Texas Society for Electron Microscopy, Inc.

JOURNAL
VOLUME 19, NUMBER 1, 1988
ISSN 0196-5662

Student-Technician Award Entries
Begin on Page 23

Bandera Photo Album
Begins on Page 35

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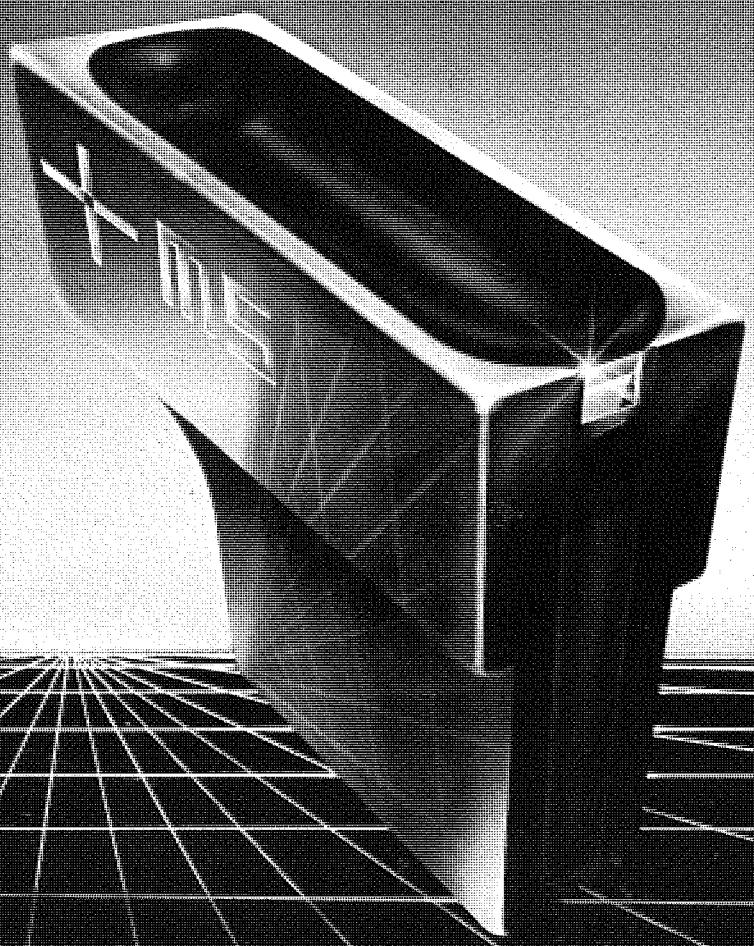

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TEXAS SOCIETY FOR ELECTRON MICROSCOPY JOURNAL
VOLUME 19, NUMBER 1, 1988
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Ronald W. Davis, Editor
Department of Medical Anatomy, Texas A&M Univ., College, Station, TX 77843

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President's Message

Recently the name of our society has been changed from The Texas Society For Electron Microscopy to The Texas Society For Electron Microscopy, *Inc.* This apparently subtle change is just the visible tip of some fairly complex, and very important, changes we are instituting in the way the society is structured and conducts its business. The reasons for doing this are straight forward. We want to protect both the membership and officers from personal liability, and we want to protect our tax-exempt status.

How we go about doing this is not so straight forward. These changes primarily involve rewriting our bylaws and articles of incorporation to more accurately describe who the society is made up of and what the purpose of the society is. Our tax-exempt status is governed by some rather complex IRS rules and regulations that stipulate just what our income can be and, more importantly, where it can come from. Also important is how we record our income and expenses, ie. how we keep our books. In a nut shell, it is tightly specified by the IRS just what *proportion* of our income can come from membership dues, ad revenue from *The Journal,* etc. As one might expect, we will be making some adjustments to our dues and to what we charge for advertising. Right now it appears that we will be increasing membership dues from $10 to $15 per year for regular members. Student and corporate members' dues will remain the same as they are now. At the same time we will probably have to lower what we charge for advertising in *The Journal.* All of this is to establish the correct proportion of income from these two main sources to conform with the rules of IRS.

Shortly after the Dallas meetings we will be mailing out revised bylaws that reflect these changes for comment and vote by the membership. Although these changes will not affect the way in which we run our meetings, choose our officers, recruit new members, or publish our journal; they will influence how the general public sees us. None of the officers have any legal expertise in these matters so we have retained the services of Mr. Chris Streifender, a tax attorney. Bob Droleskey, our Treasurer, has put in a huge amount of personal time and effort with Mr. Streifender on these changes and is deserving of our unceasing gratitude. It has not been easy and Bob has been very generous with his time.

Sincerely,

Joiner Cartwright, Jr., President
Texas Society For Electron Microscopy, Inc.
February 12, 1988

ON THE COVER

Transfer cells from a field-corn seed. There is no vascular connection between the maternal tissue (corn cob) and the developing seeds in corn. All nutrients must pass through a special groups of cells called transfer cells. These cells are characterized by having cell wall extensions that protrude into the cell lumen. The effect is to increase the plasma membrane surface area and presumably the potential for transmembrane flux. In this way, this system is analogous to *microvilli* in animals.

Corn is a very important food crop for both humans and animals. It has been called the "plant fruit fly" because so much is known about it genetically. Sweet corn is actually a mutant with a genetically induced block that prevents sugar from being sythesized to starch. Field corn is a normal corn with large amounts of carbohydrate stored in the form of starch.

All of the carbohydrates that enter the seed must pass through the transfer cells. It is not known if sugar transport, into the seeds, is an active (symplastic) or passive (apolastic) process. Micrograph by: R.W. Davis, Department of Medical Anatomy, Texas A&M University, College Station, Texas (1,500x).

Secretary's Report

Minutes of the General Business Meeting
Waco, Texas
March 6, 1987

President Randy Moore called the general business meeting of the Texas Society For Electron Microscopy, Inc. to order at 7:50 p.m., in the Top Deck of the Brazos Queen II at Waco, Texas.

Wayne Sampson read the minutes of the previous business meeting held October 17, 1986 in Houston. Bob Turner made the motion, seconded by Hilton Mollenhauer, that they be approved as read. The motion passed unanimously.

The treasurer's report was discussed by Randy Moore. He noted that the Society has approximately $12,000 in assets in the bank. Hilton Mollenhauer made the motion, seconded by Elizabeth Root that the treasurer's report be approved. It passed by unanimous vote of the members present.

Wayne Sampson reported on the election results as follows: President-Elect will be Howard Arnott. Treasurer will be Bob Droleskey and Program Chairman-Elect will be Ron Davis.

Randy Moore discussed the Executive Committee's decision concerning student and technical participation at TSEM meetings. He noted that up to three awards per meeting would be presented consisting of $100 plus a certificate or plaque. The participating student or technician must be senior author and present a two-page abstract in addition to the regular abstract. The two-page abstract must contain one page written material and one page of photographs. Randy Moore also indicated that the revision of the bylaws would be presented at the next meeting. He informed the members that the next meeting will be at Bandera October 8-10 and the first call for abstracts will be in April. The following spring meeting will be in Dallas and the fall meeting of '88 will possibly be in Galveston.

The report on new members was read by Wayne Sampson. He reported that since the Houston meeting in October, twenty-one new members joined the Society. This includes thirteen new members from the life sciences, one from the material sciences and seven from sales. Eleven of the new members are regular members, four students, and six are corporate members.

Randy Moore expressed his appreciation for all the many persons that assisted in the meeting at Waco and then gave the gavel to Joiner Cartwright. President Cartwright then thanked Randy Moore for his services as President for the past year and presented him with a plaque. The meeting was adjourned at 8:00 p.m.

Respectfully submitted,

H. Wayne Sampson, Ph.D.
Secretary

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Treasurer's Report

TSEM, Inc., has started the process of finalizing tax exemption with the Internal Revenue Service. The process requires financial records for the past three years and proposed budgets for the next two. We are currently waiting for documents to complete our 1985 records. The process is a long one, and it will take some time to get final approval from the IRS. Our legal counsel advises that he sees no problem in the society ultimately gaining IRS approval.

Sincerely,

Bob Droleskey

Bob Droleskey
Treasurer, TSEM, Inc.

TREASURER'S YEAR-END REPORT

For Calendar Year 1987

ASSETS ON 1 JANUARY, 1987:		
Certificate of Deposit No. 66177[1]	$ 2,110.61	
Certificate of Deposit No. 100-475-417-0[2]	4,086.43	
Certificate of Deposit No. 111-849-6	2,814.05	
Checking Account No. 097-212-5	3,128.43	$12,139.52
RECEIPTS:		
Registration Fees - Houston Meeting		
Biological Photographers	$1,236.41	
Waco Registration and Dues	1,345.00	
Bandera Registration and Dues	1,631.00	
Individual and Corporate Dues	4,127.00	
Journal Ad Revenue 17:1 & 2	150.00	
Journal Ad Revenue 18:1 & 2	5,990.00	
EMSA Grant	500.00	
Checking Account Interest	204.32	
Interest Earned on Certificates of Deposit	808.59	$15,992.32
EXPENSES:		
Waco Meeting Expenses	$2,357.60	
Bandera Meeting Expenses	368.69	
Student Travel/Financial Support	1,212.00	
Journal Printing	6,130.10	
Misc. Mailing Expenses	90.61	
President's Expenses	178.63	
Meeting Announcement/Program		
Printing & Mailing	1,900.00	
Treasurer's Expenses	196.04	
Checking Account Charge	85.19	$12,518.86
ASSETS ON 31 DECEMBER, 1987:		
Certificate of Deposit No. 10-7199995[1]	$2,267.03	
Certificate of Deposit No. 10-8829764[2]	4,536.73	
Certificate of Deposit No. 111-849-6	3,015.92	
Checking Account No. 015210-01	5,793.30	$15,612.98

[1]Certificate of Deposit No. 66177, University National Bank of Galveston, reached maturity on 14-3-87. The money from that certificate was redeposited in Certificate of Deposit No. 097-212-5, Lamar Savings of Bryan.

[2]Certificate of Deposit No. 100-475-417-0, United Savings Association of Texas, reached maturity on 15-12-87. The money from that certificate was redeposited in Certificate of Deposit No. 10-8829764, City Savings & Loan Association of College Station.

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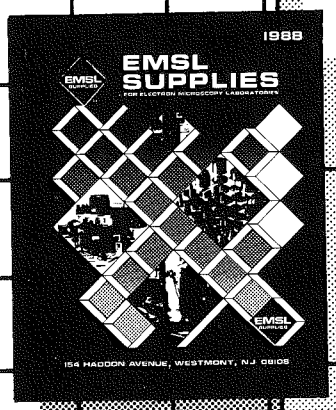

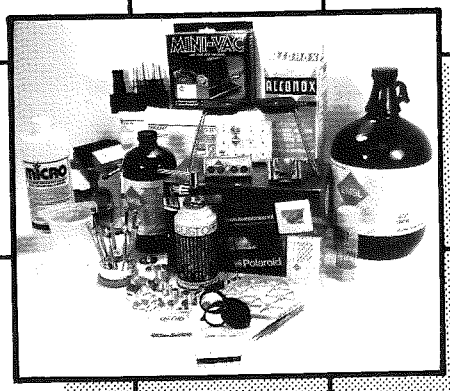

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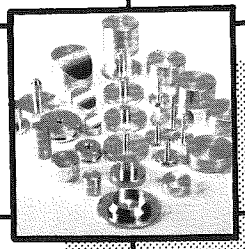
All in all, we have had a very gratifying response from you. We appreciate it, and we'll keep listening. To receive your copy of the NEW 1988 EMSL Supplies catalog, please give us a call at (800) 858-EMSL.



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TREASURER'S REPORT CONTINUED

1988 TREASURER'S REPORT
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Certificate of Deposit No. 10-8829764	4,536.73	
Certificate of Deposit No. 111-849-6	3,015.92	
Checking Account No. 015210-01	5,793.30	$15,612.98
RECEIPTS:		
Individual and Corporate Dues	$1,057.00	
Journal Ad Revenue 19:1	625.00	
Donations	20.00	
Checking Account Interest	24.57	
Interest Earned on Certificates of Deposit	17.03	$ 1,743.60
EXPENSES:		
Meeting Announcement/Program Printing & Mailing	$ 400.00	
Journal Printing	151.72	
Professional Fees	1,000.00	
Treasurer's Expenses	52.81	$ 1,604.53
ASSETS ON 4 FEBRUARY, 1988:		
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Certificate of Deposit No. 10-8829764	4,536.73	
Certificate of Deposit No. 111-849-6	3,032.95	
Checking Account No. 015210-01	5,915.34	$15,752.05

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. Guidelines for submission of articles are given below. The views expressed in the articles, editorials and letters represent the opinions of the author(s) and do not reflect the official policy of the institution with which the author is affiliated or the Texas Society for Electron Microscopy. Acceptance by this Journal of advertisements for products or services does not imply endorsement. Manuscripts and related correspondence should be addressed to Ronald W. Davis, Editor, TEXAS SOCIETY FOR ELECTRON MICROSCOPY JOURNAL, Department of Medical Anatomy, Texas A&M University, College Station, Texas 77843.

GUIDELINES: Manuscripts written in English will be considered for publication in the form of original articles, historical and current reviews, case reports and descriptions of new and innovative EM techniques. It is understood that the submitted papers will not have been previously published. Accepted manuscripts become 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 manuscripts lost in the mail.

PAGE PROOFS/REPRINTS: The editor will be responsible for proof-reading the type-set article. Reprints may be ordered from the printer.

MANUSCRIPT PREPARATION: Manuscripts should conform 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 (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 parentheses and listed at the end, as in the following examples:

(1) A. Glauert, Practical Methods in Electron Microscopy. Vol. 2 (North-Holland. Amsterdam, 1974) 82-88.
(2) P.S. Baur, Jr., G.F. Barratt, G.M. Brown and D.H. Parks. Ultrastructural Evidence for the Presence of "Fibroclasts" and "myofibroclasts" in Wound Healing Tissues. J. of Trauma. 19 (1979) 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:

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

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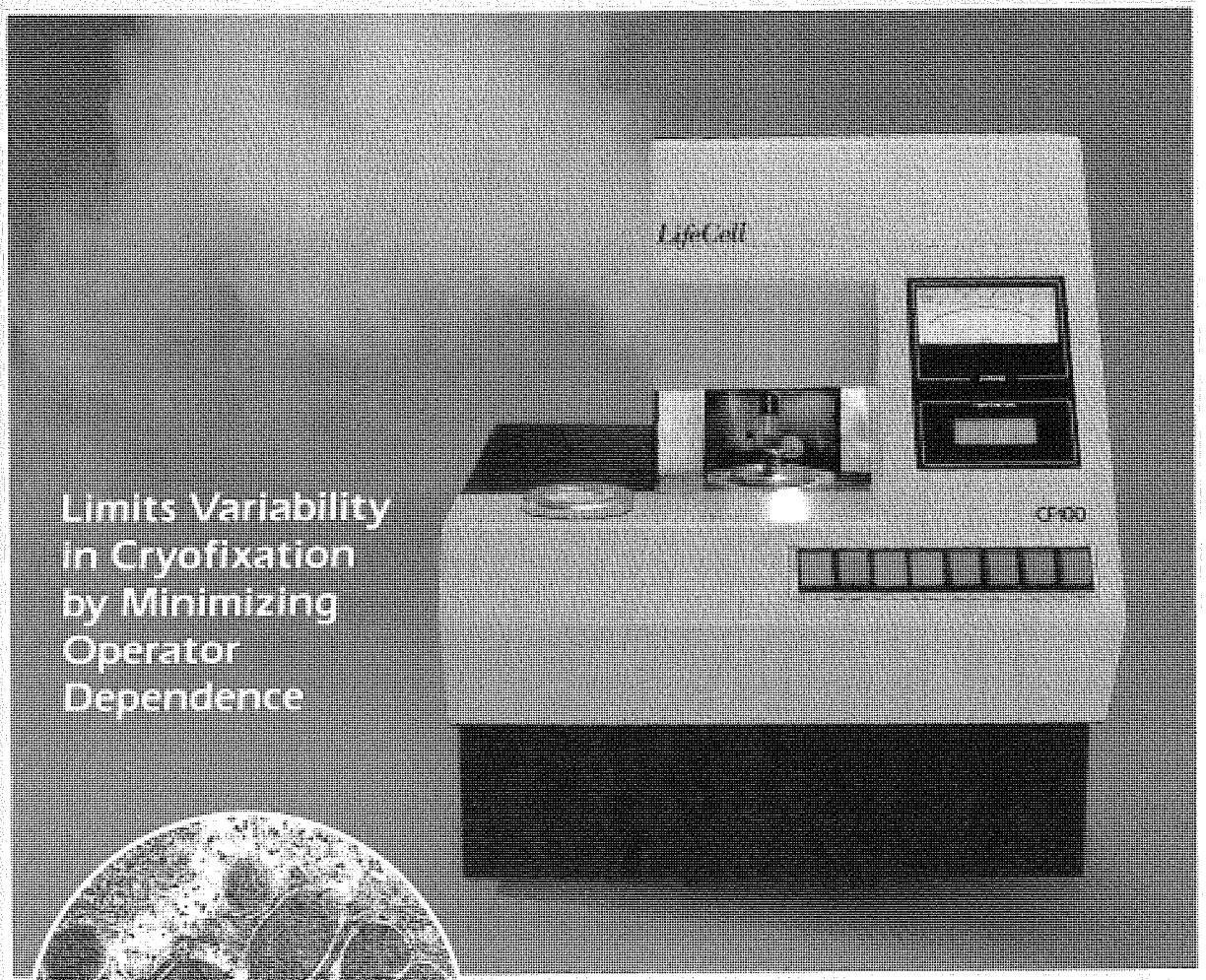

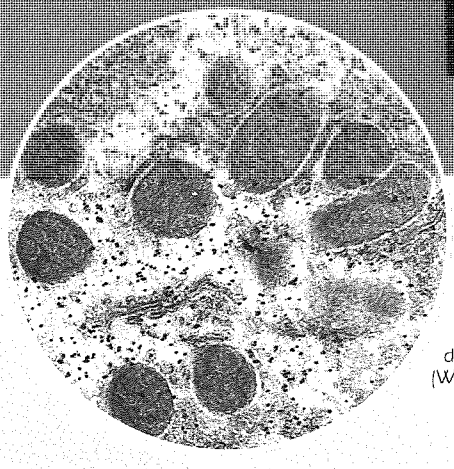

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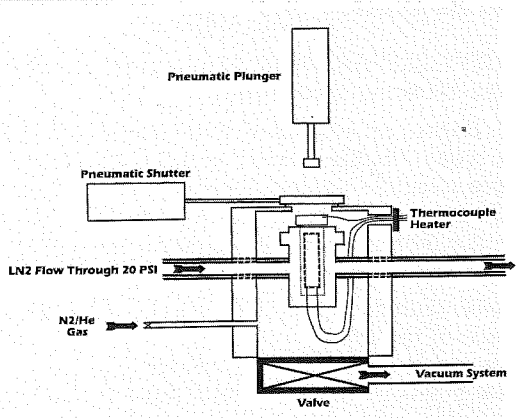

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IMMUNOGOLD LABELING IN PATHOLOGY

By

Hal K. Hawkins and Linda S. Rehm
Department of Pathology
Baylor College of Medicine
Electron Microscopy Laboratory
Texas Children's Hospital
Houston, Texas

INTRODUCTION

Colloidal gold labeling of tissue antigens provides a practical means to detect and localize macromolecules at the ultrastructural level. It promises to provide practical access to a realm of structural analysis which had been almost inaccessible. This tantalizing promise, of course, is limited by the necessity to control the many variables which affect all immunostaining methods. (17) Immunogold staining is not an easy technique and requires some investment in time for development. Furthermore, there are differing technical requirements for different antigens. However, after moderate effort in development, colloidal gold staining can be applied as a routine to the study of many antigens. We have also found that there are important benefits in sharing the technical experience of others. Indeed, the application of these methods in our lab was made possible by the consultation of Drs. Horisberger, Papermaster and Schneider in their laboratories in Switzerland and San Antonio. This paper is intended to provide the reader with some benefit from our experiences with colloidal gold.

At Texas Children's Hospital our work is divided between diagnostic pathology, research and research support. During the past few years we have selected and tested several simple, straightforward methods for immunogold staining. Most of this work involves labeling thin sections of tissue embedded in acrylic plastics. In addition, we have worked with the technique of labeling cell surfaces for transmission or scanning electron microscopy, and have tested methods for combining immunogold labeling and negative staining of viral particles.

