat heat and liver" and "Aminotriazole-insensitive catalase activity in rabbit peritoneal exudate monocytes" at the 40th Annual Meeting of the EMSA.

NEW FACULTY AND/OR STAFF

New EM tech - Lisa Gansky

OTHER NEWS OF INTEREST TO MEMBERS OF TSEM

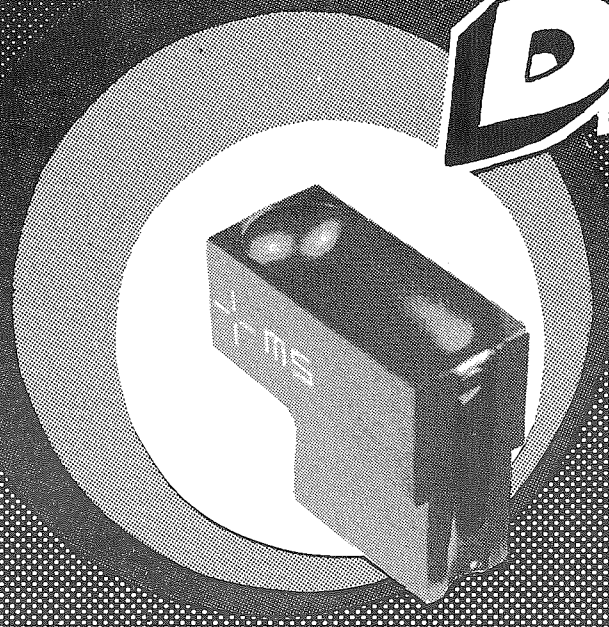
Dennis Bellotto has transferred from Dr Shannons' lab at the VA Medical Center to Dr. Haglers' lab in Pathology at the UTHSCD. Sally Bates has moved to Washington, D.C. where she will be an EM tech for Dr. Peter Andrews at Georgetown Univ. School of Medicine.

Shannon appointed to the Board of Directors of the American Diabetes Association, Dallas County. Shannon was awarded grant from Alcon Laboratories in Ft. Worth for "Ultrastructural analyses of effects of aldase reductase inhibitors in diabetic rats."

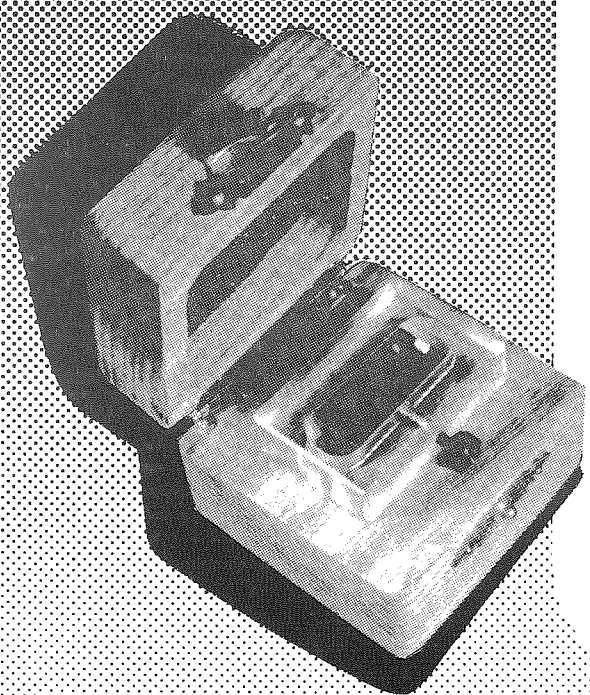
Regional Editors

DIAMOND KNIVES

for ultramicrotomy



PROMPT
COMPLETE
SERVICE
AT
LOWER
PRICES



NEW KNIVES

We produce the new MICRO STAR diamond knives in sizes between 1.5 and 4.5 mm, mounted in an oval cavity boat which fits all standard microtomes. Knives in stock are shipped the next day after receiving your order, otherwise the maximum wait is eight weeks.

RESHARPENING

We resharpen diamond knives from all manufacturers and reset them in their original or new boats, your choice. You will receive your resharpened knife in six weeks or sooner.

TRADE-IN

You can get up to $700 discount by trading-in one or two old knives from any brand when buying a MICRO STAR knife. When trading-in two knives, add the lengths to estimate your discount. The minimum charge when buying a knife with trade-in is $950.

GUARANTEE

Our new or resharpened knives are guaranteed to cut flawless silver sections without compression or scoring. You have a full month to test and ascertain the quality of a knife, and if not completely satisfied, return it for replacement, repolishing or refund. We also guarantee unlimited number of resharpenings for our MICRO STAR knives, or replacement for a new one the same size at the resharpening price.

PRICES

Knives are priced by their edge length every .1 mm. To find the price of sizes not listed, interpolate using the given increment. Terms, net 30 days.

EDGE LENGTH	MICRO STAR KNIVES	RESHARPENING ANY BRAND	TRADE-IN DISCOUNT
1.5	$1390	790	350
2.5	1790	990	450
3.5	2190	1190	550
4.5	2590	1390	650
Increment per .1 mm	40	20	10

micro engineering, inc.

Rt. 10 268 Summer Place St.
Huntsville, TX 77340
409/291-6891

AN INEXPENSIVE OVEN FOR CURING EPOXY EMBEDMENTS

By

Thomas Caceci, Ph. D.
Assistant Professor of Veterinary Anatomy

and

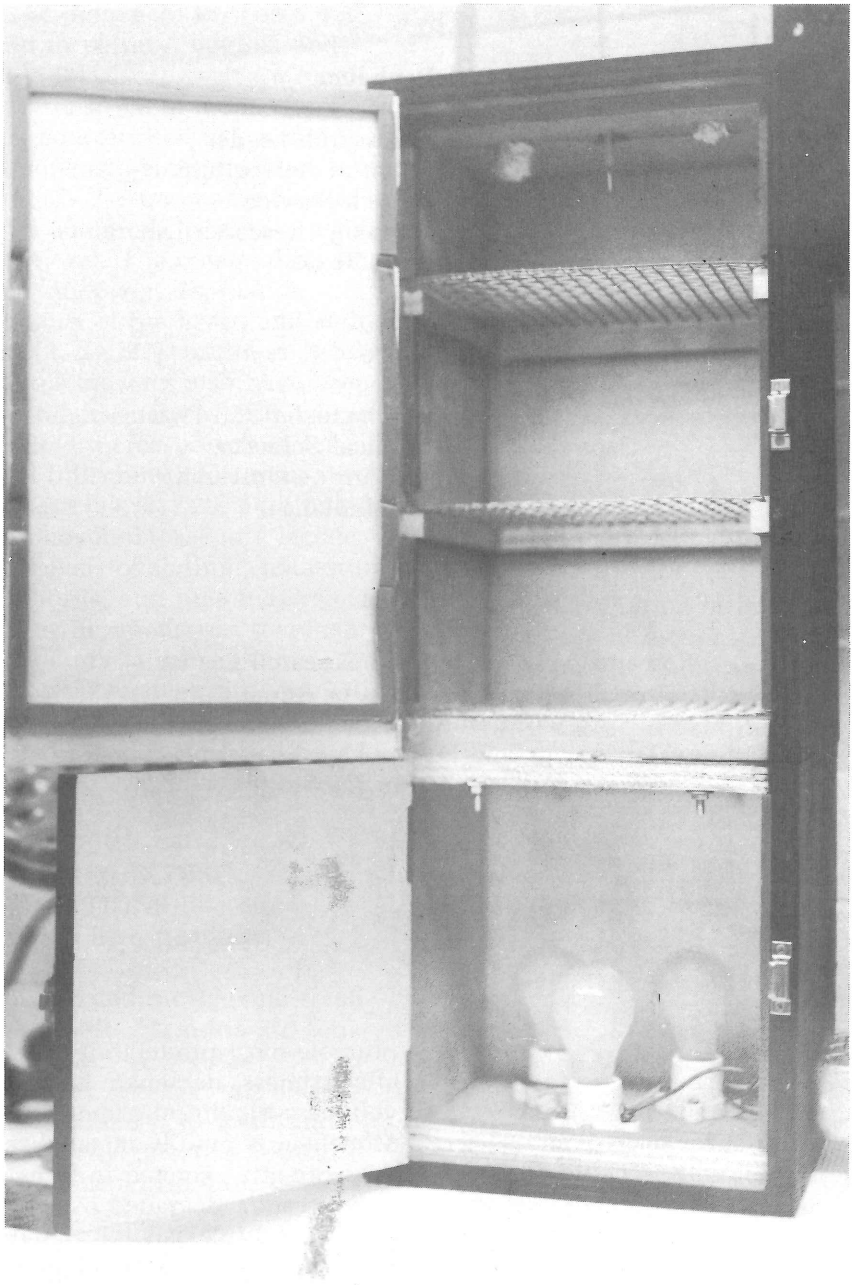
Robert W. Wagner
Technician I

Department of Veterinary Anatomy
College of Veterinary Medicine
Texas A&M University
College Station, Texas 77843
(409) 845-2828

Reprints: Reprints can be requested from Dr. Caceci at the above address or by calling him at (409) 845-4200.

Good laboratory equipment need not be expensive. With sources of grant funding becoming ever more restricted, and institutional budgets constantly being scrutinized for ways to cut costs, laboratory directors who want to stretch their research dollars as far as possible should consider the possibility of making some of their own equipment from scratch. Many pieces of laboratory apparatus are quite simple in principle, and can easily be made in any reasonably well-equipped workshop. We present here one example of such a piece of equipment: an oven we made for curing epoxy embedments in our electron microscopy facility. The total cost for this oven was less than fifteen dollars, which is a savings of more than $200 over the price of a similar oven from one of the large supply houses.

The oven is made of scrap lumber and a few electrical parts, most of which were salvaged from other pieces of apparatus that had been discarded. The heat source is three 100-watt light bulbs, wired in parallel and controlled by a rheostat switch. The rheostat (which is the only component which was purchased new) is an ordinary lamp dimmer switch that can be bought in any hardware store for about three dollars. By varying the current flow to the bulbs, the heat output can be controlled quite easily. Interestingly enough, at lower settings, there is no visible glow from the bulbs; but heat output is sufficient to maintain a temperature of about 40°C. Higher or lower wattage bulbs can also be used to increase or decrease the maximum termperature obtainable. We have found that with three 100-watt bulbs, we can attain a maximum temperature of 100°C — quite adequate for curing epoxy, drying out dessicants, or other low-temperature applications. In fact, this actually exceeds the maximum attainable temperature in most of the inexpensive commercial incubators, which cannot be heated above 70°C. Over the range of 40°-100°, the temperature response of the oven to varying settings of the rheostat control is linear, and the temperature does not fluctuate significantly. The stability of the oven at 55°C is $\pm$ 2°C, equal to the stability attained by many commercial ovens. This may be partly due to the insulating effect of the exterior walls, which are made of three-quarter-inch plywood. We painted the inside of the cabinet silver to reflect the heat inward, and the exterior a flat black for the sake of appearance. The shelves are made of half-inch mesh galvanized hardware cloth, cut to fit the shelf supports. The size and internal dimensions of our oven were dictated by the scrap plywood we had on hand,



but an oven such as this could be made any size that is convenient. Three holes about 15.0 mm in diameter were bored in the top to provide for air circulation and insertion of a thermometer. The accompanying photograph shows the details of the oven's construction.

Many other pieces of simple apparatus could be fabricated in this way, at substantial cost savings. Examples are water baths, rotary shakers, and photographic drying cabinets. There is no reason why these home-made devices should not be as durable and serviceable as their commercially-made counterparts if some care is taken in their design and manufacture.

Materials List:

3	100-watt light bulbs
3	light bulb sockets
1	lamp dimmer switch, 1875-watt for incandescent bulbs
1	outlet box
10′	16-gauge lamp cord
1	wall plug
4	2″ hinges
2	door catches
10′	roll 3/8″ x 1/4″ foam weatherstripping
	Hardware cloth
3/4″	plywood for walls (dimensions will vary with individual laboratory needs and available space)

MONENSIN TOXICITY: AN OVERVIEW

by

H.H. Mollenhauer

Microbiologist and Research Leader
United States Department of Agriculture
Agricultural Research Service
Veterinary Toxicology and Entomology Research Laboratory
College Station, Texas

D.J. Morré

Professor, Department of Medicinal Chemistry and Pharmacognosy
Department of Biological Sciences
Director, Cancer Center, Purdue University
West Lafayette, Indiana

L.D. Rowe

Research Veterinarian and Research Leader
United States Department of Agriculture
Agricultural Research Service
Veterinary Toxicology and Entomology Research Laboratory
College Station, Texas

Send reprint requests to:
Hilton H. Mollenhauer, USDA, ARS, VTERL
P.O. Drawer GE
College Station, TX 77841 USA
409/260-9374

ABSTRACT

Monensin is an antibiotic ionophore used in agriculture both as a coccidiostat for poultry and as a feed additive to promote weight gain in cattle. It is toxic to mammals if given in excess of recommended doses. The equine is particularly susceptible to monensin poisoning. A common symptom of monensin poisoning in poultry, bovine and equine is vacuolization and swelling of heart mitochondria. In rats and mice, however, diaphragm mitochondria appear to be the primary targets of monensin toxicosis. **In vitro** effects of monensin differ markedly from **in vivo** effects; namely, the Golgi apparatus (but not the mitochondria) of cells directly exposed to monensin become vacuolated and swollen.

INTRODUCTION

Monensin is a sodium-selective ionophore derived from **Streptomyces cinnamonensis** (24). It is used extensively as a coccidiostat in the production of poultry (see 3, 42) and as a feed additive for cattle to promote meat productivity (3, 24, 42). Because of its effectiveness, monensin has become one of the largest volume agricultural chemicals currently produced. Monensin is rapidly metabolized by the liver and subsequently excreted in feces (7). Excreted monensin is not readily degraded by the environment and, thus, the possibility exists for a gradually increasing level of environmental contamination.

TOXICITY

Monensin may form lipid soluble complexes with alkali ions and transport these ions across biological membranes. The ion specificity of monensin is Ag►Na►K►Rb►Cs (Calbiochem Technical Bulletin on monensin) with approximately a ten-fold selectivity for sodium over potassium (36). Transport of sodium ions through the plasma membrane results in a marked increase in the intracellular sodium ion concentration and, at low monensin concentrations, a small increase in potassium ion concentration (3.29). At high levels of monensin, however, there appears to be a net exit of potassium ions from the cell (3, 24, 29). Because of this ability to transport ions across

biological membranes, monensin can influence biological processes and cause cellular disturbances. Indeed, numerous cases of monensin toxicoses and death have been reported in poultry, bovine, and equine (3, 15, 24, 27, 29, 42).

Equine are particularly sensitive to monensin poisoning and care must be exercised to prevent them from gaining access to monensin-supplemented cattle feed. The LD_{50} for equine is 2-4 mg monensin per kg body weight compared to 50-80 mg monensin per kg body weight for cattle (24, 42). The outstanding signs of monensin toxicosis are anorexia, depression, sweating, ataxia, palpitations of the heart, and sudden death following exercise (3, 24, 27). Stiffness of hindquarters and swollen glutus muscles also have been reported (27). Clinically, blood analyses of horses poisoned by monensin show increased values for blood urea nitrogen, total bilirubin, and serum glutamic oxalo transaminase (24, 27, 29) and for the muscle enzymes creatin phosphokinase and lactate dehydrogenase (27, 29). Levels of sodium, potassium, chlorine, calcium, phosphorus, and urea may remain at near-normal levels following monensin treatment (29). Pathologically, circulatory failure and macroscopical injuries to striated muscle, liver, and kidney have been noted (3, 20, 27, 29). A consistent observation in ponies, cattle, and fowl, has been cardiac myocyte degeneration and vacuolization (3, 15, 20, 26, 29).

LIGHT AND ELECTRON MICROSCOPIC OBSERVATIONS

(Results of **in vivo** investigations)

Macroscopic injuries to striated muscle and swelling and/or disintegration of mitrochondria are commonly reported cellular aberrations (20, 23). Presumably, the most severely damaged muscles are those that are continuously active (i.e., heart and diaphragm) (26). Nephrotoxicity and, to a lesser extent, hepatotoxicity, of monensin also have been indicated (23). Kidney changes range from generalized tubule necrosis (20, 29) to swelling of glomeruli (20). Liver injuries include proliferation of smooth endoplasmic reticulum, formation of protein bodies (3, 23) and general cellular breakdown (29).

Perhaps the most consistent, as well as the most striking, ultrastructural pathology is vacuolation of striated muscle mitochondria (3, 15, 20, 26, 29). Vacuolated mitochondria are characterized by an almost total loss of matrix substance and, in most instances, severe swelling (Fig. 1). Mitochondrial cristae do not swell though they may be markedly reduced in extent or even totally absent. Typically, only some mitochondria in a particular fiber become vacuolated and these appear to be randomly distributed throughout the fiber (Fig. 2). Early stages of mitochondrial swelling have been identified in both rat and pony (23, unpublished results) but these mitochondria were seldom plentiful suggesting that

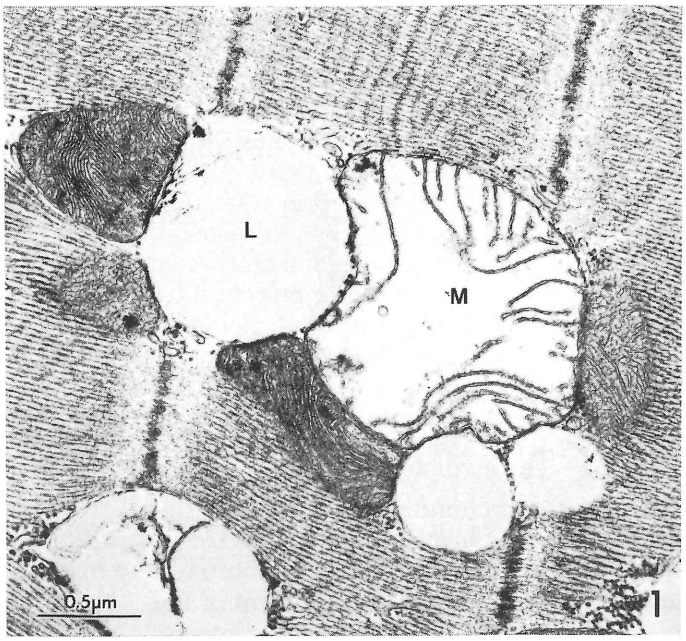


FIGURE 1. Portion of left ventricle from pony given a single dose of monensin (4 mg/kg body wt). Some mitochondria (M) became vacuolated and swollen. Cristae did not swell but were usually reduced in extent. Other aspects of the cell, including the Golgi apparatus (not illustrated), appeared normal. Lipid (L.)

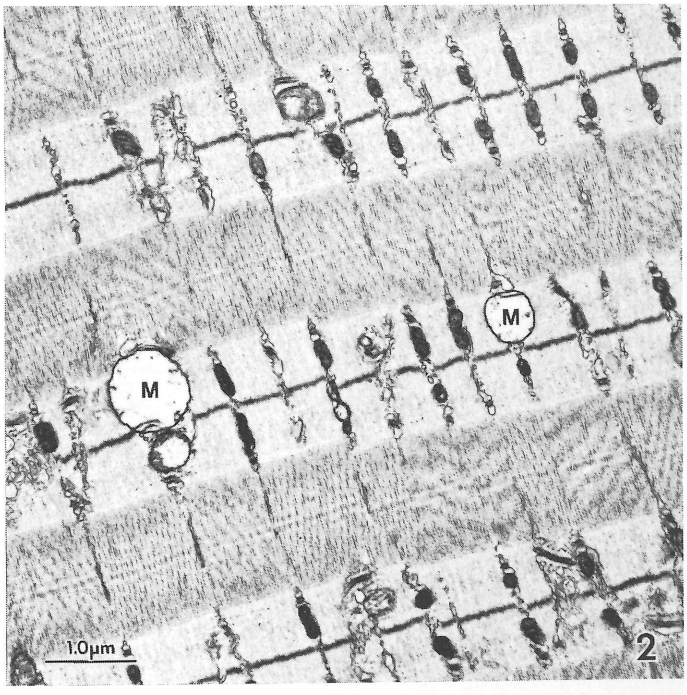


FIGURE 2. Portion of diaphragm from rat given a single dose of moninsin. Vacuolated mitochondria (M) were distributed randomly through the muscle fiber.

transition to the swollen state was relatively sudden. Except for the swollen, or partially swollen, mitochondria, the muscle fibers in these tissues appeared relatively normal (Figs. 1, 2). The percentage of swollen mitochondria varies markedly between

muscle types and species of animal. Analyses of three kinds of pony striated muscle showed that there is a much greater (50-100 times) likelihood of finding swollen mitochondria in heart tissues than in diaphragm or appendicular muscle (Unpublished results). A similar relationship existed in rats except that most swollen mitrochondria were in the diaphragm (Fig. 2). Differences in distribution patterns of swollen mitochondria also have been observed between the red and white muscle fibers of the rat diaphragm (Unpublished results). Red and white fibers were differentiated structurally by size, mitochondrial content, and Z-band configuration (11, 12, 31). Vacuolated mitochondria were present in all fiber types when the number of affected mitochondria was small. However, when large numbers of vacuolated mitochondria were present, the distribution pattern was heavily skewed toward the white muscle fibers (Fig. 3). These distribution patterns coupled with the characteristic form of the degenerating mitochondria were unique and are sufficient to be diagnostic for monensin poisoning.

We have not observed vacuolated mitochondria in any of the nonmuscle cells of the heart, diaphragm, or appendicular tissues or in liver, adrenal, or kidney cells. Thus, monensin administered to mammals **in vivo** induces mitochondrial changes only in selected tissues and/or muscle fiber types. The mechanism for these mitochondrial changes and reason for the specificity are not known. These problems are compounded by the fact that mitochondrial vacuolization does not occur in cells exposed directly to monensin (see section on Cell Cultures and Other Model Systems, immediately below).

