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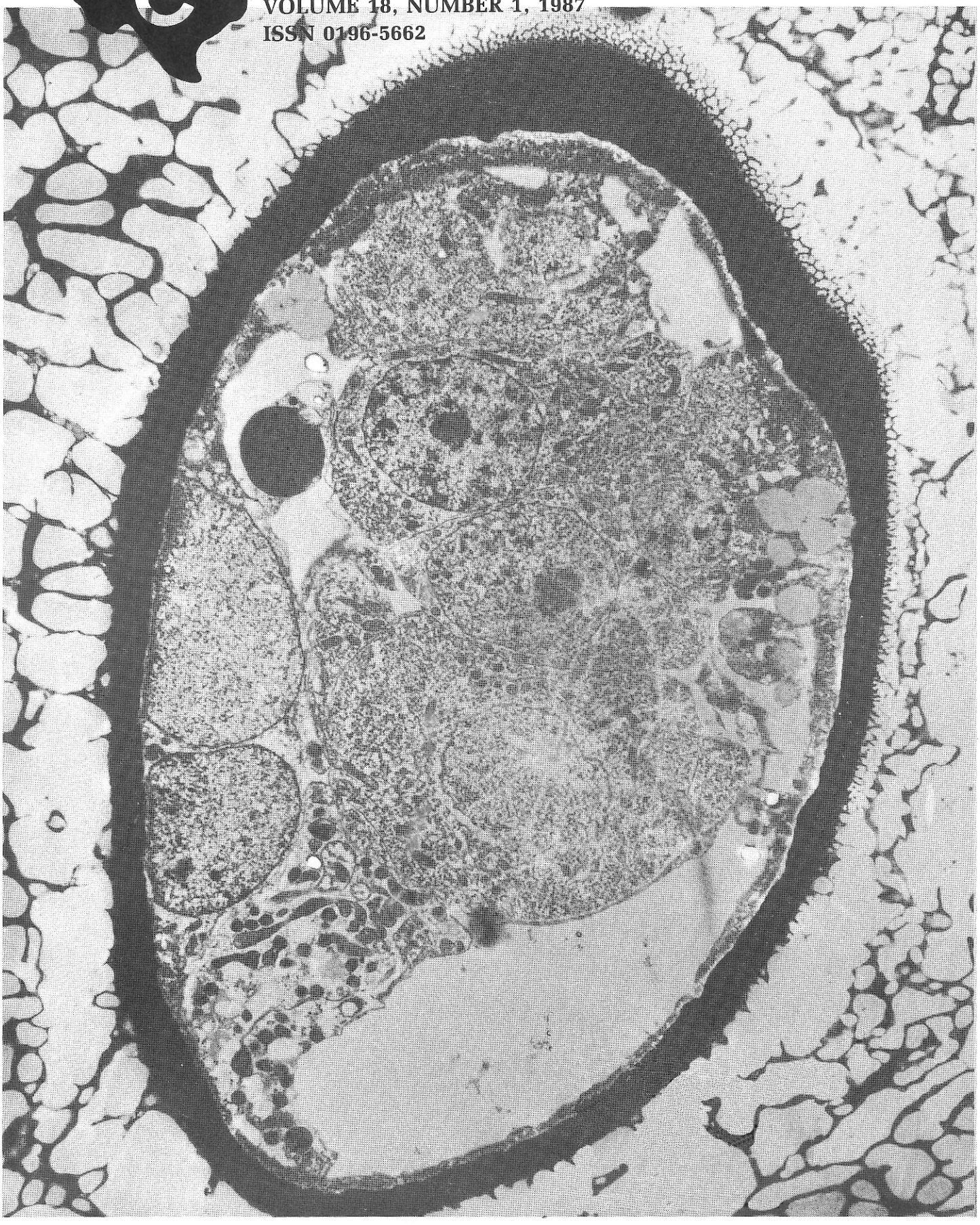