MATERIALS AND METHODS

Preparation of Gold Sols

Colloidal gold sols are commercially available in a variety of sizes. For a small investment in time and roughly 50¢ in materials, however, it is possible to prepare colloids of any desired size in any laboratory. Use of very clean, perferably reverse osmosis water and clean, siliconized glassware or plasticware (see Editor's Note 1) is advisable. The method we found best for preparing particles 4-30 nm in diameter is a variant of Ostwald's tannic acid method developed at Utrecht. (16) The method is simple and produces particles with a very narrow spread of sizes; the standard deviation of diameter is less than 10% of the mean. For larger particles, gold chloride is reduced with trisodium citrate using the method of Frens. (3) The size of the particles produced can be determined by examining the sol by EM after drying on a Formvar-coated grid, or by using a pseudoreplica method. (Fig. 1)

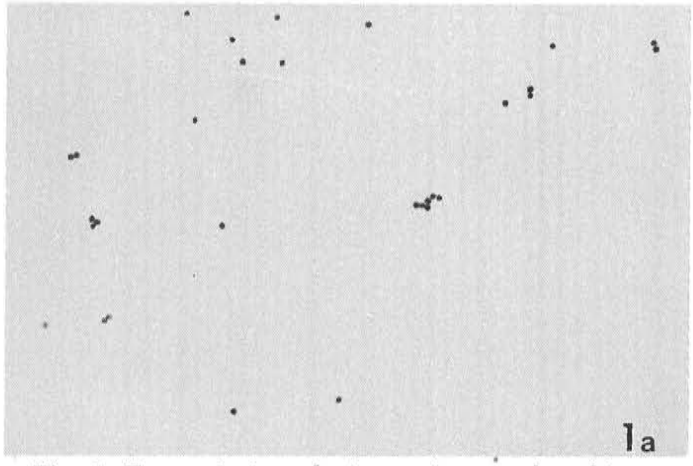
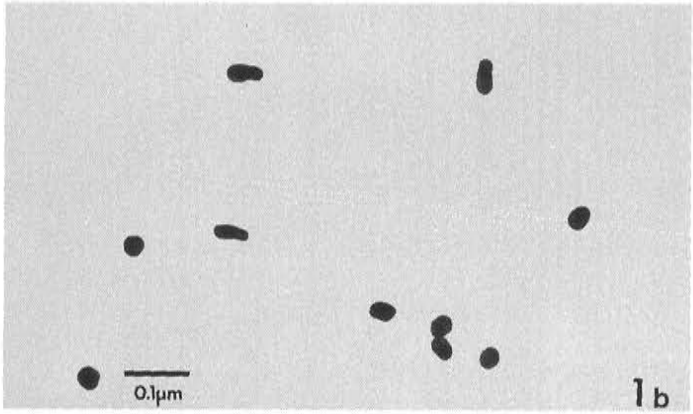


Fig. 1. Transmission electron micrographs of two colloidal gold preparations, taken at the same magnification. (a, upper) prepared with the tannic acid method, (b, lower) prepared with citrate. The larger particles, suitable for scanning EM work, tend to be more elongated.



Multiple Techniques of Labeling

The major advantage of the colloidal gold technique is its extraordinary versatility. (5) Many different types of macromolecules can be adsorbed on gold particle surfaces, to take advantage of a variety of methods of labeling. A primary antibody of interest can be bound directly to gold particles for a one-step labeling method. Alternatively, a second antibody such as goat anti-rabbit IgG can be bound to gold and used to mark sites of binding of a primary antibody. Protein A-gold can be used to recognize complete IgG molecules or avidin-gold can be prepared to bind any biotin-labelled ligand. (12) The latter method provides a very widely useful probe, since nucleic acids, small peptides, carbohydrates or lectins can be tagged readily with biotin in a light-activated reaction. (Vector Photoprobe). Commercially available histologic reagents can be used (ABC kits). Multiple labeling can be done, blocking unreacted binding sites on avidin with free biotin between steps. For preparation of colloidal gold probes, streptavidin (available from Enzo, Boehringer-Mannheim or Tago) offers an advantage over egg white avidin due to its neutral isoelectric point.

A number of other creative techniques have been devised for labeling with colloidal gold, but have not been tried in our lab. A hormone, drug or carbohydrate moiety can be linked to a macromolecule such as albumin and bound to gold particles, to detect cellular binding sites or receptors. An antigen can be bound directly to gold particles, with use of the antibody molecule as a bridge to link cellular and gold-bound antigens (the GLAD technique). (9) A particularly clever variant is to use an enzyme-gold complex to localize the enzyme's specific substrate. (10)

Binding Macromolecules to Gold Particles

Colloidal gold sols have a rich dark red color. They are very sensitive to the presence of dissolved ions, rapidly turning dark blue and precipitating in the presence of 0.1 M or more salt. When the gold particles are coated with protein, they are resistant to dissolved ions and remain stable in concentrated salt solutions. This property makes it very easy to determine the optimum quantity of macromolecule needed to optimally coat a new batch of colloidal gold. We do a series of twelve two-fold dilutions of the protein ligand in a microtiter plate, using 100μl each. An equal volume of the gold sol is added to each well and mixed, followed by 20μl of 10% NaCl. The color changes from pink to blue with greater dilution of protein. (Fig. 2) One simply observes the last well with no color change to determine the quantity of protein required (the nth well contains one volume protein: 2 volumes gold sol). Then the gold colloid should be poured into a small excess of protein. If desired, one may use a spectrophotometer to select the proper ratio. We then add 1/20th volume of 1% polyethylene glycol (PEG-20 or PEG-20M) and centrifuge the resulting probe for 10 to 30 minutes at 10,000 to 30,000 X G in an angle rotor, carefully aspirate the liquid phase and resuspend in stabilizing buffer (0.15M NaCl, 0.05M Tris pH 7.0, 0.5 mg/ml PEG-20 plus $CaCl_2$, MgCl and $MnCl_2$, .001M each) (7) Dr. Horisberger has shown in quantitative work that the optimum quantity of protein selected in this way also produces the most stable and effective probes. (8) To avoid clumping of the gold preparation, it is essential to bind the gold to protein which is completely dispersed. The protein must be dialyzed overnight against water or .005M HEPES buffer, and should be spun in an airfuge or at least passed through a micropore filter to remove aggregates. The gold sol should be titrated to ph 7.6 for IgG or streptavidin, or just above the protein's isoelectric point, using 0.2M potassium carbonate and pH paper. To prepare protein A-gold, the gold sol is titrated to pH 6. (6)

Embedding in Polar Resins

Several hydrophilic resins have been made available recently for embedding tissue in hard plastic with minimal denaturation. At least two of these, LR White and Lowicryl K4M, are practical for widespread use. LR White (medium grade), a clear acrylic resin with very low viscosity (8cp), is particularly simple to use. The manufacturer recommends dehydrating tissues only to 70% ethanol, then transferring directly to several changes of the resin (see Editor's Note 3).

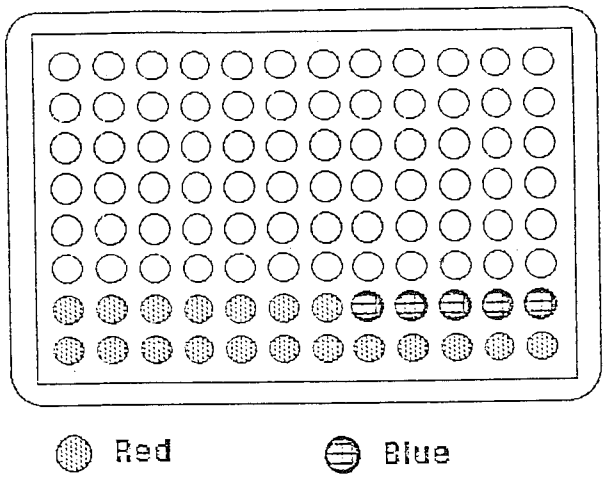


Fig. 2. Diagram of the microtitration method for determining the optimal ratio of protein to colloidal gold sol. The bottom row contains the gold sol mixed with an equal volume of water, as a color reference. In the next row, serial two-fold dilutions of protein have been mixed with equal volumes of gold sol and 1/10th volume of 10% NaCl, and mixed. Wells 8-12 have turned blue, while well 7 remains pink. Since well 7 contains a volume ratio of protein to gold sol of 1:128, addition of 100 parts of the gold sol to 1 part of dialyzed protein should produce a stable colloidal gold probe.

After overnight infiltration, blocks are hardened at 55-60°C for 24 hours in full, tightly closed gelatin capsules. (14) The resulting block can be sectioned easily, but should be picked up on Formvar-coated grids for extra stability. Coated slot grids are very useful for locating scarce antigens. Gold and gilded grids should be used to avoid corrsion caused by copper grids and astigmatism caused by the magnetic properties of nickel. (13) Lowicryl K4M can also be recommended, particularly when used with the simplified method of Dr. Papermaster and his colleagues. (1,15) Lowicryl is slightly more difficult to section then LR White, but provides very low background staining and may preserve some antigens better than LR White. For immunochemical work, tissues are ordinarily fixed in picric acid-glutaraldehyde, (see Editor's Note 2) osmium is avoided in processing.

RESULTS

The Endocrine Pancreas

One of the most interesting subjects amenable to study by EM immunochemical methods is the endocrine pancreas of infants with persistent hypoglycemia. Several babies have been referred to TCH recently who had very low blood glucose levels and required constant intravenous infusion of glucose in spite of pharmacologic therapy. Three required surgical therapy in the form of subtotal pancreatectomy. Their tissues were immediately fixed for immunoelectron microscopy. A group of abnormalities has been described in such patients (''nesidioblastosis''), but much work remains to define the differences from age-matched controls and to clarify the abnormality of endocrine regulation responsible for the disease. The immunogold technique is well adapted for such studies. Antisera are commercially available which bind to insulin, glucagon, somatostatin and pancreatic polypeptide. Using colloidal gold techniques and LR White embedding, individual endocrine granules can be unequivocally identified. Our usual technique is listed in Table I. On large thin sections, the distribution of endocrine cells can be mapped, and cells can be identified which contain more than one hormone, using adjacent thin sections or a double-labeling technique. (Figs. 3 and 4) We have been able to label endocrine granules using both conventional and monoclonal antibodies and both second-antibody and streptavidin detection methods. Insulin granules seem

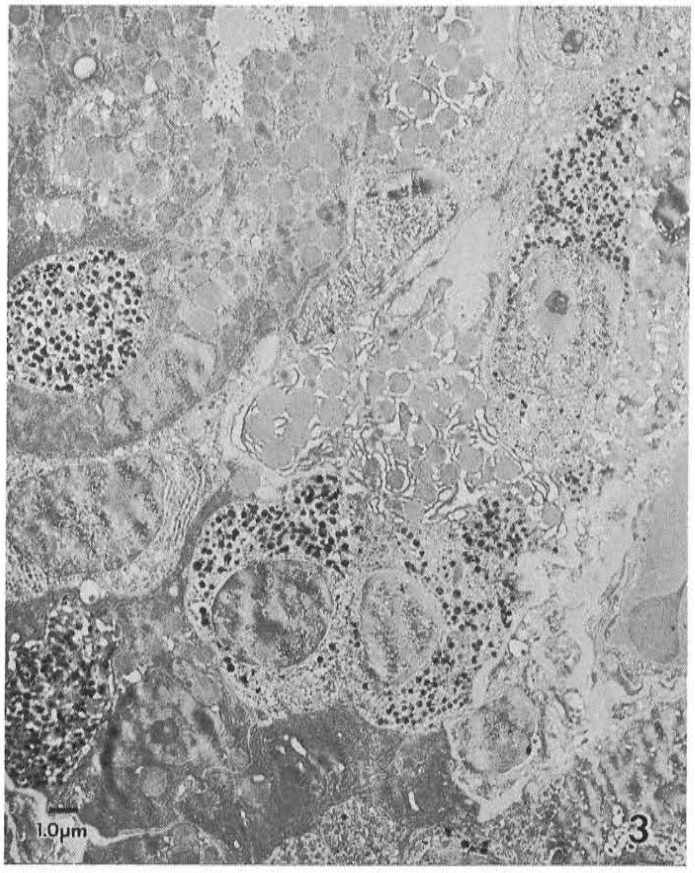


Fig. 3. Human pancreas embedded in LR White, reacted with antiserum to glucagon, marked with goat-anti-rabbit-gold (17 nm) and stained with uranium acetate. Five cells stain prominently as well as small parts of others, while other endocrine cells and exocrine pancreatic cells are not labeled.

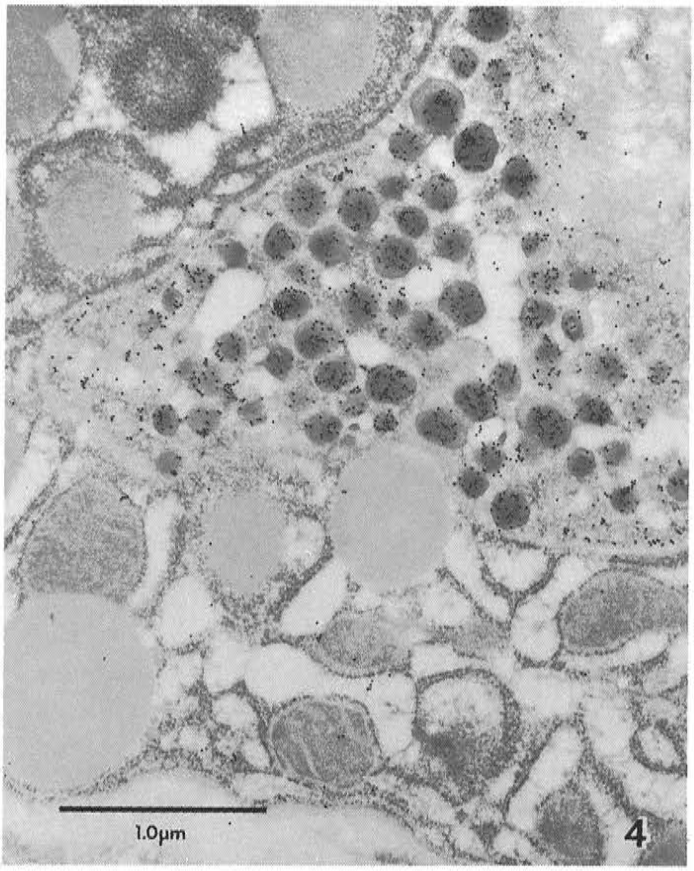


Fig. 4. Human pancreas, glucagon granules labeled with goat-anti-rabbit-gold (11 nm). In LR White-embedded tissue, membranes are clearly visible with reversed contrast.

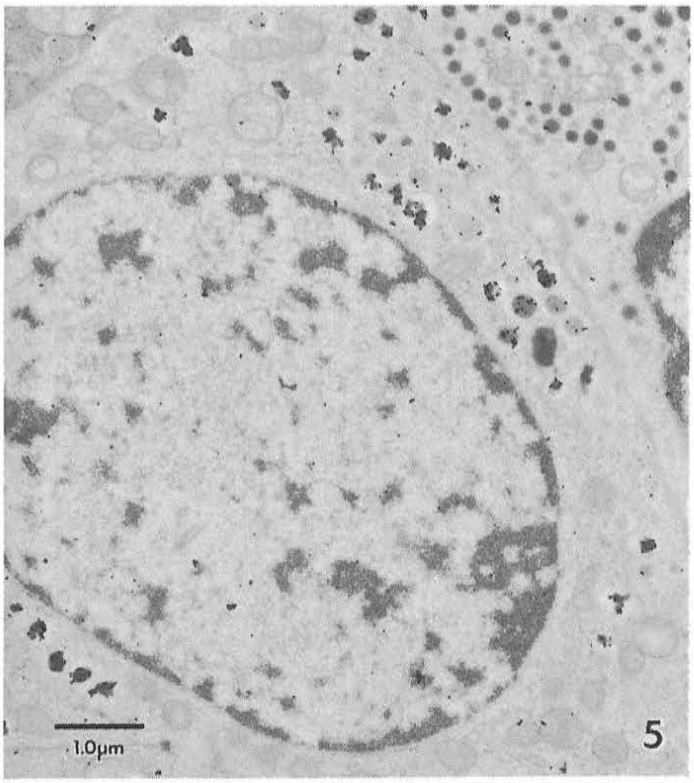


Fig. 5. Human pancreas fixed in glutaraldehyde and osmium tetroxide and routinely embedded in Araldite 502. Sections were treated with saturated sodium metaperiodate for one hour to remove osmium, then stained with guinea pig anti-insulin and rabbit-anti-guinea-pig-gold (24 nm).

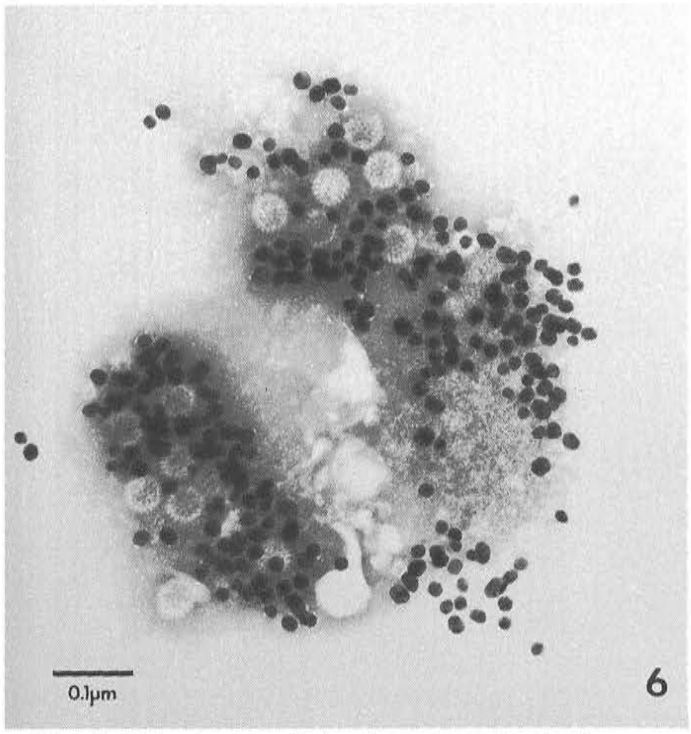


Fig. 6. Rotavirus particles from human stool specimen, negatively stained with phosphotungstic acid and reacted with antiviral antibody and protein A-gold.

to be especially resistant to denaturation, and can be labelled in tissues routinely fixed in glutaraldehyde and osmium tetroxide and embedded in Araldite resin. (Fig. 5)

Labeling Virus Particles

Detection and identification of viral particles remains one of the areas of practical application of electron microscopy. An elegant technique is available in which aggregates of viral particles clumped by specific antibody are simultaneously negative-stained and marked with protein A-colloidal gold. (4) The authors claim that this method provides greater sensitivity of detection than negative staining, and it clearly offers the possibility of distinguishing different viruses with a similar appearance, for example herpes simplex virus and cytomegalovirus. An example of gold-tagged human rotavirus is shown in Fig. 6.

Surface Labeling and Scanning EM

One of the most straightfoward applications of colloidal gold labeling is the detection of antigens and receptors on cell surfaces. For this purpose it is not necessary to embed or even fix the cells before application of the ligand. Monoclonal antibodies, which may be more sensitive to antigen denaturation than conventional antibodies, can be used in most cases. After surface labeling, cells can be conventionally processed and sectioned. They also can be examined by scanning electron microscopy. Recently, we have studied cells stimulated by the chemoattractant fMLP; in some cases albumin-coated latex beads have been used to identify adhesion sites. Cells are fixed in suspension in 1% glutaraldehyde, rinsed and allowed to settle onto polylysine-coated coverslips. (11) Monoclonal antibody directed against the adhesion protein Mac-1 is then added, followed by affinity-purified goat anti-mouse IgG (Boehringer-Mannheim or Tago) bound to 30-40 nm colloidal gold. Cells are critical-point dried and heavily coated with carbon. Colloidal gold particles can be imaged selectively using the backscattered electron (BSE) method, due to the sensitivity of the BSE signal to atomic number. (2) With our SEM equipment (JEM 100CX STEM) optimal results are obtained with BSE imaging in the differential mode, which images gold particles vividly at bright spots with dark shadows. (Figs. 7 and 8)

Positive Control Tissue: Mouse Pancreas

At the editor's request, we selected a readily available tissue which readers could use to test these methods in their own laboratories. Since the pancreatic hormones have polypeptide sequences which are well conserved among mammals, the commercially available antisera react well with animal pancreas. Thus the mouse pancreas provides a tissue which can be used for positive control purposes, which is available in almost any laboratory. Fig. 9 illustrates the results which can be expected when mouse pancreas is fixed overnight by immersion in BGPA, embedded in LR White, and the sections are reacted overnight in a refrigerator with Ortho guinea-

pig anti-insulin diluted 1:100 from the prediluted kit supplied by Ortho. This is a representative result, and should be reproducible anywhere.