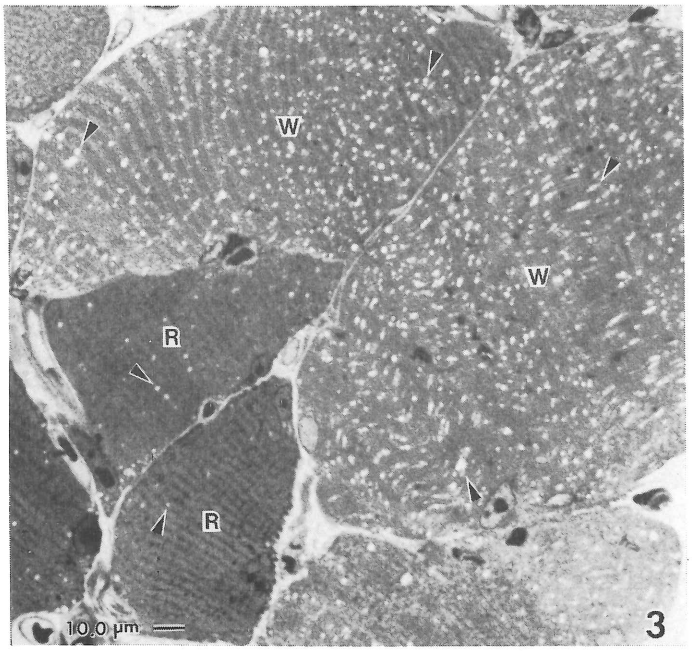


FIGURE 3. Light micrograph of diaphragm from a rat given a single dose of monensin. Most vacuolated mitochondria (arrowheads) were in the large, white muscle fibers (W) and very few were in the red muscle fibers (R).

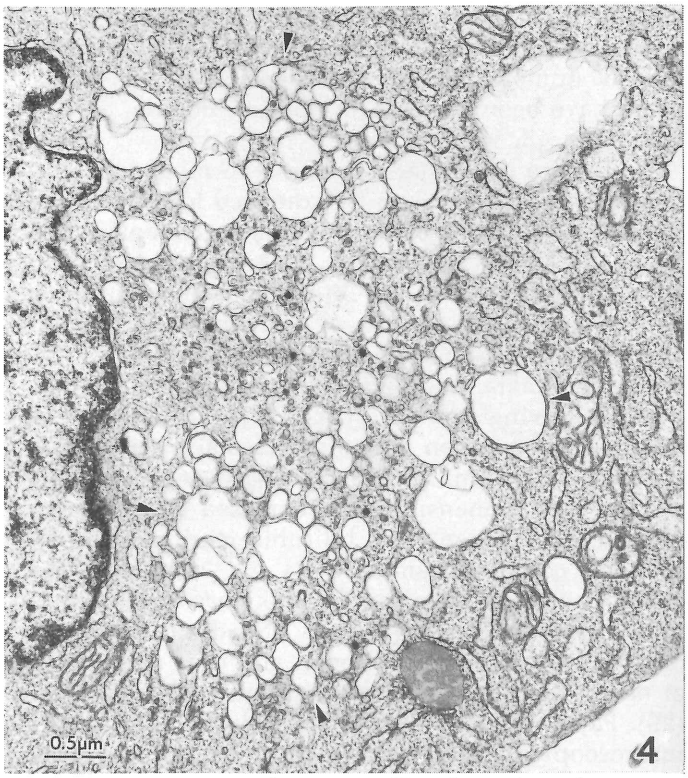


FIGURE 4. Portion of a V79 cell cultured in the presence of 10^{-7}M monensin for 1 hr. Under these conditions, the Golgi apparatus vesiculated (arrowheads) and lost its identity.

CELL CULTURES AND OTHER MODEL SYSTEMS

Cellular responses to monensin differ markedly from the **in vivo** responses indicated above when the cells or tissues are exposed directly to monensin; namely, mitochondria do not swell when cultured cells are exposed to monensin even when the cultured cells are from striated muscle. Numerous **in vitro** effects of monensin toxicosis have been reported (16, 19, 38); however, a primary effect is inhibition of cellular secretion (6, 19, 38). Ultrastructurally, Golgi apparatus cisternae of monensin-treated cells appear vesiculated after glutaraldehyde and osmium tetroxide fixation and the dictyosomes lose integrity (Fig. 4). Less vesiculation occurs when either potassium permanganate or osmium tetroxide (without glutaraldehyde) are used as fixative but loss of dictyosome integrity still occurs (22). Though the entire Golgi apparatus may respond to monensin, the major effect appears to be associated with the mature poles of the dictyosomes (21, 38). This can be clearly illustrated in appropriate models such as **Euglena** (Fig. 5).

In animal cells, changes in Golgi apparatus occur at a minimum monensin concentration of about 10^{-7}M (18, 38, unpublished results). In plant cells, the minimum effective monensin concentration is about 10^{-5}M (22, 25). In both plant and animal cells, the monensin effect is quite rapid; i.e., changes in Golgi apparatus are easily observed after only two minutes of treatment (22, 25). These early effects have been

documented particularly well in suspension cultures of carrot (**Daucus carota** L.) (25). When these carrot cells were exposed to 10^{-5}M concentration, an increased number of cisternae in the dictyosome stacks was observed. An average of one additional cisterna per stack was formed within the first 2-4 min of monensin treatment and, in some experiments, a second cisterna was formed within about 6 min. Thereafter, the dictyosome cisternae began to vesiculate as observed in other model systems and the number of cisternae per stack was reduced to the control level. The mechanism postulated for this momentary increase of dictyosome cisternae was that monensin, acting on the mature pole of the dictyosome, temporarily blocked the normal release of mature cisternae without blocking the formation of new cisternae.

EFFECT ON PLANT GROWTH

When the root tips of maize seedlings were submersed in monensin at concentrations of 10^{-5}M or higher, distinct ultrastructural changes occurred in the outer cells of the root cap and in those epidermal cells directly exposed to the monensin. The ultrastructural changes were essentially the same as those reported above in the "**in vitro**" studies; i.e., the Golgi apparatus cisternae visiculated and dictyosomes lost integrity (22, 30). This results in diminution of secretion of hydrated polysaccharides by rootcap cells and cessation of cell plate formation in epidermal cells (22) as well as a reduction in the rate of root growth (unpublished results). Comparable results on root elongation were obtained on rye grass. In both corn and rye, shoot elongation was less inhibited than root elongation by monensin. For example, growth of rye roots was reduced by 30% when the root tips were exposed to 10^{-5}M monensin, but the rate of shoot elongation was reduced only about 10% (Unpublished results). At 10^{-4}M concentration, monensin completely inhibited root growth of rye grass while limiting shoot growth to about 30% of normal. The lower inhibition of shoot growth by monensin probably reflects poor penetration of monensin into the root and/or poor transport of monensin to the shoot axis. This hypothesis is supported by our observations on the maize root tip which showed that a monensin effect was demonstrable for only 2-3 cells into the rootcap and only on epidermal cells of the root proper (i.e., those in direct contact with monensin) (22). Interior cells of the root or rootcap did not demonstrate distinct morphological responses to monensin (22).

MODE OF ACTION OF MONENSIN

In vitro studies clearly demonstrate that monensin alters or inhibits numerous membrane-oriented phenomena including transfer of α2-macroglobulin from coated pits to receptosomes (5), pinocytosis (43), membrane turnover (6), transfer of product from endoplasmic reticulum to Golgi apparatus (18), and cellular secretion (22, 25, 38). Swelling of Golgi apparatus cisternae is, perhaps, the most consistent visual **in vitro** demonstration of a monensin-induced membrane effect on a membranous cell compartment. Monensin, like other carboxylic acid ionophores, has the ability to form lipid-soluble complexes with alkali metal cations (usually sodium) and, in some examples, with biogenic amines (3). These complexes are freely soluble in the lipid components of biological membranes and thus accessible to the cellular milieu (3, 36). Ions are transported rapidly across membrane barriers at rates estimated as high as 500 ions/sec (3). After entering a cell through the plasma membrane, the alkali ion is released, after which the monensin molecule picks up a proton to form an undissociated molecule which then retraverses the plasma membrane releasing the proton to the outside of the cell. The net effect is a transmembrane exchange of alkali ions for protons (3).

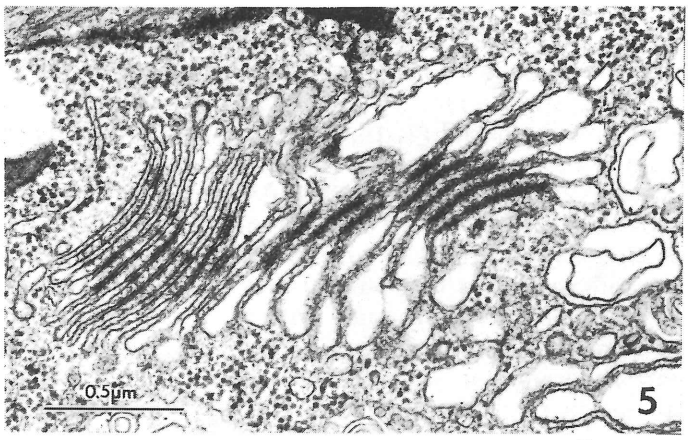


FIGURE 5. Portion of **Euglena** exposed to 10^{-5}M monensin for 1 hr. The cisternae of the mature poles of the Golgi apparatus swelled but the forming poles remained normal even after prolonged monensin treatment.

Intracellular ionic imbalance could account for some of the observed effects of monensin poisoning, particularly those effects related to myocardial dysfunction. Monensin is known to inhibit Ca^{2+} accumulation in myocytes and to release Ca^{2+} from microsomes (33, 36). Moreover, the force developed by the myocardium is modulated by extracellular Na^+ and Ca^{2+} (36). Drugs such as cardiac glycosides (which cause myocardial contraction) appear to act primarily by altering intracellular Na^+ concentration (see 36). Thus, Na^+ balance could play a critical role in modulating myocardial function. However, the different responses observed in cell organelles between whole animals and cell models (see section below entitled MITOCHONDRIAL VS GOLGI APPARATUS SWELLING) very much clouds the issue at this time.

As already mentioned, monensin has a striking effect on the Golgi apparatus of a wide range of plant and animal species. Action is exerted near the middle

of the stacked cisternae (10, 19, 28) or near the point of exit of secretory vesicles at the mature face of the stacked cisternae (13, 14, 18, 21, 25). Intracellular transport is blocked (2, 14, 16-19, 34, 35, 37, 38, 41, 43) and transport units (secretory vesicles or entire distended cisternae) may accumulate near the Golgi apparatus (22, 25, 37). The early effects of monensin on Golgi apparatus (1 to several hours at 10^{-5}M or less) are reversible (25, 30, 43). In carrot cells, normal secretory activity is resumed within 20 minutes after transfer of cells to a monensin-free medium (Unpublished results). With prolonged treatment, monensin causes swelling or dilation of Golgi apparatus cisternae (13, 14, 16-19, 21, 25, 30, 37, 38). Dilated membranes remain dilated upon withdrawal of monensin even though secretory activity may resume.

The swelling of the Golgi apparatus cisternae appears to be osmotic, due to a K^+/H^+ exchange across the membranes leading to the net uptake of salt. However, in order for sufficient salt to accumulate to allow swelling, an influx of protons is required. Inhibitors that disrupt proton gradients as well as secretion (17), such as carbonylcyanide-p-trifluoromethoxyphenylhydrazone (FCCP), prevent monensin-induced swelling of Golgi apparatus cisternae (4) as do lysosomotropic amines and, to a lesser extent, quercetin, a putative inhibitor of lysosomal proton-translocating ATPase (8), and vanadate (results unpublished). Organic acids, by providing a readily permeable counterion, promote monensin-induced swelling.

While a proton gradient seems to be involved, it is not certain that, as suggested by Geisow and Burgoyne (9), monensin-induced swelling of membranes is always dependent upon active proton pumps. In chloroplasts, swelling of thylakoids (inner membrane compartments) but not of the space between inner and outer plastid membranes was observed along with dilation of mitochondrial cristae and Golgi apparatus cisternae following monensin treatment (32). Thylakoid swelling, but not that of mitochondria and Golgi apparatus, was reduced upon incubation in darkness, suggesting a relationship between swelling in the presence of monensin and the light-driven proton gradient used for photophosphorylation. As suggested by Schnepf (32), monensin could be a useful tool in cell biology to visualize proton gradients.

Not only do the monovalent ionophores block transport and surface expression of several secretory glycoproteins (18, 35, 41) in normal cell functioning but also the transport of membrane glycoproteins or enveloped viruses (7, 16, 17, 23). The site of the block for the latter remains problematic. Griffiths et al. (10) have shown that monensin inhibits the transport of viral membrane proteins from medial to trans Golgi apparatus cisternae, thus indicating a monensin block between the medial and trans cisternae. Srinivas et al. (34) report failure to process simple endo-H sensitive to complex endo-H resistant oligosaccharides and reduced efficiency of cleavage of the PrENV glycoprotein precursor to gp70 for Eveline mouse cells infected with Friend murine leukemia virus, thus indicating a block prior to entry into the Golgi apparatus. With BHK-21 cells and chicken embryo fibroblasts infected with vesicular stomatitis virus or Sindbis virus, transport of viral glycoproteins appears to be arrested between the Golgi apparatus and plasma membrane (1, 7). Also, in cultured hepatoma cells, transport of vesicular stomatitis virus G protein is arrested after endo-H resistance has been acquired in contrast to transferrin, which is arrested prior to acquisition of endo-H resistance (35). The accumulation of incompletely processed glycoproteins indicates either an up-stream accumulation of secretory materials behind a Golgi apparatus blockage by monensin or an additional monensin block near the exit site from endoplasmic reticulum (34). The effect must be on the transport since oligosaccharide processing of those glycoproteins that reach the appropriate site occurs normally even in monensin-treated cells (34, 37, 39, 40, 41). The possibility is raised also that processing of oligosaccharide chains of different secreted glycoproteins occurs at different sites, some sensitive to monensin and others insensitive to monensin (34).

MITOCHONDRIAL VS GOLGI APPARATUS SWELLING

The swelling of Golgi apparatus cisternae observed **in vitro** and in the maize root tip is not a characteristic aberration of the cells of animals poisoned by monensin. In all of the tissues from monensin-poisoned animals that we have studied, the Golgi apparatus has appeared normal (i.e., not swollen) even though other aspects of the cell may have been grossly affected. This lack of Golgi apparatus swelling infers that cells from monensin-poisoned animals are bathed in body fluids containing less than 10^{-7}M monensin (10^{-7}M monensin is approximately the minimum effective dose of monensin on cultured cells). Similarly, lack of swollen mitochondria **in vitro** implies that the swollen mitochondria observed in striated muscle from monensin-poisoned animals are secondary effects of monensin poisoning; i.e., a metabolite of monensin causes the mitochondrial swelling, or monensin acts on a yet-unknown target system within the animal which then causes the mitochondrial swelling. Numerous metabolites of ingested monensin have been identified (7) and could account for the different experimental results. However, our initial tests using microsomal fractions in tissue culture preparations to metabolize monensin were negative. Thus, we currently favor the hypothesis that the mitochondrial swelling observed **in vivo** is a secondary effect of a toxic action which has not been identified.

(1.) F.V. Alonso and R.W. Compans, Differential effects of monensin on enveloped viruses that form at distinct plasma membrane domains. J Cell Biol 89 (1981) 700-705.

(2.) S.K. Basu, J.L. Goldstein, R.G.W. Anderson and M.S. Brown, Monensin interrupts the recycling of low density lipoprotein receptors in human fibroblasts. Cell 24 (1981) 493-502.

(3.) B.E. Beck and W.N. Harries, The diagnosis of monensin toxicosis: A report on outbreaks in horses, cattle and chickens. Am Assoc Vet Lab Diagnosticians 22nd Ann Proc (1979) 269-282.

(4.) W.F. Boss, D.J. Morre'and H.H. Molenhauer, Monensin-induced swelling of Golgi apparatus cisternae mediated by a proton gradient. Plant Physiol (In Press).

(5.) R.B. Dickson, R. Schlegel, M.C. Willingham I. Pastan, Binding and internalization of α2-Macroglobulin by cultured fibroblasts. Exptl Cell Res 142 (1982) 127-140.

(6.) K.A. Doeg and A.H. Phillips, Effect of monensin, chloroquine, and cyclohexamide on rapid membrane turnover and secretion by MOPC 41 myeloma cells. J Cell Biol 91 (1981) 401a.

(7.) A. Donoho, J. Manthey, J. Occolowitz and L. Zornes, Metabolism of monensin in the steer and rat. J Agric Food Chem 26 (1978) 1090-1095.

(8.) M. Geisow, Lysosome proton pump identified, Nature 298 (1982) 515-516.

(9.) M.J. Geisow and R.D. Burgoyne, Effect of monensin of chromaffin cells and the mechanism of organelle swelling. Cell Biol Int Reports 6 (1982) 933-939.

(10.) G. Griffiths, P. Quinn and G. Warren, Dissection of the Golgi complex. I. Monensin inhibits the transport of viral membrane proteins from medial to trans Golgi cisternae in baby hamster kidney cells infected with Semliki forest virus. J Cell Biol 96 (1983) 835-850.

(11.) G.F. Guathier and S. Lowey, Distribution of myosin isoenzymes among skeletal muscle fiber types. J Cell Biol 81 (1979) 10-25.

(12.) A.W. Ham and D.H. Cormack, Histology, ed. 8 (J.B. Lippincott Company, Philadelphia & Toronto, 1979) 547-548.

(13.) R. Hammerschlag and G.C. Stone, Membrane delivery by fast axonal transport. Trends NeuroSciences 5 (1982) 12-15.

(14.) R. Hammerschlag, G.C. Stone, F.A. Bolen, J.D. Lindsey and M.H. Ellisman, Evidence that all newly-synthesized proteins destined for fast axonal transport pass through the Golgi apparatus. J Cell Biol 93 (1982) 568-575.

(15.) L.A. Hanrahan, D.E. Corrier and S.A. Naqui, Monensin toxicosis in broiler chickens. Vet Pathol 18 (1981) 665-671.

(16.) D.C. Johnson and M.J. Schlesinger, Vesicular stomatitis virus and Sinbis virus glycoprotein transport to the cell surface is inhibited by ionophores. Virology 102 (1980) 407-424.

(17.) L. Kääriäinen, K. Hashimoto, J. Saraste, I. Virtanen and K. Penttinen, Monensin and FCCP inhibit the intracellular transport of alphavirus membrane glycoproteins. J Cell Biol 87 (1980) 783-791.

(18.) P.W. Ledger, N. Uchida and M.L. Tanzer, Immunocytochemical localization of procollagen and fibronectin in human fibroblasts: Effects of the monovalent ionophore, monensin. J Cell Biol 87 (1980) 663-671.

(19.) J.D. Lindsey and M.H. Ellisman, Histochemical analysis of the effect of monensin on the Golgi apparatus. J Cell Biol 91 (1981) 96a.

(20.) F.M. Mallon, J.F. Amend, A.S. Ramos, C.J. Morrow and W.B. Wren, Mechanisms of experimental monensin toxicosis in ponies. Fed Proc 40 (1981) 752.

(21.) H.H Mollenhauer, D.J. Morre'and R.E. Droleskey, Monensin affects the trans half of Euglena dictyosomes. Protoplasma 114 (1983) 119-124.

(22.) H.H. Mollenhauer, D.J. Morre'and J.O. Norman, Ultrastructural observations of maize root tips following exposure to monensin. Protoplasma 112 (1982) 117-126.

(23.) H.H. Mollenhauer, L.D. Rowe, S.J. Cysewski and D.A. Witzel, Ultrastructural observations in ponies after treatment with monensin. Am J Vet Res 42 (1981) 35-40.

(24.) T. Matsuoka, Evaluation of monensin toxicity in the horse. J Am Vet Med Assoc 169 (1976) 1098-1100.

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

(26.) E. Muylle, C. Van Den Hende, W. Oyaert, H. Thoonen and K. Vlaeminck, Delayed monensin sodium toxicity in horses. Equine Vet J 13 (1981) 107-108.

(27.) J. Nuytten, D. Bruynooghe, E. Muylle, C. Van Den Hende, K. Vlaeminck and W. Oyaert, Acute en subacute verschijnselen bij monensin intoxicatie bij paarden. VLAAMS Diergeneeskundig Tijdschrift 50 (1981) 242-249.

(28.) P. Quinn, G. Griffiths and G. Warren, Dissection of the Golgi complex. II. Density separation of specific Golgi functions in virally infected cells treated with monensin. J Cell Biol 96 (1983) 851-856.

(29.) M.F. Raisbeck and R.B. Miller, An episode of monensin intoxication in pastured calves. Am Assoc Lab Diagnosticians, 24th Ann Proc (1981) 373-384.

(30.) D.G. Robinson, The ionic sensitivity of secretion-associated organelles in root cap cells of maize. Eur J Cell Biol 23 (1981) 267-272.

(31.) R.W.D. Rowe, The ultrastructure of Z disks from white, intermediate, and red fibers of mammalian striated muscles. J Cell Biol 57 (1973) 261-277.

(32.) E. Schnepf, Light-dependent, monensin-induced thylakoid swelling. Naturwissenschaften 70 (1983) 260.

(33.) M. Shlafer, P. Somani, B.C. Pressman and R.F. Palmer, Effects of the carboxylic ionophore monensin on atrial contractility and Ca^{2+} regulation by isolated cardiac microsomes. J Molecular Cellular Cardiology 10 (1978) 333-346.

(34.) R.V. Srinivas, L.R. Melsen and R.W. Compans, Effects of monensin on morphogenesis and infectivity of Friend murine leukemia virus. Virology 42 (1982) 1067-1075.

(35.) G.J.A.M. Strous and H.F. Lodish, Intracellular transport of secretory and membrane proteins in hepatoma cells infected by vesicular stomatitis virus. Cell 22 (1980) 709-717.

(36.) J.L. Sutko, H.R. Besch, Jr., J.C. Bailey, G. Zimmerman and A.M. Watanabe, Direct effects of the monovalent cation ionophores monensin and nigericin on myocardium. J Pharmacol Exptl Therapeutics 203 (1977) 685-700.

(37.) A.M. Tartakoff and P. Vassalli, Plasma cell immunoglobulin secretion: Arrest is accomplished by alteration of the Golgi complex. J Exptl Med 145 (1977) 1332-1345.

(38.) A. Tartakoff and P. Vassalli, Comparative studies of intracellular transport of secretory proteins. J Cell Biol 79 (1978) 694-707.