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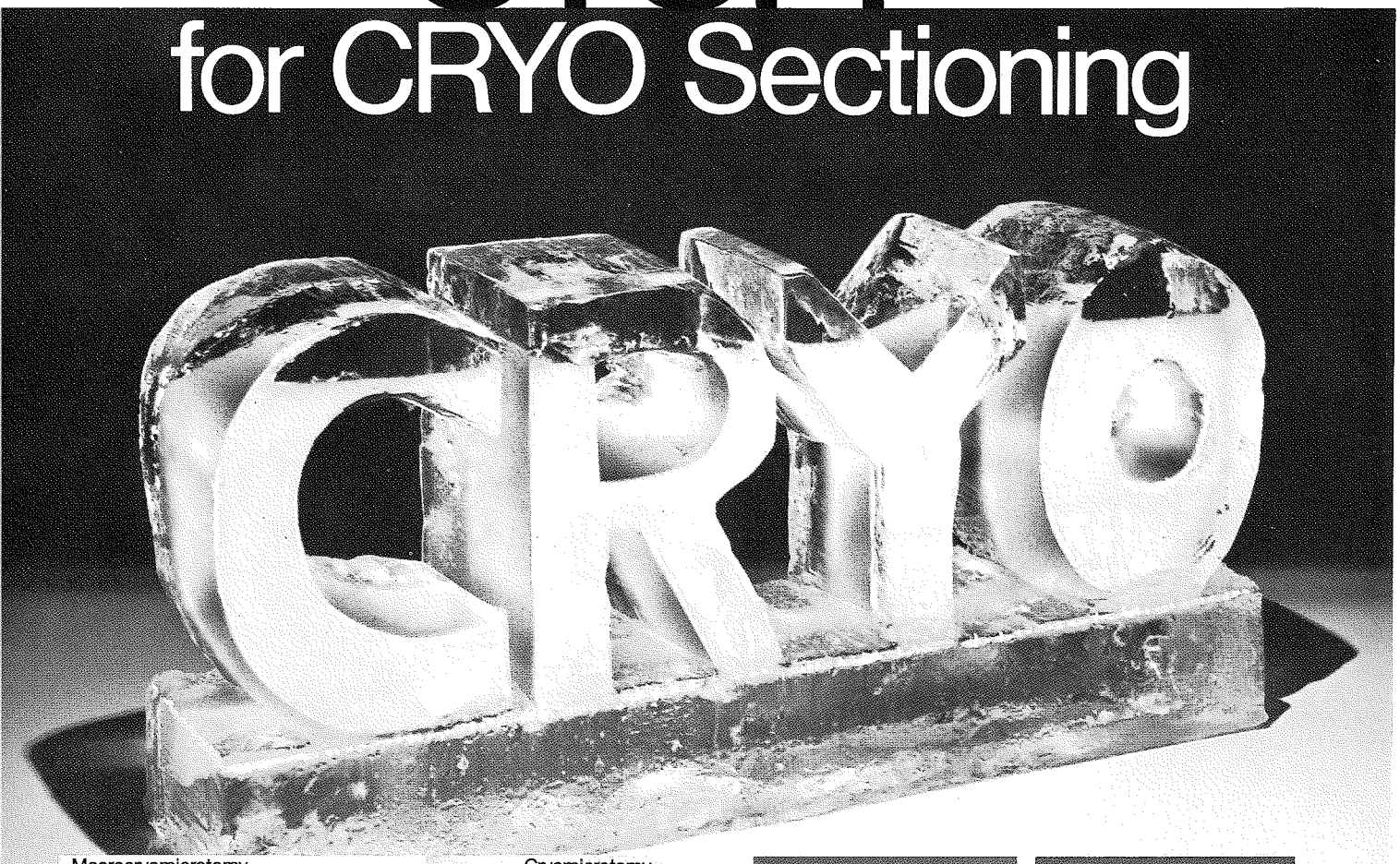

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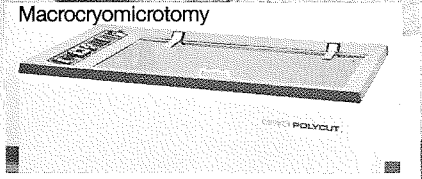

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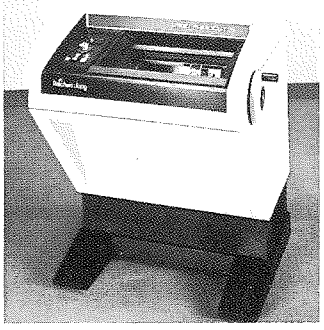