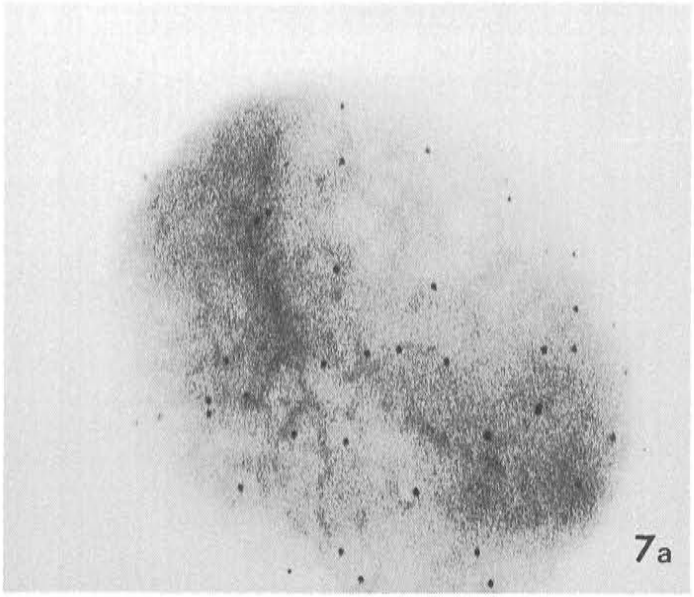
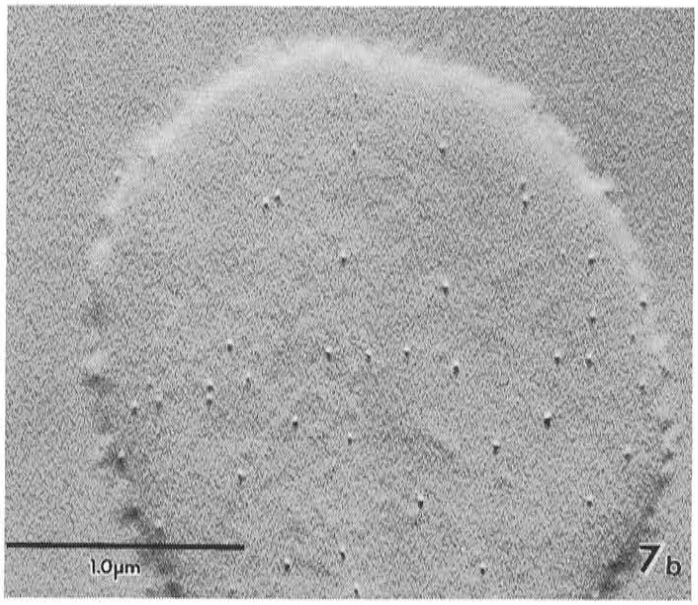
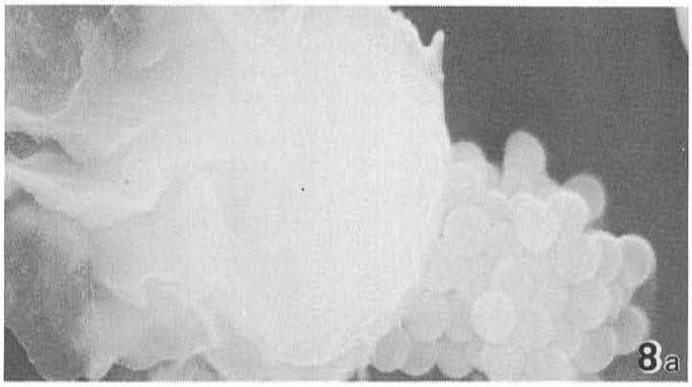


Fig. 7. Human polymorphonuclear leukocytes exposed to monoclonal antibody to the Mac-1 adhesion glycoprotein after fixation, then labeled with goat-anti-mouse-IgG-gold. (a, upper) Backscattered electron signal, reversed polarity. (b, lower) Same cell, signal processed in differential BSE mode.





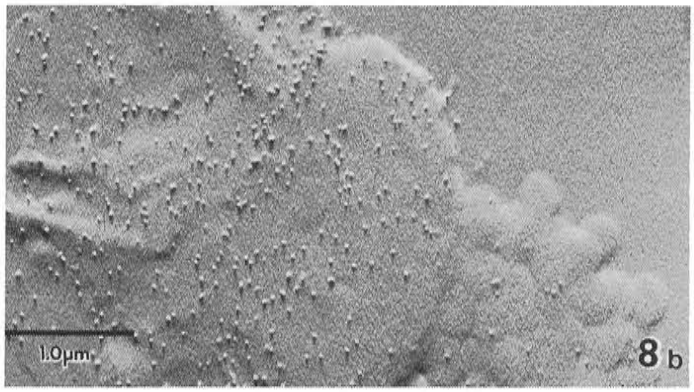


Fig. 8. Human polymorphonuclear leukocytes treated in suspension with 0.1 nm fMLP, then 5 nm fMLP, then incubated with albumin-coated latex beads to mark adhesion sites and fixed. Cells were incubated, as above, with monoclonal anti-Mac-1, then with goat-anti-mouse-IgG-gold. Scanning electron microscopy. (a, upper) Secondary electron signal, (b, lower) Differential backscattered electron signal.

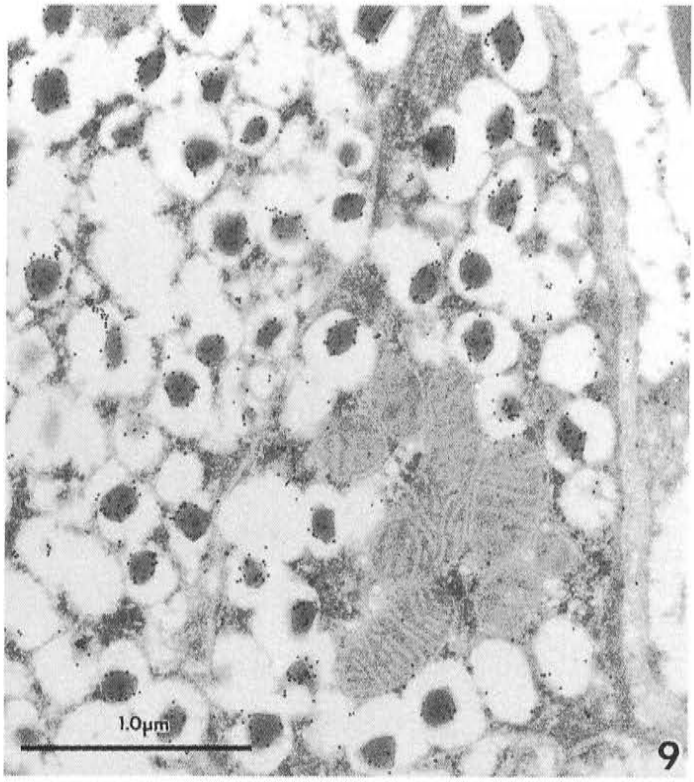


Fig. 9. Mouse pancreas, fixed in BGPA, incubated overnight with a 1:100 dilution of Ortho guinea pig anti-(human)-insulin, followed by rabbit-anti-guinea-pig-IgG-gold. The rectangular insulin granules are marked by gold particles, while mitochondria and red cells are not labeled.

SUMMARY

A wide variety of techniques is available for labeling macromolecules with colloidal gold particles. Many of these methods are not difficult, may be useful as routine procedures in many laboratories, and can provide uniquely distinct and satisfying results.

ACKNOWLEDGEMENTS

In addition to the invalubale advice of many colleagues in the Texas Medical Center and the members of the local "Gold Balls Club," the authors are grateful to the principal investigators in the collaborative work cited here, Drs. C. Wayne Smith, Donald C. Anderson, and Kenneth Gabbay, to Dr. Jim Kelly for help with Macintosh graphics, and especially to Dr. Milton Finegold for his support and encouragement of our work with the immunogold technique.

TABLE ONE

Indirect Immunogold Staining Procedure

1. Collect thin sections of tissue on Formvar-coated gold grids.
2. Pre-wash grids on drops of PBS for 5 minutes.
3. Incubate in 4% BSA for 10 minutes, then wash X 6 in PBS.
4. Place grids on drops of multiple dilutions of primary antibody on Parafilm, and incubate overnight in a refrigerator in closed, humidified chambers. (we use Lab-Tek square Petri dishes.)
5. Wash grid on drops of PBS X 6.
6. Place grids on drops of secondary antibody / gold reagent at desired dilutions in PBS, usually 1:4 - 1:64, for 30 minutes.
7. Wash grids in drops of PBS X 6.
8. Wash grids by dipping 20 times in each of 3 beakers of water.
9. Stain sections in 2% aqueous uranium acetate for 5 minutes and dry.

Notes:

- Use reverse osmosis water.
- Centrifuge antibody solutions before use. (airfuge or microfuge)
- Filter gold solution before use.(we use 0.45-micron S&S Uniflo #46-02340 filter units.)
- Filter PBS in advance. (we use Nalgene disposable filter flasks.)
- Prepare strips of parafilm, uncover when ready to use, and organize reagents in rows of drops.
- Float grids on drops section side down; keep backs of grids dry.
- Draw off excess fluid with filter paper between solutions.

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EDITOR'S NOTES

(1) What is siliconized glassware? After talking to Dr. Hawkins and some of the people I know at TAMU, here is what I found out.

Proteins have a tendency to attach to glass or plastic surfaces through various hydrophilic, hydrophobic or charge attractions. This has the effect of reducing the concentration of the protein in

solution. To avoid this, the glassware or plasticware can be "siliconized." This puts a hydrophobic, amorphous coat on the container which retards protein binding to the surfaces.

There are various ways to siliconize, and one of those is to use a product called Sigmacote, sold by Sigma. It comes as a liquid and is reusable. It can be swirled around, or the vessel to be coated can be filled or immersed for several hours to overnight. The coating will not last forever, but I was told that as long as water beads up on the surface it is still intact.

(2) Buffered Glutaraldehyde - Picric Acid (BGPA) has the following concentrations: Buffer .1M, Glutaraldehyde 1.0%, and Picric acid 0.2% W/V.

(3) Common problems with LR White are: Mixing LR White with 70% alcohol turns the solution cloudy. The alcohol may not actually be 70%. Try to raise the alcohol concentration a little at a time until the resin and alcohol stay clear. Dr. Hawkins tells me that dehydration no farther than to 70% appears to be a good routine procedure. Remember, if your 70% alcohol stock turns cloudy with LR White you will have to adjust the concentration of the 70% dehydration step also. Dr. Hawkins also says that several changes of pure resin are also critical.

Been capsules vs Gelatin capsules? Beem capsules are more convenient, but do not seem to work all the time. Air is believed to permeate the plastic capsules and the oxygen inhibits polymerization. Gelatin capsules seem to work consistantly.

Vacuum embedding? It is probably not a good idea. Dr. Mollenhauer feels that there is increased evaporation of the monomers and that there can be a change in the component balance. Dr. Hawkins has tried it, but does not use it.

Flat embedding? Several of us have tried various ways, but without consistant success. There is a definite need for this. Does anyone have a consistantly good technique you can give us?

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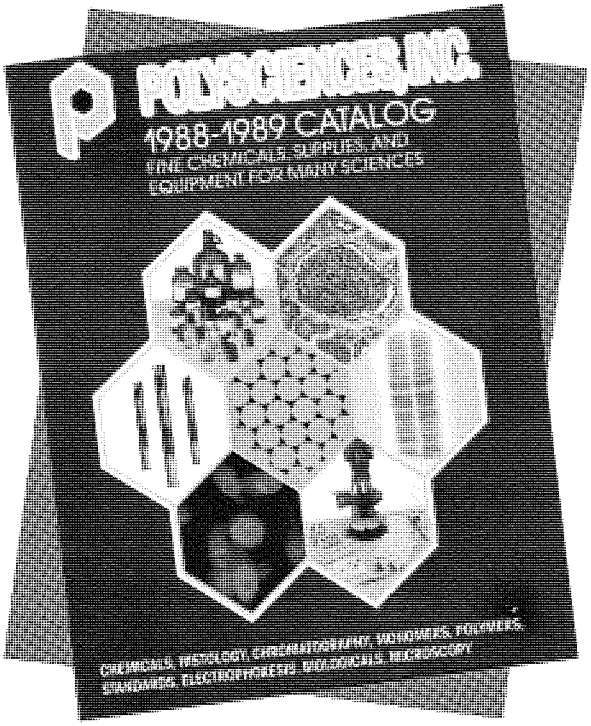

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"Student-Technician Award Entries Fall, 1987"

CRYPTOSPORIDIOSIS IN A CALIFORNIA KINGSNAKE (LAMPROPELTIS GETULUS CALIFORNIAE)

By

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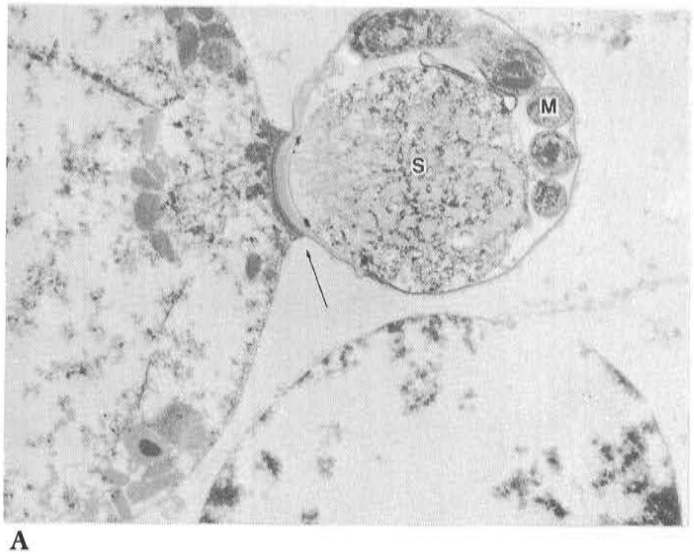
Cryptosporidiosis is a zoonotic disease found in many species of animals including man. This protozoan, coccidian parasite inhabits the respiratory, digestive and intestinal epithelium of their hosts. Though usually asymptomatic in guinea pigs, rats and other species, this parasite may be fatal to calves and humans. It is not host-specific and is usually transmitted by the fecal-oral route. Upon review of H & E slides, *Cryptosporidium* sp. was evident in the stomach and intestine of a California Kingsnake that was necropsied in our laboratory. Specimens of the kingsnake's intestine were prepared for transmission electron microscopy using routine procedures.

Upon examination of the intestinal tissue, different stages of *Cryptosporidium* sp. were observed in the intestinal lumen. Many round and oval organisms were adhered at attachment zones to the microvillar borders on the surface and pit epithelium. Also noted was residual oocyst material consisting of clear globules of different sizes. The complete life cycle of this parasite is not known; the different stages found in this sample are presented.



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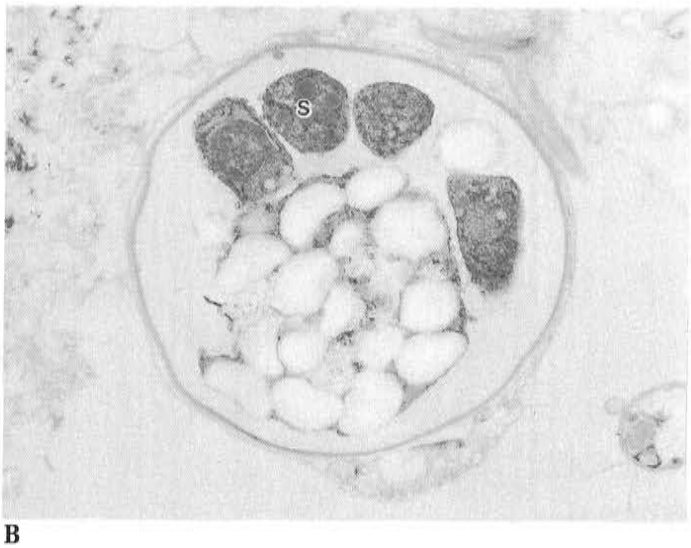

B

Electron micrographs of cryptosporidia-infected snake intestine. **A** - First generation schizont (S) adhered to epithelium at attachment zone (arrow) with five developing merozoites (M). (15,625x) **B** - A mature oocyst containing four electron-dense sporozoites (S). (33,333x)

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THE URTICATING HAIRS IN TWO SPECIES OF JUMPING SPIDERS

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The presence of defensive or urticating hairs of spiders has only been described from members of the infraorder Mygalomorphae (the tarantulas). These hairs may be an important component of the anti-predator repertoire. We utilized scanning electron microscopy to examine two species of jumping spider (*Phidippus apacheanus* and *P. audax*; Salticidae) from the other infraorder of spiders Araneomorphae for the presence of urticating hairs. Urticating hairs were found on the dorsal surface of the abdomen in both sexes as well as in both species of these spiders. These hairs correspond to Cooke *et al*'s (1972) Type II category of urticating hairs found in tarantulas. The urticating hairs of *P. apacheanus* and *P. audax* were numerous along the periphery of the abdomen, decreased in number towards the center, and appeared to be attached directly to the exoskelton by a well defined socket.

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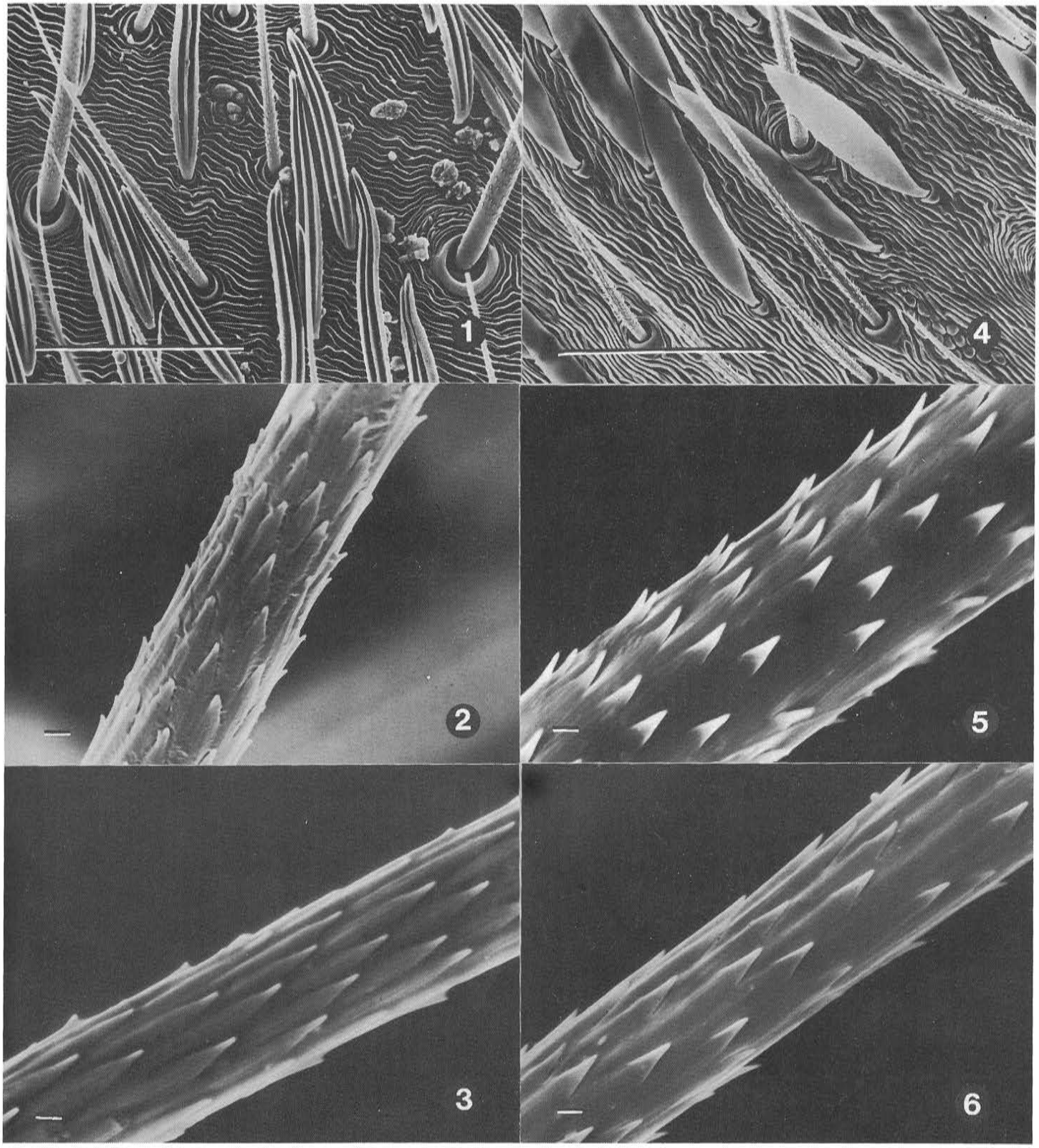

Figures 1–3. Phidippus apacheanus. Fig. 1. Survey of abdominal and urticating hairs. Bar = 100 um. Fig. 2. Urticating hair on male. Bar = 1 um. Fig. 3. Urticating hair on female. Bar = 1 um.
Figures 4–6. Phidippus audax. Fig. 4. Survey of abdominal and urticating hairs. Bar = 100 µm. Fig. 5. Urticating hair on male. Bar = 1 um. Fig. 6. Urticating hair on female. Bar = 1µm.