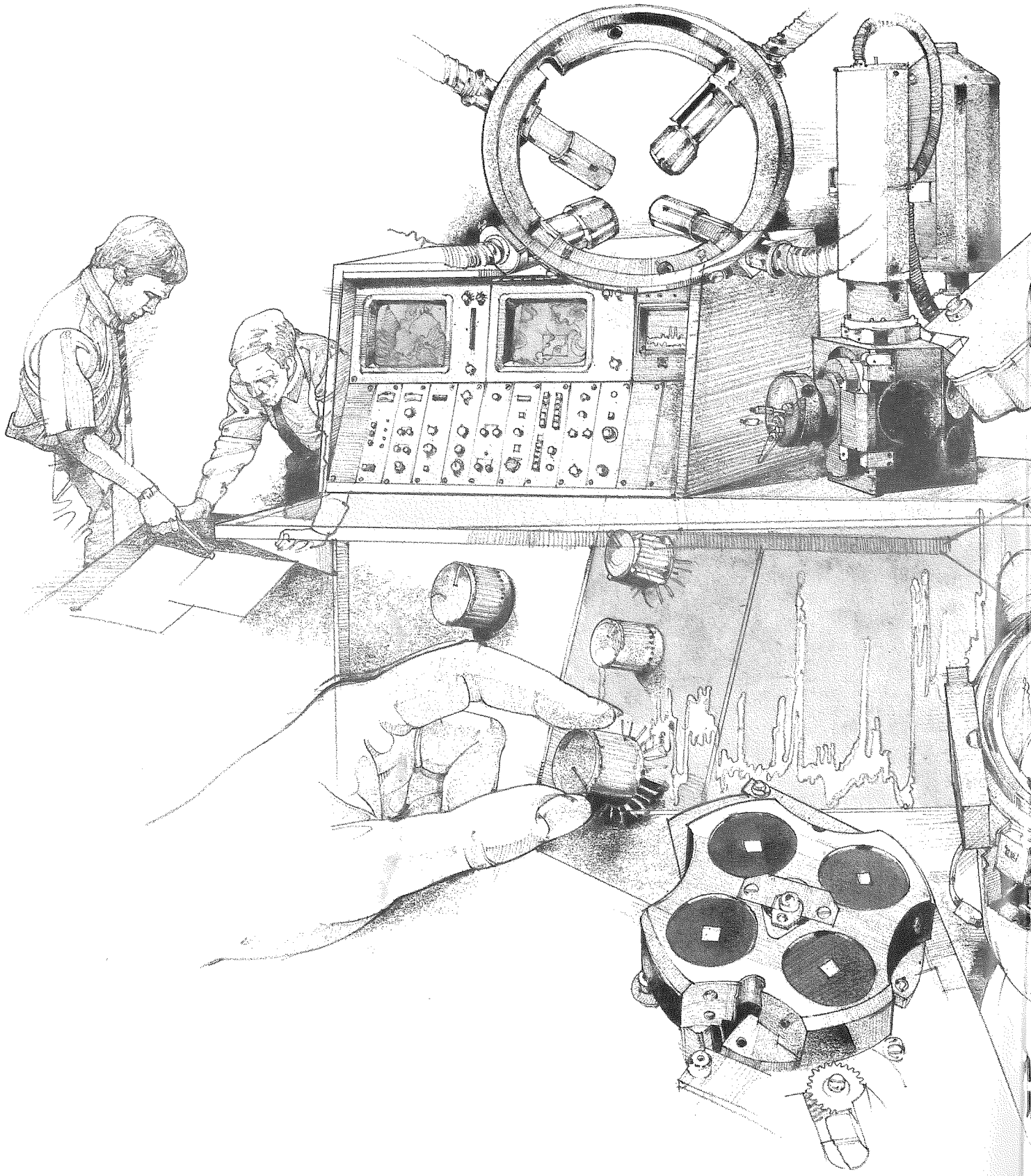
(39.) A. Tartakoff, D. Hoessli and P. Vassalli, Intracellular transport of lymphoid surface glycoproteins. J Mol Biol 150 (1981) 525-535.

(40.) K. Tajiri, N. Uchida and M.L. Tanzer, Undersulfated proteoglycans are secreted by cultured chondrocytes in the presence of the ionophore monensin. J Biol Chem 255 (1980) 6036-6039.

(41.) N. Uchida, H. Smilowitz and M.L. Tanzer, Monovalent ionophores inhibit secretion of procollagen and fibronectin from cultured human fibroblasts. Proc Natl Acad Sci USA 76 (1976) 1868-1872.

(42.) R.H. Whitlock, N.A. White, G.N. Rowland and R. Plue, Monensin toxicosis in horses: Clinical manifestations.

(43.) D.K. Wilcox, R.P. Kitson and C.C. Widnell, Inhibition of pinocytosis in rat embryo fibroblasts treated with monensin. J Cell Biol 92 (1982) 859-864.

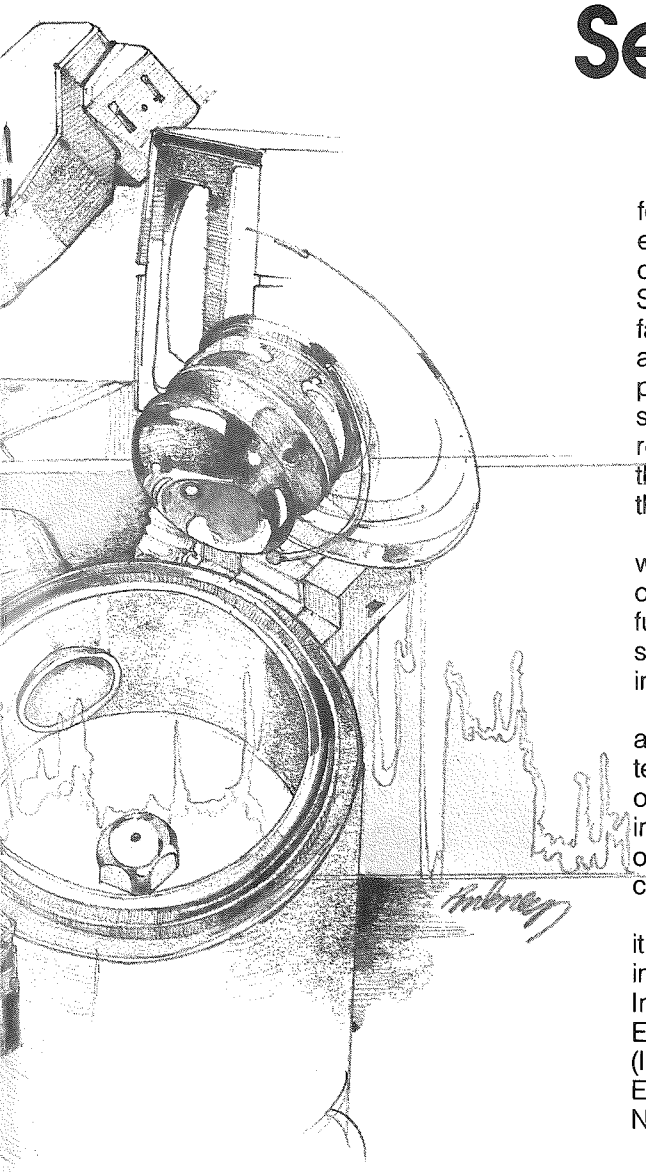


PHILIPS

Scientific &
Analytical Equipment

Observe the true nature of your specimen.

See it with Philips.



The superior imaging and analytical capabilities found in the world famous Philips transmission electron microscopes are now also available in our scanning electron microscopes. The Philips SEM 505 enables users to examine specimens in far greater detail than ever before. Real time images are enhanced by the β^+ electron source, which, particularly when using very low kV, provides superior imaging. This gives microscopists high resolution, truly representative images, while insuring that specimens are neither destroyed nor altered by the electron beam.

With the added capabilities offered by energy and wave length dispersive spectrometers, the SEM 505 can serve as a complete analytical system. These functions enable the user to perform everything from simple imaging to in-depth studies of a sample's internal structure as well as its surface.

This highly professional microscope is remarkably easy to operate. The use of computer-type technology and machine intelligence allows the operator to concentrate on the meaning of the image, instead of on the image acquisition itself. This operating system is ideal for external interactive control for advanced image quantification.

To learn more about the Philips SEM 505 and how it can help you observe the nature of your specimen in greater detail, contact: N.V. Philips Science & Industry, Electron Optics Department, TQ-III-4, Eindhoven, The Netherlands. Tel.: (40) 788582. (In U.S.A.) Philips Electronic Instruments, Inc., Electron Optics Group, 85 McKee Drive, Mahwah, NJ 07430. Tel.: (201) 529-3800.

PHILIPS

Abstracts

CYTOCHEMICAL METHODS FOR ELECTRON MICROSCOPY by B.A. Nichols, F.I. Proctor Foundation, University of California, San Francisco, CA 94143

Cytochemical methods for electron microscopy provide a means for localizing certain enzymes or other chemical substances within cells. These techniques have greatest value when used to identify organelles that cannot be distinguished morphologically or biochemically. For example, the secretory granules of human monocytes are identical in appearance, and they have not been isolated and analyzed biochemically. Therefore, it was not recognized that there are two distinct populations of granules with different enzymatic contents until they were studied cytochemically. These facts emphasize the unique value of cytochemical methods.

There are several technical requirements for a successful cytochemical experiment. First, the chemical reaction must yield an electron dense reaction product so that it forms an electron-opaque image. In addition, to provide the substrate with access to the enzyme in the tissues, it may be necessary to cut 20-40 μm sections with a tissue chopper before cytochemical incubation. Fixation must be minimal to retain adequate enzyme activity, yet also sufficient to produce adequate structural preservation.

Controls should include, when possible: 1) tests using specific enzyme inhibitors, 2) tests for non-specific binding of capture agents, 3) biochemical confirmation of localization and other tests as indicated. Common problems include loss of enzymatic activity by fixation or holding tissues in buffer before testing, lack of substrate penetration, cross-reactivity of enzymes, and loss of reaction product during specimen preparation following cytochemical incubation. Although cytochemistry is tedious and demanding, it yields rewards that are unique and worthy of pursuit. For further details, see "Use of Ultrastructural Histochemistry" by B.A. Nichols, In: Methods for Studying Mononuclear Phagocytes (Eds. Adams et al.) 1981, p. 413

GLUTARALDEHYDE FIXATION CHEMISTRY: A CROSSLINKING SCHEME, T. J. A. Johnson and J. E. Rash, Dept. of Anatomy, Colorado State University, Ft. Collins, CO 80523.

Although glutaraldehyde has been widely used for the fixation of biological tissue, the chemical mechanism of fixation has not been established. Rapid α,ω-bis-Schiff base formation is responsible for the rapid production of acid but not crosslinking. Pyridine derivatives are the major reaction products of amine-aldehyde reactions and require oxygen for their formation. The stoichiometry of oxygen consumption is 0.5 moles O_2/mole amine; the rate of oxygen uptake is first order with respect to [amine]. The chemical uptake of oxygen competes with respiration for available oxygen during the course of tissue fixation and is a major concern in the preservation of tissue morphology.

A variety of products distinguished by molecular size are rapidly synthesized in amine-glutaraldehyde reactions. The molecular size range of the products varies with the specific amine and with the glutaraldehyde/amine ratio. Higher molecular weight products predominate when the ratio is 2:1. These high molecular weight products are rapidly and irreversibly synthesized, and the major reaction products share physical and chemical characteristics with the highly substituted pyridine compounds.

The addition of formaldehyde to glutaraldehyde fixatives alters amine-glutaraldehyde chemistry by decreasing both oxygen uptake and the yield of UV absorbing products. Other additives to glutaraldehyde-based fixatives, such as hydrogen peroxide and sodium azide, have been reported to improve preservation of cell ultrastructure. Our data suggests that these additives may have a common function. In the presence of these additives, more oxidizing equivalents are available for relief of tissue hypoxia and for irreversible chemical oxidation processes.

Based upon these data and upon precedents in the chemical literature, we now propose a new scheme for the synthesis of pyridine derivatives (and branched pyridine polymers) from precursor primary amines and glutaraldehyde. We also propose that the branched polymers are a major class of crosslinks in glutaraldehyde fixed tissue.

IMMUNOCYTOCHEMICAL METHODS IN DIAGNOSTIC ELECTRON MICROSCOPY. Barbara G. Crawford and Juan Lechago, Department of Pathology, L.A. County Harbor-UCLA Medical Center, Torrance, California 90509.

Immunocytochemical investigations must on occasions be carried out at the electron microscopic level, either because no adequate tissue is available for light microscopic examination, or because ultrastructural features must be taken into account for diagnostic purposes. Our laboratory has been involved for several years in the fine structural localization of hormonal peptides in digestive endocrine cells and their tumors. In our experience, immunocytochemical methods, particularly those which involve carrying out pre-embedding immunoperoxidase techniques, often result in less than ideal preservation of ultrastructural detail, a serious drawback when tissues are evaluated under the electron microscope. Our efforts, therefore, have been directed at evolving post-embedding immunocytochemical techniques that can be applied to tissues previously processed in a more or less routine fashion for electron microscopy. A "sandwich" approach was developed which made it possible to concurrently visualize individual cells by light microscopic immunocytochemistry, electron microscopic immunocytochemistry, and conventional electron microscopy in tissues fixed with glutaraldehyde, with or without postosmication, and embedded in Epon 812. Our observations have resulted in the immunolocalization of serotonin, gastrin, somatostatin, and glucagon-like immunoreactivity both, in normal human digestive endocrine cells and in some carcinoid tumors, derived from such cells. The results of: a) indirect immunoperoxidase, b) PAP immunoperoxidase, c) avidin-biotin complex immunoperoxidase, and d) colloidal gold-avidin D complex methods were compared and evaluated for specificity, sensitivity, and reproducibility. It is anticipated that these or similar techniques may also prove useful in the ultrastructural immunolocalization of other peptides and related substances.

This work was supported by NIH grants AM 17328 and RO1 AM 30523.

A NOVEL INTERACTION BETWEEN CELLS: FILOPODIA FROM GROWTH CONES INSERT INTO OTHER CELLS AND INDUCE COATED PITS AND VESICLES, M. BASTIANI AND C.S. GOODMAN, Dept. of Biol. Sciences, Stanford University, Stanford, Ca 94035

Serial section TEM analysis of identified embryonic neurons in the grasshopper has revealed a novel interaction between developing cells. Filopodia from the MP1 growth cone contact the growth cone of the pCC (a putative "landmark" cell), insert deep within the growth cone, and induce coated pit and vesicle formation specifically at the tips of these insertions. Other filopodia from MP1 that contact the cell body of the pCC, although closely adherent, do not penetrate into the cell nor induce coated pit formation. The interaction between the MP1 filopodia and the pCC growth cone is quite specific-- filopodia from other nearby growth cones contact the pCC growth cone, but do not penetrate or induce coated pit formation. This novel interaction between filopodia and growth cones may be a general mechanism mediating specific cell-cell communication necessary for normal growth and differentiation.

HRTEM INVESTIGATION OF INFINITELY ADAPTIVE SERIES $Ba_{1+x}Fe_2S_4$, Andrea Holladay and LeRoy Eyring, Department of Chemistry, Arizona State University, Tempe, AZ 85287.

The microstructure in the infinitely adaptive series $Ba_{1+x}Fe_2S_4$ has been examined by HRTEM. A number of samples in the range $0 \leq x \leq .135$ where the stoichiometry of Ba was accurately determined by powder diffraction patterns were provided by Professor Hugo Steinfink. The range of Ba composition has allowed the comparative investigation of the local reordering which occurs in this system. Comparison of observed images and electron diffraction patterns with those calculated from models as well as with structural models derived from X-ray crystallographic data on the stoichiometric $BaFe_2S_4$ species will be made.

THE OCCURRENCE OF MALLORY BODIES IN VITAMIN A DEFICIENT MICE, S. Akeda, K. Fujita, Y. Kosaka, N. Benson, and S. W. French, First Dept. of Internal Medicine, Mie University School of Medicine, Tsu, Japan, and Dept. of Pathology, University of California, Davis, and VA Medical Center, Martinez, CA. 94553

It has recently been postulated that Mallory bodies (MBs) seen in the liver of patients with alcoholic liver disease are caused by vitamin A deficiency. This hypothesis is based on the concept that vitamin A is required for normal differentiation of cytokeratin filaments in hepatocytes. MBs are intracytoplasmic aggregates of filaments in part composed of cytokeratin polypeptides. To test this hypothesis 12 Swiss albino male mice weighing 20-25 gm were divided into two groups. Seven were fed a vit. A deficient diet. Five were fed a diet supplemented with vit. A. After 18 months on this diet the mice were killed and the livers were examined by light and electron microscopy. Mallory bodies were found in two of the three surviving vit. A deficient mice livers and in none of the five surviving control mice. The MBs were morphologically the same as those seen in man. Type II randomly oriented MB filaments were seen by EM. Coincidental amyloid deposits were found in the space of Disse in all three vit. A deficient mice and in none of the controls. The amyloid stained with congo red and was birefringent by polarized light. EM examination of the amyloid deposits revealed thin parallel filaments occupying the space of Disse and impinging on hepatocytes and sinusoids. The reason for the amyloid deposits is not known. One hyperplastic nodule was found in the liver of a vit. A deficient mouse in which numerous megamitochondria were found. The results support the hypothesis tested that MBs are the result of faulty differentiation caused by vit. A deficiency. MBs are commonly associated with cholestasis in man. Cholestatis may cause malabsorption which could cause vit. A deficiency which leads to MB formation.

SCANNING ELECTRON MICROSCOPIC STUDIES OF INFLUENZA AND SENDAI VIRUS INFECTED TISSUE CULTURES, Z. Apelian, L. Hong, and J.T. Seto, Department of Microbiology, California State University, Los Angeles, CA 90032

The ultrastructure of influenza and Sendai virus infected cells, examined in the TEM, is similar. However, little is known concerning the surface morphology. Therefore, it was of interest to examine virus infected cells in the SEM, particularly cells that were persistently infected. We have shown that there are fewer microvilli present in MDBK and MDCK cells persistently infected with Sendai virus. Minor differences were observed in cells infected with stock virus, and the surface morphology of MDBK cells infected with a ts mutant of Sendai virus was similar to control cells. Cells infected with two strains of influenza virus, PR8 and WSN, were examined. WSN infected cells showed a dramatic loss in the number of microvilli and structures interpreted to represent budding were numerous. On the other hand, very little budding was seen in PR8 infected cells. It should be noted that MDBK cells are permissive for WSN but nonpermissive for PR8. Likewise MDBK and MDCK cells are nonpermissive for Sendai virus.

EFFECTS OF THIOCARBOHYDRAZIDE (TCH) ON MAMMALIAN CILIA, J. A. Blixt and G. P. Epling, Dept. of Veterinary Science, Montana State University, Bozeman, MT 59717.

These studies were designed to compare bovine tracheal ciliary diameters utilizing different preparation techniques.

High resolution SEM studies of ciliary diameters in specimens sputter coated with gold were compared with specimens treated with thiocarbohydrazide (TCH) linked with osmium tetroxide. Gold sputtered cilia averaged 200 nm in diameter whereas TCH treated cilia averaged 277 nm in diameter.

In TEM studies, TCH treated cilia averaged 348 nm in diameter and conventionally fixed and processed cilia averaged 278 nm in diameter.

In both studies these differences were significant according to the Student's t test ($P < 0.001$).

The variations in ciliary diameters in this study were probably indicative of internal structural change. TEM studies of TCH prepared material did not demonstrate membranes well. A possible explanation could be that TCH linked with osmium has permeated structures in the cell equally and not differentiated them.

GLUTARALDEHYDE FIXATION: BIOLOGICAL APPLICATION, Janet Boyles, Gladstone Foundation Laboratories and Department of Pathology, University of California at San Francisco, P.O. Box 40608, San Francisco, CA 94140.

Glutaraldehyde fixation is insufficient for the preservation of many negatively charged elements of cells and tissues. Actin filaments, for example, even after glutaraldehyde, are destroyed by the breakage of peptide bonds during postfixation in OsO_4 (P. Maupin-Szamier & T.D. Pollard, J. Cell Biol. 77:837, 1978). Other highly hydrated structures, such as the cell surface glycocalyx, have few amine groups available for extensive cross-linkage during aldehyde fixation and may collapse during dehydration. Pellets of pure actin filaments have served as a model system in our search for a better fixative. We explored the potential of several amines for improving fixation, because in our experience, tissues soaked in diaminobenzidine (DAB) were better fixed, and two studies suggested that amines improved fixation (J.W. McLean & P.K. Nakane, J. Histochem. Cytochem. 22:1077, 1974; M. Hauser, Cytobiology 18:95, 1978). Aldehyde and amine at molar ratios of 2:1 to 12:1 were used together as an initial fixation for 10 min, followed by aldehyde alone for 1 hr, postfixation in 1% OsO_4 in barbital buffer at 4°C for 15 min, uranyl acetate in bloc stain, acetone dehydration, and embedding. In these experiments, primary monoamines (amonia and ethanolamine), which interact with glutaraldehyde to give a polymer, improved the preservation of filaments: filaments were bent but not broken. Monoamines that do not form such compounds (Tris) or contain negatively charged sites (glycine, proline, serine) do not protect filaments that are broken beyond recognition by OsO_4. Excellent preservation is seen, however, when primary diamines (cadaverine, putrescine, 1,8-diaminooctane, lysine, or arginine) are used. In more complex systems, lysine has been extensively studied. It is effective with cytoskeletons, single cells, and tissues fixed by perfusion. In addition to preserving actin within cells, it appears to act as a fixative for negatively charged sites in general. Therefore, surface glycocalyx and basement membranes are well preserved. The positively charged lysine may accomplish this by interacting with the negatively charged sites of tissues, initiating aldehyde cross-linkage around these sites. The use of diamines in glutaraldehyde has applications in the preservation of lipid components as well. It has proved effective in preserving lipoproteins in cells. In conjunction with tannic acid and uranyl acetate, it preserves isolated membranes through critical point drying, producing images comparable to freeze-dried samples.