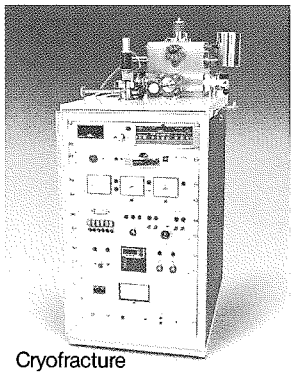

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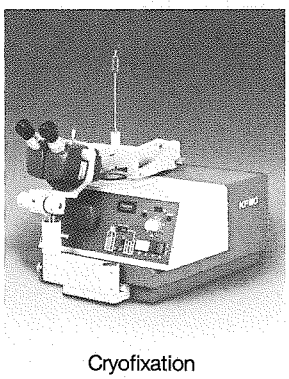
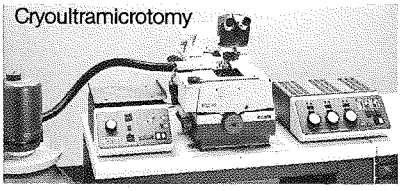


Cryofixation

Cryoultramicrotomy



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Ronald W. Davis, Editor
Department of Medical Anatomy, Texas A&M Univ., College, Station, TX 77843

Texas Society for Electron Microscopy

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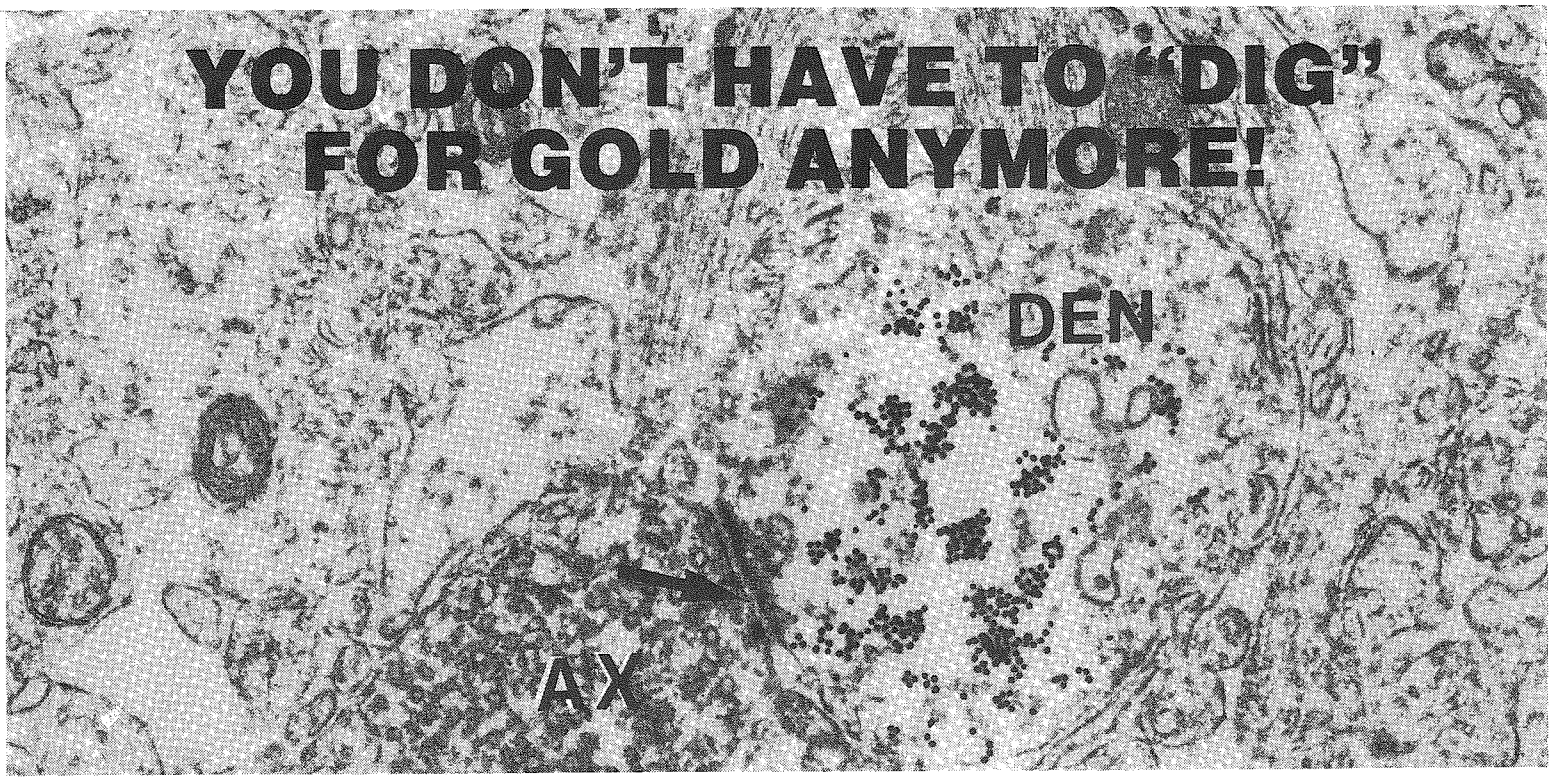
ON THE COVER