A SCANNING ELECTON MICROSCOPE STUDY OF *PNEUMONYSSUS SIMICOLA*

By

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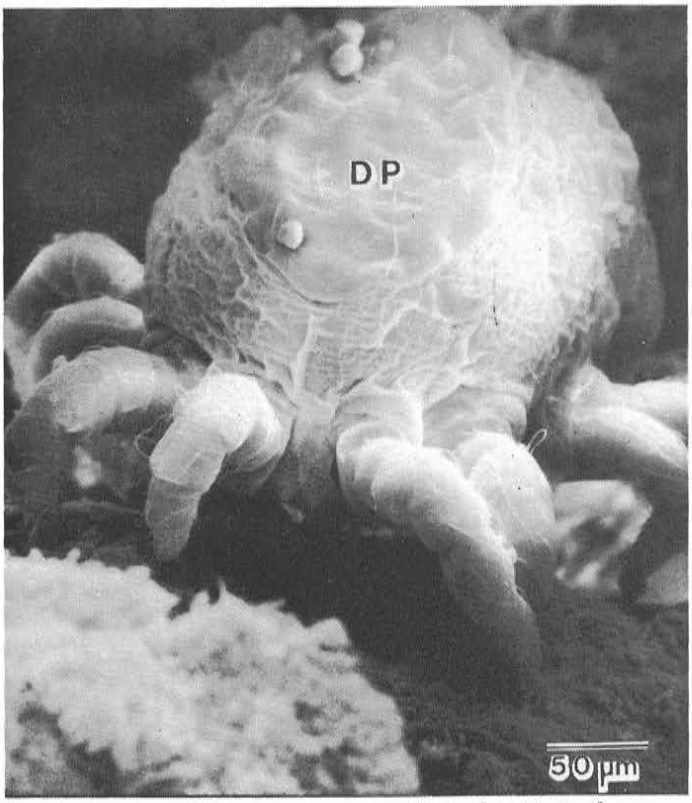
USAF School of Aerospace Medicine
Brooks AFB Texas 78235-5301

Endoparasitic respiratory mites occur in many species of animals which include bats, whales, walruses, seals, birds, dogs, rabbits, reptiles, amphibians, and bears, as well as in nonhuman primates. Respiratory mites are known to infect at least twenty-two species of primates. Although these infections are usually asymptomatic and clinically undetectable, severe infestations have been known to cause death. Lung mites have also been known to complicate experiments involving pulmonary research. *Pneumonyssus simicola* is recognized as the genus and species found in rhesus monkeys *(Macaca mulatta)*.

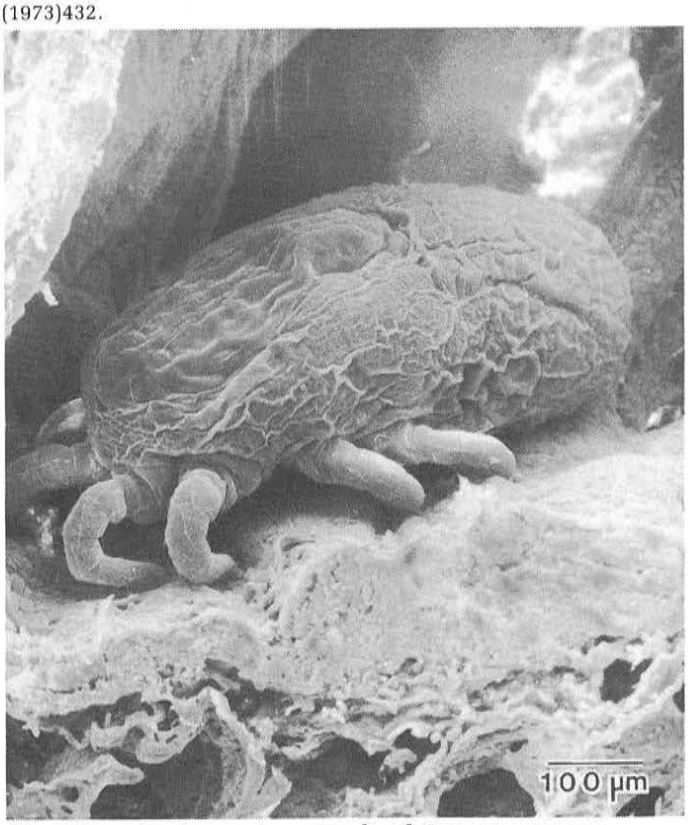
The lung tissue containing mites to be examined by scanning electron microscopy (SEM) had been fixed and stored for approximately one year in 10% buffered formalin. Tissue was prepared for examination by scanning electron microscopy. Individual mites were isolated and removed from tissue and mounted on seperate stubs.

In tissue the mites were found in terminal bronchioles where the host reaction was quite severe. Examination of mites revealed a roughened integument. The general structure of legs, gnathosoma and dorsal shield was also studied.

Examination of the lung mite by scanning electron microscopy provides a three-dimensional view of the parasite, as well as insight into the host-parasite relationship.



Frontal view of *Pneumonyssus simicola*. Note the dorsal plate (DP) with numerous setae. (x263)



Scanning electron micrograph of *Pneumonyssus simicola* in the bronchiole of a rhesus monkey. (x125)

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SATELLITE CELL INVOLVEMENT IN EXERCISE-INDUCED INCREASES IN MUSCLE MASS OF CATS

By

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Exercise-induced increases in muscle mass result from enlargement of existing muscle fibers (hypertrophy) and from increases in muscle fiber number (hyperplasia) (1). The mechanisms involved in these processes are not known. Burleigh (2) postulated that the growth potential of a muscle fiber may be dependent upon the number of nuclei within the fiber. Two populations of nuclei are associated with the muscle fiber (fig. 1). One population, myonuclei, are peripherally located beneath the muscle fiber sarcolemma and are post-mitotic (3). The second nuclear population reside in small mononucleated cells, known as satellite cells, which are found between the muscle fiber basal lamina and the sarcolemma (4). Satellite cells in adult muscle appear quiescent with a heterochromatic nucleus and scanty sytoplasm. Satellite cells have known myogenic potential. and become activated in response to injury to participate in muscle fiber regeneration (5). During early postnatal muscle growth, satellite cells fuse with growing muscle fibers to provide a source of additional myonuclei (6).

This study examined the role of satellite cells in exercise-induced muscle enlargement in seven adult cats, which performed weight-lifting exercise for an average of 194 weeks (fig. 2). Trained and control wrist flexor muscles were perfused with glutaraldehyde. Three cats received tritiated thymidine intravenously at various intervals before sacrifice for autoradiographic studies. Small muscle fiber bundles from each muscle were embedded in Epon. All myonuclei and satellite cell nuclei were counted in cross-sections from the midregion of each fiber bundle using electron microscopy. Muscle fiber areas were measured on light micrographs using a Summagraphics digitizing tablet. Myonuclei per unit area and satellite cell frequency were calculated. For autoradiographic studies, slides were coated with Kodak NTB-2 emulsion and exposed for 4 weeks at 4°C. Autoradiograms were examined using light microscopy to identify labeled nuclei. Serial ultrathin sections were used to identify the nuclei type (6).

Training resulted in an average 16.8% increase in muscle mass (range 0-67%). Trained muscles possessed both larger and smaller fibers than were found in control muscles (fig. 3). Myonuclei number per unit area in trained muscle was not significantly different from control muscle, implying that additional myonuclei were added to muscle fibers undergoing hypertrophy (fig. 4). Satellite cell frequency was not different between groups indicating that their numbers were not depleted by fusion with muscle fibers (fig. 5). The autoradiographic studies confirmed that satellite cells are stimulated to undergo DNA synthesis in response to weight-lifting exercise (fig. 6,7). Labeled satellite cells were observed in autoradiograms from trained muscles. Basal lamina was observed intervening between the satellite cell and muscle fiber (fig. 8). This suggests that satellite cells may migrate away from the muscle fiber to participate in repair processes or in new fiber formation. Cells which resembled satellite cells, but were outside the basal lamina and contained developing myofilaments were found in trained muscle (fig. 9).

In conclusion, weight-lifting exercise induces satellite cell activation and division. Satellite cells may fuse with muscle fibers undergoing hypertrophy, providing a source of additional myonuclei which are necessary to maintain the nucleus to cytoplasm relationship as the fiber enlarges. In addition, satellite cells may also leave the muscle fiber basal lamina and participate in new fiber formation.

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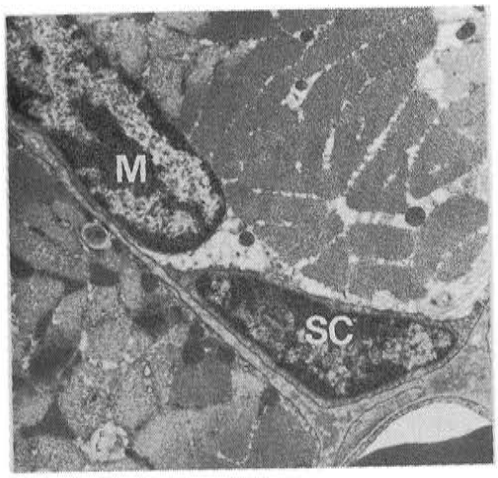

Fig.1 Electron micrograph of a myonucleus (M) and satellite cell (SC).

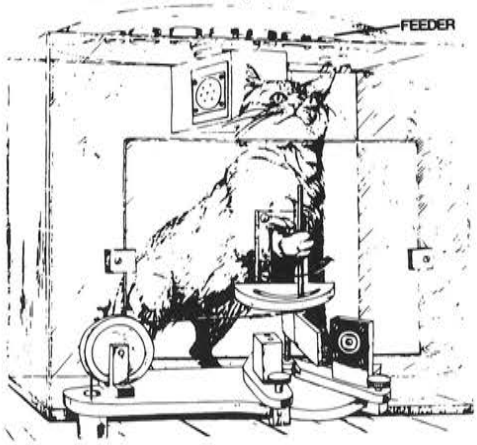

Fig.2 The cat must move the bar between stops to receive a food reward. Weights are lifted as the bar is moved.

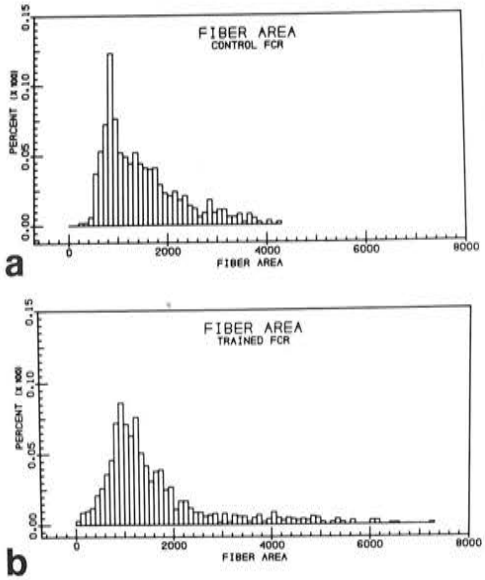

Fig.3 Muscle fiber area histograms.

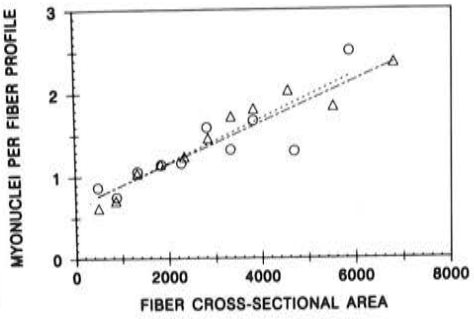

Fig.4 Plot of myonuclei to fiber cross-sectional area in control (circle) and trained (triangle) muscles.

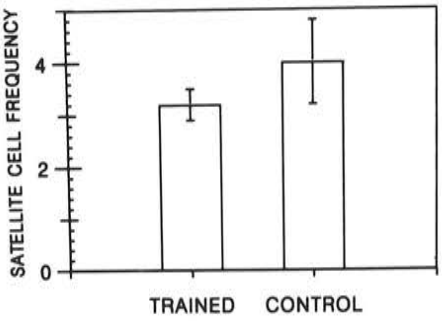

Fig.5 Bar graph of satellite cell frequency in trained and control muscles.

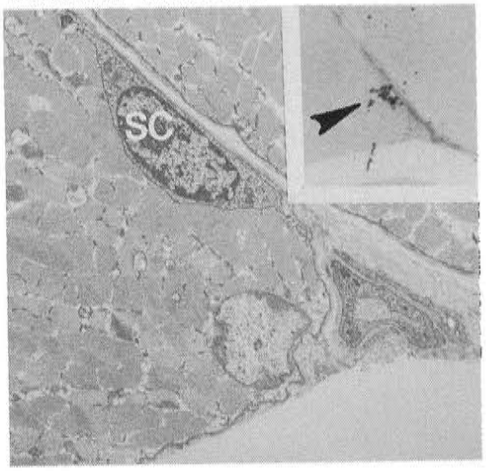

Fig.6 Inset: Light micrograph of a labeled nucleus in autoradiogram from trained muscle (arrow). Electron micrograph confirming that the labled nucleus resides in a satellite cell (SC).

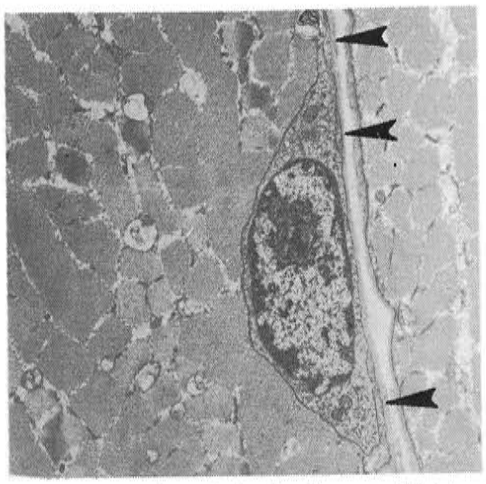

Fig.7 The labeled satellite cell has increased cytoplasm and organelles. Muscle fiber processes appear to be engulfing the cell (arrows).

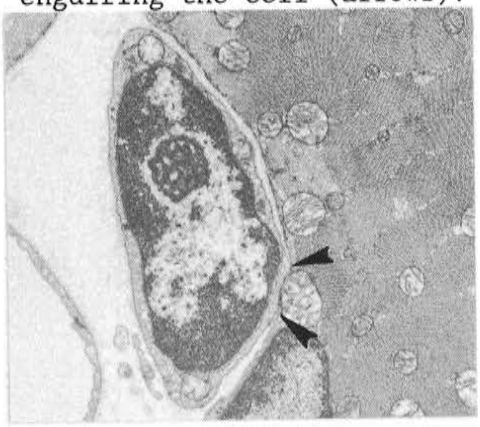

Fig.8 Satellite cell separated from muscle fiber suggesting that it may leave the basal lamina.

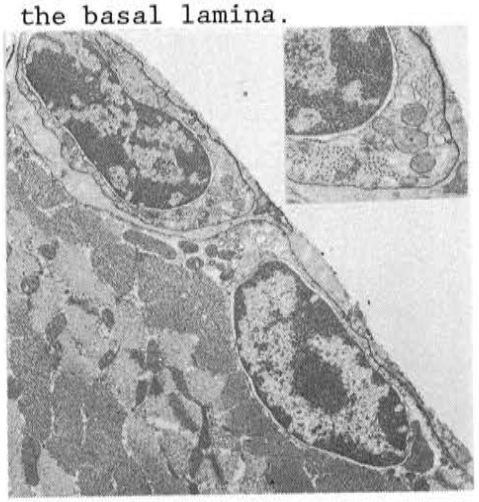

Fig.9 Satellite-like cell which contains myofilaments (inset). Fusion of such cells may result in new fiber formation.

ELECTRON MICROSCOPY OF LIPID DEPOSITION IN CYCLOSPORINE-TREATED MICE BEARING CARDIAC ALLOGRAFTS

By

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INTRODUCTION

Chronic rejection in cardiac allografts is cyclosporine A (CYA) resistant and shows an accelerated atherosclerotic process.[1] CYA is associated with increased plasma cholesterol in those taking CYA as an immunosuppressive against kidney rejection as well as in those using CYA as a treatment for certain other autoimmune diseases.[2] Whether the pathologic mechanism of chronic rejection is heart transplants results from a low-level inflammatory response or is caused by a change in lipid metabolism is unknown. To address these questions we utilized an ectopic murine cardiac allograft model in a recipient mouse strain that has been shown to develop high plasma cholesterol on a normal diet.

MATERIALS AND METHODS

Adapting a method utilized previously in our laboratory, we grafted newborn murine hearts into adult mouse ears by incising the pinna of the ear and placing a newborn heart subcutaneously with a trocar.[3] CYA or an olive oil, alcohol, and saline vehicle were administered daily intramuscularly at a concentration of 30mg/ml while some animals were grafted with no treatment. Graft survival was determined by observing pulsatility in the mouse's ear with a dissecting microscope. High plasma cholesterol (HPC) mice were used as the recipients and a low plasma cholesterol strain were used as donors.[4] Periodically, the grafts were removed and frozen sections were cut and stained for lipid with oil red O. Glutaraldehyde-fixed grafts were stained for lipid using the OTO method and processed for electron microscopy.[5] To test plasma cholesterol in the grafted and ungrafted mice, 50 ul of plasma was taken to assay by spectrophotometric method.

RESULTS

Animals grafted and given CYA had 50%, 75%, 75% and 100% pulsatile allografts at D7, D14, D21, and D28 respectively while allografts of untreated and vehicle-treated animals were no longer pulsatile by D28. Oil red O staining of frozen graft sections showed significantly more lipid staining in CYA-treated HPCs than in vehicle-treated and untreated allografts. Before grafting or treatment, plasma cholesterol levels for normal mouse (C57BL6) serum was low whereas HPC cholesterol was higher. After two weeks of CYA or vehicle treatment alone, the serum cholesterol rose significantly for the HPC mice. Electron microscopic inspection of the graft site showed osmophilic lipid droplets in the greatest concentration intracellularly in fibroblasts, mononuclear cells, and cardiac myocytes. Extracellular lipid was detected near or within interstitial connective tissue. The vehicle-treated animals showed fewer inclusions in each of these categories and allograft tissues showed minimal lipid accumulation in the tissues and times surveyed.

DISCUSSION

Our data suggests that CYA and /or its vehicle change lipid metabolism in treated HPC mice and cause increased lipid deposition in their cardiac allografts. It is possible that the liver's cholesterol sythesis is altered by CYA or that the vehicle is converted into cholesterol and other lipids which appear in the blood serum. The lipids also appear in the graft and it is possible that this lipid deposition could eventually devitalize the graft. This may be the same kind of CYA-resistant chronic rejection which produces accelerated atherosclerosis in human transplant patients.

CONCLUSIONS

A model for CYA-resistant chronic allograft rejection is suggested in which murine cardiac allografts in CYA-treated HPC mice develop an accelerated lipid deposition in graft tissues and high serum cholesterol. More study needs to be devoted to the question of why vehicle-treated HPC mice grafts do not develop as much graft lipid despite elevated serum cholesterol levels.