A NANNOCHLORIS-TYPE GREEN ALGA FROM OFF OAHU, HAWAII, U.S.A.
Susan A. Brady, Department of Botany, University of California, Berkeley, Calif. 94720.

Nanoplankton community composition at depths of 50m and 110m from a station 5 miles off Kahe Point, Oahu, Hawaii was investigated by means of enrichment cultures. Several naviculoid diatoms were common, as well as Nitzschia sp. and Entomeis sp. In addition a Pyramimonas sp. was present in some cultures. The most abundant organism in all cultures, however, was a small (2 um) spherical Nannochloris-type green alga that is typically associated with nutrient-rich waters and coastal communities. Examination of this cell by scanning electron microscopy revealed a tough, smooth cell wall. The ultrastructure of this organism is characteristic of Nannochloris and other very small eucaryotes, with a single nucleus, chloroplast and mitochondrion, and numerous starch granules.

CYCLING OF PHOTORECEPTORAL MEMBRANE IN EYES OF A SNAIL, HELIX ASPERSA, J.L. Brandenburger and R.M. Eakin, Department of Zoology, University of California, Berkeley, CA 94720

We investigated turnover of photoreceptoral membrane in eyes of a garden snail through a series of EM studies on localization of horseradish peroxidase (HRP) and acid phosphatase (AcPase) in combination with light and dark adaptation.

Degradation. Long slender microvilli borne at the distal end of a type I sensory cell are the light-sensitive organelles. Degradation of them is indicated by the villar tips becoming swollen and more electron lucent. Sometimes membranous debris is found in the humor. These products of degradation appear to be internalized by pinocytic vesicles and tubules, judging by our study of HRP uptake. Eyes of dark-adapted snails exhibited more endocytosis than light-adapted ones. Uptake of HRP varied among the different retinal cells: more in type II sensory cells and pigmented supportive cells than in type I sensory cells. Pinocytosis also occurs basally where the retinal cells rest on the capsule of the eye. Acid phosphatase studies suggest that primary lysosomes packaged by GERL cisternae fuse with vacuoles to form secondary lysosomes in the somata of the sensory cells. This lytic activity increases in snails maintained in the dark for up to four months.

Restoration. Resynthesis probably occurs within the Golgi and ER of type I sensory cells that receive small molecules from the pigmented cells and type II cells, new materials brought in basally, and products of its own autophagy. These materials are synthesized into macromolecules, including the photopigments rhodopsin and retinochrome, needed for regeneration of the microvillar membranes. The new molecules are sequestered into numerous vesicles of uniform size (80nm) and shape called photic vesicles that migrate distad where we believe they fuse with one another and with the ER. The submicrovillar ER appears to be the origin of new villar membrane.

The authors are grateful for the support of USPHS Grant GM 28778.

TEM AND SEM OF CEREBRAL ARTERIES IN DIFFERENT ISODOSE RANGES OF STEREOTACTIC GAMMA RADIATION OF PITUITARY. W. Jann Brown, R.W. Rand, H.D. Snow, F.E. Holly and A. Cassady. Depts. of Neuropathology, Neurosurgery and Radiology, UCLA Med. Ctr., Los Angeles, Calif.

A non-invasive treatment of hypersecretion syndromes of pituitary should be advantageous. Conventional radiation cannot ablate the gland in this sense since there is the large problem of particle scatter and secondary injury to adjacent neural and vascular structures. In this study the Leksell Gamma unit was used to discretely, by stereotaxis, destroy canine anterior pituitary with radiation doses as high as 30,000 rads with no ill effects. Ultrastructural studies of adjacent arteries and microvasculature of the optic chiasm within computer predicted isodose distributions in two planes will be presented.

ULTRASTRUCTURAL EVALUATION OF RETRIEVED TISSUE NOT ORIGINALLY PROCESSED FOR ELECTRON MICROSCOPY,
K. Chien, R.L. Van de Velde and R.C. Heusser,
Special Anatomic Pathology, Cedars-Sinai Medical Center,
Los Angeles, CA 90048

The need for ultrastructural evaluation of tissue to confirm or improve upon a diagnosis is common in pathology. Ideally, tissue is made available for electron microscopic analysis by routinely fixing a small sample in glutaraldehyde. However, there are times when it is necessary to use tissue originally processed for other uses. In some instances tissue prepared by these other means prove very informative when analyzed by electron microscopy. We have successfully retrieved and obtained diagnostic information from re-embedded plastic one micrometer sections, liquid nitrogen preserved tissue, tissue from frozen or paraffin blocks and slides and cytospin cell suspensions. Processing has been facilitated by modifying methods for re-embedding tissue and by using specially designed embedding molds. This presentation will describe these above techniques and evaluate electron microscopic information obtained from retrieved tissue samples.

PROTEIN A-GOLD IMMUNOELECTRON CYTOCHEMISTRY OF ISOCITRATE LYASE IN COTTONSEEDS, Diane C. Doman and Richard N. Trelease, Dept. of Botany-Microbiology, Arizona State Univ., Tempe, AZ 85287

Shortly following seed imbibition, glyoxysomal enzyme activities rapidly increase. Isocitrate lyase (ICL) is an exception; activity is detectable only after a 16 h lag. The purpose of this study is to: 1) adapt postembedding immunochemical methods to enzyme localization in glyoxysomes, 2) determine whether ICL protein is present before activity can be assayed, 3) detect the site of synthesis.

Fixation of cotyledon segments in 3% glutaraldehyde and embedment in either Spurr's resin or Epon provided optimal ultrastructural preservation and maintenance of antigenicity. Optimal staining was achieved when sections were floated on BSA-15', Ab (1:30)-20', BSA-10', protein A-gold (1:10 in BSA)-15'. Ab was purified by DEAE Affi-gel: pAg was from E-Y Labs. This technique resulted in the deposition of gold particles over all sections through glyoxysomes at 24, 48, and 68 h postimbibition (70-130 particles/um^2 glyoxysome section. Background depositions were essentially nil.

The indirect labeling technique with protein A-gold has been adpated successfully for the visualization of an enzyme within glyoxysomes. The staining pattern indicates that ICL is distributed uniformly throughout the matrix. Significant numbers of particles outside the glyoxysome (indicating the site of synthesis) were not observed at any age; other embedding media (GMA) and frozen thin sections will be tried. It appears that a low level of ICL protein is present in immature-seed glyoxysomes prior to development of the measurable spectrophotometric activity observed following germination of mature seeds. Supported by NSF Grant PCM 8204666.

STUDIES OF CRYSTALLINE DNA BY ELECTRON CRYSTALLOGRAPHY, Kenneth H. Downing, Donner Laboratory, Lawrence Berkeley Laboratory, University of Calif., Berkeley, CA 94720

Thin, plate-like crystals of highly polymerized DNA can be grown by gradual cooling of a supersaturated, ethanolic DNA solution. The form of these crystals makes them quite well suited to study by electron diffraction and microscopy. I have recently been working to determine the conformation of the DNA in the crystals using electron crystallography combined with other techniques. Because of the large amount of solvent, including ethanol, in the crystals, they must be examined in the frozen-hydrated state to preserve their structure in the vacuum of the microscope. Some technical problems remain in preparing these specimens, but the results to date have been quite promising. Electron diffraction patterns extending to 3.5 Å have been obtained, as well as images with resolution to about 8 Å. The phases determined from these preliminary images are not reliably defined, however, and thus give little structural information. On the other hand, the regular hexagonal form of the crystals and the 6-fold symmetry of the diffraction patterns indicate that the DNA strands, seen in axial projection, must have at least 3-fold symmetry. From circular dichroism measurements made on single crystals, we expect that the DNA is in a conformation of the B family. The crystallization properties of a number of synthetic DNAs and RNAs support this prediction. Altogether, our data is most consistent with the DNA in a B conformation with 12 base pairs per turn, a conformation which has not previously been seen in polymeric DNA.

INTERACTIONS BETWEEN CULTURED ALVEOLAR MACROPHAGES AND PARTICLES AS REVEALED BY CORRELATIVE MICROSCOPIC TECHNIQUES; Gregory L. Finch, Thomas L. Hayes, Gerald L. Fisher*, and Karen L. McNeill*, Donner Laboratory, Lawrence Berkeley Laboratory, Berkeley, CA 94720, and *Battelle Memorial Laboratory, Columbus, OH 43201.

A technique is described which allows electron microscopy of cultured alveolar macrophage (AM) cells known to be live or dead at the time of fixation as determined by light microscopic vital staining. Bovine AM are lavaged from an excised lung lobe, assayed for viability and differential count, then cultured in a serum-containing medium on glass coverslips with attached formvar-coated finder grids. Cells are allowed to attach then exposed to either nontoxic glass beads or toxic nickel subsulfide (Ni_3S_2) particles for periods ranging from minutes to several hours. Following exposure, coverslips with grids are inverted into trypan blue dye for viability assessment. Cells are identified as live or dead by location on the finder grid then immediately plunged into glutaraldehyde fixative. Cultures are post-fixed with osmium, stained with uranyl acetate, dehydrated through ethanol, critical point dried in CO_2, and carbon coated by evaporation. Cells thus prepared are viewed in the scanning electron microscope, and particle identification is achieved using an energy-dispersive x-ray microanalyzer. High voltage transmission EM can also be performed on the whole cell mounts.

Data are currently being collected on AM exposed and prepared for EM using this technique. A preliminary study examined AM exposed to Ni_3S_2 for one to three hours; associations between cell viability and particle content were tested using contingency tables and Chi-squared statistics. A highly significant association was observed between particle internalization and cell death.

In conclusion, we have described a technique combining light microscopic vital staining with scanning and transmission EM on the same cells. This technique may prove particularly useful when dealing with complex mixtures, allowing information on elemental burdens to be combined with viability and morphology on an individual cell basis. The authors wish to acknowledge the support of the U. S. Department of Energy and the Electric Power Research Institute.

MEMBRANE LIPID CYTOCHEMISTRY, D.S. Friend, Department of Pathology, University of California, San Francisco, CA 94143

Lipids, which constitute about half the dry weight of biological membranes, confer to the membrane the properties of stability, fluidity, and selective permeability, as well as influencing the enzymatic and receptor functions of integral membrane proteins. Biophysical principals would predict a random localization of each lipid (neutral phospholipids, aminophospholipids, sphingolipids, and free sterols) in the plane and depth of the bilayer. In fact, however, most lipids are asymmetrically distributed across the bilayer in all cells and form planar mosaic patches in polarized-cell membranes. The disposition of specific lipids can be cytochemically detected by applying lipases and lipid-seeking antibiotics. Sphingomyelinase hydrolyzes the sphingosine group from the outer-leaflet lipid, sphingomelin, yielding pits and particle-cleared circles in the plasma

membrane when examined in freeze-fracture replicas. Filipin, demonstrable by fluorescence microscopy and by electronmicroscopy of thin sections and freeze-fracture images, interacts with β-OH-sterols. Another antibiotic, adriamycin, fluoresces when combined with anionic phospholipids in either membrane leaflet; whereas polymyxin B perturbs membranes containing anionic phospholipids in the outer leaflet. And neomycin, conjugated to horseradish peroxidase and colloidal gold, establishes the site of the calcium-binding phosphatidylinositol derivative, triphosphoinositide. Used in concert, these reagents become detailed maps of plasma-membrane lipid distribution in both resting and stimulated cells. (Supported by NIH Grant HD-10445.)

APPLICATION OF IMMUNOELECTRON MICROSCOPY TO VIRAL INFECTION, R.S. Garrett, M. Rodriguez, Dept. of Pathology, University of California, San Diego, La Jolla, CA 92093.

We have used immunoelectron microscopy techniques to further study the pathogenesis of persistent lymphocytic choriomeningitis virus (LCMV) infection in mice. The immunoperoxidase technique (PAP) was used to identify viral antigen in persistently infected neurons. Vibratome sections from the central nervous system (CNS) were sequentially stained with; 1) 10% egg albumin, 2) guinea pig anti LCMV IgG, 3) rabbit anti guinea pig IgG and 4) guinea pig peroxidase-anti-peroxidase. The peroxidase was reacted with Hanker-Yates reagent in the presence of hydrogen peroxide to produce a brown-black product which can be visualized with the electron microscope. These studies show that specific neuronal populations in the cerebral cortex, hippocampus and hypothalamus express viral antigen. Thin sections of these PAP labeled vibratome sections reveal that the viral antigen is associated with host ribosomes.

Same strains of mice infected with LCMV develop the clinical picture of pituitary dwarfism. Immunoperoxidase staining shows that LCM viral antigen is localized to the adenohypophysis. Electron microscopy reveals LCM virions budding from the surface of anterior pituitary cells. An immunocolloidal gold labeling technique was used to identify specific hormone secreting cells of the pituitary. Thin sections of the infected pituitary were placed on gold grids and the grids were stained sequentially with 1) monkey anti growth hormone, and 2) rabbit anti monkey IgG bound to colloidal gold. Using this technique, we show that LCMV specifically infects growth hormone secreting cells.

In conclusion, these studies demonstrate the application of ultrastructural immunolabeling techniques to persistent viral infection of the CNS and pituitary.

Ultrastructural Contents of the Gut in Anchovy Larva. R. S. Garrett, Julio Cesar Solis, Department of Pathology, University of California, San Diego, La Jolla, CA 92093, and C.I.C.E.S.E., Ensenada, Baja, California.

The northern anchovy (Engraulis mordax) is an important constituent of the marine food chain that exists off the Pacific coast of the United States and Mexico. As such, knowledge of its dietary preferences is important in the cultivation and husbandry of this fish. Presently attempts are being made to produce a defined diet which can be used to raise this fish in a captive environment. If successful, these efforts could produce a cheap source of high quality protein to improve the diets of underdeveloped nations.

Past histological analysis at the light microscopic level reveals that the gut consists of a straight tube whose walls consists of columnar cells having a brush border projecting into the lumen of the tube. Ultrastructural analysis further demonstrates that this brush border consists of microvilli (sometimes bifurcated) and occasional cilia. These cells do not show extensive endocytotic or exocytotic activity.

Anchovy feed on plankton. It is thought that the dinoflagellate Gymnodinium is a major constituent of the food intake of the larva living in the coastal habitat.

The major formed elements found in the gut are round unicellular organisms and bacteria. The unicellular organisms seemingly remain relatively undegraded. The remaining gut contents consist of debris resembling multilaminar membranes.

There are two prominent types of configurations present in the gut cavity. One is an extensive electronluscent linear unilaminar membrane often conforming to the gut wall. At greater magnification this membrane consists of honeycomb-like fenestrations. The second are electrondense, coiled bilaminar membranes resembling the mainsprings of clocks and watches. These membranes do undergo degredation.

The sources of this bilaminar membrane is currently being investigated.

THE EFFECTS OF ASCORBIC ACID ON THE GROWTH RATE OF ESTABLISHED TRANSPLANTABLE COLON TUMORS IN RATS, R. L. Grayson, R. DeMoville and L. R. Shaver, Biological Sciences Dept., California Polytechnic State Univ., San Luis Obispo, CA 93407

Twelve Fischer 344 rats were given 1.0 cc subcutaneous inoculations of Ward's R 4047 transplantable tumor in the back region below the shoulder area. After 12 days a palpable mass could be detected and within 18 days noticeable mucinous adenocarcinomas were evident beneath the skin. Daily injections of 1.1 cc of ascorbic acid, or saline, were given for a 10-day period. Initial measurements of tumor size were taken and measurements recorded at 3-day intervals. Results indicate that ascorbic acid has the effect of slowing down or inhibiting tumor growth rates. On a cellular basis there were dramatic differences in the quantity of collagen produced in the treated versus the controls based on light microscopy, electron microscopy and other techniques.

CHLAMYDIA AND CYTOMEGALOVIRUS COMPLICATIONS IN AN AQUIRED IMMUNO-DEFICIENCY SYNDROME PATIENT

M. SUE HERTWECK, A.B.,EMT(EMSA) and VICTOR J. ROSEN, M.D.
Department of Pathology, Brotman Medical Center, Culver City, California

Lung and liver specimens were submitted to the laboratory from a patient with a final diagnosis of Aquired Immuno-Dificiency Syndrome after extensive evaluation and testing at Brotman Medical Center, an institute in Boston, and at UCLA.

The lung biopsy revealed an acute, extensive cytomegalovirus infection, illustrating CMV in all stages of viral replication within the alveolar lining cells.

The liver biopsy revealed an organism having the appearance of bacteria in a triadal region in and among several histiocytic cells as well as within the liver parenchyma and the lumen of a bile duct.

The ultrastructure of the organism is consistant with Chlamydia and has not previously been reported in an A.I.D.S. patient.

THE ORGANIC MATRIX OF SEA URCHIN SPICULES, E. Jones, F. Wilt, Dept. Zoology, Univ. of Calif., Berkeley, CA 94720, N. Benson, S. Benson, Dept. Biology, Calif. State Univ., Hayward, CA 94542.

Developing sea urchin embryos possess an endoskeleton composed of two spicules contained within the blastocoel cavity. The spicules are composed of calcite ($CaCO_3$) microcrystals. The spicules begin to appear at 36 hours and can easily be visualized by polarized microscopy as triradiate structures. As development proceeds, the spicules elongate until by the larval stage they extend the full longitudinal length of the embryo. The purpose of our study was to determine if an organic matrix was associated with the spicules. Spicules were isolated from 96 hour S. purpuratus embryos by exhaustive extraction with DOC-Triton X-100 followed by treatment with SDS-urea. For SEM the spicules were fixed, placed on poly-lysine coated coverslips, treated with EDTA, dehydrated and critical point dried. The spicules were examined by TEM after sectioning or negative staining. Uranyl acetate was used to concommittently decalcify and stain the organic matrix. By both SEM and TEM, the observations suggested a granular or fibrillar substructure associated with the $CaCO_3$. Cross sections of the TEM preparation revealed a lamellar substructure with adherent crystals. Initial biochemical evidence suggests that the matrix material is not collagen.

NUCLEAR AND CHROMATIN STRUCTURE IN RAT SPERMATOZOA, James K. Koehler and Utta Würschmidt, Department of Biological Structure, University of Washington School of Medicine, Seattle, WA 98195.

The nuclei of mature mammalian spermatozoa contain a highly ordered, lamellar substructure presumably constituting the nucleoprotein of the haploid chromosomal complement. Although this fine structure has been known for many years through incidental freeze fracture observations on sperm from several species, little effort has been expended on a more detailed analysis. With a view toward constructing a plausible model of chromatin packing in sperm, we have determined some of the quantitative parameters associated with these "nuclear lamellae" in rat spermatozoa. Epididymal sperm from white, Sprague-Dawley rats were examined by conventional sectioning methods, freeze fracture of fixed and unfixed specimens and by whole mount replica techniques. Fixation and glycerolation did not significantly alter nuclear structure as seen by freeze fracture. Numerical data obtained from cross fractures of sperm heads indicates that the number of lamellae are quite constant at 10.4 ± 1.8 and that the linear measure of the lamellae is 7.2 ± 2.3 microns per cross fracture. The total area of cross fracture, assuming an elliptical profile, is 2.3 ± 0.7 μ^2 and the thickness of the lamellae is 18.2 ± 3.5 nm with a range of 13.5 to 25.5 nm. An estimate of the total surface area of the nuclear lamellae could be made from measurements of projected nuclear area (from replicas and sections) as 115 ± 11 μ^2. From this data and the known amount of DNA in the rat sperm nucleus, a model can be proposed for the organization of the nucleoprotein in these lamellar sheets. It is suggested that the chromatin is arranged in a coiled-coil configuration closely associated together in a side-by-side fashion and continuous in extent somewhat similar to a TV raster diagram. Approximate calculations based on this simple model are within a factor of 2 or 3 of predicting the correct amount of DNA in the sperm nucleus.
Supported by a grant from the National Science Foundation (PCM-77-01138).

EFFECT OF FIXATIVE OSMOLARITY ON THE ULTRASTRUCTURE OF PATHOLOGICAL TISSUE: A STUDY ON PIGEON AORTA, O. Mathieu and M.L. Costello, AMES-Bioengineering, University of California, San Diego, La Jolla, CA 92093.

The osmolarity of glutaraldehyde (GA) fixatives influences the dimensions of tissue and cellular structures. Results from this laboratory indicated that 2.5% GA solution in 0.1M Na cacodylate buffer (total osmolarity, 600 mOsm) led to a generally well fixed appearance of vessel wall ultrastructure in rabbit aortas, whereas isotonic (350 mOsm) and 500 mOsm fixatives led to cellular and mitochondrial swelling; tissue shrinkage occurred in samples fixed with 730 and 1030 mOsm fixatives. Dorsal and ventral segments of the aorta are not equally susceptible to experimental and/or pathological damage. We compared the effect of different GA fixatives (600 and 750 mOsm) on the ultrastructure of different portions (normal vs lesion sites) of the aorta of 6-9 months old White Carneaux (atherosclerosis susceptible) pigeons. Our hypothesis was that the same fixative solution may induce different ultrastructural changes in the same tissue type depending on changes in membrane properties in a given pathological situation. Vessel preparation was: perfusion under physiological pressure of GA fixative, immersion in same fixative, rinse in same buffer (430 mOsm), postfixation in 1% osmium tetroxide solution in same buffer (total 360 mOsm), and standard processing for EM. In dorsal segments of the aorta (no lesion) taken 1 cm below the diaphragm, the vessel wall was generally well fixed with 600 mOsm GA solution, whereas tissue shrinkage occurred in comparable samples fixed with 750 mOsm GA solution. In ventral segments (lesion sites) of the aorta taken at the same level, cellular and mitochondrial swelling or disruption occurred after fixation with 600 mOsm GA solution, while ultrastructural preservation was considerably improved in comparable samples from animals perfused with 750 mOsm GA solution. Our conclusion is that normal and atherosclerotic portions of the arterial wall may respond differently to the same fixative. Dimensional and/or structural changes in pathological tissue may be secondary events induced by preparation, whereas primary changes occurred elsewhere, e.g. in cell or mitochondrial membrane properties. This is of considerable importance for description or morphometry of pathological events.
Supported by Grant-in-Aid from American Heart Association, Calif. Affiliate.

OsFeCN-URANIUM STAINING IMPROVES ULTRASTRUCTURAL PRESERVATION AND VISUALIZATION IN A VARIETY OF CELL TYPES. Kent McDonald, Botany Dept., University of California, Berkeley, CA 94720.