Low power electron micrograph of a freeze substituted *Schistosoma mansoni* egg. Conventional chemical fixatives have proven unsatisfactory in preserving these parasite eggs. By using a process of freeze substitution, the internal egg structures of this immature egg are successfully preserved for transmission electron microscopy. The surrounding tissue has extreme freeze damage, suggesting a natural cryoprotectant in the parasite egg. x8000. Photo submitted by Pamela J.G. Neill and Jerome H. Smith, Department of Pathology and Laboratory Medicine, Texas A&M University, College Station, Texas 77843-1114.



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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.

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(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.

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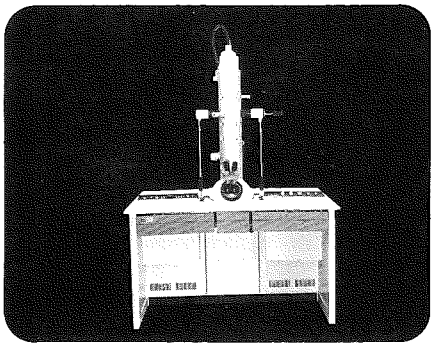

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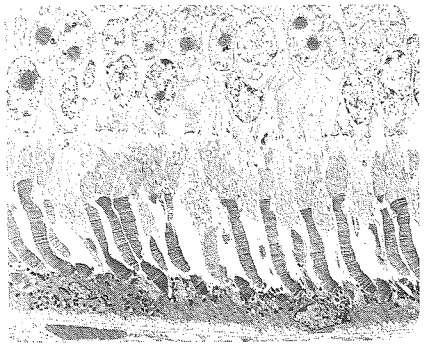

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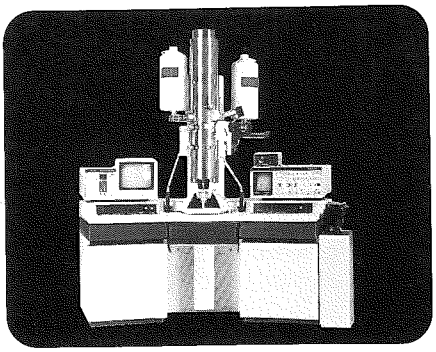

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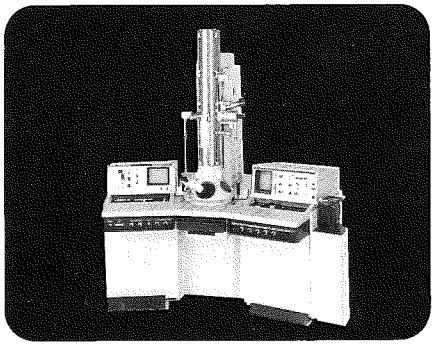

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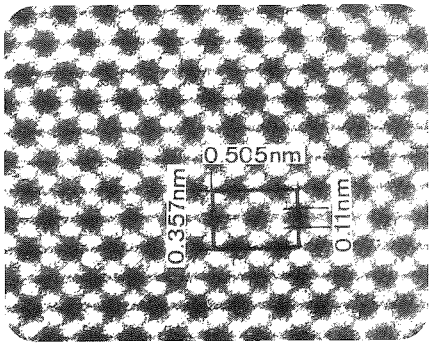