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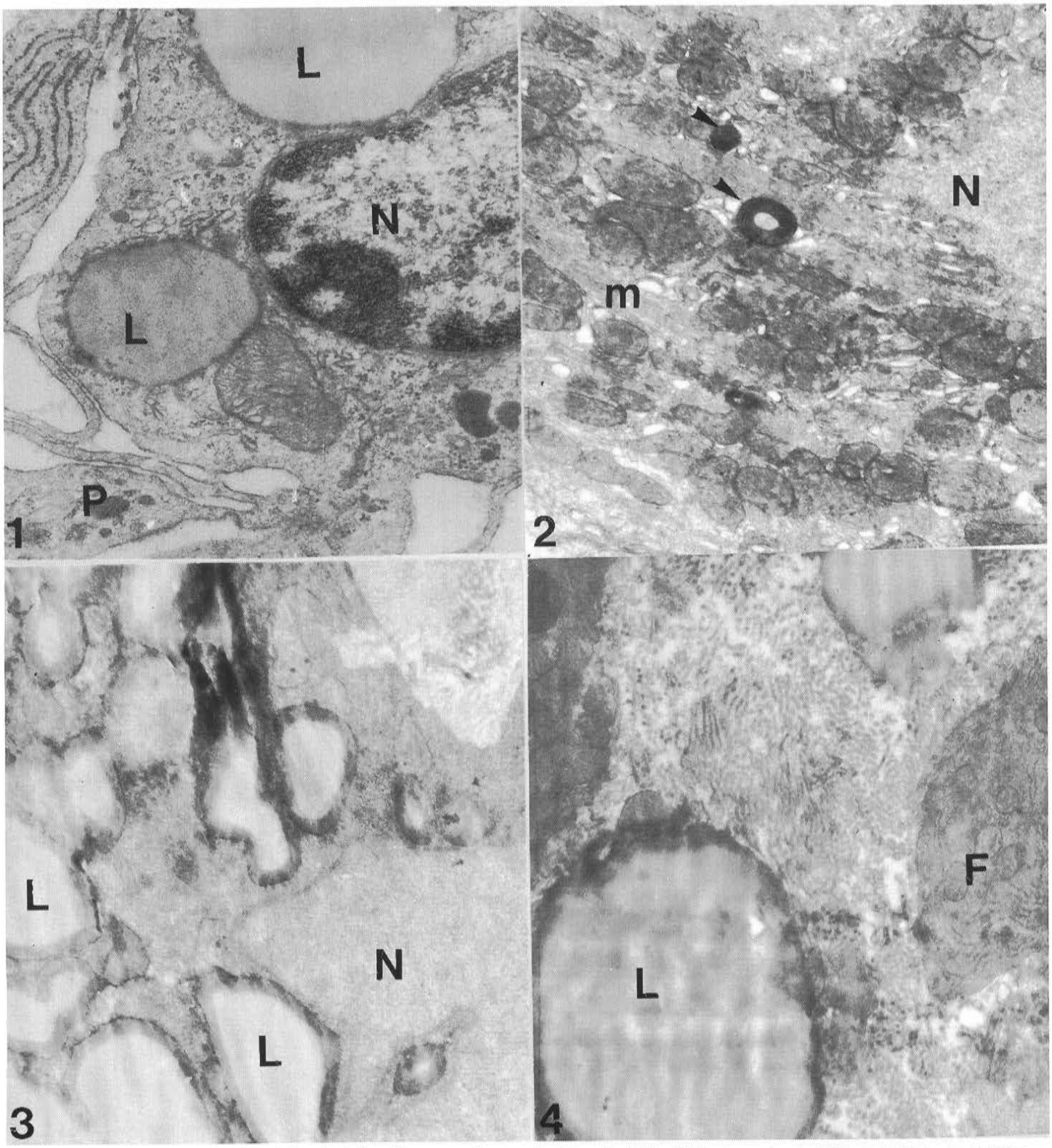

1. Intracellular lipid within Mononuclear Cell of D12 CYA-treated HPC murine cardiac allograft. L = Lipid Inclusion N = Nucleus P = Process of Mononuclear Cell.
2. Intracellular lipid within Myocardial Cell of D12 CYA-treated HPC murine cardiac allograft. m = myofibrils. Arrows point to OTO-philic lipid.
3. Intracellular lipid within Fibroblast of D12 CYA-treated HPC murine cardiac allograft. Note: Lipid droplet is in the fold of an invaginated nucleus.
4. Extracellular lipid near collagen of D12 CYA-treated HPC murine cardiac allograft. F = Fibroblast

SYNAPTONEMAL COMPLEX RECONSTRUCTION FOR KARYOTYPING *ACHLYA RECURVA*

By

H.R. Williamson and J.T. Ellzey

The karyotype for *Achlya recurva* was established by Win—Tin and Dick (1975) using light microscopy and the haploid chromosome number for *Achlya recurva* is said to be n = 6. Since the light microscopic methods for karyotyping have been questioned for organisms with small nuclei (Tanaka et. al. 1982), electron microscopy and synaptonemal complex reconstruction are needed to accurately count all the chromosomes in *Achlya* spp.. Cultures of *Achlya recurva* have been grown for two days on Barksdale #5 liquid medium and transferred to sterile distilled water and sterile tap water (1:1) which sychronizes the cultures in the sexual cycle. Cultures 24 hours after inoculation on water show pachytene prophase I nuclei with synaptonemal complexes, therefore these cultures were fixed for electron microscopy. Serial sections of oogonia and antheridia have been taken and photographed on the Zeiss EM-10A. The synaptonemal complexes in these photographs have been traced and analyzed using an HP Vectra with a digitizer and the "Metamorphosis" program to create a three dimensional image of meiotic nuclei with synaptonemal complexes. The reconstructions are being utilized to accurately determine the correct karyotype for the fungus. Other characteristics of the synaptonemal complex in Oomycetes have not been confirmed, so further investigations concerning the synaptonemal in Oomycetes are in progress.

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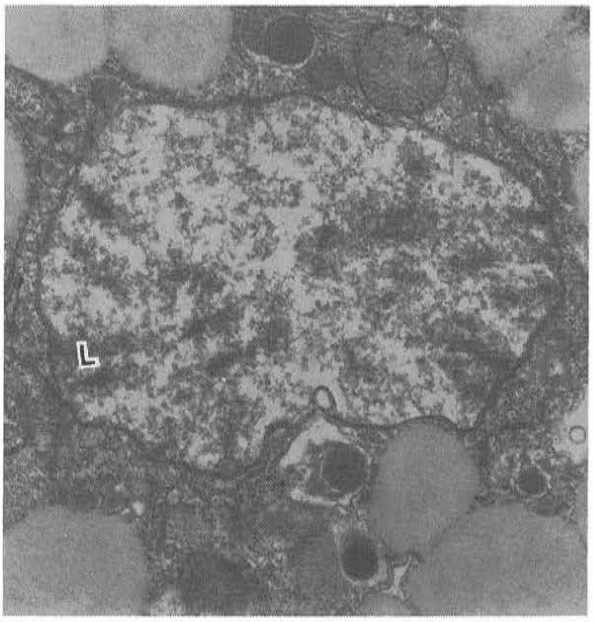
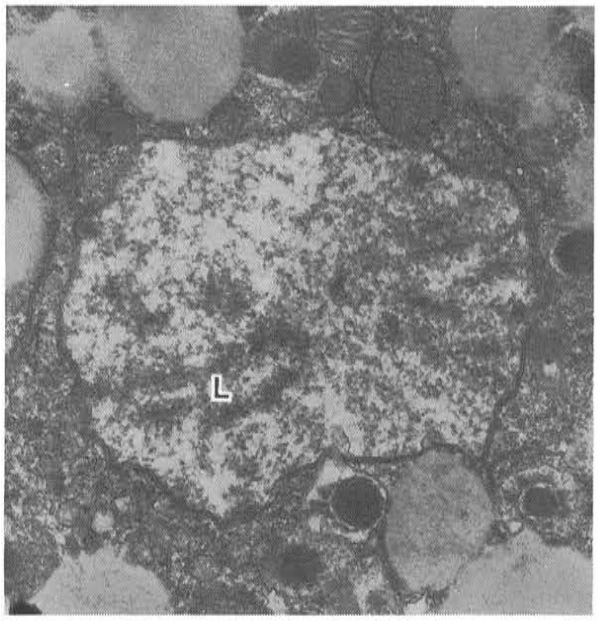
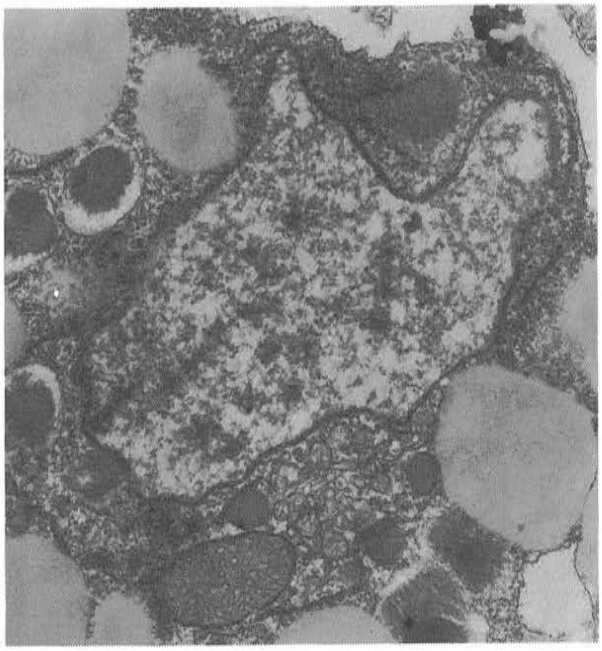
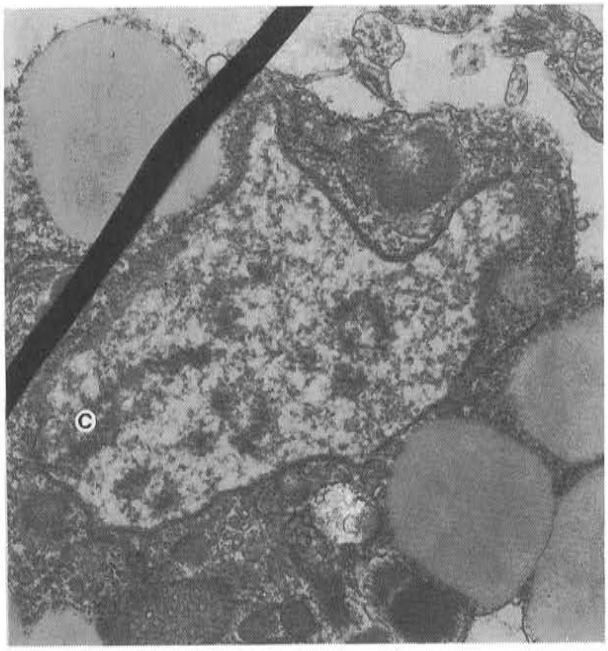




Figures 1 and 2. Micrographs of two successive sections of a meiotic nucleus in *Achlya recurva*. Paired lateral elements of the synaptonemal complex (L) show continuity between the two sections and extend half the distance across the nucleus from the nuclear envelope (sections 117 and 118 of 230, 20,000x).





Figures 3 and 4. Micrographs of two successive sections of another meiotic nucleus in *Achlya recurva*. Synaptonemal complex lateral elements are seen with no distinct central element. The twisting of the elements shows an area which may be part of the central element (C). (Sections 176 and 177 of 230, 20,000x).

ADVERTISERS INDEX

Editor's Message

USE IT OR LOSE IT

By

R.W. Davis
TSEMJ Editor
TSEM Program Chairman Elect
Research Associate, TAMU

About a year ago, the TSEM Executive Council decided to offer cash awards to students and technicians that presented papers demonstrating superior work using electron microscopy. The idea was to offer 0-3 cash awards of $100.00 each, and hopefully increase both student and technician participation in the Society. This proposal received much thought and discussion before being implimented. I know, because I was present during much of the discussion.

The first competition was during the Bandera meeting in October, 1987. There were six entries, and three awards were given. This time, for the Dallas meeting, there were no entries submitted by the deadline. There were, however, about six inquiries after the deadline. Personally, I find it amazing that there is such a lack of interest in this program. A common complaint by both students and technicians is that they feel they are not included in the proceedings of the professional organizations. In the case of TSEM, we, as a Society, have bent over backwards to invite this kind of participation, but by anyone's measure it has, so far, met with limited success.

TSEM has offered students both travel assistance and an opportunity to get a cash award for doing only two things: 1) showing up at a meeting and 2) giving a paper. I do not care how you look at it, that is a good deal — and for doing no more than what you are supposed to be doing in the first place! The deal is not quite as good for technicians, but then we are supposed to professionals and not as poor as when we were students.

The real point is, however, that what is being offered is an opportunity to present *your* work in a professional atmosphere. Very often when I have encouraged students or technicians to present papers at meetings, I get the same old lame excuse, "I don't like to get up in front of people." Well, in TSEM, that excuse does not hold water. The Society has a long standing and clearly demonstrated history of being supportive with the student and technician members. If you feel uneasy, there is no better place to get started and to learn to feel comfortable.

Realistically, I know that it is going to take student advisor and researcher support and insistance to get this program going. In my opinion the potential rewards are too great to let it drift away. Students need the experience of organizing and delivering scientific papers. If they are afraid to get up in front of a TSEM group, what do they think it will be like at a thesis defense, a job interview, or a national meeting?

If technicians want to be treated as professionals they are going to have to act like professionals and, like it or not, speaking to groups is part of it. Benefits from this type of participation should be realized by both the technicians and the researchers, but encouragement and support from the latter will be necessary.

If students and technicians do not take advantage of the opportunities they have in TSEM, the opportunities will, in time, disappear. Worse than that, the people that helped create the opportunities will wonder why they should bother if no one appreciates their efforts. If things continue in the direction they have been going, I will not be able to blame them. Worse than that, I may even be one of them.

STUDENT/TECHNICIAN PRESENTATION COMPETITION

Meetings in Dallas, March 1988

★ ★

Students and/or technicians who present papers from platform at the T.S.E.M. meeting in Dallas may compete for a cash award and sponsorship by the Society for an Electron Microscopy Society of America Presidential Scholarship. *Up to three (0-3) of these awards may be presented at the Dallas meeting.*

ELIGIBILITY

You must be a member of T.S.E.M. *when you submit your abstract.* You must be a student in good standing or a technician. Neither corporate members nor regular members with doctorate degrees, or the equivalent, are eligible. You must be the senior author of the paper you present. You must have prepared the judged abstract (see below), and the slides you show, yourself. You may have other junior authors on the paper. Students who compete must be sponsored with a signed statement from a regular T.S.E.M. member. Submit this statement with your special abstract.

REQUIREMENTS

You must submit the usual abstract, in the regular format, by the deadline (January 29) for publication in the T.S.E.M. Journal. *In addition,* you must submit a special abstract, which will be judged, and which will have the following requirements:

1. It will be on two pages, each 8½'' x 11'' with a margin of ½'' all around.
2. The first page will have text only which will include title, paper summary, and references.
3. The second page will have pictures and text (picture captions, etc.).
4. This special abstract will be submitted to the Secretary, Dr. Wayne Sampson, and must be in his hands no later than *January 29, 1988.* Submit the regular abstract, by the same date, to Ron Davis as instructed on the bottom of the abstract form. As Ron and Wayne work in the same lab, there should be no mix-up.

You will present your paper from platform in the usual format, with the usual time constraints. You will have time to answer questions from the audience.

JUDGING

You will be judged by a panel of regular T.S.E.M. members. You will be judged 50% on the quality of your special abstract and 50% on the quality of your presentation, including how well you answer questions from the audience. The regular abstract you submit for publication in The Journal will not be judged.

NOTICE TO STUDENT & TECHNICIAN COMPETITORS

★ ★

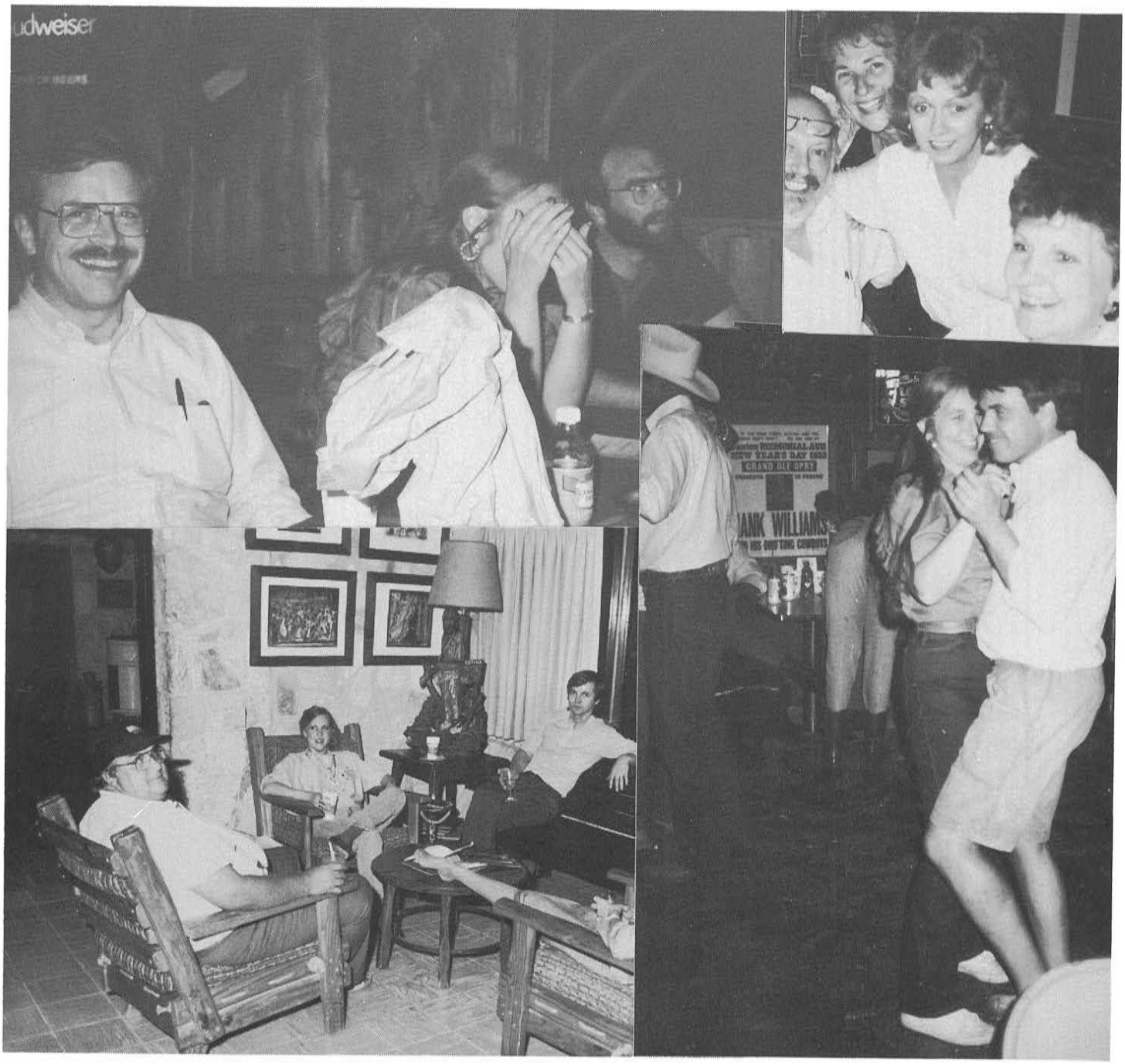
In order to be eligible for the student/technician presentation competition, you must be a member of the Texas Society for Electron Microscopy *at the time you submit your abstract.*