Karnovsky (1971) first suggested adding potassium ferrocyanide to osmium tetroxide during post-fixation as a general contrasting agent. This technique has come to be known as the osmium tetroxide-potassium ferrocyanide (or ferricyanide) method (abbr. OsFeCN) and has been used to selectively stain plant and animal membrane systems as well as gap junctions, glycogen, low density lipoprotein molecules, invertebrate photoreceptors and Leydig cells. Following Hepler's (1980) successful application of the OsFeCN method to membranes of the barley mitotic apparatus, we used his fixation schedule on dividing PtK_1 cells. The OsFeCN not only improved the contrast of membranes but seemed to improve the preservation and/or visualization of microfilaments and intermediate filaments as well. We then tried the method on a variety of cell types (mammalian tissue culture cells, Xenopus egg cytoplasm, teleost retina cells, and several algae and higher plants) and found that all animal cells were improved but not the plant cells. Experiments to determine the

cause of the improved ultrastructural preservation have shown that en bloc staining with uranyl acetate prior to dehydration is critical and addition of 5 mM $CaCl_2$ to the buffer further improves the final image. The exact mechanism of fixation/staining by OsFeCN and uranium is not known but it is likely that the added deposition of heavy metals prior to dehydration further protects the cells from the destructive effects of dehydration and/or embedding.

Karnovsky, M.J. 1971. Abstr. Amer. Soc. Cell Biol. Meeting, New Orleans. p. 146.
Hepler, P.K. 1980. J. Cell Biol. 86:490-499.

PSEUDOTUBERCULOMA SILICATICUM INVESTIGATED BY X-RAY MICROANALYSIS, C. W. Mehard and W. L. Epstein, Department of Dermatology, University of California - San Francisco, San Francisco, California, 94143.

Identification of the causative agent as the initiator of cutaneous granuloma is frequently a perplexing problem. Granulomas may arise as a foreign body response to a colloidal substance involving a simple phagocytic process or as a hypersensitivity phenomena. Elemental identification of a presumed causative agent or foreign body within a tissue can be identified by X-ray microanalysis. We reported on an unusual case of silica induced granulomatous inflammation-of a patient in which the granuloma was manifested 36 years following a broken glass injury to the foot. The silica crystalloids were identified within the primary site of the foot injury. Subsequently, additional lesions developed on the same foot closely followed with superficial lesions extending up to the knee of both legs which do not contain crystalloids of silica. However the secondary leg lesions developed following a tetanus toxoid vaccine injection which appeared to be unrelated at that time. To determine if the patient had developed a hypersensitivity reaction to silica, a patch test to the arm was done but was negative. An intradermal silica injection test was also done which resulted in the development of body lesions in addition to the granuloma at the injection site. X-ray microanalysis of the freeze-dried frozen thin sections of one area of the arm lesion showed many foreign body granules composed of Al (aluminum) and few of Si (silicon). The SiO_2 used to determine the hypersensitivity reaction was devoid of Al. It appeared that the Si intradermal test was performed unknowingly at the same site on the arm where the tetanus toxoid vaccine was previously injected. The tetanus toxoid vaccine is carried on alumina but appeared to contain a small amount of Si which may have resulted in hypersensitivity response in the observed leg lesions. Indeed, the microanalysis of tetanus toxoid vaccine showed that the Al carrier contained small amounts of Si. This study has demonstrated that the analytical electron microscope played a major role in the diagnosis of silica induced granulomatous inflammation and in explaining the chronological events of a pathological process in a clinical case study. Supported by PHS Grant No. 5 R01 CA 29440-03 awarded by National Cancer Institute, DHHS.

COMPARISON OF THE ULTRASTRUCTURE OF INCLUSIONS FOUND IN THE NUCLEI OF OSTEOCLASTS OF PAGET'S DISEASE OF BONE WITH OTHER LESIONS CONTAINING GIANT CELLS, B. G. Mills and F. R. Singer, Orthopaedic Hospital/USC Sch. of Dentistry, Los Angeles CA 90007

A characteristic nuclear inclusion which can be demonstrated by electron microscopy is found in the osteoclasts of Paget's disease of bone. These have been well described and measured by several workers. These microtubular structures must be distinguished from the tubulin protein of the mitotic spindle and from various types of simple bodies found in tumor nuclei or cells infected by viruses. It is not known what the incidence of microtubules of the Paget's disease variety is in other pathologic conditions in which giant cells are also found. This study was designed to study the incidence of typical Paget's bone disease inclusions in giant cell tumors and other diseases where giant cells are found. The population studied included 45 patients with Paget's disease of bone, 2 patients with giant cell tumors arising in Paget's bone, 7 patients with "true" giant cell tumors of bone, 1 malignant giant cell tumor, 6 patients with simple bone cysts containing giant cells, 2 osteoid osteomas, 1 giant cell tumor of tendon sheath and 4 patients with primary or secondary hyperparathyroidism. About 25 patients not known to have giant cell lesions but undergoing total hip surgery were also studied.

Methods included: glutaraldehyde fixation, decalcification with neutral EDTA post fixation with OsO_4, embedment in Spurr, sectioning with a diamond knife, staining with uranyl acetate and lead citrate, and TEM on an AEI Corinth 500 electron microscope at 60 kv.

Results showed that all 45 of the Paget's bone lesions contained the typical 12.5 nm diameter microtubular structures. Both of the giant cell tumors arising is Paget's lesions contained the inclusion. Of the 43 other patients studied which were not known to have Paget's disease, in only the patient with the malignant giant cell tumor could Paget's type microtubules be demonstrated.

We conclude that a search should continue for the inclusions found in Paget's disease of bone in other bone diseases, because there are very few studies in the world literature in which such a study has been made. It would be useful to correlate these inclusions with prognosis & therapy once the data base is established.

AN ULTRASTRUCTURAL STUDY OF MITOCHONDRIAL ALTERATIONS IN A RABBIT MODEL OF REYE'S SYNDROME, R.Mixon, Dept. of Pathology, E-M Lab, Providence Medical Center, Portland, OR 97213

Studies of Reye's syndrome have shown certain pathologic alterations including short chain fatty acidemia (Trauner et al: Neurol. 25:296-298, 1975), hyperammonemia (Shannon et al: Pediatrics 56:999-1004, 1975), and central hyperventilation. Ultrastructurally, mitochondria have been shown to exhibit abnormal structure including swelling, loss of intramitochondrial particles, reduction of cristae integrity, and increase in granular matrix material. Although the exact etiology of Reye's syndrome is still unknown, it has been shown experimentally that the following agents will produce similar characteristic changes in an animal model when used alone or in combination: short chain fatty acids, Influenza B, and aspirin. The naturally occuring fatty acid, octanoic was chosen for infusion in the rabbit model because of its consistently high elevation in children with Reye's syndrome. In the current study electron microscopy was done on muscle biopsies taken from rabbits before and after they were infused with sodium octanoate. Intracranial pressure was also monitored for the duration of the experiment (Daley et al: Neurosurgery 11: 617-621, 1982). Ultrastructural changes including swelling and cristae degradation were noted in mitochondria after the rabbits had been infused with sodium octanoate for several hours. These changes are similar to the mitochondrial abnormalities reported in Reye's syndrome (Partin et al: N Eng J Med 285: 1339, 1971).

IMAGE ANALYSIS OF PLATELET GIANT AND FUSION GRANULES IN PATIENTS WITH NEOPLASTIC MYELOPROLIFERATIVE DISORDERS, C.M. Payne and L. Glasser, Dept. of Pathology, AHSC, University of Arizona, Tucson, AZ 85724.

The normal human platelet is ultrastructurally complex and consists of many cell organelles which include α-granules (contain fibrinogen and platelet factor 4), dense bodies (contain serotonin, ADP, ATP and calcium), mitochondria, microfilaments and microtubules. In normal platelets, giant α-granules, which appear azurophilic on Wright's stain and spherical by electron microscopy, can occasionally be seen. Giant fusion-type granules have been reported to occur in neoplastic myeloproliferative disorders (MPD), and consist of irregularly-shaped fusion granules by electron microscopy. A qualitative assessment, however, of what constitutes an abnormal granule is subjective. We have morphometrically analyzed over 1,600 giant and fusion granules from 38 patients with MPD and 112 control subjects with no evidence of a MPD. It was determined that the maximum granule area in normal subjects was $0.51\mu^2$ compared with $1.54\mu^2$ for the diseased control groups, and $2.23\mu^2$ for the MPD groups. The area of a granule and its degree of deviation from a perfect circle (Form Factor or Granule Contour Index) could not be used independently to discriminate between the neoplastic MPD group and the normal and diseased control groups. The Megagranule Index (Granule Contour Index X Area), which takes into consideration both the shape and size of a given granule, can be used to discriminate between the MPD groups and the normal ($p < .001$) and diseased control groups ($p < .001$). Abnormal granules with a Megagranule Index (MGI) > normal subjects was observed in 7% of the diseased control subjects with no evidence of a MPD. Abnormal granules with a MGI > normal subjects was observed in 47% of all patients with neoplastic MPD. Ultrastructural examination of circulating platelets therefore has predictive value in the diagnosis of neoplasic MPD.

METAL DEPOSITION FOR ELECTRON MICROSCOPY, K.-R. Peters, Sect. Cell Biology, Yale University, School of Medicine, New Haven, CT 06510.

In electron microscopy metals are deposited on the surface of specimens to generate contrast, to increase electrical and thermal conductivities, and - less importantly - to mechanically support surface fine structures. Continuous metal films of ~5-20nm thickness are used in low magnification microscopy (~<20,000 times), and may be deposited with conventional techniques (i.e., evaporation; diode or bias sputtering - high voltage). For high magnification work (~50,000-200,000 times), in TEM as well as in SEM (new SE-I image mode), only very thin metal films of 1-2nm average mass thickness are required, since thicker films cover up fine details and reduce resolution. During metal deposition, the atoms aggregate on the substrate surface (specimen) and form crystalites which grow together into a continuous film at a certain average mass thickness (critical thickness). This thickness is determined by the specific surface mobility of the metal atoms (type of metal), and by the deposition technique (energy parameters, deposition conditions). If the mobility of the metal atoms is promoted (electron beam evaporation), large crystals form and tend to grow on high-rised fine structures (decoration). If the mobility is reduced (magnetron sputtering - low voltage, ion beam sputtering), small crystals form, covering the surface with an even coat (coating). Highest topographic resolution is obtained by coating the specimens with continuous films of critical thickness. Under favorable conditions (Penning sputtering), metal films may be continuous at a thickness of only 1.0 to 1.5nm. Such very thin continuous films are also applied in experimental physics.

EXTERNAL MORPHOLOGY OF SHUTTLE FLOWN HONEY BEES AS SHOWN BY SCANNING ELECTRON MICROSCOPY, Delbert E. Philpott, Jaime Miquel, Katharine H. Kato*, Charles Turnbill, and Rosemarie Binnard, NASA Ames Research Center, Moffett Field, CA 94035, *University of California at San Francisco, San Francisco, CA 94143.

Honey bees were flown on board the Space Shuttle Mission #3 in March, 1982, in order to investigate the effects of weightlessness on insect biology. Three species of insects were on board: Worker honey bees (Apis mellifera), Velvet Bean caterpillar moths (Anticarsia gemmatalis), and houseflies (Musca domestica). This flight experiment was designed by Todd Nelson, an 18 year old senior from Southland Public Schools, Adams, Minnesota. Following the Shuttle flight, three of the bees were frozen and sent to NASA Ames Research Center for examination. These Shuttle flown bees along with controls, provided by a local apiarist, were prepared for scanning electron microscopy in the following manner: Bees were exposed to 4% OsO_4 vapors overnight at 0°C. The following day, the bees were dehydrated in graded concentrations of ethanol (75%,95%,100%). The bees were left in a fresh solution of 100% ethanol overnight, then critical point dried (Ladd Critical Point Dryer) or freeze dried (Balzars Freeze Fracture Apparatus). The dried bees were put on stubs and sputter coated (Polaron SEM coating unit E5100) with 18.0 nm of platinum. The bees were studied on an Amray 1200 scanning electron microscope. The electron micrographs revealed a normal exoskeleton, including antennas, wings and body appearance. The stingers of the flight bees were supposedly cut to prevent injury to the astronauts if the bees were to escape from their cage. Video tapes of the insects taken during flight showed inactivity of th bees. The bees tended to "float" in their cage and tumbled end over end when their wings were activated. Except for the abnormal behavior, the space flight did not p duce any damage to their external organs.

WIRING IN THE RETINA OF TIGER SALAMANDER. Arthur S. Polans, Grant D. Nicol, James B. Hutchins and Frank S. Werblin, Graduate Group in Neurobiology, U. of Calif., Berkeley, CA. 94720.

By determining the membrane properties of individual neurons and the synaptic connections they make with other cells, we hope to understand how visual information is processed through the retina. A unique preparation, the retinal slice, allows us to examine connectivity and correlate the findings with electrophysiological data. Along with conventional EM methods for establishing identifiable synaptic contacts, we also examine putative transmitters as a means of establishing functional contacts between cells.

Earlier observations of the effects of acetylcholine (ACh) on horizontal cells of the salamander retina led us to attempt localization of the site of cholinergic action. Using the Karnovsky method, we found precipitate resulting from acetylcholinesterase (AChE) activity localized at the EM level in discrete extracellular patches we call stain laminae. The laminae are found predominantly adjacent to horizontal cell axon terminals, but were never seen near horizontal cell body processes. No extracellular precipitate was found in the presence of the specific AChE inhibitor BW284C51.

Additionally, we have examined putative ACh receptors both biochemically and by light microscopy and autoradiography using 3H-propylbenzilylcholine mustard (PrBCM) and 125I-α-bungarotoxin, which bind to muscarinic and nicotinic ACh receptors, respectively. PrBCM binding sites are found in both plexiform layers, although primarily in the inner plexiform layer, while α-BGT binding is exclusively in the inner plexiform layer. The ultrastructural characterization of some of these binding sites has been undertaken using monoclonal antibodies specific for ACh receptor.

Subsequent to elcetrophysiological recording, horizontal and photoreceptor cells have been injected with the enzyme horseraddish peroxidase and processed for EM analysis, since several lines of evidence suggest a role for these cells in cholinergic transmission. These combined physiological and anatomical data are providing a more accurate picture of visual pathways in the vertebrate retina.

A TEM STUDY OF INVASION OF THE INTESTINE OF THE CHICK BY SALMONELLA, I. Popiel* and P.C.B. Turnbull, Depts. of Tropical Pathology and Microbiology, S.A.I.M.R., Johannesburg, S. Africa.

Results are presented of an investigation of the morphological events which took place in the caeca of day-old chicks 12-14 h after inoculation of Salmonella enteritidis. A preliminary study by Turnbull and Richmond (Br. J. Exp. Path. 59, 64, 1978) established that entry of bacterial cells into the undifferentiated epithelial cells lining the caeca is by endocytosis and sequestration into membrane bound vesicles. We have since observed many accompanying changes which included the development of Golgi complexes and the production of lysosome-like vesicles and multivesicular bodies. Apparent fusion of bacteria containing vesicles with each other and with smaller Golgi-derived vesicles took place throughout the epithelial cell cytoplasm.

Although free bacterial cells were observed in spaces which traversed breaks in the basal lamina, it is suggested that the organism is more frequently taken across this barrier intracellularly. Cells containing bacteria, some of which have been identified as phagocytes, were observed spanning the epithelial and lamina propria regions through breaks in the basal lamina.

A continuing investigation is aimed at further elucidation of the events which take place during passage of bacterial cells from the caecal epithelium to the lamina propria.

DIAGNOSTIC EM IN SURGICAL PATHOLOGY, Harold M. Price, Path. Dept., Valley Med. Center, Fresno, CA 93702.

Electron microscopy now plays a well-established diagnostic role in surgical pathology. Its first practical application came in the diagnosis of renal diseases where its routine use has made it possible to more precisely classify these diseases as well as to be more accurate in terms of prognosis and more straightforward in the means of evaluating therapeutic results. More recently, ultrastructural information of neoplasms has accumulated to the point at which the data can now be used for the diagnosis of tumors which are poorly defined by routine light microscopic techniques. It has proved to be effective in the differential diagnosis of a group of neoplasms characterized as "small round cell tumors". It has made it easier to identify poorly differentiated squamous cell carcinomas, amelanotic melanomas, various poorly differentiated endocrine tumors, some sarcomas and certain lymphoreticular tumors. Identification of these neoplasms is possible because the EM affords us the resolving capabilities to identify specific cytoplasmic and extracellular structures which serve to specify types of cellular differentiation. These include membrane bound granules, melanosomes, cell junctional complexes, basement membranes, intracytoplasmic fibriles, and specific types of tubules. Although the increased resolution of the EM has improved our perception of cellular differentiation, the limited area which can be surveyed in a particular case can introduce significant sampling problems when dealing with the variety of changes possible in pathologic material. In addition to having a proven diagnostic track record in the evaluation of renal diseases and certain neoplastic processes, it has found some limited use to-date in evaluating some skeletal muscle disorders, some hematopoietic processes and in certain viral disorders. Obviously in the time that we have for presentation, it will be difficult to review all of the applications of EM to diagnostic work. Emphasis in this discussion will be examples of EM application to the diagnosis and evaluation of neoplastic processes including recent concepts about the classification of lung tumors.

DIMORPHISM OF Sporothrix scheckii, H.D. Raj and S.S. Sekhon, Dept. of Microbiology, California State University, Long Beach, and Veterans Administration Hospital, Long Beach, CA 90840

Morphogenesis of this fungus depends upon cultural conditions which include the nutritional regime, proper pH and temperature. In a less nutritional medium containing glucose and glutamate with acidic pH (5.4) and incubation at 25 or 35°C, it grows mostly in the hyphal phase. Here, the conidia germinate as germ tubes which continue to grow apically into hyphae until sporulation (conidiation); the conidia are formed either singly along the hyphal filament or in clusters at the ends. But when grown in the above medium with an alkaline pH (8.0) at 35°C, its hyphal phase is transformed into yeast-like budding phase. Here, the hyphal cells swell, produce bud-like protrusions and subsequently fragment completely. Also, budding occurs directly from conidia. In its budding phase, the fungus is known to parasitize living host tissues.

Electron microscopic studies of cells of the hyphal phase and budding phase show ultrastructural dimorphic differentiations. Unlike the budding phase cells, the hyphal cells contained septa with simple pores, Woronin bodies, pincocytotic vesicles and other organelles. During the transformation, some of the hyphal cells give rise to a unique pattern of multiple budding system.

SEM AND TEM OF DETRITUS FROM THE SURFACE AND DEEP OCEAN, M. W. Silver and M. M. Gowing, Center for Coastal Marine Studies, University of California, Santa Cruz, CA, 95064.

During the last decade, oceanographers have learned that most photosynthesis in the open sea is accomplished by unicells no larger than a few micrometers in diameter, and that these are usually consumed by protozoans under 10 micrometers. The taxonomic affinities, physiological characteristics, and ecological roles of these microorganisms are very poorly known because light microscopy has been inadequate to describe them. Problems have been particularly severe in studying microorganisms associated with oceanic detritus, sites of intense microbial activity, because of the abundance of non-living debris of the same size as the associated organisms, and the lack of culture techniques for most of these organisms.

We have been studying natural populations of unicellular organisms in the water and on detritus using SEM, TEM, and STEM-EDS. These methods have allowed us to recognize groups of organisms that are poorly known and, in a few cases, to find new ones. For example, we have noted abundant 0.5 to 2.0 micrometer cyanobacteria and green algae on detritus between 0 and 2000 meters depth, and have made the first discovery of purple photosynthetic bacteria and metal-precipitating bacteria in the pelagic zone. We discuss various organisms, their characteristics, and some ecological relationships revealed by our EM studies. Formidable problems still beset such work, and we discuss fixation under high pressure (up to 200 bars), and other difficulties related to the study of natural populations from oceanic environments.

UPTAKE OF HORSERADISH PEROXIDASE BY COCHLEAR HAIR CELLS. R.L. Snyder and P.A. Leake-Jones. Epstein Laboratory, Dept. of Otolaryngology, University of California, San Francisco, CA 94143.

The present study examines the morphologic mechanisms involved in the uptake of horseradish peroxidase (HRP) into cochlear hair cells of cats. HRP was introduced into the cochlear perilymph of the scala tympani via a micropipette through the round window membrane. 5 to 10 uL of 10% HRP in artificial perilymph was slowly infused using a stepping motor piston drive to control the infusion rate (0.18 uL/min.). Post-injection intervals ranged from 1 to 72 hrs. In most experiments the cochlear microphonic at 1 kHz, frequency following response at 1 kHz, and the click evoked ABR were recorded before, during and after introduction of the HRP. All of these measures indicate that the functional status of the hair cells to be unaltered. HRP was localized by incubation of bulk cochlear specimens in 3,3' diaminobenzidine tetrahydrochloride, followed by post-fixation in OsO4.

Following short post-infusion intervals (1-3 hrs.), HRP reaction product was found filling the small intercellular spaces between cells and coating the outer surfaces of all supporting cells. However, the lateral surfaces of the outer hair cells were completely free of reaction product. In addition to extracellular labeling, we observed HRP reaction product within many membrane bound compartments of all cochlear hair cells. These compartments included coated pits, coated vessicles and smooth ER cysternae at the base of the cells and multivesicular bodies, secondary lysosomes, and smooth ER cysternae at the cells' apex. Outer hair cells were more lightly labeled than the inner hair cells and their labeled compartments were located primarily in the basal third of the cells. The labeled compartments in the inner hair cells were more evenly distributed throughout the cells. In addition to the uptake of HRP in membrane bound compartments, we observed diffuse, uniform labeling of the entire hair cell cytoplasm. Such labeling occurred without significant change in the functioning of the hair cells as demonstrated by our three physiological measures. There was significant variation in the number of these diffusely labeled cells in each cochlea and this variation may be related to the level of acoustic stimulation given to the cochleae during the post-infusion interval.