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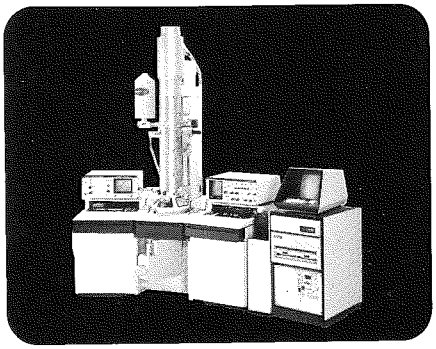

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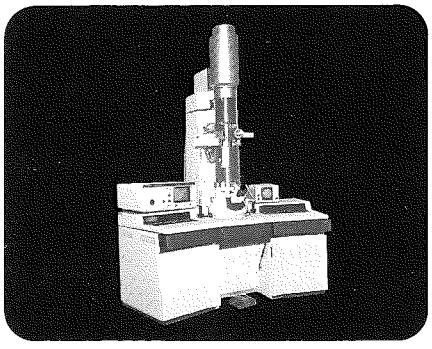

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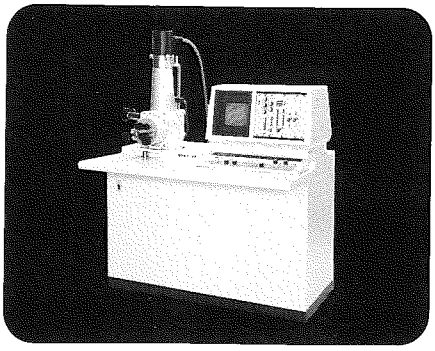

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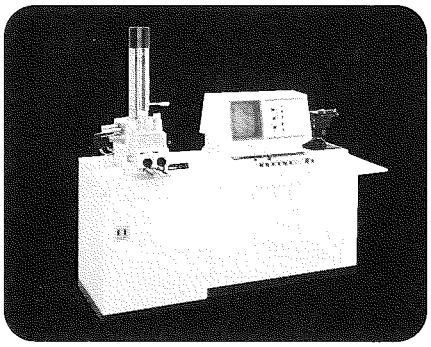

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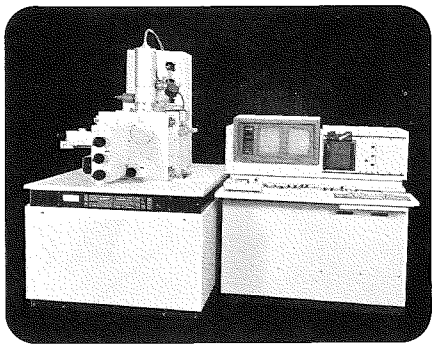

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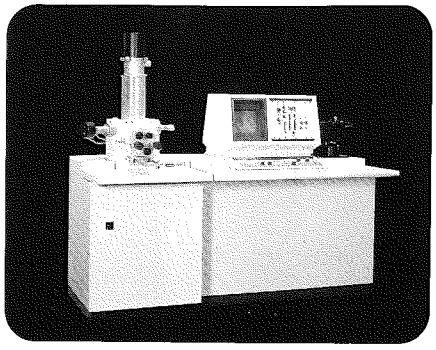

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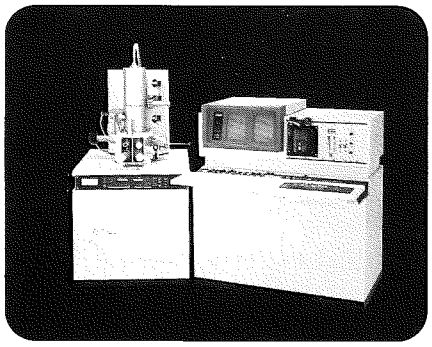

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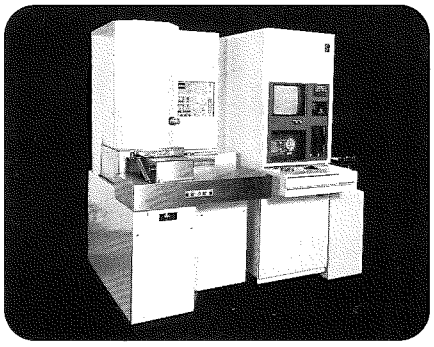