TSEM Photo Album

October 1987

Bandera, Texas

Photos by Joiner Cartwright

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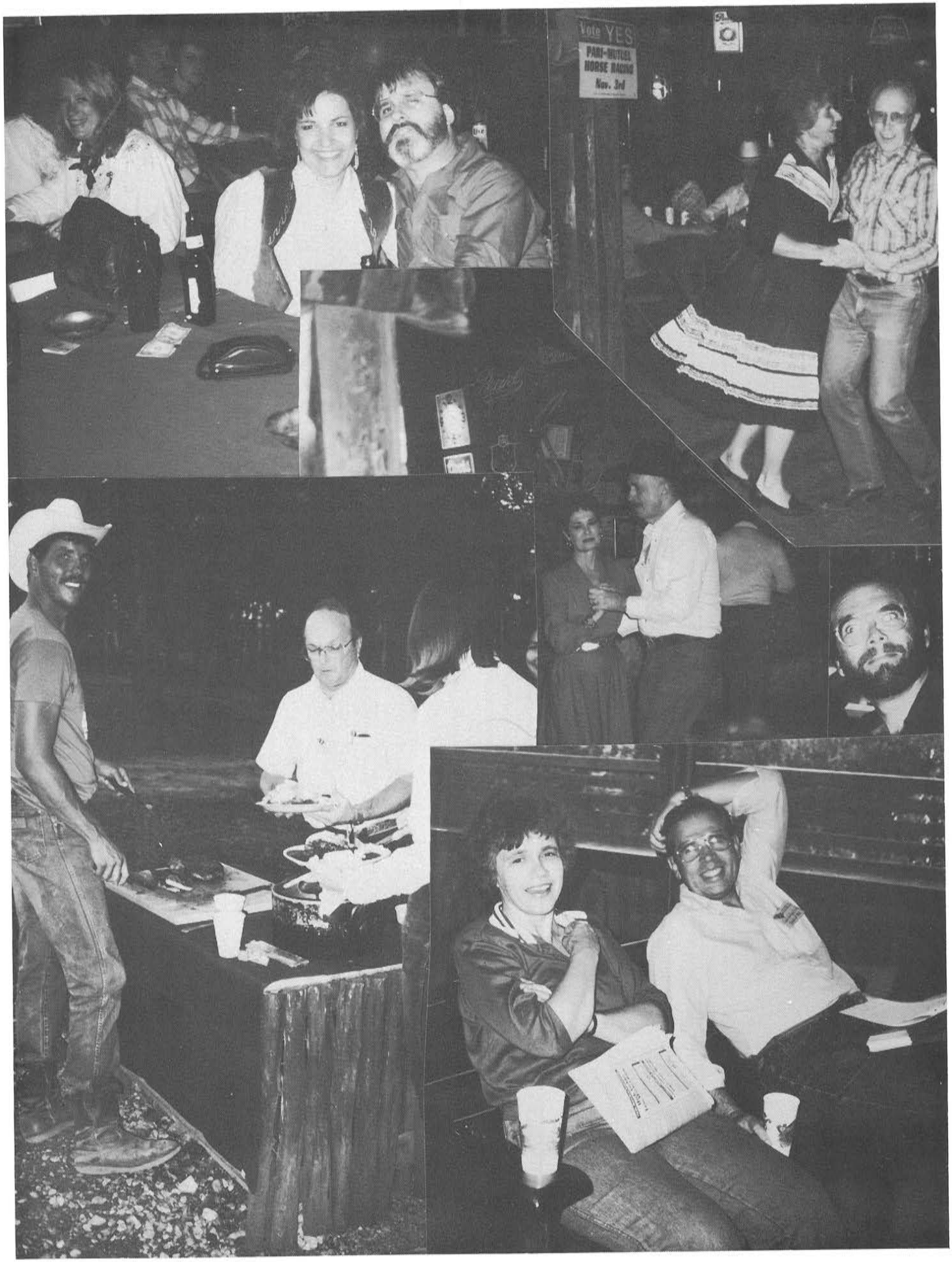
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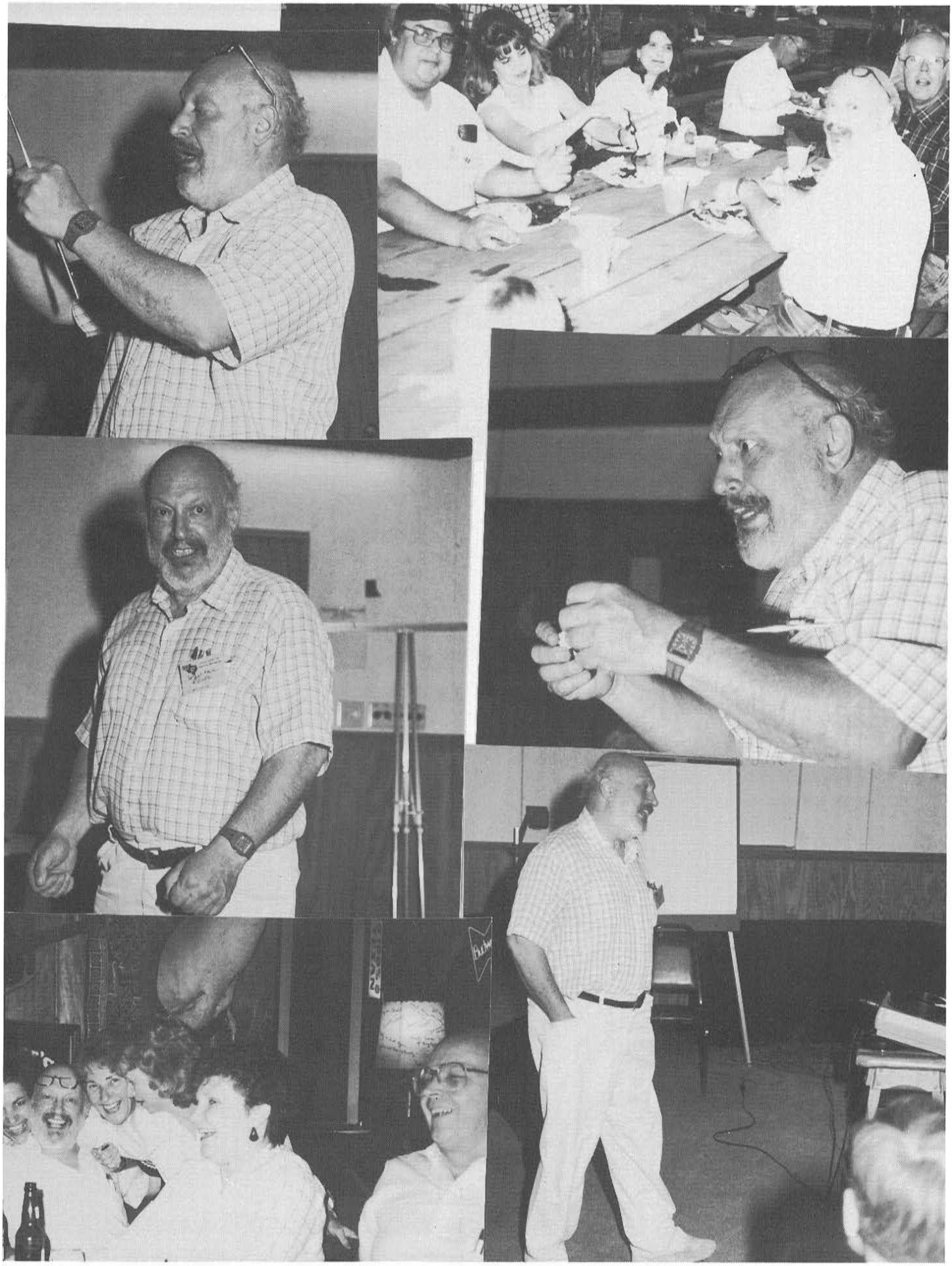

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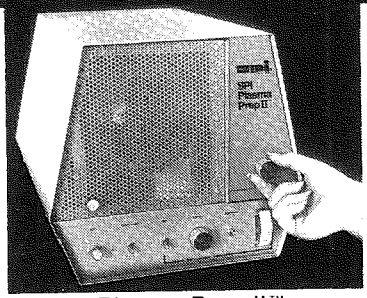

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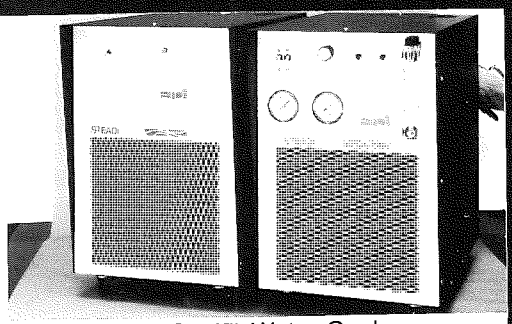

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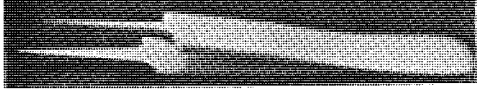

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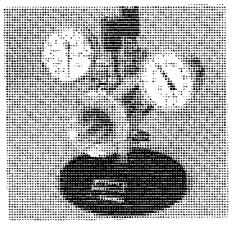

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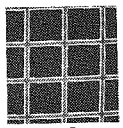

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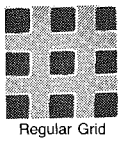

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LOW TEMPERATURE S.E.M.

By

David Cowell
Emscope Laboratories
and
***John Fitzpatrick**
International Electron Optics

LET'S DO CRYO!

Conventional preparation techniques for scanning electron microscopy (SEM) necessarily involve dehydration which, in turn, usually necessitates chemical treatment. The main advantage of low temperature methods is that they offer the opportunity of observation and microanalysis of biological specimens under conditions which are closely related to the natural state. This can be important for structural investigation and may be imperative for analytical research.

In addition to allowing the observation of specimens which may collapse or distort, it is possible to view specimens such as emulsions and suspensions — not previously a practical application for SEM.

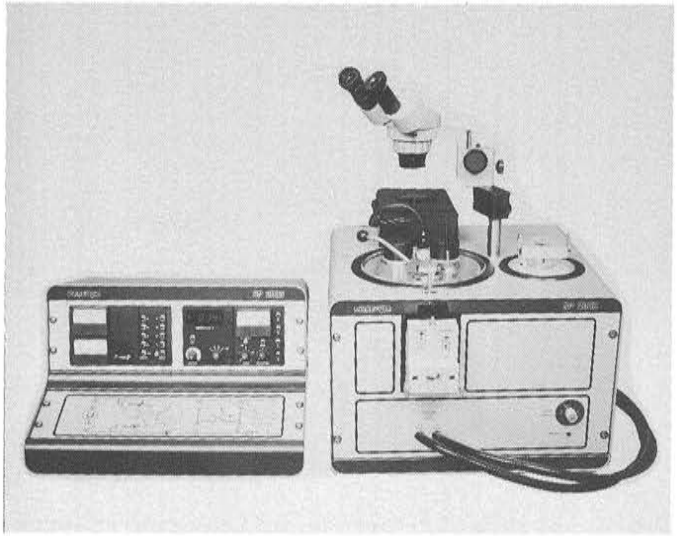

Figure 1. A Cryosem system indicating Control Unit and Preparation Unit.

SO WE WANT TO FREEZE IT!

As a first approach it would seem reasonable to wish to freeze the specimen as rapidly as possible, so that ice crystal damage is minimal, and to as low a temperature as practical, to avoid the possibility of sublimation. Indeed a range of specialized freezing techniques has been developed for fast freezing of very small specimens. However, for the bulk specimens we are considering, the cooling rate will be relatively slow, and the choice of cryogen less critical. The poor thermal conductivity of ice would also suggest that the thermal capacity of the specimen support stub may not significantly affect the cooling rate and subsequent size of ice crystals at the specimen surface. This would allow the use of a relatively large stub and the advantage of good thermal stability, while for special applications smaller specimen mounts could still be used and catered for on the larger stub.

It would seem reasonable to consider liquid nitrogen with a boiling point of -196°C to be an essentially satisfactory cryogen; this is more valid when used in its sub-cooled state (slushy nitrogen) at -210°C, to reduce the effects of an insulating gas layer being formed when freezing.

WHAT NOW?

Having frozen a specimen it is necessary to carry out preparation and viewing activities to attempt to achieve at least equivalent validity to more conventional techniques. The unnatural environment in which the specimen has to be contained must be considered.

It is necessary to prevent contamination of the specimen with condensing water vapour while inhibiting sublimation of water from the specimen.

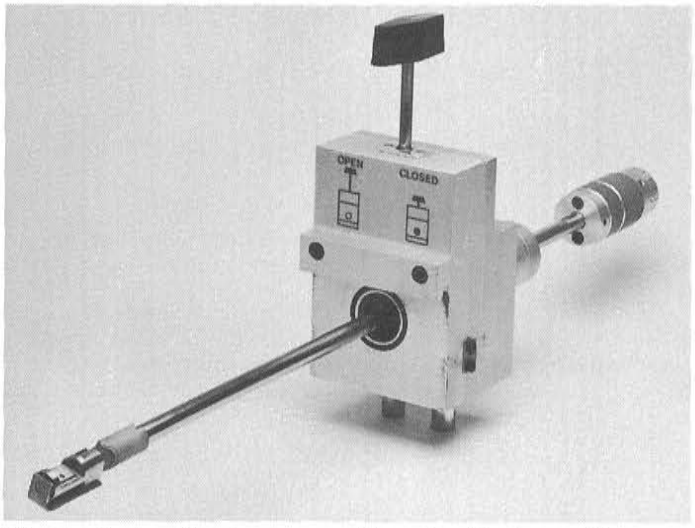

Figure 2. A valved vacuum Transfer Device with Specimen Stub attached.

A SEPARATE PREPARATION CHAMBER

This chamber is held under vacuum and the temperature of the stage is held at better than -160°C, and equipped with the following facilities:

(i) Macro fracture device for fracturing the specimen, using a cooled knife with adjustable verticle feed.
(ii) Macromanipulator (pointed needle).
(iii) Sputter coating stage with ultra low thermal input, high resolution (magnetic deflection) sputtering (less than 15°C temperature rise during sputtering for 4 minutes).
(iv) Carbon evaporation stage for carbon fibre flash evaporation.
(v) Metal evaporation (from a tungsten wire basket).
(vi) Etching stage (conductive heating and/or radiant heating).

S.E.M.

The coldstage is custom built to suit the make and model of the electron microscope. The metal pallet onto which the specimen stub is mounted is attached to the existing SEM specimen stage. This metal pallet is connected via a flexible copper rope to the cold finger of a liquid nitrogen dewar, which provides the cooling and is maintained at less than -160°C.

Access for the specimen stub using the transfer device is via a valve port to suit the S.E.M.

DOES IT WORK?

Its use covers the range of current SEM applications and are continuously being developed, typically:

- Biological sciences — frozen hydrated specimens.
- Microelectronics — observing devices at low temperature.
- Oil exploration — examination of core samples, tar, sand, etc.
- X-ray microanalysis — diffusible substances.
- Cathodoluminescence.

*Correspondence or reprint requirements, should be made to: John Fitzpatrick, International Electron Optics Inc., Suite 28-145, 4444FM, 1960 West, Houston, Texas 77068. Tel. (713) 893-2067. Telex: 166554 HILL HOU.

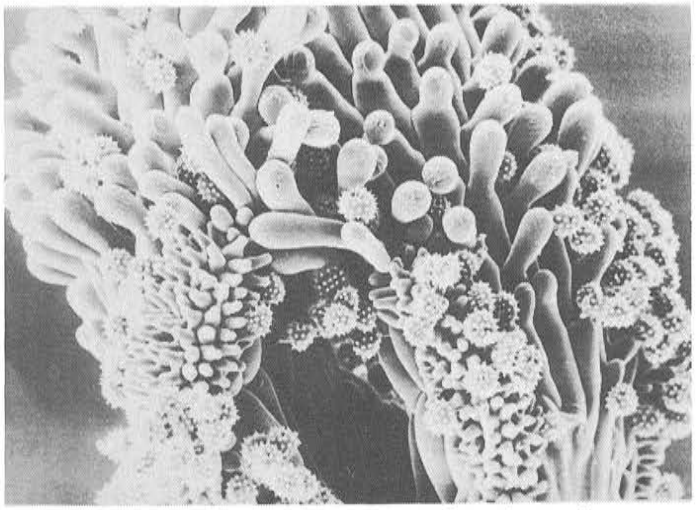

Figure 3. Frozen hydrated stigmatal surface of alyssum coated with gold at 15mA and 0.1 Torr for 3 mins. (x750) *Courtesy of Dr. A.J. Wilson - University of York, England.*

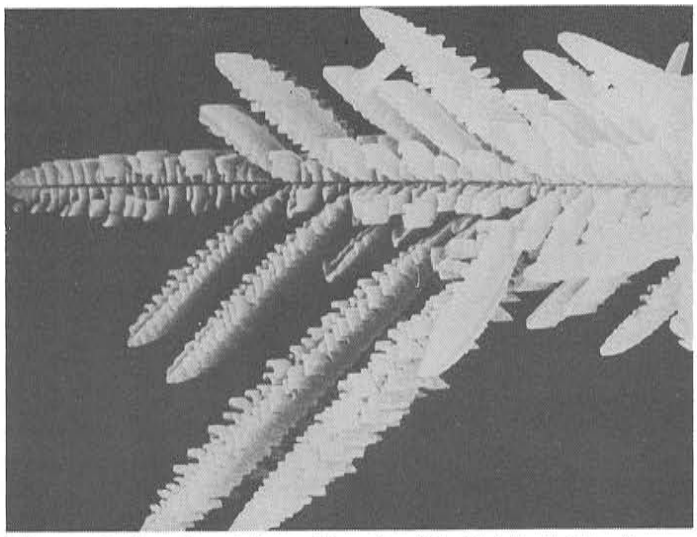

Figure 4. Ice Crystal sputtered with Gold at 15mA and 0.1 Torr for 3 mins. (x5,000) *Courtesy of Dr. A.J. Wilson, University of York, England.*

EDITOR'S NOTE

This article makes reference to "nitrogen slush", but there may be a number of you that do not know how to make it. Here is how it is done:

Put liquid nitrogen into a large styrofoam cup or insulated plastic beaker and pull a rough vacuum on it. I use our vacuum evaporator with the mechanical pump only. The liquid will begin to boil and in a few minutes it will freeze. As soon as you vent the chamber the slush will begin to melt. I have not found a way to maintain it as an actual "slush" at atmospheric pressure.

The temperature of the slush is reportedly -210°C. There does seem to be a difference between this and regular liquid nitrogen. You can demonstrate it by making the slush and dipping a pair of forceps or other room temperature object in it. There appears to be considerably less boiling around the object as compared to regular liquid nitrogen. Presumably, the insulating gas layer that is normally produced is reduced and you get more efficient freezing. The nitrogen slush warms up to normal liquid nitrogen temperature over a 5-10 minute period.

If anyone out there in TSEM-land knows more about nitrogen slush, or wants to do a review on freezing agents, let me know.

WOMEN IN EMSA

By

Marcelle A. Gillott, Ph.D.
Center for Electron Microscopy
74 Bevier Hall, MC-188
905 S. Goodwin Avenue
Urbana, IL 61801

An informal informational meeting to assess the interest in establishing a "Women In EMSA" forum was held at the annual meeting in Baltimore. Approximately 45 women (and a few men) attended the session and the consensus was that the formation of such a group would be beneficial. Our aim is to provide women with a framework for networking, as well as, support and encouragement as they pursue their scientific careers in electron microscopy. One of the major activities of this group would be to sponsor a talk or panel discussion at the annual EMSA meeting. Various topics either scientific or general interest (e.g. strategies for getting a postdoctorate, a job, a grant ...) would be considered. Other activities, including a newsletter may be instituted at a later date.

At this point we need your input. Please take a few minutes to answer the following questions and send them to me at the address listed above.

1. Would you be interested in joining this group?

2. Would you be willing to pay nominal membership dues ($5, $10) to support a newsletter and/or provide honoraria for speakers?

3. Are there any specific topics you feel this group should address?

4. Would you, or someone you know be willing to present a talk or participate in a panel discussion at the Milwaukee meeting?

NOTE: This is not a firm commitment on your part — but do let us know if you would be interested enough to present on a specific topic. Please include your name, address and LAS affiliation, if any. Thank you very much for your support.

Abstracts

BIOLOGICAL SCIENCES

POSTER PRESENTATION — FALL 1987

SYNOVIAL SARCOMA: AN ULTRASTRUCTURAL STUDY
Soheir Mahfouz, Mannie Steglich and Bruce Mackay, Dept. of Pathology, University of Texas System Cancer Center, Houston, Tx. 77030.

A synovial sarcoma is a malignant soft tissue tumor composed of gland-like structures within a background of spindle cells. The tumor is of interest to pathologists for a number of reasons. A biphasic pattern is uncommon among human neoplasms and raises questions concerning the histogenesis of the tumor. Despite a fancied resemblance by light microscopy to normal synovium, only a few of the reported cases have been related to synovial joints, and a monophasic variant composed only of spindle cells has been described. We have studied 55 synovial sarcomas by electron microscopy, including 7 biphasic and 48 monophasic tumors, and have compared the neoplastic epithelial cells to those of normal human synovium, and the spindle cells to those of sarcomas that resemble monophasic synovial sarcoma by light microscopy. There is not a close resemblance between the epithelial cells of biphasic synovial sarcomas and the lining cells of normal synovial membrane, suggesting that the designation is a misnomer. The spindle cells of monophasic synovial sarcomas are similar to those in the biphasic tumors supporting the concept that the monophasic form is a synovial sarcoma variant. The spindle cells of the tumors differ ultrastructurally from neoplastic fibroblasts, and electron microscopy is consequently useful to confirm the diagnosis of monophasic synovial sarcoma.

BIOLOGICAL SCIENCES

PLATFORM PRESENTATION — SPRING 1988

SCANNING ELECTRON MICROSCOPY OF ERYTHROCYTES RECOVERED FROM THE GUT OF STABLE FLIES AFTER FEEDING ON BOVINE BLOOD. HEINZ KIRCH, J.R. DeLOACH, AND ELLEN MOORE, USDA-ARS, Veterinary Toxicology and Entomology Research Laboratory, College Station, Texas 77841

The digestion of red blood cells (RBC) in the blood meal of blood feeding insects is not understood. In fact, the mechanism of hemolysis of RBC remains elusive. To better understand the mechanism of RBC hemolysis by digestive enzymes, we have studied morphological changes in RBC occurring in the gut of flies. RBC were recovered from anterior and posterior midgut of stable flies at intervals between 30 min and 18 hr post feeding and prepared for scanning electron microscopy. Within 30 min after feeding, RBC from anterior midgut are mainly echinocytes III. RBC progressively become sphero-echinocytic. RBC from posterior midgut never appeared as echinocytes III but were mainly sphero-echinocytes I. Morphological changes in RBC from in vivo experiments are markedly different from changes that occur in vitro. By studying the morphology of RBC in vivo and correlating shape changes with enzymatic activity in the gut, we have a better understanding of the hemolytic process. And possibly we can infer mechanistically the causal factors responsible for hemolysis of the RBC.

ULTRASTRUCTURAL LOCALIZATION OF CALBINDIN-D AND CALCITONIN IN CHICK ULTIMOBRANCHIAL GLANDS. N. Inpanbutr and A.N. Taylor, Dept. of Anatomy, Baylor College of Dentistry, Dallas, Texas.

Calbindin-D, a vitamin D-dependent calcium binding protein (CaBP), first isolated from chick intestine has been reported in other chick tissues and tissues of higher organisms. CaBP was recently colocalized with calcitonin (CT), at the light microscopic level, in chick ultimobranchial gland (UBG) cells (Anat Rec 219:86, 1987). This study reports for the first time, the ultrastructural colocalization of CaBP and CT in the cord cells of UBGs by using the amplified protein A-gold technique (J Histochem Cytochem 34:569, 1986). Chick UBGs were fixed in 4% paraformaldehyde, processed and embedded at -20 °C in Lowicryl K4M. Specific antiserum raised against CaBP from chick intestine and antiserum specific for salmon CT were used as the primary antisera. CaBP was found in the nucleus and areas of organelle-free cytoplasm of UBG secretory cells. The protein was present in both the small-granule and the large-granule type of secretory cells. The nucleus often exhibited a higher gold bead intensity than the adjacent cytoplasm. This ultrastructural distribution of CaBP has also been reported in enterocytes. The same cell types demonstrated a positive reaction for CT, which was localized in the small and large granules of the cells. In the large-granule cells, gold beads, the immunomaker for CT, were observed more frequently over the darker staining granules compared to the lighter staining granules. The functional significance of CaBP, an end-organ marker for the vitamin D endocrine system, in CT-secreting cells is not understood, but it suggests a possible direct influence on CT secretion. (Supported by NIH grant DE07916).