After longer post-infusion intervals (20 to 72 hrs.), most of the extracellular HRP had disappeared, there was no diffuse labling of the hair cells and the membrane bound label within the hair cells had increased. In outer hair cells HRP was observed only in the extreme apical end of the cell in secondary lysosomes, extending only 2-4 um below the cuticular plate. In inner hair cells similar labeled compartments were more dispersed, and were observed around the inner hair cell nucleus in association with the more dispersed Golgi apparatus of the inner hair cells.

A New Ultra High Vacuum Freeze Etch Machine. E. Sohm, Product Manager for EM Preparations Systems, BALZERS, 8 Sagamore Park Road, Hudson, New Hampshire 03051

Freeze etch replicas which have been produced under conditions of ultra high vacuum and very low specimen temperatures appear to show greater detail of biological structures that seem possible with standard high vacuum produced replicas. The unit to be described features an automatically controlled cryo pumping system having a pumping speed for air of 2700 liters/second. The pumping speed for water vapor is 3,500 liters/second, and the attainable ultimate vacuum of the system is 5×10^{-10} mbar. The helium refrigerator specimen cold table maintains a specimen temperature between +20°C and -265°C with ±0.2°C precision and accuracy without using liquid helium. A turbomolecular pumped high vacuum airlock having an LN_2 cooled specimen table can be used for freeze drying, or for allowing UHV cycles of less than 15 minutes. A one button evaporation control unit deposits the platinum/carbon or tantalum/tungsten and carbon films automatically at pre-set evaporation rates. A quadrupole mass spectrometer provides a mass scan of the residual gas composition and can accurately measure the partial pressure of water vapor in the UHV chamber. In this way, the mass spectrometer can be used to determine when a sample has been completely freeze dried or sufficiently etched.

It appears that the very low specimen temperature is the important factor in producing these improved quality replicas by limiting the amount of surface diffusion of the coating material upon deposition. In addition, these low specimen temperatures are thought to reduce the amount of plastic deformation which occurs upon fracturing.

VITAMIN E AND VISION, R. J. Stephens, D. J. Buntman, and D. W. Thomas, Cell Biology Program, Life Sciences Division, SRI International, Menlo Park, CA 94025.

The amount of vitamin E in the retinas of rat eyes markedly influences the structural integrity of the rods and cones and thus determines the animal's ability to see. The studies reported here combine a newly developed microbiochemical analysis of vitamin E using mass spectrometry with ultrastructural observations made on adjacent areas of the same retina. These data are then correlated with computer-analyzed performance data from the same animal.

Fischer rats 40 days of age, obtained from Simonsen Laboratories, were used in this study. Prior to placing these animals on formulated vitamin E diets, they were trained to respond to visual stimulation. Once the rats were capable of responding at an established level of performance, they were placed on diets containing 0, 200, and 3000 mg of vitamin E/kg of food. Their performance was monitored twice weekly for up to 8 weeks. At sacrifice, each eye was prepared for structural observations and microbiochemical determination of the amount of vitamin E in adjacent areas of the retina. A positive relationship was found between the amount of vitamin E in the retina, the structural integrity of the rods and cones, and the animal's ability to respond to visual stimuli.

This research was supported by SRI International's Research and Development fund.

DATA MANAGEMENT FOR TISSUE SAMPLES IN AN ULTRASTRUCTURE LABORATORY, Joann Stevenson, Lonnie Chrisman, Douglas Silkwood, NASA Ames Research Center M/S 239-14, Moffett Field, CA 94035.

Data organization for electron microscopy laboratories are mandatory. Due to the large number of tissue samples handled, a centralized and uniform classification system is necessary to prevent loss of material and to permit easy retrieval of tissues at a later date. Each animal is assigned a number. In turn, each organ of that animal also is assigned the left or right (L or R) designation, e.g. left retina = #1204 L Ret. Data management consists of using the following: A binder is kept near the site of specimen preparation, a master book is kept in the office, the boxes containing the embedded blocks are kept in drawers, identification is kept with the sections, an electron microscopy (E.M) card (5"x6") is kept at the microscope and filled out for microscope data, and later a stamp is used on the prints to insure complete print data. Details: The data book by the hood receives the immediate details of the experiment. This information is transferred to the master book plus any additional data. The boxes which contain the embedded blocks are labelled with animal number, date of experiment, animal and organ type, and treatment. The blocks from a box are sectioned, keeping the identifying number with the sections. The data from the E.M. is filed on cards. All the pertinent data is placed in a stamped form inked on the back of each print. Our laboratory is in the process of computerizing the files using a Compupro Computer with which the master records and specimen preparation will be filed. This will facilitate retrieval of information in seconds, just by inputting the animal or slide number. Error in data keeping should be at a minimum and little storage space is needed. This system guarantees: 1) Easy retrieval of any animal or organ data at any future date. 2) Control over a large number of samples, regardless of their point

of origin. 3) A uniform documentation of all samples in the laboratory and centralized files, allowing researchers to retrieve the work of other researchers with a minimum of time and effort.

MARINE DINOFLAGELLATES AS REVEALED BY EM, B. M. Sweeney, Dept. of Biological Sciences, University of California, Santa Barbara, CA 93106.

Dinoflagellates as a group have a number of unique features which cannot easily be observed by light microscopy, since they are unicellular, many are small and the cytoplasm is densely packed with heavily pigmented chloroplasts in the photosynthetic forms. I shall describe and illustrate with electron micrographs three species of dinoflagellate, each of which represents a different type of morphology: Gonyaulax polyedra, a typical motile dinoflagellate with girdle and sulcus, Noctiluca scintillans, a heterotrophic form without cellulose plates which feeds by means of a tentacle, and Pyrocystis fusiformis, in which the vegetative cell is non-motile with a cystlike heavy wall and a large central vacuole. Structures which are particular to the dinoflagellates will be emphasized. The nuclei of dinoflagellates contain chromosomes which are always "condensed" in which the DNA is wound in a supercoil. There is no conventional mitotic figure, chromosomes separating at mitosis by means of microtubules which remain outside the nuclear envelope in cytoplasmic invaginations. Most dinoflagellates have trichocysts, organelles resembling the trichocysts of ciliates, the contents of which can be ejected to the exterior of the cell. Many dinoflagellates are found in symbiosis with other organisms, notably coelenterates, but also foraminifera and the mollusc Tridachna. The Noctiluca in S. E. Asia contains small algae motile in the vacuole. I have identified these as Pedinomonas noctilucae. A number of dinoflagellates are capable of bioluminescence, including the three species above. The bioluminescent organelles in Gonyaulax and Noctiluca have not been identified. However, there is evidence that the microsources of bioluminescence in Pyrocystis fusiformis are particles found in the center of the cell during the day and dispersing to all parts of the cytoplasm at night. Circadian rhythmicity in cell division, photosynthesis and bioluminescence is common in photosynthetic dinoflagellates.

LOCALIZATION OF SOLUBLE PLANT PRODUCTS, S. S. Thayer, Plant Growth Lab, University of California, Davis, CA 95616

The field of electron microscopic localization of soluble plant products as well as ions, elements, proteins and enzymes will be reviewed. Brief descriptions of methods such as autoradiography freeze-substitution, rapid fixation and embedding, X-ray analysis, immunohistochemistry, enzyme histochemistry and others will be presented along with pertinent plant applications. Limitations and problem areas of the above methods in relation to plant studies will be discussed.

LOW DENSITY LIPOPROTEIN (LDL) RECEPTOR LOCALIZATION USING A COLLOIDAL GOLD LABEL AND SURFACE REPLICATION; R. Thrift, T.M. Forte and R.W. Nordhausen, Donner Laboratory, Lawrence Berkeley Laboratory, Univ. of California, Berkeley, CA 94720.

Recently, much attention has been directed to cell-surface binding sites for the various blood lipoproteins. These receptors figure prominently in the transfer of cholesterol and other lipids between cells, particularly in the process of atherosclerosis. Studies using thin sections of fibroblasts incubated with LDL-ferritin and LDL-gold have shown that more than two-thirds of the LDL binding sites are located in coated regions. However, each thin section depicts only a minute portion of the cell's surface.

With the technique of surface replication we can visualize the location, distribution and surface density of ligands bound to the whole exposed cell surface. To allow conclusive identification of the LDL (and thus binding site), the lipoproteins were labelled with colloidal gold according to Handley et al. (PNAS, 78:368, 1981). Negative staining showed individual gold particles (approx. 20 nm dia.) to be surrounded by LDL, forming rosettes. Chinese hamster ovary and Hep G2 human hepatoma cell monolayers on glass cover slips were incubated 1 hour with LDL-gold, then fixed with 2.5% glutaraldehyde (1/2 hr), 1% osmium (overnight) and 2% uranyl acetate (overnight). After critical point drying, the monolayers were shadowed using a combination of fixed and rotary replication. (This technique provides even "illumination" yet retains depth cues.) Although the organic material was cleaned from the replica, the gold particles remained in situ, providing spectacular contrast. Binding of the complexes is specific; it is abolished by reductively methylating the LDL prior to gold conjugation, and also by incubating cells with LDL-gold in the presence of heparin. (Both procedures are known to inhibit the binding of LDL to its receptor.) Moreover, only small amounts of bovine serum albumin-gold are bound by the cells. Binding is also saturable, being inhibited by an excess of unlabelled LDL. This method permits ready assessment of the distribution of LDL binding sites over the cell surface under various metabolic conditions.

STRUCTURAL ANALYSIS OF THE SURFACE LAYER PROTEIN OF SPIRILLUM SERPENS BY HIGH RESOLUTION ELECTRON MICROSCOPY, W. H. Wu and R. M. Glaeser, Dept. of Biophysics and Medical Physics, and Donner Laboratory, Lawrence Berkeley Laboratory, University of California, Berkeley, CA. 94720.

In order to understand the detailed association of the surface layer protein of Spirillum serpens, it is necessary to do a high resolution structural analysis. Single layered crystalline arrays of the protein of the size of 20 μm^2 have been obtained by extensive heating (in high $CaCl_2$), a procedure derived from that of Buckmire and Murray (1970). Low dose, low temperature electron microscopy has been applied to the large arrays.

As a first step, samples were negatively stained with neutralized phosphotungstic acid, and the specimens were imaged at 40,000 magnification by use of a high resolution cold stage on a JEOL 100B. Low dose images were recorded with exposures of 7-9 electrons/$Å^2$. The optical diffraction patterns obtained with these specimens show a marked "handedness" (i.e. absence of mirror symmetry), in contrast to the results which are obtained with isolated vesicles.

Selected areas of these images were subjected to computer image processing procedures. A resolution of at least 12 Å for the in-plane projection map was obtained. The computer processed image reveals a pore about 10 Å diameter at the 6-fold symmetry center. The individual molecular envelope of the protein monomers now seems to be apparent, and details of the protein-protein contact at the three-fold lattice positions are beginning to emerge.

Work has also been done in which samples were sandwiched between two carbon films to make a thin, wet specimen, which was then frozen in liquid nitrogen (Jaffe and Glaeser, 1982). Micrographs have been obtained which show optical diffraction patterns to a resolution of 12 Å.

INTERMEDIATE FILAMENT-RICH VARIANT OF BASAL CELL EPITHELIOMA-AN ELECTRON MICROSCOPIC STUDY, Gloria S.M.Yu, C.Kokes, J.W.Carson, R.Schwartz, S.W.French, Dept. of Pathology, VA Medical Center, Martinez & University of California, Davis.
Skin appendage tumors probably comprise the most complex group of skin tumors, with the variable combinations of hair, sebaceous, eccrine and apocrine differentiations. We have examined a skin nodule with unusual light and electron microscopic finding: The tumor occupying the upper dermis is composed of circumscribed lobulated masses of medium-sized polygonal cells with eosinophelic cytoplasms("K"). Scattered cells with foamy to completely vacuolated cytoplasm are seen. Their nuclei are vesicular with distinch nucleoli. A second cell type ("M") with abundant eosinophilic cytoplasm and pynotic nuclei are seen among these tumor cells and in the surrounding stroma. Cytoplasm of "M" and globular areas of occasional "K" cells show positive PAS staining with diastase. Immunoperoxidase stain shows "M" to be positive for lysozyme and "K" for CEA. Electron microscopy reveled "K" showing abundant tonofilaments and well developed desmosomes . Occasional cells show a central core of fine filamentous material surrounded by fundles of tonofilaments indistinguishable from those seen in the signet ring type of basal cell carcinoma. A few cells contain lipid droplets. Occasional cells show vacuolated cytoplasm. "M" cells uniformly show similar ultrastructural appearance. No cell junctions are seen. The cytoplasm is distended by distinct masses of fine filamentous material similar to filaments in "M" cells. However granules and vesicles are also seen between these masses. The nuclei are small with abundant heterochromatin. No myoepithelial cells are seen. Correlating the light microscopic, histochemical, immuno-histochemical and electron microscopic findings,"K" cells are best defined as keratinocytes showing focal sebaceous differentiation and "M" cell as macrophages which presumable have phagocytosed the products of "K" upon lysis. Before EM studies, "M" cells were thought to represent granular cells which have similar light microscopic findings and special stain reactions. Comparison of EM findings of these cells with granular cell tumor will be discussed.

ACUTE ADULT CYTOMEGALOVIRUS INFECTION - AN ELECTRON MICROSCOPIC STUDY - Gloria Yu, T.Steinkirchner, L.Lefevre, DA Katzenstein,& MC Jordan, Dept. of Pathology and Medicine, VA Med Center, Martinez,CA. and Univ. of California, Davis, CA
Cytomegalovirus (CMV) infection is common in immunocompromised individuals. However, no detailed ultrastructural studies have been published in the literature to date. Blood and tissue samples from a fatal case of disseminated CMV infection in an adult male patient was studied by electron microscopy prior to death and at autopsy. This 69-year-old man, with a diagnosis of chronic lymphocytic leukemia, on chemotherapy, presented with low platelet count (16,000 per mm^3) and pulmonary infiltrates. Initial diagnosis was made on bronchial washing cytology. CMV was observed in peripheral blood granulocytes within phagocytic vacuoles as well as in a rare platelet. At autopsy, enlarged cells with typical intranuclear inclusions are seen in various organs and cell types. Under the electron microscope these inclusions are made up of bands of granular and filamentous material showing a cribriform pattern. Naked virions, measuring around 90 nm diameter are seen among this meshwork and in the "halo" around this inclusion. The majority of the DNA core show a classical donut-shape with an outer diameter of 47 nm. Abnormal forms of DNA cores are frequently seen. Defective virions without DNA cores comprise about 45%. The inner nuclear membrane is generally thickened with irregular protrusions. Detached pieces of this membrane are seen showing C-and S-shapes, enclosed within the outer nuclear envelope. Virions can be seen in association with these structures. Smooth membrane-bound, enveloped and naked virions are seen in the cytoplasm or in association with prominent lysosomal granules. These typical infected cells are seen most abundantly in the splenic reticuloendothelial cells (80 per HPF on thick sections) pulmonary alveolar lining cells and endothelial cells (10/HPF). Thirty percent of the renal glomeruli are involved. The liver shows mainly periportal involvement of hepatocytes and Kupffer cells. Bone marrow shows involvement of endothelial cells & cells compatible with megakaryocytes. The reticuloendothelial system stands out as the most extensively involved cell type, and probably as a reservoir (e.g. the spleen) and circulating PMN's disseminate the virus. No virions are seen in lymphocytes which presumably carry the viral genome.

ALTERED DERMAL ELASTIC FIBERS FROM HYDROQUINONE BLEACHING CREAMS IN AMERICAN BLACKS, K.G. Zimmerman, M.D. and R.A. Hoshaw, M.D., Departments of Pathology and Dermatology, University of Arizona Health Sciences Center, Tucson, Arizona 85724.

Two black American women are presented who developed deeply pigmented areas of the face after using non-prescription 2% hydroquinone bleaching creams. This has been previously described in thirty five South African Bantu women also using hydroquinone creams.(Findlay, 1975) Routine light microscopy of the skin shows homogeneous golden-brown fibers in the papillary dermis. These fibers are remarkably similar to the changes seen in ochronosis, an uncommon systemic disease that results in deposition of homogentisic acid in skin, cartilage and articular tissues. Transmission electron microscopy reveals the fibers to have two morphologic components unlike the punctate electron dense deposits seen in ochronosis. The two components are identical to those present in elastic fibers normally found in the dermis. The altered elastotic fibers are enlarged, fragmented and have a prominence of the electron dense component. It is suggested that prolonged use of these bleaching creams leads to alteration of the dermal elastic fibers. The resultant disfiguring pigmentation of the skin seen clinically resolves slowly and incompletely.

STEM STUDIES OF DEFECTS AND DISORDER, J.M. Cowley, Department of Physics, Arizona State University, Tempe, AZ 85287.

The special attributes of STEM which make it valuable for studies of defects and disorder in crystals include the capabilities for combining microdiffraction and microanalysis with high resolution imaging. The possibility of obtaining microdiffraction patterns from regions as small as 3Å in diameter offers a new approach to the study of the structure of individual defects but interpretation of the patterns involves complications associated with the use of coherent incident convergent beams. These complications appear in patterns from regions 10-20Å in diameter and, in themselves, offer convenient means for characterizing defects. In studies of disordered systems microdiffraction patterns from regions 10-20Å diameter give direct evidence of local ordering in that the patterns are spotty. Beyond a subjective estimate of spottiness, the interpretation of these patterns in terms of ordering parameters requires extensive statistical analysis. A method involving pattern recognition techniques for the detection of particular atomic configurations is being evolved as an alternative approach. The study of thin surface layers on small particles is also possible. The surface potential distribution is a complicating factor which itself may be an interesting subject for investigation. Energy analysis of electrons passing close to or through surface layers can provide information on surface energy states.

A TEM ANALYSIS OF REPEATED PRECIPITATION ON CLIMBING DISLOCATIONS IN AL-4Cu,* U. Dahmen, M. Wall and K.H. Westmacott, University of California, Materials and Molecular Research Division, Lawrence Berkeley Laboratory, Berkeley, CA 94720

Heterogeneous precipitation on dislocations is generally thought to result from the elastic interaction of the strain field of the precipitate with that of the dislocation. The present investigation studies the role of the dislocation in detail. Using the process of repeated precipitation of θ' on climbing dislocation loops in Al-4Cu, first reported by Guyot and Wintenberger (1) and thoroughly analyzed by Headley and Hren (2), a TEM contrast analysis shows that by dissociating into a/2 <100> partials the dislocation can accommodate not only the strain field but also the crystal structure of the growing precipitate. The experimental evidence is shown to be consistent with a recent structural model for the formation of θ' precipitates.

1. P. Guyot and M. Wintenberger, J. Mat. Sci. 9, 614 (1974).
2. T.J. Headley and J.J. Hren, Phil. Mag. 34, 101 (1976).

EXTENDING THE TECHNIQUE OF STEREO ELECTRON MICROSCOPY, J.F. DeNatale, J.S. DeNatale+, R.H. Geiss*, D.G. Howitt, V.E. Hanchett*, D.K. McElfresh, and P.J. Hood, Department of Mechanical Engineering, University of California at Davis Davis, CA, +Department of Civil Engineering, University of California at Davis, Davis, CA, and *I.B.M. Research Labs., San Jose, CA.

The limitations of the low angle projection and the difficulties associated with depth measurements in stereo electron microscopy are inherent to the human brain and not to the technique of image reconstruction in three dimensions. Employing a simple algorithm we can relieve the microscopist of the responsibility and pleasure of visualizing stereo projections.

MORPHOLOGICAL ASPECTS OF α"-$Fe_{16}N_2$ PRECIPITATION IN α-Fe,* P. Ferguson, U. Dahmen and K.H. Westmacott, University of California, Materials and Molecular Research Division, Lawrence Berkeley Laboratory, Berkeley, CA 94720.

Previous studies of α"-$Fe_{16}N_2$ precipitation in α-Fe have reported the precipitate as a disc-shaped "rosette" which has a $\{001\}_\alpha$ habit plane (see 1). Recently a theoretical analysis (2) suggested that a habit plane shift occurs to $\{102\}_\alpha$ as the aspect ratio of the precipitate increases to a value >11.34. A limited experimental verification of this apparent phenomenon has subsequently been reported (3). A comprehensive study of the quench-aging of nitrogen-ferrite (4) has shown that α" precipitation occurs via nitrogen-atom cluster (nitrogen GP zone) formation by a continuous growth and ordering process. Lattice imaging has resolved the cluster as an undulating platelet which lies about {001} matrix planes and that this morphology is retained during α"-$Fe_{16}N_2$ formation. The present work examines the reasons why the nitrogen-atom clusters and α" precipitates adopt this particular morphology and adequately explains the characteristic rosette appearance of α" precipitates.

1. P.A. Beaven and E.P. Butler, Acta. Met. 28, 1349, 1980.
2. M. Hong, M.S. Thesis, Department of Materials Science, U.C. Berkeley, 1978.
3. Y.C. Shih and J.W. Morris, Jr., 40th. Ann. Proc. Electron Microscopy Soc. Amer., Washington, D.C., 1982, G.W. Bailey (ed.), p. 728.
4. P. Ferguson, Ph.D. Thesis, The University, Newcastle upon Tyne, U.K., 1981.

THE STRUCTURE OF GRAIN BOUNDARIES: MODELS AND MICROSCOPY, E.A. Kamenetzky and R. Gronsky, Materials and Molecular Research Division, Lawrence Berkeley Laboratory, and Department of Materials Science and Mineral Engineering, University of California, Berkeley, CA 94720.*

Our current understanding of grain boundary (GB) structure in cubic metals is based on geometrical or structural unit models. Much of the evidence for each model comes from transmission electron microscopy. Diffraction contrast and lattice images of GB dislocations predicted by O-lattice theory have been obtained in polycrystalline materials and bicrystals of controlled geometry respectively. Stacking fault contrast of rigid body GB displacements and many beam lattice images of group of atoms gave evidence for the structural unit model. The interpretation of images is complicated by the fact that the recorded micrographs have to be explained in light of the model of boundary structure that has been used to establish experimental conditions in the first place. A number of examples taken from the literature will be analyzed to show the relationship between modeling and microscopy.