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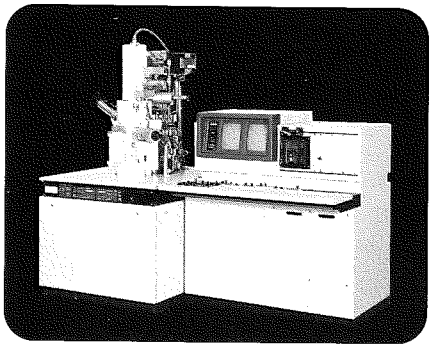

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SUGGESTED PROCEDURES AND GUIDELINES FOR AN ELECTRON MICROSCOPE LABORATORY IN A MEDICAL FACILITY

By

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Department of Surgical Pathology

Scott and White Memorial Hospital and Clinic
Scott, Sherwood and Brindley Foundation
Texas A&M University College of Medicine

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ABSTRACT

Various electron microscopy laboratory methods to improve the quality of micrographs and decrease the turnaround time are presented for a diagnostic, surgical pathology laboratory.

DEVELOPMENT OF AN ELECTRON MICROSCOPY LABORATORY

Sixteen years ago, after three to four months of "start-up" time, the electron microscopy (EM) laboratory at the Scott and White 500-bed medical facility was established. Over the years and during 7,700 cases, supplies and equipment have been added to the EM facility which include: 1) Hitachi HS-8 electron microscope; 2) darkroom equipment for both negatives and prints; 3) microtomes (Sorvall, Porter-Blum MT-2B, MT-1 used for 1μm and thinner sections); 4) chemicals, glassware, and miscellaneous supplies. In the last ten years, we have added a Hitachi H-600, and a Reichert Ultracut E Microtome with the use of a Diatome diamond knife to cut high quality sections.

In addition to the initial and more practical concerns of establishing the laboratory, we were concerned about the more difficult task of "selling" the service to the physicians in medical areas other than pathology such as nephrology, hematology, and neurology. The response to our service in these specialties has grown over the years to the point that our present-day electron microscopy facility processes approximately 400 specimens annually.

For those first few years, the medical electron microscope was considered to be in its infancy, in part due to the lack of EM interpretive knowledge. Common questions in those early years included: "What is this structure and what does it mean?" Pathologists responded to these questions and others by quickly becoming aware of the potential for the microscope as an effective diagnostic tool in pathology, especially in the diagnosis of kidney disease and certain tumors.

Improved engineering designs have simplified the use of electron microscopes for the physician and have favorably affected the number and quality of processing techniques available to the pathologist. Because of these improvements, the application of electron microscopy has gained acceptance as an essential diagnostic tool for certain types of tissues.

Unfortunately, there are many laboratories equipped with electron microscopes that are underutilized for their potential capabilities and in some situations, there are insufficient personnel to operate them effectively. In other examples, the EM facility is located away from the tissue processing laboratory, making access inconvenient. For whatever reason, underutilization of electron microscopy laboratories is a common problem. With the increasing development and use of less expensive immunohistochemical techniques, this may worsen.

There seems to be pressure put on most EM labs today to emphasize cost effectiveness and rapid turnaround time. Because of this, and increasing charges to patients, it is becoming more important for the surgical pathologist to obtain alternate diagnostic methods such as immunohistochemical techniques that are faster and more economical.

Although most hospital EM laboratories have evolved through independent efforts to become self-supporting and to serve physicians, adequate funding remains an increasingly urgent problem. In this climate of austerity, it is even more important to provide quality micrographs in the shortest possible time to the pathologist users.

This is accomplished in many ways in order to

achieve quality results for diagnostic pathology. Lab personnel must not only be qualified and competent, but they must be conscientious. The best equipment and materials must be used. It is advantageous to buy chemicals such as fixatives and buffers from reputable commercial sources rather than make in-house stock solutions. Of utmost importance is using a purified grade of water for collecting thin sections in the diamond knife boat and for rinsing grids during post-staining with uranyl acetate and lead citrate. The selection, use, and care of a diamond knife used to thin section is critical to the resultant quality of thin sections.