ULTRASTRUCTURE OF THE MINERALIZING METACARPOPHALANGEAL JOINT OF PROGRESSIVE ANKYLOSIS MICE. H. W. SAMPSON, DEPARTMENT OF ANATOMY, TEXAS A&M UNIVERSITY COLLEGE OF MEDICINE, COLLEGE STATION, TEXAS 77843.

Progressive ankylosis mice (ank/ank) spontaneously develop a joint disorder characterized by calcium hydroxyapatite deposition and bony ankylosis of the joints of the axial and the appendicular skeleton. These mice were used to study the process and extent of mineralization of the metacarpophalangeal (MP) joint and to determine their suitability as a model for studying disorders of ankylosis and cartilage mineralization. The MP joints of the forepaw were examined at four, six and eight weeks of age using electron microscope techniques for studying undecalcified tissues. These studies describe the progression of the disorder and they reveal the three overlaping phases of pannus proliferation, chondrophyte formation and mineralization and articular cartilage mineralization. The chondrophyte had the appearance of a disorganized bone growth plate that mineralized in a manner fairly similar to endochondrial bone. Mineralization was first seen associated with matrix vesicles along longitudinal septa, between large hypertrophic appearing cells. Unlike endochondrial bone formations, it then proceeded along transverse septa, ultimately surrounding the chondrocyte. The articular cartilage mineralized by advancement of a calcification front across the cartilage. Matrix vesicles were not identified in this process. The calcification front eventually spanned the cartilage, and crystals were shed into the synovial space. Synovial crystal phagocytosis was also observed. This mouse should be an important model for studying the mechanism of both crystal deposition and abnormal articular cartilage calcification.

ELECTRON MICROSCOPY IN THE DIAGNOSIS OF SMALL ROUND CELL TUMORS. M.C. Steglich, N.G. Ordonez, B. Mackay, Department of Pathology, University of Texas System Cancer Center, Houston

Small round cell tumor is an appropriate designation for a heterogeneous group of neoplasms composed of uniform small cells with scanty cytoplasm, often forming diffuse sheets. The differential diagnosis is frequently difficult by light microscopy, though an architectural pattern is occasionally present to suggest the diagnosis, such as rosettes in a neuroblastoma. Immunocytochemistry can be of significant value: immunoreactivity for neuron specific enolase will suggest a neuroblastoma or neuroendocrine carcinoma, while desmin or myoglolin positivity indicates a rhabdomyosarcoma. Electron microscopy is the most consistently successful method to classify a small round cell neoplasm. The differential diagnosis differs in the pediatric and adult age groups, and clinical data must be taken into consideration in evaluating the ultrastructural findings. Neuroblastomas in children and neuroendocrine carcinomas in adults have similar features by electron microscopy, though the number of dendritic processes is greatly increased in the younger patients. Rhabdomyosarcoma is indicated if the cells contain skeletal muscle myofilaments, but they are often sparse and sometimes absent: certain other ultrastructural features may nevertheless indicate the diagnosis. Ewing's tumor mainly occurs in young patients, particularly as a bone neoplasm although soft tissue variants have also been described. The ultrastructural spectrum of Ewing's tumor is not well defined: classical cases are readily identified, despite some overlap with neuroblastoma, but atypical forms occur. Some small cell malignant tumors can not be placed in a defined category but electron microscopy has an important role in defining the detailed structure of these neoplasms.

CORRELATING THE ULTRASTRUCTURE WITH THE MICROSTRUCTURE OF THE MANDIBULAR ORGAN OF THE LOBSTER, HOMARUS AMERICANUS. ERNEST F. COUCH AND JAMES K. BUTLER, Department of Biology, Texas Christian University, Fort Worth, Texas 76129 and Department of Biology, The University of Texas at Arlington, Arlington, Texas 76019.

The mandibular organ is known to produce methyl farnesoate (MF), a molecule closely related to insect juvenile hormone. We have previously shown that MF is produced only in the anterio-lateral region of the gland. Initially, the ultrastructural picture was confusing because of the different morphological appearances of the cells examined. Examination of large area light microscopical glycol methacrylate (GM) sections showed the presence of a variety of morphologically distinct cell types in different regions of the gland. The cells of the MF-synthesizing region are substantially different from those in the remainder of the gland. In order to dependably obtain samples of each different cell type identified by light microscopy for ultrastructural examination, we have made use of the following method: Glands, fixed whole, were hand cut into 2 mm thick slices. Before embedding these slices in GM for light microscopy, an 18 gauge needle punch was used to remove plugs from selected areas. The plugs were osmium post fixed and prepared for EM. By first examining the high resolution LM sections, the type of cells surrounding and likely making up each plug can be identified and their ultrastructure and microstructure readily correlated. (Supported by the TCU Research Fund)

PLEOMORPHIC CRYSTAL SHAPES OF CALCUIM OXALATE ASSOCIATED WITH LITTER FUNGI. D.C. GARRETT, Dept. Biology, University of Texas, Arlington, TX 76019.

Calcium oxalate crystals were examined, using scanning electron microscopy techniques, from a Juniper (Juniperus sp.) forest litter sample collected in Murray County, Oklahoma. Numerous shapes of calcium oxalate crystals form in association with hyphal strands. The pleomorphic crystals range from long acicular to short spindle shapes labelled type I and type II respectively. The crystals are arranged along a central axis with either mid or end point attachments. The axis appears to be the hyphal strand although this relationship is sometimes difficult to demonstrate. Crystal bearing hyphae have been found on the surface of leaves, branches, and seeds.

ARCHITECTURE OF PULMONARY SMALL-GRANULE CELL (SGC) CLUSTERS IN THE ADULT SYRIAN GOLDEN HAMSTER. A.D. Pearsall, Department of Anatomy, Baylor College of Dentistry, Dallas TX, 75246.

The epithelial lining of the respiratory system contains SGC's, singly or in clusters, which tentatively have been classified as APUD cells. Previous descriptions of archetypal rabbit clusters have focused upon their neural associations and putative chemoreceptor function. However, studies in the adult hamster suggest significant species variability in that 5 subclasses have been identified and serial reconstruction of an entire cluster demonstrated that some may be non-innervated. Therefore, the present study was designed to further characterize the organization of SGC clusters in adult hamster lung. Animals were vascularly perfused with buffered aldehydes and routinely prepared for electron microscopy. Within the clusters, differences existed in the cytological profile of the individual cells which permitted the identification of two types, "dark" and "light". This distinction was due to differences in the electron density of the cytoplasmic matrix. Both types were normal as judged by uniform spherical nuclei, chromatin organization and distribution of cellular organelles. The "dark" cells, however, presented the profile of a cell actively involved in synthesis, with a markedly dilated perinuclear cisterna and endoplasmic reticulum. Additionally, the "dark" cells contained membrane-deliminated structures containing concentric membranous whorls, clear vacuoles and lipofuscin granules. Occasionally, cells were observed to contain features of both cell types, suggesting that they may represent a continuum of the same cell type. Finally, it appears that some cell clusters in the adult hamster are intimately associated with nerve endings.

POLLEN MORPHOLOGY IN THE GENUS PENSTEMON (SCROPHULARIACEAE) AND ITS TAXONOMIC SIGNIFICANCE. M.L. MOORMAN, University of Texas at Arlington, Arlington, TX 76019.

Pollen from eleven species of Penstemon was examined by scanning electron microscopy. The principle pollen grain morphology identified was tricolpate with the exine being microreticulate. Overall the pollen morphology was fairly homogeneous, however, the pollen grains differed in size and shape between the various species. A few species exhibited unique exine or colpus characteristics. Pollen morphology was of limited taxonomic value in delimiting specific taxa. However, comparing the pollen morphology of supposedly closely related species proved interesting. Penstemon buckleyi exhibited little affinity in pollen morphology with other members of the section Coerulei. Pollen morphology may prove useful in establishing relationships within the various sections of Penstemon.

SCANNING ELECTRON MICROSCOPY OF ERYTHROCYTES EXPOSED TO DIGESTIVE ENZYMES FROM THE POSTERIOR MIDGUT OF STABLE FLIES IN VITRO. ELLEN MOORE, HEINZ KIRCH, AND J.R. DeLOACH, USDA-ARS, Veterinary Toxicology and Entomology Research Laboratory, College Station, Texas 77841

To better understand the digestive processes of blood feeding insects, the progressive morphological changes in erythrocytes (RBC) (human and bovine) were studied at timed intervals after incubated exposure to lumen contents extracted from the posterior midgut of Stable Flies. The bovine RBC were immediately converted to sphero-echinocytes I upon addition to the incubation mixture and progressed through spherocytosis to sphero-stomatocytes II entrapped in RBC stroma. The echinocytic changes were not present when soybean trypsin inhibitor (SBTI) was added to the incubation mixture before adding RBC. In this case, the morphology progressed from normal discocytes through stomatocytes to spherocytes. Human RBC converted to various stages of echinocytosis immediately upon addition to the incubation mixture. The morphology then changed back through echinocytes I to stomatocytes, sphero-stomatocytes, and ultimately to spherocytes before total hemolysis. The process was apparently unaffected by the addition of SBTI to the incubation mixture. The difference in response between bovine and human RBC is thought to be due to the differences in lipid content of the RBC membrane.

IMMUNOGOLD LOCALIZATION OF SIMIAN VIRUS 40 LARGE TUMOR ANTIGEN USING COLLOIDAL GOLD-STREPAVIDIN. L.S. Stein and R.C. Burghardt, Dept. Veterinary Anatomy, Texas A&M University, College Station, TX 77843

A clonal line (DC3) of SV40-transformed, rat granulosa cells is being studied as a potential model system for use in studies of granulosa cell differentiation and ovarian carcinogenesis. DC3 cells are characterized by an undifferentiated morphology, rapid and apparent indefinite growth in culture without luteinization. In order to analyze these properties and the effects of transformation on the properties of juvenile granulosa cells, it was necessary to confirm the presence of the virus in the cell line. The large tumor antigen, which is encoded by the double stranded DNA genome of the virus, is important in both the initiation and maintenance of transformation. Cells were analyzed by immunofluorescence for the expression of SV40 large T and small t antigens and a predominantly nuclear localization with two monoclonal antibodies support the presence of the viral genome within the cells. Immunogold labelling also confirmed the intranuclear labelling and negligible cytoplasmic labelling. Immunofluorescence analysis of other developmentally expressed proteins in granulosa cells including the regulatory subunit (RII) of cyclic AMP-dependent protein kinase, cytochrome P_{450} cholesterol side-chain cleavage enzyme, and prostaglandin synthase indicate that the DC3 cells exhibit characteristics of undifferentiated granulosa cells. (Supported by a BRSG).

FIBRONECTIN LOCALIZATION IN EMBRYONIC CHICK HEART DEVELOPMENT: AN IMMUNOFLUORESCENCE STUDY. E.B. Van Gorkom and D.A. Hay, Dept. of Biology, Stephen F. Austin State University, Nacogdoches, TX 75962.

During cardiac development, various glycoproteins accumulate in the extracellular matrix of prevalvular mesenchyme of atrioventricular (AV) endocardial cushions. Many of these glycoproteins are believed to be secreted by the AV myocardium; one such glycoprotein, fibronectin, is thought to be involved in the activation and transformation of cushion endothelial cells into migratory mesenchymal cells.

The AV regions of embryonic chick hearts were frozen in Freon 22 chilled in liquid nitrogen, processed by freeze-substitution, embedded in paraffin and sectioned. Selected sections were processed for light immunocytochemistry utilizing a fibronectin-specific primary antibody and an appropriate FITC-conjugated secondary antibody.

Two forms of fibronectin were localized: a long, filamentous type and a finely particulate or granular form. The filamentous type was observed throughout several stages of heart development, whereas the particulate type appeared before activation and disappeared shortly thereafter. This evidence lends credence to the suggestion that the particulate form of fibronectin may be in some way involved in the epithelial mesenchymal transformation process.

ANATOMY OF THE TRANSFER CELL REGION IN CORN. Ronald W. Davis, Dept. of Medical Anatomy; B. Gregg Cobb, Dept. of Horticulture; J. D. Smith, Dept. of Genetics, Texas A&M University, College Station, Texas 77843.

No vascular connection exists between the cob and developing caryopses of corn. Nutrients entering the seeds must pass through, by symplastic or apoplastic methods, specialized cells called transfer cells (TC). These are of primary importance in the process of grain filling, but little is known about their anatomy or physiology. Various stages of caryopsis development in TX5855, were examined from 4 days post pollination until the development of a black layer. Samples of grains at 23 days were chosen to illustrate mature, functioning TC. These were examined in two planes of section, longitudinal section (ls) and cross section (xs). The two planes of section showed that the typical TC had extensive cell wall ingrowths, all of which were bounded by plasma membrane. In LS, the TC had the greatest cell wall proliferation in the basal portions of the cells and progressively less toward the apices. The interior of the cells had no cell wall material and were filled with cytoplasm containing normal organelles, including numerous vesicles and some crystals. In XS, the ingrowth connection to the primary wall was easily observed.The most striking feature was that wall ingrowths, of contiguous cells, often arose from common loci on the primary wall. Areas between ingrowths contained numerous plasmodesmata. The most basal TC had the most cell wall proliferation. Some wall extensions could be found in cells 6 layers deep in the endosperm. Each successively deeper cell layer had less proliferation and there was a complete transition to typical endosperm cells. There was also a transition between the transfer cells and the aleurone layer.

CRYOFIXATION AND MOLECULAR DISTILLATION DRYING, AN OPTIMUM TISSUE PREPARATION METHOD FOR RETENTION OF WATER SOLUBLE CELLULAR COMPONENTS. S. Livesey, J. Linner, D. Harrison, and T. Schifani, Univ. of Texas Health Science Center at Houston

Localization of labile cellular components using electron microscopy requires methods of sample preparation which minimize alteration of cellular antigens and displacement or removal of highly water soluble proteins and ions. Physical or cryofixation without preceding chemical crosslinking, cryoprotection or aqueous contact is now considered the optimal first step. In order to maintain this state, however, all subsequent sample processing should avoid any aqueous or solvent contact as loss and redistribution is still liable to occur.

Metal mirror cryofixation of fresh noncryopretected tissue followed by molecular distillation drying, direct resin embedment with Lowicryl K_4M and dry sectioning avoids aqueous or solvent contact at each processing step. EDS probing of subcellular compartments, e.g., parotid granule, cell nucleus, mitochondria and neutrophil granules show highly localized and characteristic spectra. In contrast, aqueous contact, even at the final step of sample sectioning results in the extraction of water soluble ions.

For immunolabelling, stabilization of the sample is necessary to minimize the effect of aqueous extraction by buffer solutions. Use of this method with vapor phase osmication and direct embedment in Spurr's resin has been shown to stabilize and retain water soluble enzymes. A comparison of labelling using anticalmodulin antibody in conventionally prepared and cryofixed and molecular distillation dried rat liver highlights the effect of processing on the end result.

OKM1-COLLOIDAL GOLD TRANSLOCATES TO THE UROPOD OF HUMAN POLYMORPHS STIMULATED WITH fMLP. H.K. HAWKINS, C.W. SMITH, B.J. HUGHES, and L.S. REHM, Depts. of Pathology and Pediatrics, Baylor College of Medicine, Houston, TX 77030.

Isolated adult human polymorphonuclear leukocytes (PMN), when stimulated with fMLP in suspension (formyl-met-leu-phe, 10^{-7} M), change to a polarized shape, forming broad flat pseudopods and narrow uropods at their opposite ends. Glutaraldehyde-fixed cells were incubated with 38-nm colloidal gold particles coated with an IgG fraction of the monoclonal antibody OKM1, which binds to the α_m subunit of the CD18 adhesion protein "Mac-1." By scanning electron microscopy in the backscatter mode, gold particles were found to be distributed evenly over the cell body and pseudopod, sparing the uropod. In contrast, when living, unfixed fMLP-stimulated cells were exposed to OKM1-gold for 10-15 minutes and then fixed, gold particles were found predominantly over the uropod. Binding of gold was dependent upon specific antigen-antibody binding, since little or none was seen in the presence of excess OKM1 antibody in solution, or when gold particles coated with nonimmune mouse IgG were substituted. The observed translocation of binding sites following attachment of OKM1-coated gold to viable human PMN is interpreted as a response of polarized cells to crosslinking of the Mac-1 adhesion protein. This or a related mechanism may provide the basis for PMN locomotion.

EFFECT OF METHODS OF PREPARATION ON THE PRODUCTION OF METHOTREXATE CARRIER ERYTHROCYTES. R.E. DROLESKEY, K. ANDREWS, C. KRUSE*, AND J.R. DeLOACH, USDA-ARS, Veterinary Toxicology and Entomology Research Laboratory, P.O. Drawer GE, College Station, Texas 77841 and *Denver General Hospital, Division of Surgical Oncology, Denver, CO 80204.

Although erythrocytes have been used as carriers for many drugs, not all drugs are suitable for entrapment. In this study, it was found that the suitability of using the erythrocyte as a carrier of the drug methotrexate (MTX) may depend on the method used for carrier production. Addition of MTX to erythrocytes prior to dialysis resulted in an increase in the number of stomatocytes when examined using transmission electron microscopy. Addition of MTX after dialysis did not result in stomatocyte formation. Common among carrier erythrocytes prepared by both procedures was the presence of microvacuoles within the erythrocytes. Vacuoles were also present within control carrier erythrocytes but not within nondialyzed control cells. The amount of vacuolization may be increased by the addition of Ca, Mg, and ATP to the annealing medium. Carrier erythrocytes prepared by addition of MTX after dialysis may have a longer survival time *in vivo* than for those prepared by premixing due to their more normal structual appearance to the spleen.

THE INFLUENCE OF MICROGRAVITY ON THE STRUCTURE OF MERISTEMATIC CELLS IN ROOT AND SHOOT TIPS OF ZEA MAYS. RANDY MOORE, Department of Biology, Baylor University, Waco, TX 76798

The objective of this research was to determine the influence of microgravity on the structure of meristematic cells in root and shoot tips of Zea mays. We did this by comparing tissues of Earth-grown plants with those of plants grown aboard flight 61-C of the space shuttle Columbia. Microgravity increased the relative volume of lipid bodies and hyaloplasm, and decreased the relative volume of mitochondria, proplastids, and dictyosomes. The relative volume of nuclei was not affected by microgravity. Cells of flight-grown plants had thinner cell walls than those of Earth-grown controls. These results indicate that microgravity exerts an organelle-specific effect on the structure of meristematic cells of Zea mays seedlings. This research was supported by a grant from the Life Sciences Program of the National Aeronautics and Space Administration.

BIOLOGICAL SCIENCES

POSTER PRESENTATION — SPRING 1988

ENHANCEMENT OF FERRITIN AND MEMBRANE CONTRAST USING OSMIUM TETROXIDE-FERRICYANIDE POSTFIXATION. Hilton H. Mollenhauer, Rae D. Record*, and Lawrence R. Griffing*. Veterinary Toxicology and Entomology Research Laboratory, USDA-ARS, P.O. Drawer GE, College Station, TX 77841 and *Department of Biology, Texas A&M University, College Station, TX 77843.

Electron dense markers such as cationized ferritin (CF) are of relatively low contrast and may be difficult to see in sections that are not counterstained. Counterstains, however, can produce dense particulates (e.g., ribosomes) which may be confused with CF. We have found that osmium-ferricyanide postfixation (de Bruijn, J. Ultrastruct. Res. 42:29-50, 1973) greatly increases the contrast of both CF and membranes in unstained sections over that obtained with postfixation procedures that do not contain ferricyanide. An additional advantage of the osmium-ferricyanide fixation procedure is that cellular particulates such as ribosomes appear with low contrast. However, the procedure may not be useful for tissues containing significant quantities of glycogen since ferricyanide preserves glycogen which may appear with high contrast in sections. This procedure for increasing membrane contrast and lowering background noise can be used to advantage in immunocytochemistry, particularly where methacrylate-based resins such as Lowicryl K4M or LR White are required and where membrane contrast is low. Preliminary experiments using protein A-colloidal gold indicate that ferricyanide postfixation increases membrane contrast with only a partial reduction in the level of antibody recognition of tissue antigens from that usually obtained. However, because of the good membrane clarity and low background noise produced by osmium-ferricyanide fixation and the low background signal of the hydrophillic resins, some reduction in antigen recognition may be tolerated.