VOLTAGE CONTRAST TESTING ON SILICON-ON-SAPPHIRE (SOS) CIRCUITS: REVIEW AND PREVIEW, R.E. Johnson, L.R. Tocci, M.J. McNutt, J.K. Hakhu, Microelectronics Research and Development Center, Rockwell International, Anaheim, California 92803

Todays advanced VLSI circuits have thousands of internal nodes which are routinely tested with a few tens of external electrical terminals. Internal measurements are always essential during the development phase of an I.C. The main limitations of mechanical probing these nodes include capacitive loading (as well as disrupting the metal lines), the time required to relocate to individual test points, and the inability to monitor more than two or three points simultaneously. These problems are escalating as the technology advances to near and submicron geometries.

Voltage contrast between two areas at different potentials when scanned with an electron beam is a viable alternative to mechanical probing. Voltage contrast in bulk silicon has been successfully used for the diagnostics of integrated circuits for over sixteen years. However, charging effects in silicon-on-sapphire (SOS) wafers increases the probablity of error in detecting voltage contrast. A low voltage technique has been developed to overcome this problem. Also, preliminary studies have identified and minimized electron beam and subsequent X-ray irradiation damage to the I.C. MRDC's newly installed Stroboscopic Voltage Contrast Tester capabilities will be previewed (eg. real time device testing, node to node waveform monitoring). Voltage contrast progress on SOS circuits will be reviewed and demonstrated by micrographs and a video presentation.

CHEMICAL AND STRUCTURAL ANALYSES OF γ' PRECIPITATES IN Al-Ag ALLOYS BY EDS AND CBED, J. Howe, M. Sarikaya and R. Gronsky, Materials and Molecular Research Division, Lawrence Berkeley Laboratory, Berkeley, CA 94720

Al-Ag alloys are model systems to study the diffusion-controlled growth of the transitional precipitates, i.e. γ', which are characteristic of age-hardening alloys. Most studies on this alloy system have concentrated on conventional contrast analysis of the interfacial structure and growth kinetics of the γ' phase by electron diffraction and of the crystal structure by x-ray diffraction. Recent research has further employed high-resolution TEM to study the interfacial structure and mechanisms of growth at the atomic level. However, there have not been any well-defined studies on the chemical composition or structural changes which accompany the growth process. In this investigation we intend to present composition and structural data obtained on well-formed γ' precipitates in an Al-15wt% Ag alloy which was solution heat treated, quenched and aged for 30 min at 350°C. Quantitative analysis of the chemical compositions were performed by energy dispersive x-ray (EDS) microanalysis, and convergent beam electron diffraction (CBED) was used to identify the crystal structure and any symmetry changes during the transition in a STEM instrument. All analyses were performed on γ' precipitates which were separated from the matrix using carbon extraction replicas which were prepared using several different etchants.

Alloys were provided by Professor H. I. Aaronson, Department of Metallurgy and Materials Science, Carnegie-Mellon University. This work was supported by the Director, Office of Energy Research, Office of Basic Energy Sciences, Division of Materials Science of the U. S. Department of Energy under Contract No. DE-AC03-76SF00098.

USES OF BACKSCATTERED ELECTRONS (BSE) AT 100 keV, R.H. Geiss, IBM Research Division, Dept. K32/281, 5600 Cottle Road, San Jose, CA 95193.

We have built a BSE detector for use in the Philips 301 S(TEM) transmission electron microscope. This allows us to use BSE up to 100 keV. The detector consists of two silicon diodes, 2.5 x 4.5 mm, mounted equidistant from and normal to the incident electron beam in the microscope. Specimens are carried via the standard side entry goniometer rods and are approximately 2.5 mm below the plane of the detectors.

Two applications of the use of high energy BSE will be described. (1) The use of Type II magnetic contrast in conjunction with a synchronous detection technique to study magnetic domain wall dynamics in inductive magnetic recording heads. (2) The use of small area (i.e., less than five microns in diameter) electron channeling patterns to study defects in single crystal silicon films grown by lateral nucleated epitaxy using scanning laser and electron beams.

X-RAY FLUORESCENCE ANALYSIS IN SCANNING ELECTRON MICROSCOPES, M. Kotera and D. B. Wittry, Departments of Materials Science and Electrical Engineering, University of Southern California, Los Angeles, CA 90089-0241.

It is well known that fluorescence excitation of characteristic X-rays can sometimes provide higher signal to background ratios than direct excitation by electron bombardment if the geometry is suitably optimized. This is true because the background in XRF consists of scattered radiation while the background for direct excitation is due to the X-ray continuum (bremsstrahleng) which is always produced by electron bombardment. For these reasons, it has sometimes been possible to use indirect (fluorescence) excitation in scanning electron microscopes to provide better limits of detection than are obtained with direct excitation when using energy dispersive X-ray spectrometry (1), (2). In the present paper, the results of theoretical calculations for the efficiency of excitation of characteristic X-ray by monochromatic X-rays are given and the advantage of excitation by monochromatic X-rays vs excitation by electrons is discussed. The case of partial monochromatization of the X-rays by thin foil targets is also considered.

(1) H. R. Zulliger and W. D. Stewart, American Laboratory, April 1977.
(2) A. van Riessen and K. W. Terry, JEOL News, Vol. 20E, No. 3., pp. 19-23, 1982.

IMAGE PROCESSING FOR MATERIALS CHARACTERIZATION: W. Krakow, IBM T.J. Watson Research Center, P.O. Box 218, Yorktown Heights, N.Y. 10598

A television rate frame store device has been interfaced to a large mainframe computer which permits time shared image processing to be performed from remote instrument sites such as: TEM's, SEM's light microscopes, etc. The system is particularly useful for on line determination of high resolution TEM's performance by obtaining the power spectrum of a low light level image in excess of 10^6x magnification in real time (∿ sec.). Examples of algebraic and geometric image manipulations, psuedocolor, contrast enhancement, densitometry, as well as particle and feature analysis will be shown. The system is also a text processor and can make final labeled slides and transparencies of high quality.

While this system has recently been developed it is now being applied to a number of materials problems such as the structure of amorphous to crystalline Si boundaries the nucleation and growth of Au on NaCl, disorder in Ni supper lattices, surface structure of thin Au crystals and surface plasmon modes of small Al spheres.

ELECTRON MICROSCOPY STUDIES OF OXIDATION, K. R. Lawless, Dept. of Materials Science, University of Virginia, Charlottesville, VA 22901.

Various techniques of electron microscopy, including HVEM, HREM, AEM and associated microdiffraction procedures, provide the most powerful means available for studying the details of the interaction of oxygen with a metal or alloy. This interaction, depending on the reaction conditions and the material being oxidized, may be primarily a surface reaction, or an internal phase transformation, or a combination of both surface and internal effects.

This paper will discuss briefly some early studies on the oxidation of copper, nickel, and copper-nickel alloys, which under most conditions do not involve internal oxidation, and in more detail the oxidation of tantalum and vanadium metals, and tantalum-hafnium and vanadium-titanium alloys which involve extensive internal oxidation effects. In particular low pressure in-situ oxidation studies of the early stages of the reaction of oxygen with Ta, V, and V-20Ti will be discussed.

The early stages of the oxidation of Ta involve an ordering of oxygen, and the formation of internal sub-oxide structures. With V, the early stages of the oxidation do not show oxygen ordering, but subsequent stages show sub-oxide and long period superlattice formation. In V-20Ti alloys, the earliest stages of oxidation are characterized by a "tweed" structure, and a possible explanation for this structure will be given.

TEM STUDIES OF CRYSTALLIZATION IN METALLIC GLASSES, A.F. Marshall, A.R. Pelton, Y.S. Lee and S. Yoshizumi, Center for Materials Research, Stanford University, Stanford, CA 94305

Characterization of crystallization behavior in metallic glasses is of much current interest and can benefit greatly from TEM studies of the microstructures obtained at different stages of annealing. Results are presented for amorphous alloys prepared by vapor deposition in the Cu-Ti, Ni-Ti and Mo-Ge alloy systems.

The crystalline phases obtained by heating these alloys through the isochronal crystallization temperature were analyzed by convergent beam diffraction, lattice imaging and energy dispersive spectrometry. In the case of $Cu_{60}Ti_{40}$ and $Ni_{56}Ti_{44}$ more than two intermetallic phases were identified, indicating that the material had not reached equilibrium. These phases were often closely related in structure and could be finely intermixed as shown by lattice imaging. $Mo_{65}Ge_{35}$ crystallized

by direct nucleation and growth of the Mo_5Ge_3 phase and the final structure was a mixture of this phase and the Mo_3Ge phase.[3] Studies of the partially crystallized microstructure of $Mo_{65}Ge_{35}$ are being correlated with superconducting flux pinning measurements.

$Cu_{60}Ti_{40}$ formed a simple metastable structure as an intermediate crystallization step during continuous heating but not during isothermal annealing at lower temperatures. This was also observed for a liquid-quenched alloy of the same composition. TEM analysis of incubation times and growth rates as a function of isothermal annealing temperature for the equilibrium Cu_3Ti_2 phase were correlated with thermal data for both alloys. Comparison of the microstructures showed distinctly different crystallite orientation and morphology; this is interpreted as due to differences in amorphous short range order resulting from the different synthesis methods.

TEM OF SEMICONDUCTOR INTERFACES, R. Sinclair, Department of Materials Science and Engineering, Stanford University, Stanford, CA 94305.

The most profitable way of examining interfaces in semiconductor structures and devices is by cross-section transmission electron microscopy. The specimen preparation is particularly difficult, but if successful this approach provides a means of investigating the interface topography, cystallography, chemistry and defects at the working resolutions of a TEM system. Examples from our recent work will be presented to illustrate the power of the technique. Specific disadvantages of TEM will also be discussed and alternative experiments to overcome its limitations will be cited. The materials in question include Si-SiO_2, Si-$TiSi_2$, CdTe-CdS. The application of videotape recording of dynamic events will also be outlined.

An Optimized Twin Objective Lens for the Philips Transmission Electron Microscopes. R. L. McConville, Electron Optics Applications Laboratory, PHILIPS ELECTRONIC INSTRUMENTS, INC., 85 McKee Drive, Mahwah, N. J. 07430.

A number of modifications to the illumination system and objective lens assembly has lead to improvements in the performance of the microscope in TEM, STEM, and analytical modes.

The importance of a properly designed condenser system and objective lens will be discussed. Advantages of the symmetrical objective for TEM, STEM, and analytical work will be proven with practical applications from many branches of research interest. Emphasis will be given to discussing the "cleanliness" of x-ray data, diffraction information, and hybrid diffraction techniques such as rocking beam, double-rocking beam, and conical scanning.

AN ELECTRON MICROSCOPY STUDY OF EXSOLVED PHASES IN NATURAL AUSTRALIAN SAPPHIRE.
A.R. Moon and M.R. Phillips, Dept. of Physics,
The New South Wales Institute of Technology, Sydney 2007, Australia.

Natural Australian Sapphire (α - Al_2O_3 with around 1% Fe and up to .03% Ti) occurs in many colours, generally with an abundance of exsolved phases. Such "silky" stones, cut en cabochon, exhibit the well-known phenomenon of asterism. For stones with a well-defined "star" needle-like precipitate phases can be seen under an optical microscope. The needle axes are perpendicular to the star axes and generally parallel to the prism a-axes. (For artificial star sapphire the precipitate needle axes are perpendicular to the prism axes, e.g., Phillips, D.S., Heuer, A.H., and Mitchell, T.E., 1980, "Precipitation in Star Sapphire", Phil.Mag. A42: 385-404.) Although many authors have assumed that these precipitate phases in natural sapphire were needles of rutile (TiO_2), as is the case for artificial sapphire (Phillips, D.S., et al, op.cit.), our studies confirm the existence of a variety of iron and titanium rich phases, including rutile.
Sample specimens were prepared by illuminating a polished basal plane with a He-Ne Laser to obtain the star directions and then cutting slices of the stone parallel to the star axes. The polished sections were then ion-beam thinned and examined in a JEOL 100CX (S)TEM, equipped with an energy dispersive X-ray spectrometer. Precipitate phases were identified by electron diffraction and from their X-ray spectrum.
This work has been supported in part by an Australian Research Grant. Use of the facilities at the Sydney University Electron Microscope Unit is gratefully acknowledged.

THE INFLUENCE OF GRAIN BOUNDARIES ON INTERSTITIAL PHASE PRECIPITATION IN AN AUSTENITIC ALLOY, A.R. Pelton, Department of Materials Science and Engineering, Stanford University, Stanford, CA 94305.

Precipitation of interstitial phases in a quenched and aged austenitic stainless steel was studied using various transmission electron microscopy techniques. Incipient intragranular precipitation involved the formation of vacancy-carbon (V-C) and vacancy-phosphorus (V-P) complexes on {100} matrix planes. These interstitial phases are homogeneously distributed within the grains with densities of approximately 10^{16} and 10^{15} cm^{-3} respectively. Discontinuities in the densities of the precipitates are observed near grain boundaries. Uniform depleted zones formed in the regions adjacent to grain boundaries due to vacancy-solute migration during the quench. The width of these denuded zones near disordered, high-angle boundaries varies with the thermal history of the sample and with the type of precipitate. There are no significant differences in zone width with the geometry of the non-special boundaries. Typical depleted regions are 200 nm for V-C and 500 nm for V-P which indicates the relative magnitudes of the vacancy-solute binding enthalpies. In contrast to the precipitation behavior near non-special boundaries, no precipitate-free zones are observed in the proximity of coherent or semi-coherent $\Sigma = 3$ (twin) boundaries. Analysis of grain boundaries in samples aged at higher temperatures also reflects the differences between disordered and twin boundaries. Phosphorus enrichments are measured by EDS at triple junctions and incoherent facets of twin boundaries. Equilibrium phosphide precipitates are subsequently observed at these solute-rich sites. These phenomena will be discussed in terms of the relationships among grain boundary structure, sink strength and vacancy-solute binding enthalpies.

This work was partially supported by the Director, Office of Energy Research, Office of Basic Energy Sciences, Division of Materials Sciences of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098.

THE USE OF A RULED RETICLE FOR STEREOLOGY DIRECTLY OFF THE FLOURESCENT SCREEN: MEASUREMENT OF RADIATION EFFECTS, D. E. Philpott, NASA Ames Research Center, Moffett Field, CA 94035

A number of stereology methods have been devised for quantitative measurement of tissue changes. Cutting out the areas of interest from the prints and weighing, overlaying the print with various grid patterns and counting the points of intersection, outlining the objects on graphic tablets or with a point of light on the screen and using computers for recognition drawing and calculation are general methods currently in use. Using a ruled reticle in the binocular of the electron microscope has an advantage over its use in the light microscope because the magnification of the electron microscope can be varied over a much greater range. Thus, the magnification can be adjusted to allow the visable field to include enough organelles for a better statistical evaluation. This reduces the need for a large number of different grid overlays in order to match the print magnification which also reduces the necessary fields of view to achieve an acceptable accuracy. Reticles are available from several manufacturers, but satisfactory ones can easily be made providing exactly what the operator wants. One method is to mark the desired periodic grid spacings on graph paper, overlay this with white paper and trace to obtain a grid pattern without the graph paper lines. Copy this sheet with a camera adjusting the distance to produce the correct image size for the binocular eyepiece. The film can now be cut out and used if it is polaroid or direct positive. Otherwise, it must be reversed to provide a clear background. By making the grid system, the desirability of its use and the necessary grid spacings can be tested before any investment is made in a commercial reticle. While computers do the statistics much faster, this simple system has the advantages of extremely low cost, no need for negatives or prints, and rapid results. Therefore, it may function as an adjunct to another system or provide a method for those who cannot afford the much more expensive systems. This method is currently being used to measure the percentage change in organelles after high energy particle radiation.

AN IMAGE STORAGE AND COMPUTER SYSTEM FOR STATISTICAL ANALYSIS, D.E. Philpott, M. Edgerton*, Mike Hallesy**, and Paul Gracie**, NASA Ames Research Center, Moffett Field, CA 94035, *Computer Sciences Corporation, Ames Research Center, Moffett Field, CA 94035, **MicroDoctors, Palo Alto, CA 94306.

An image storage and analysis system has been devised for high resolution recording on disks for later retrieval and analysis. This method obviates the time and costs necessary for taking and printing pictures before statistical analysis can be carried out. The shorter time frame also allows a more rapid feedback for changes in experimental protocol. The hardware consists of a Philips TV camera attached to a Philips 300 electron microscope, a TeleVideo terminal 925, a Summagraphics bitpad and an Epson MX80FT printer. A separate video camera makes it possible to work from electron micrographs as the system will work with any standard source of EIARS 170 video.

Images are selected on the electron microscope and immediately transferred to a disk for storage. Over thirty pictures can be stored on a double-density double-sided 8 inch disk. Statistical analysis can be performed at any time without further interaction with the microscope by an untrained individual following a "cookbook computer procedure." The image is brought up on the viewing screen and organelles of interest are marked or outlined using a bitpad and bitpad pen. The software includes statistical analysis of particles, areas, lines and tests for significance of results, e.g., student "t" tests. This image storage and analysis capability increases efficiency and accuracy in determining the statistical significance in experimental procedures while reducing photographic expenses and time-consuming operations. The computer can also be used for other tasks when not being utilized for image storage and analysis.

PROBLEMS RELATED WITH NITROGEN DETECTION IN SIALONS BY EELS*

M. Sarikaya, P. Rez, T. Dinger, and G. Thomas, Materials Science and Mineral Engineering, and Materials and Molecular Research Division, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720

High temperature properties of SiAlONs are very susceptible to the nature of the amorphous phase formed at the grain boundaries and the grain junctions. This is a result of the sintering process and its presence is caused by several factors including a change in the chemistry of this phase at high temperatures, which, in addition to impurity segregation, also involves nitrogen partitioning which probably occurs during solution/reprecipitation. Therefore, to fully interpret this process, the detection and, if possible, quantification of nitrogen both in the crystalline and the glassy regions is necessary. Since the problem of nitrogen detection involves high spatial resolution, Electron Energy Loss Spectroscopy was used to determine quantitatively the amount of nitrogen present in both phases and to give an estimate of the error involved in the analyses. The analyses were performed in the TEM image, and TEM and STEM diffraction modes; the advantages and the disadvantages of each mode are described. Deconvolution routines based on earlier models are described and illustrated.

TEM OF SEMICONDUCTOR INTERFACES, R. Sinclair, Department of Materials Science and Engineering, Stanford University, Stanford, CA 94305.

The most profitable way of examining interfaces in semiconductor structures and devices is by cross-section transmission electron microscopy. The specimen preparation is particularly difficult, but if successful this approach provides a means of investigating the interface topography, cystallography, chemistry and defects at the working resolutions of a TEM system. Examples from our recent work will be presented to illustrate the power of the technique. Specific disadvantages of TEM will also be discussed and alternative experiments to overcome its limitations will be cited. The materials in question include Si-SiO_2, Si-$TiSi_2$, CdTe-CdS. The application of videotape recording of dynamic events will also be outlined.

PHILIPS NEW BACKSCATTERED ELECTRON DETECTION SYSTEM (MFD) AND SIGNAL MIXING TECHNIQUES IN SEM, Stewart Spiers, Philips Electronic Instruments, 3000 Scott Blvd., Santa Clara, California 95050

Discussion on a new form of backscattered electron detection system utilizing four scintillation type detectors, fibre optics and optical switching.

Examples will be shown of the effects of subtracting backscattered electrons from secondary electron images and imaging backscattered electrons from different detectors.

High Resolution Observation of PbS growth on clean Ge (111) surfaces, N. Tanaka, Dept. of Appl. Phys. Nagoya Univ. Chikusa-ku, Nagoya, 464 Japan, Now at Dept. of Physics, Arizona State University, Tempe, AZ 85287

Characteristic surface structures and adsorbed structures on semiconductor surfaces such as Ge and Si have been elucidated by LEED and RHEED. These methods however, can not give the information about atomic arrangement of the structures and adsorbed sites. We have tried to observe the surface and adsorbed structures by high resolution transmission electron microscopy. In the present experiment, a few atomic-height PbS islands and Pb clusters grown on clean Ge (111) surface

are observed with axial bright field lattice imaging technique by JEM-200CX. The Ge (111) surfaces are prepared by vacuum deposition on CaF_2 (111) cleaved surfaces, PbS of less than 1 nm in mean thickness is deposited on the surface at 1×10^{-8} torr in clean vacuum. The growth process of PbS on Ge (111) surface is clarified as follows:

PbS deposited on Ge (111) surface shown firstly $\sqrt{3}$ adsorbed structures which is due to Pb atoms, secondly with increasing thickness, it shows PbS pseudomorphic layers and finally it grows up in normal 111 epitaxial islands. Pb islands are also detected due to the difference of vapor pressure between Pb and S atoms. The growth processes of PbS or Pb are interpreted from the point of view of simple geometrical fitting conditions.

MEASUREMENT OF X-RAY COUNT RATES IN THE TEM TO ESTIMATE SPECIMEN THICKNESS

J. E. WITTIG, Department of Materials Science and Engineering,
Stanford University, Stanford CA 94305

Knowledge of the specimen thickness is an important parameter for quantitative x-ray analysis of thin foils. The widely accepted technique of two-beam convergent beam electron diffraction (CBED) patterns for thickness determination can produce measurements which are accurate to +-2% for optimum conditions. However, the CBED method requires the specimen to be positioned exactly at a specific two-beam condition. The intensity variations in the diffracted disc must be subsequently measured and analyzed. This procedure is extremely time consuming if the thickness must be determined at many different locations. It was this experimental obstacle which prompted the search for an easier and faster method to estimate local foil thickness.

X-ray production from an electron probe is a function of the accelerating voltage, the probe size, the current density in the probe, the material composition, the density of the material, the x-ray take off angle, the crystallographic orientation of the specimen, contamination build-up, and of the specimen thickness. Thus, the count rate can be directly related to the foil thickness when the other parameters are maintained constant. The accuracy of this correlation depends on the reproducibility of the experimental conditions. Initially, the effect of increasing thickness on x-ray production must be calibrated using some other technique i.e. two beam CBED patterns. Once this calibration procedure has been completed for a given material and experimental parameters, the count rate alone can be used to estimate the foil thickness. The pupose of this study is to determine the precision of the thickness measurement which can be obtained using this technique.

Funding for this research from the Electrical Power Research Institute is gratefully acknowledged.