RECOMMENDED EM LAB PROCEDURES

Knowing that there are no established rules in the EM field to standardize quality and to achieve results, our goal is to provide the highest quality micrographs possible with a 48-hour turnaround time from the fresh tissue to the finished print. In order to obtain adequate tissue samples, a good understanding between the clinician and electron microscopist is essential. In our medical facility we are fortunate to have the operating suites adjacent to a frozen section surgical pathology laboratory. As surgical tissues are received in the frozen section area, a pathologist grossly examines the specimen to determine if any of the sample is to be examined by EM, in which case the pathologist will carefully excise a small piece of the affected area and immediately immerse it into a small vial containing the appropriate EM fixative (2.5% PO_4-glutaraldehyde). The time lapse from the patient's blood supply until the specimen is immersed into the EM fixative to less than five minutes, which, under hospital surgical conditions, is commendable. The pathologist cuts representative frozen sections, makes a diagnosis using a light microscope and, using an intercom system to the surgeon in the operating room with the patient. This expedient course of action enables the surgeon to complete the operation or to take additional tissue.

Tissue for ultrastructural evaluation is received from either the operating or treatment rooms, and an EM representative is usually present at the time of excision. A major problem we experience with small regional hospitals is receiving tissues that are improperly fixed (e.g., saline, formalin, water, or some unknown liquid). Final electron micrographs reveal the inferior technical results of these inadequate fixatives which often make the specimen of no diagnostic use.

Our routine procedure in procuring and processing specimens, either from surgery or from the treatment room, depends on the type of tissue. After removal from the patient, all tissues are immediately immersed in phosphate buffered 2.5% glutaraldehyde for a 2 to 4-hour period to allow sufficient penetration time. The following day the vials of EM specimens containing the fixative are changed to a PO_4 buffer and allowed to refrigerate (4°C) for approximately one hour. Our laboratory examines three categories of tissues which are handled in one of the following ways:

A. Muscle and kidney biopsies—0.1 M glutaraldehyde (sic) buffered with Millonig's PO_4 formula.
B. Tumors and other tissues—2.5% glutaraldehyde plus 0.1 M cacodylate buffer.
C. Nerve biopsies—nerve fixative containing .025 M cacodylate buffer plus 2.5% glutaraldehyde.

Probably the most common buffer used in ultrastructural studies is HCL-sodium cacodylate, although phosphate buffers are also popular. S-collidine is widely used for osmium tetroxide solutions. The function of the buffer is to maintain the pH of the cell and its components as close to the natural level as possible. The choice of buffers includes S-collidine, veronal acetate, Millonig's phosphate, and cacodylate; no one buffer has a clear advantage over the others.

TECHNICAL CONSIDERATIONS

Tissues may remain in their appropriate fixative overnight. The following morning these tissues are placed in their appropriate buffer for two changes at 15 minutes each. After the buffer rinse, the tissues are allowed to post-fix in 2% OsO_4 with the appropriate buffer for one hour at 4°C. The fixative is then decanted and 20% ethyl alcohol at 4°C is added for five minutes. The specimen is next placed for 15 minutes into a solution of 5% ETOH with 1-2% (W/V) uranyl acetate. This en bloc staining further enhances the tissue contrast for final electron micrographs.

After the en bloc staining, the tissues are rapidly dehydrated through a series of cold 70%, 95%, and absolute alcohols at 4°C, for five minutes each, with the exception of absolute alcohol which is left cold for one hour and then allowed to warm to room temperature. Following rapid dehydration, the tissues are processed through an exacting embedding mixture infiltration using the following method:

A. Embedding Mixture. Weigh the following and mix thoroughly for five minutes with applicator sticks:
   Spurr's Low Viscosity Embedding Medium
   1) vinyl cycohexene dioxide (VCD) 10 g.
   2) Diglycidyl ether of polypropylene glycol (DER 736) 6.0 g.
   3) Nonenyl succinic anhydride (NSA) 26.0 g.
   4) Dimethylaminoethanol (DMAE) 0.4 g.
B. Add one part of the above mixture to one part absolute ethyl alcohol and mix thoroughly.
C. Pour off the alcohol from the specimen and add 2 ml of the alcohol/plastic mixture for one hour (infiltration may be enhanced by agitation).
D. After one hour decant 1 ml of the mixture from specimen and add 1 ml of 100% Spurr's embedding medium.
E. After one hour pour off this mixture and add 100% Spurr mixture for a period of two to four hours.
F. Place Beem capsules into holder and

appropriately label all capsules, by inserting a small, typed, piece of paper with project numbers.