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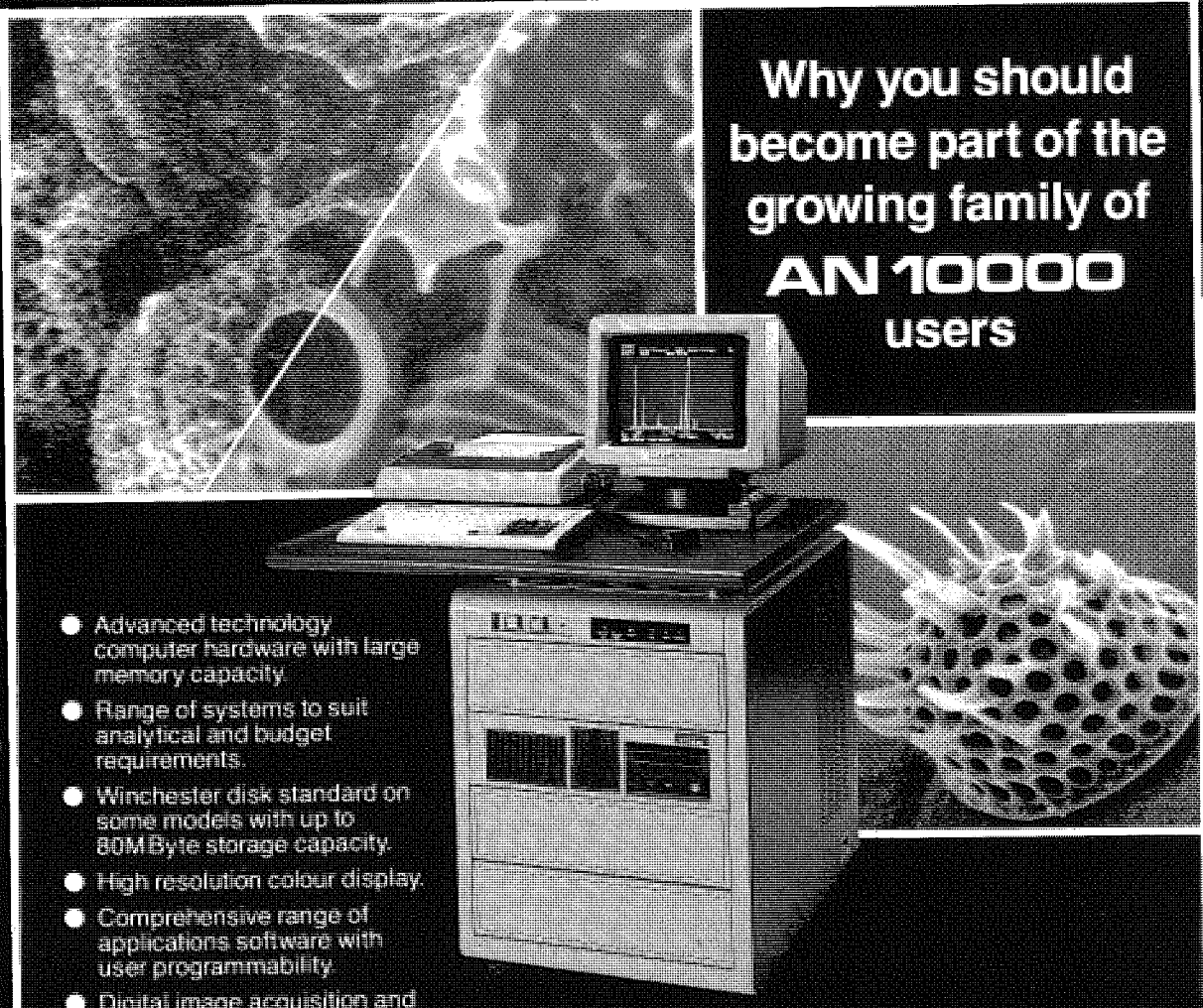

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Contact: Dr. Henry Aldrich
1059 McCarty Hall
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Gainsville, FL 32611
(904) 392-1096

JUNE, 1988

June 13-17, 1988 . . . . . Scanning Electron Microscopy and X-Ray Microanalysis

Contact: Dr. John Abel
Department of Biology
Lehigh University, Williams Hall #31
Bethlehem, PA 18015
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June 20-23, 1988 . . . . . Quantitative Biological Electron Microscopy and Analysis

June 20-23, 1988 . . . . . Analytical Electron Microscopy

Contact: Dr. David Williams
Department of Materials Science & Engineering
Lehigh University, Whitaker Lab #5
Bethlehem, PA 18015
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June 20-23, 1988 . . . . . Semiconductor Device Characterization by SEM
Advanced Imaging Techniques in SEM
X-Ray Microanalysis of Bulk, Partial and Thin Film Specimens

Contact: Dr. Joseph Goldstein
Department of Materials Science & Engineering
Lehigh University, Whitaker Lab #5
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JULY, 1988

July 26-29 . . . . . Cryotechniques in Electron Microscopy

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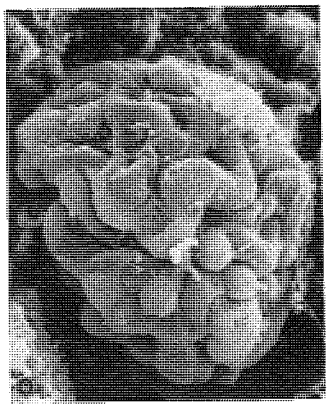
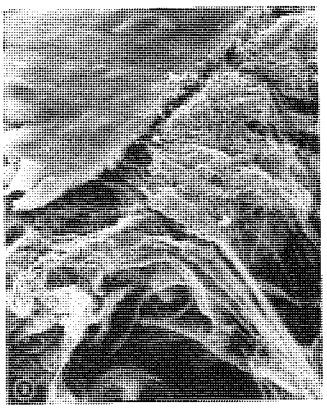
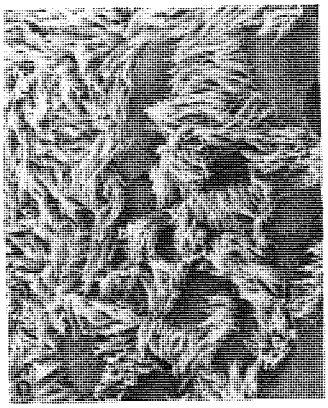
The George Washington University Medical Center
Department of Anatomy
2300 I St., N.W.
Washington, D.C. 20037
(202) 994-2881



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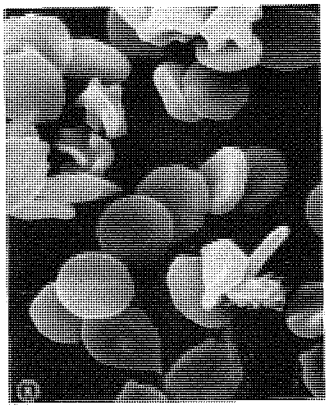

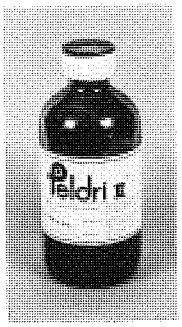

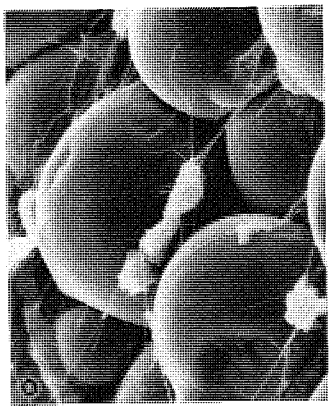

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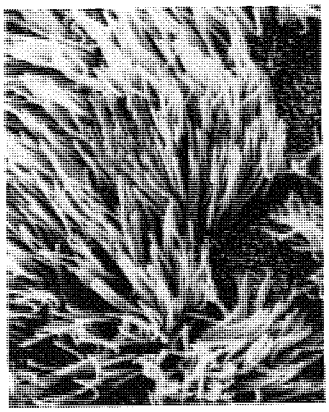

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July 19-22, 1988
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Some time ago I was wandering through the brain of a primate and came across this Pac-Man erythrocyte closing in on a platelet. Although we know such mystical encounters occur, they are not ordinarily seen. Perhaps you can use this somewhere in one of the issues of *The Journal.*

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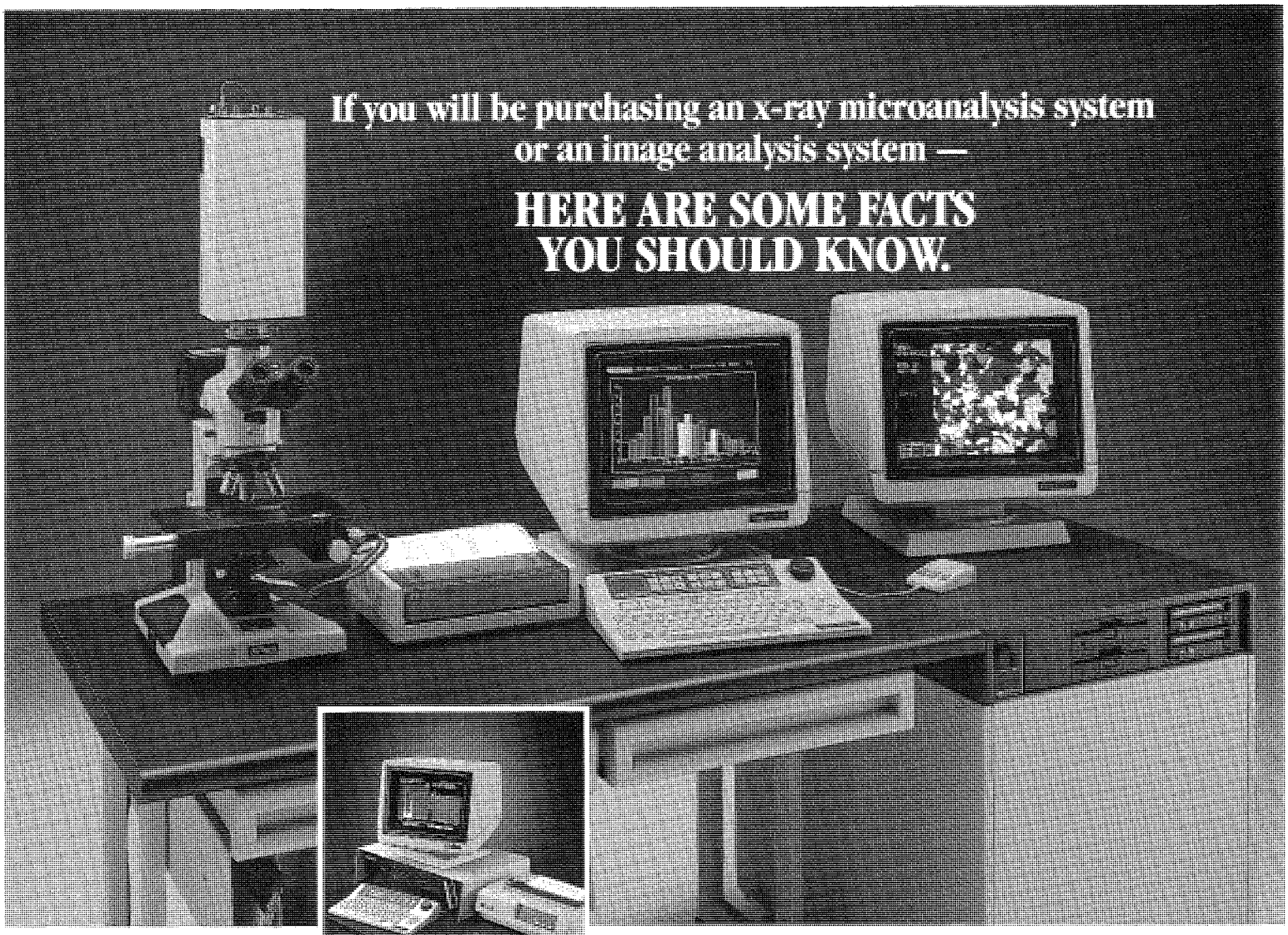
Joe G. Wood, Ph.D.

Thanks, Joe. You described it better than I ever could.
Ron Davis, Editor

EDITOR'S NOTE

Interpretation of three dimensional structure from sections is always an interesting problem for microscopists. It is amazing all the forms that a "simple" red blood cell can assume in sectional view.

My favorite discussion of this problem is "Three-Dimensional Structure Identified from Single Sections" by Hans Elias, *Science,* Vol. 174, pp 993-1000. 1971. This was required reading for all my microscopy students.



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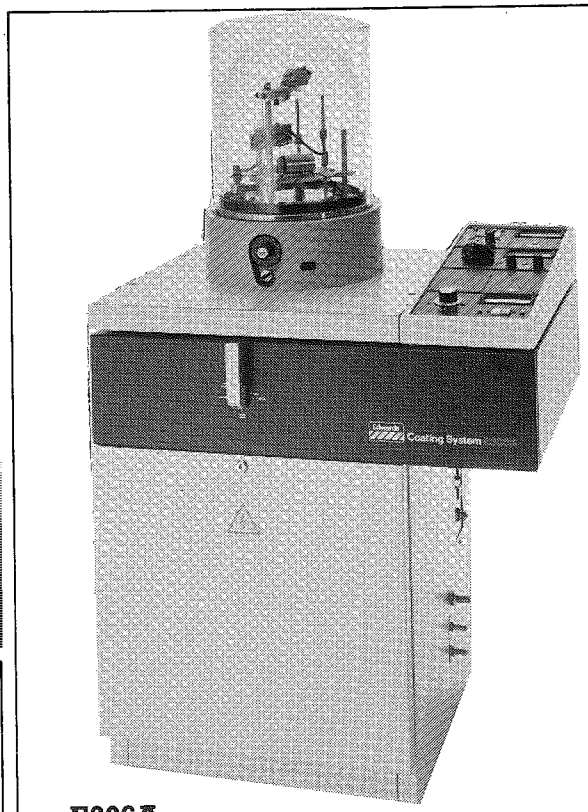

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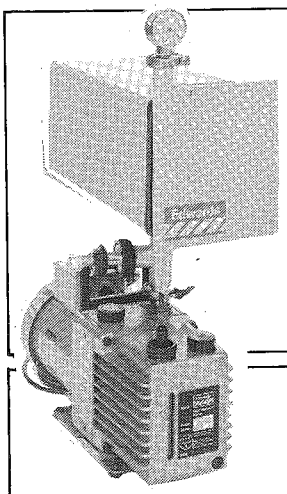

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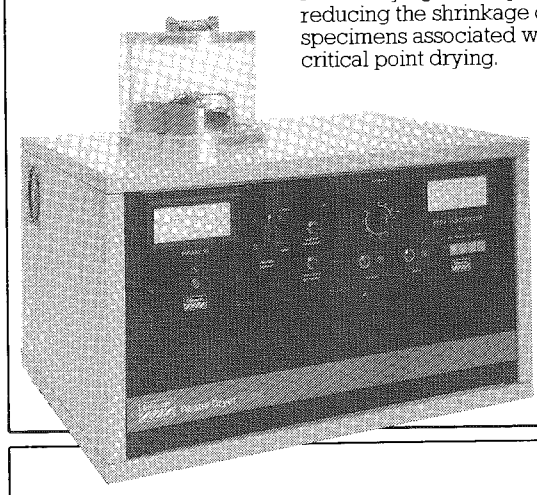

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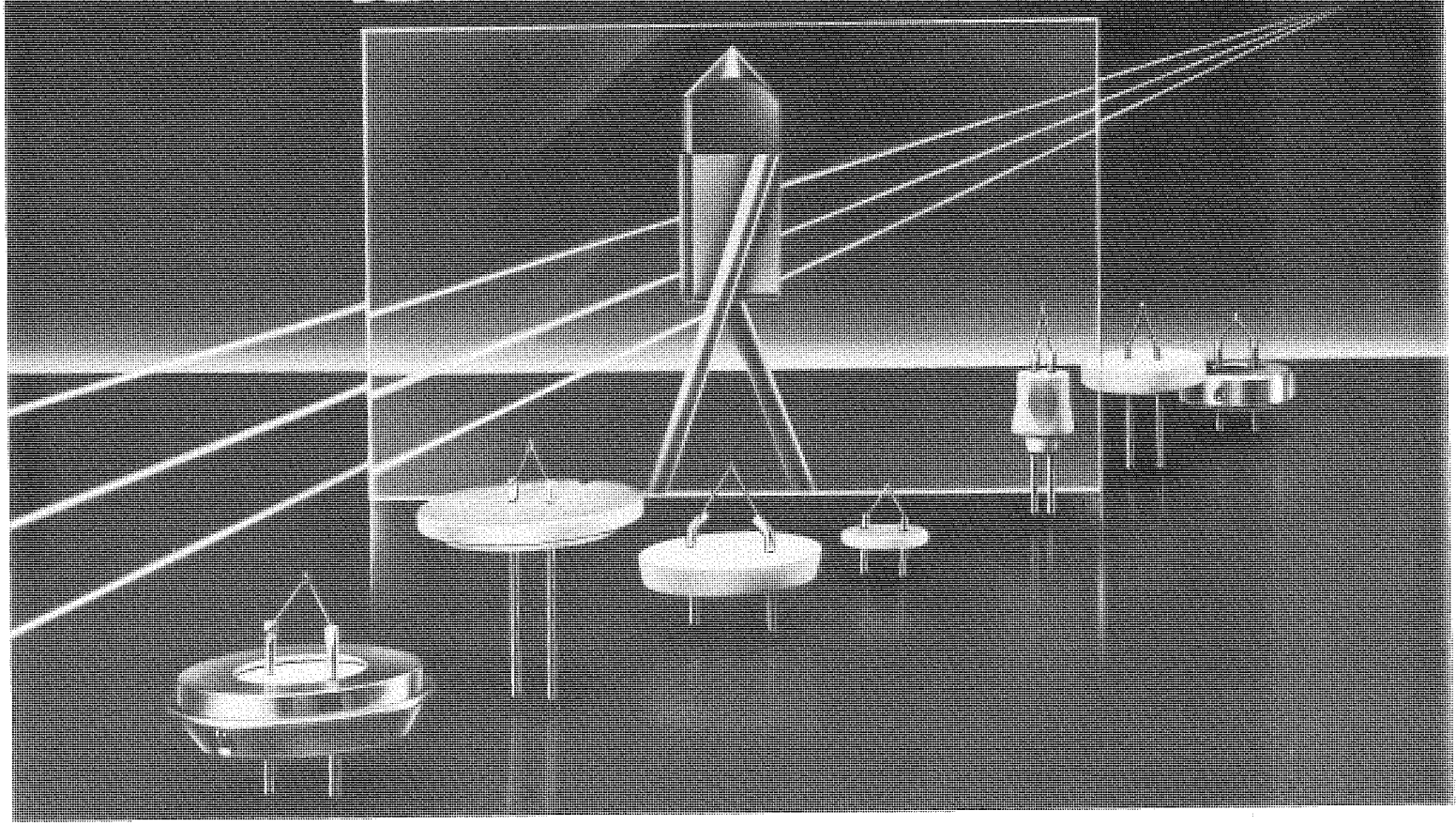

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Micrographs submitted for cover photos should be marked as such. The choice of photographs will be made by the editor. Photograph receipt and/or dispensation will not be acknowledged. Photographs will not be returned. Electron micrographs to be used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

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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.

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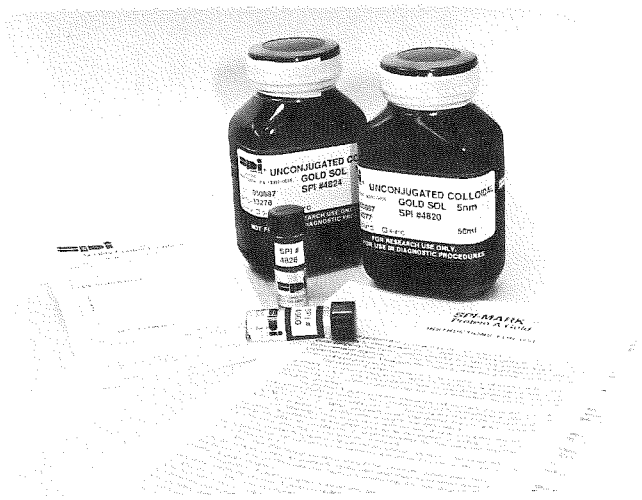

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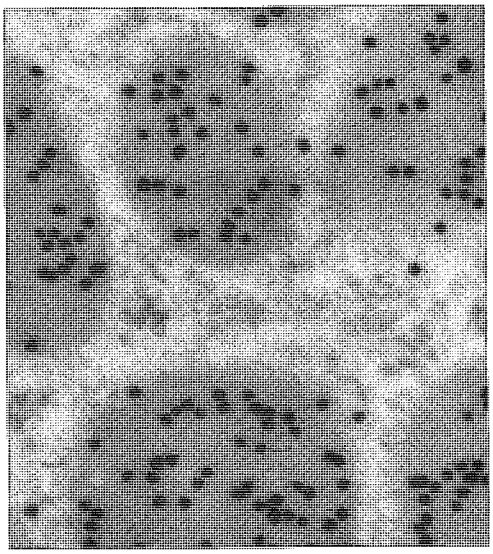

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