LATTICE RESOLUTION MOVIE OF ELECTRON BEAM-INDUCED PROCESSES IN CdTe

T. Yamashita, L. Plano and R. Sinclair

Department of Materials Science and Engineering, Stanford University
Stanford, California 94305

CdTe is a II-VI compound semiconductor material which is being studied for use as solar cells and as substrate material in infra-red detectors. The crystal has the largest lattice parameter of all II-VI materials, and as a consequence high contrast lattice images in the <110> projection are quite easy to obtain. However, the material is suceptible to electron beam damage, which is related to the beam-induced heating of the sample and enhanced oxidation of the surface due to presence of reactive oxygen species that are produced by the electron beam. At lattice resolution, the damage process is quite interesting to observe. During the initial stages, a rapid appearance and disappearance of image spots, which correspond to a pair of cadmium and tellurium atoms in projection, can be observed at the edge of the crystal. Formation of crystalline facets of various configurations accompany this process. Somewhat later, small crystallites can be seen growing at the surface of the CdTe, and they eventually replace the CdTe altogether. These crystallites have been identified as CdO. In the bulk of the crystal, motion of dislocations can be often observed. Two basic types are of interest. The motion of Shockley partial dislocation is common, and they tend to move quite rapidly. Another type is the climb of Frank dislocation, which is much slower. These dynamic phenomenon were recorded onto a video tape using a TV camera attached to the microscope. The magnification on the TV monitor was 17 million times. The images are quite noisy, but digital image processing can be employed to improve the quality of the image. Some of the representative sequences of the variety of dynamic phenomena observed in CdTe will be shown, and the advantages which the TV system offers for high resolution lattice imaging will be discussed.

STRUCTURE OF THE CdS/CdTe INTERFACE

T. Yamashita, J.G. Werthen*, R.H. Bube and R. Sinclair

Department of Materials Science and Engineering, Stanford University
Stanford, California 94305

*XEROX Palo Alto Research Center
Palo Alto, California 94304

The structure of electrically characterized CdS/CdTe solar cell interfaces have been analyzed by high resolution lattice imaging and by conventional and analytical TEM techniques. Undoped CdS was thermally evaporated to a thickness ranging from 0.2 to 1.0 m onto p-CdTe. The effect of substrate orientation and surface treatments on the CdS/CdTe interface and CdS structure have been analyzed. Specimens were prepared in cross-section, and lattice images were obtained in the CdTe <110> projection. Some aspects of specimen preparation will be discussed. Results from CdS grown on (110), (112) and (111) CdTe substrate orientations will be shown. In each case the interface and CdS structures were considerably different. CdS grown on cleaved (110) CdTe surface had the cubic sphalerite structure whereas on (112) and (111) substrates the structure was the expected hexagonal wurtzite type. The large lattice mismatch present at all of these interfaces (10%) appears to be accommodated by either defects (stacking faults) in the CdS layer, steps at the interface or by rotation of the CdS lattice with respect to the CdTe lattice. In the case of CdS deposited on (111) CdTe surface, the observed interface structure cannot be explained simply by a purely elastic model involving "misfit" dislocations. Some bond rearrangement must be proposed. The images were obtained at 3Å resolution using Philips EM400ST microscope. Image simulations of the interface structure will be also shown.

Ultrastructural aspects of macrophage and lymphocyte congregation in primary tissue cultures of human and animal lungs.

V. Richters, A. Richters, and R.P. Sherwin. Department of Pathology, University of Southern California, School of Medicine, Los Angeles, CA 90033.

Macrophage and lymphocyte congregation, defined as three or more spread lymphocytes or macrophages on the surface of a single target cell, is believed to represent one aspect of cell surveillance. This report presents the findings from ultrastructural-cytochemical studies of the cellular interactions. The main findings with respect to the macrophage investigations are: 1) macrophage congregation (MC) occurs on the target cell surface facing the substrate; 2) identification of the macrophage and its interacting cytoplasmic processes is facilitated by the use of a horseradish peroxidase label; 3) congregating macrophages are not functionally distinct subtypes; 4) adenosine triphosphatase is ubiquitous in the macrophage populations at the ultrastructural level whereas it serves to identify a subpopulation at the light microscopy level; 5) membrane to membrane apposition of macrophage and target cell occurs without membrane fusion; 6) MC is commonly associated with cellular debris and may in part be related to necrobiosis and degeneration; 7) retrovirus expression appears to be more frequent in target cells of MC than in lung cells without MC; 8) nitrogen dioxide (NO_2) increases the frequency of MC for reasons presently unknown. Cell surface alteration following NO_2 exposure, and the facilitation of expression of virus and other indigenous infectious organisms, are two of many factors that may underlie the phenomenon of MC.

With respect to lymphocyte congregation, many characteristics are shared in common with MC. The findings suggest that activated lymphocytes are involved.

Supported by EPRI Contract RP 1437 and NIH Grant CA 05297-01.

Information for Authors

GENERAL INFORMATION

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 are preferred. Guidlines for submission of articles are given below. The views expressed in the articles, editorials and letters represent the opinions of the author(s) and do not reflect the OFFICIAL POLICY OF THE INSTITUTION with which the author is affiliated or the Texas Society for Electron Microscopy. Acceptance by this Journal of advertisements for products or services does not imply endorsement. Manuscripts and related correspondence should be addressed to Randy Moore, Editor, TEXAS SOCIETY FOR ELECTRON MICROSCOPY JOURNAL, Department of Biology, Baylor University, Waco, Texas 76798.

GUIDELINES: Manuscripts written in English will be considered for publication in the form of original articles, historical and current reviews, case reports and descriptions of new and innovative EM techniques. It is understood that the submitted papers will not have been poreviously 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 8½ x 11 white paper, leaving ample margins. Number each page and identify the article by placing, at the top left of the page, a shortened form of the title, followed by the last name of the first author.

TITLE PAGE: Include:

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; MATERIALS; AND DISCUSSION.

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

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

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

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

(2) P.S. Baur, Jr., G.F. Barratt, G.M. Brown and D.H. Parks. Ultrastructural Evidence for the Presence of "Fibroclasts" and "myofibroclasts" in Wound Healing Tissues. J. of Trauma. 19 (1979) 774-756.

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

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 should appear as a footnote which will appear at the top of the first page of the article.

TSEM By-laws

Article I — NAME

The name of the Society shall be the Texas Society for Electron Microscopy.

Article II — PURPOSE

This Society is organized exclusively as a scientific and educational organization. The purpose of this Society shall be (a) to increase and disseminate knowledge concerning the biological and physical applications of electron microscopy and related instrumentation and (b) to promote free exchange of ideas and information among electron microscopists and interested participants. Not withstanding any other provision of these articles, this society shall not, except to an insubstantial degree, engage in any activities or exercise any powers that are not in furtherance of the purposes of this society. No substantial part of the activities of the Society shall be the carrying on of propaganda, or otherwise attempting to influence legislation, and the Society shall not participate in, or intervene in (including the publishing or distribution of statements) any political campaign on behalf of any candidate for public office.

Article III — MEMBERSHIP

Membership in the Society shall be open to individuals who share the stated purpose of the Society. The Society shall consist of regular members, student members, corporate members, and honorary members.

An applicant, other than a corporate organization, having an interest in electron microscopy may be considered for regular membership. An applicant enrolled in an academic undergraduate or graduate program will be considered for student membership. Students wishing to become more involved in the Society may elect to apply for regular membership. Any applying commercial organization having an interest in electron microscopy shall be considered for corporate membership. A corporate membership shall entitle that corporation to designate one representative who shall receive membership benefits as a regular member. Other representatives of the same organization may apply for regular membership to receive Society privileges. Honorary membership shall be restricted to either (a) distinguished scientists who are not members of the Society, but who have made significant contributions to this Society or (b) to Society members for extended and outstanding service to this Society.

Application for regular, student, and corporate membership shall require nomination by any regular member in good standing and shall be made to the Secretary, who, with the approval of the Executive Council, shall report same at the next business meeting of the Society. A two-thirds vote of the regular members present shall elect applicants to membership.

Nominations for honorary membership may be made by any member of the Society. Nominations shall be made in writing to any member of the Executive Council and must be accompanied by written evidence of the nominee's eligibility. The member of the Executive Council shall present the nomination at the next meeting of the Executive Council for consideration. The Executive Council shall act upon the nomination within one year of its presentation and shall notify the nominator of the final action taken on the nomination.

Only regular members shall have the right to vote, to nominate new members, to hold office, or to serve on committees. Corporate members may exhibit at the Society's meetings (additional exhibition charges may be levied by the Executive Council). An honorary member shall be exempt from dues and shall be entitled to all privileges of regular membership. All members shall receive Society mailouts except for ballots which will be mailed only to regular members.

The amount of dues shall be set by the Executive Council. Dues shall become payable on January 1 of each year. Members unpaid by the Spring meeting shall be notified and if still unpaid will be dropped from membership after the Fall meeting.

Article IV — OFFICERS

A. Elected Officers

The elected officers of the Society shall be President, President-Elect. Immediate Past President, Secretary, Treasurer, Program Chairman, and Program Chairman-Elect. The President-Elect shall serve one year as such, one year as President, and one year as Immediate Past President. The Secretary shall be elected in even-numbered years and serve for a two year term. The Treasurer shall be elected in odd-numbered years and serve for a two year term. The Program Chairman-Elect shall serve one year as such, followed by one year as Program Chairman. The installation of incoming officers shall be at the Spring meeting. All officers shall arrange for the orderly and timely transition of their offices within 30 days after the installation of officers. However, all officers shall continue until relieved by their successors. The duties of the officers shall

1. **President:** shall preside at all business meetings of the Society and at meetings of the Executive Council. The President shall represent the Society at the annual meeting of the Electron Microscopy Society of America. The President shall conduct the business of the Society between Executive Council meetings.
2. **President-Elect:** shall assist the President and substitute for him in his absence and perform such duties as assigned by the President.
3. **Immediate Past President:** shall assist the President and Executive Council.
4. **Secretary:** shall maintain the records of the Society other than financial, and distribute announcements to the membership.
5. **Treasurer:** shall be custodian of the Society funds and shall account for them in accordance with accepted business practice. The Treasurer shall be bonded and the cost of such shall be borne by the Society. The Treasurer shall have his records examined annually by an internal audit committee chosen by the Executive Council at the Winter meeting. A written report of the internal audit shall be presented to the Executive Council and the membership at the Spring meeting.
6. **Program Chairman:** shall be responsible for organizing the various scientific activities of the Society. The Program Chairman shall not commit any funds of the Society unless authorized by the Executive Council via an approved budget or as authorized by the President and Treasurer under conditions of exigency.
7. **Program Chairman-Elect:** shall assist the Program Chairman and substitute for him in his absence and, additionally, extend the planning of programs into his own term of office as Program Chairman.

B. Appointed Officers

The appointed officers of the Society shall be the Newsletter Editor and the Student Representative who shall be appointed by the Executive Council.

1. **Newsletter Editor:** shall publish a Newsletter three times a year promoting the purpose of the Society, unless otherwise ordered by the Executive Council. The term of appointment shall be for two years and may be renewed.
2. **Student Representative:** shall represent the student membership of the Society on the Executive Council. The term of appointment shall be for one year.

Additionally, the officers of the Society shall perform the duties prescribed by the Bylaws and, as appropriate, by the parliamentary authority adopted by the Society. No part of the net earnings of the Society shall inure to the benefit of, or be distributable to its members, trustees, officers, or other private persons, except that the Society shall be authorized and empowered to pay reasonable compensation for services rendered and to make payments and distributions in furtherance of the purposes set forth in Article Two hereof.

Article V — MEETINGS

There shall be three scientific meetings per year: fall, winter, and spring, unless otherwise ordered by the Society or by the Executive Council. Exact times and places of these meetings shall be designated by the Executive Council. A business meeting will be held at each scientific meeting of the Society. Parliamentary procedures to be followed in the business meeting shall be those specified in the current edition of **Robert's Rules of Order Newly Revised.** Ten percent of the regular

members, or 35 regular members, whichever is smaller, shall constitute a quorum at a business meeting.

Article VI — EXECUTIVE COUNCIL

The Executive Council shall be responsible for the scientific and administrative obligations of the Society. It shall determine policies for the good of the Society in accordance with these By-laws; it shall plan scientific and business meetings, it shall authorize the expenditure of Society funds, and it shall conduct other duties as required for the benefit of the Society. The Executive Council shall meet prior to the business meeting at each scientific meeting of the Society. Special meetings of the Executive Council can be called by the President and shall be called upon the written request of three elected members of the Executive Council.

At each spring meeting the Executive Council shall appoint a Student Representative who shall represent the student membership of the Society on the Executive Council the following year as a voting member. The Executive Council shall also appoint Local Arrangements Chairmen for each of various meetings and in so doing shall duly consider the recommendations of the PROGRAM Chairman and the President. Local Arrangements Chairmen are **ad-hoc**, non-voting members of the Executive Council.

Executive Council meetings are open to the membership.

The elected and appointed officers shall constitute the Executive Council. The President and four other elected officers or the President-Elect and four other elected officers shall constitute a quorum.

Article VII — COMMITTEES

Standing or special committees shall be appointed by the President as directed by these By-laws or as the Society, or the Executive Council, shall from time to time deem necessary to carry on the work of the Society. The President may appoint advisory committees at any time without prior consultation with the Executive Council. The President shall be **ex officio** a member of all committees except the Nominating Committee.

Article VIII — ELECTIONS AND INTERIM VACANCIES

In February of each year the Executive Council shall appoint three regular members to serve on the Nominating Committee with the President-Elect, and the Secretary. The Secretary shall serve as chairman of the Nominating Committee. The Nominating Committee shall nominate two candidates for each officer position becoming vacant that year. In preparing the slate of nominees, due consideration shall be given to the geographical area and fields of interest represented by the membership of the Society and to the nominees previous participation in the Society's affairs. The Nominating Committee shall also ascertain the willingness of each nominee to serve if elected. The report of the Nominating Committee shall be announced to the regular membership by March 1.

Additional nominations may be initiated by the membership by a petition to the Secretary signed by a minimum of ten of the regular members. Such petitions must be received by the Secretary by March 15.

Ballots shall be mailed to the regular members in March and completed ballots shall be accepted by the Secretary until April 15. The Secretary shall count the ballots on the next appropriate day and announce the results of the election at the spring business meeting and by mailout to the regular membership. Any regular member may examine the ballots at the spring business meeting.

The candidate receiving the largest number of votes shall be the winner. In the event of a tie vote, the Executive Council shall decide the winner. The ballots shall be examined by the Executive Council at the spring meeting.

A two-thirds vote of the entire membership of the Executive Council shall remove any officer or appointee derelict in their duties. The Executive Council shall accept resignations in good faith.

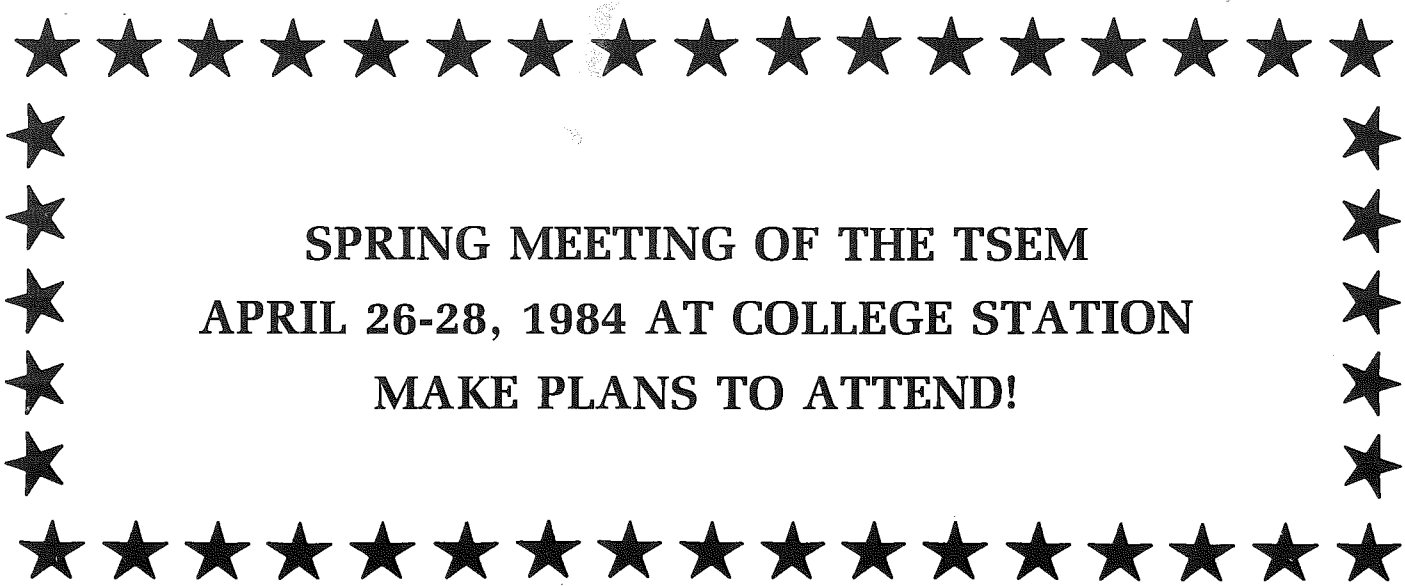
An interim vacancy in the presidency shall be filled by advancement of the President-Elect, who will go on to serve his anticipated term as President and Immediate Past President. In the event there is no President-Elect to advance, the Executive Council shall elect one of its members as acting President to serve until the completion of the next regular election. An interim vacancy in the office of Program Chairman shall be filled by the Program Chairman-Elect, who will go on to serve his anticipated term as Program Chairman. If there is no Program Chairman-Elect to advance, the Executive Council shall appoint a Program Chairman to serve until the completion of the next regular election. Interim vacancies in the offices of Secretary or Treasurer shall be filled by appointment by the Executive Council until the completion of the next regular election. Interim vacancies in the offices of Newsletter Editor or Student Representative shall be filled by an appointment made by the Executive Council.

Article IX — DISSOLUTION

Upon the dissolution of the Society, the Executive Council shall, after paying or making provision for the payment of all the liabilities of the Society, dispose of all of the assets of the Society exclusively for the purposes of the Society in such manner, or to the Electron Microscopy Society of America. Any such assets not so disposed of shall be disposed of by the Court of Common Pleas of the county in which the principal office of the Society is then located, exclusively for such purposes or to such organization or organizations, as said Court shall determine, which are organized and operated exclusively for such purposes.

Article X — AMENDMENTS

Amendments to these By-laws may be initiated by individual members of the Executive Council or by petition to the Secretary signed by ten regular members of the Society. Amendments must be approved by a two-thirds majority of the Executive Council, the proposed amendment shall then be promptly submitted by mail to the regular membership by the Secretary with statements of support and/or opposition by the Executive Council. The ballots shall be accepted by the Executive Council for one month after the date of mailing. The Executive Council shall count the ballots; the amendment(s) shall be ratified if it receives a favorable two-thirds majority of the votes cast. Any regular member can, if he or she so desires, be present at the counting of the ballots.



ELECTRON MICROSCOPY SOCIETY OF AMERICA
NOMINATION FOR MEMBERSHIP

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

Name of corporation nominated _____
person
P.O. Address _____

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

_____

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

_____
Signature of EMSA Member making nomination

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

Presented to the Council at _____ meeting. Date _____

Action _____

Remarks _____

Send Nominations to:

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

WE NEED YOU

TO SUBMIT A PAPER FOR PUBLICATION IN THE TSEM JOURNAL

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 _____

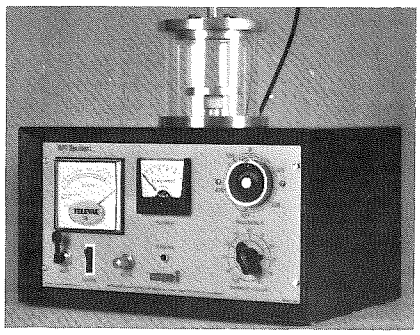
Send Application to:

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

INSTRUMENTS

SPI Sputter™
Sputter Coaters

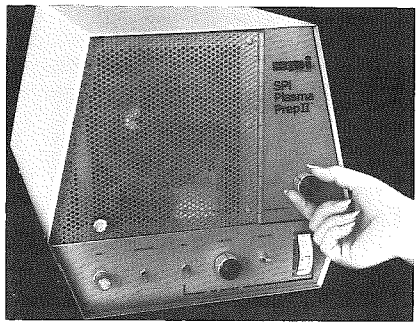
- 200Å gold in less than 10 secs.
- Cool, accurate, reproducible
- Pump: choose built-in or separate
- Uniformly coats all surfaces, shapes
- Magnetron cathode system



FAST

PLASMA-PREP II™
Asher/Etcher

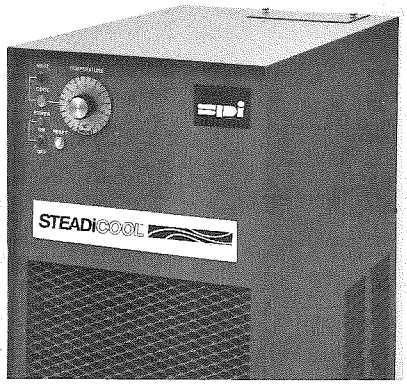
- Low-temp plasma processing gently removes organics from inorganics
- Audio alarm system monitors operation
- Extra-durable chassis design
- Optional strip chart recorder



COOL & SELECTIVE

STEADiCOOL™
Water Recirculator

- Range: +5°C to +35°C; Stability ±1.0°C
- Max. cooling capacity @20°C: 2100 Watts
- Built-in features: external temperature readout, hi-temp cutoff valve, large 4 gpm (15.2 l/min.) pump
- Safety cutoff valve



ADJUSTABLE

SPI® SUPPLIES

For further product and ordering information, call Toll-Free
(800)345-8148 (In PA (215)436-5400)

In Canada: P.O. Box 44, Station "S", Toronto, Ontario MSM 4L6 • (416)887-9534

Secretary
Texas Society for Electron Microscopy
GEA 115
The University Of Texas At Austin
Austin, Texas 78712

Non-Profit Organization
U.S. POSTAGE
PAID
Permit No. 1774
Austin, Texas 78710

THIRD CLASS

ADDRESS CORRECTION REQUESTED
FORM 3547 REQUESTED