G. Using a 12 cc disposable syringe to dispense embedding medium, fill all capsules.

H. With the aid of a wooden applicator stick, transfer tissue pieces from the vial to the epoxy-filled Beem capsules.

I. Place holder containing the Beem capsules into a 60°C oven overnight for polymerization.

Following overnight polymerization, the holder containing the capsules is removed and allowed to cool to room temperature. Selected blocks are chosen for "thick sectioning" (1μm). While using a dissecting microscope, the blocks are trimmed with a clean razor blade and thick sectioned with a Porter-Blum MT-1 microtome using a dry glass knife. As suitable sections are obtained on a glass knife, they are carefully transferred to a drop of water on a clean 1″ x 3″ glass slide. The sections are allowed to dry and adhere to the slide by using an 80°C hot plate. The sections are then stained with toluidene blue and a few drops of a solution of 1% sodium borate solution for 10 to 15 seconds while still on the hot plate, washed with a stream of distilled water, cover slipped, and examined by the pathologist.

After selecting one of the aforementioned slides, the appropriate block is then "thin sectioned" on a Reichert Ultracut E Ultramicrotome. The sections are picked up on an uncoated 200 mesh copper grid, post-stained with uranyl acetate and lead citrate, and observed in a Hitachi H 600 electron microscope.

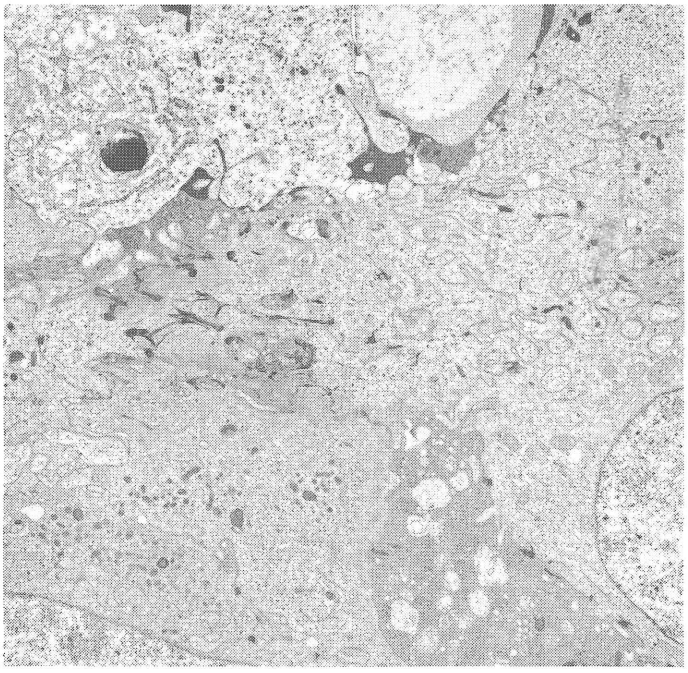

Figure 1. Breast tumor showing electron-dense tonofilaments. This lesion is classified as a neuro-endocrine carcinoma with squamous metaplasia due to transition between squamous cell and those with neuro-endocrine granules. × 9000

Because the Hitachi H 600 has the capacity for 30 negatives, general procedure includes taking ten negatives of three patients cases. Once the negatives are taken, they are removed from the microscope and processed in an adjacent negative developing room. After the negatives are processed and dried, they are placed into a clear Kodak film sleeve for viewbox examination to check quality and focus. The negatives are then printed on a Ilfospeed RC paper and developed in Ilfospeed developer and fixative. Advantages to using the Ilfospeed products include shorter developing, fixing, and washing times. To reduce drying time to 20 seconds per print, we use the Durst hot air print dryer. Once the prints are dry, they are labeled on the back in a series of information spaces. The finished prints are then placed in a manila folder along with the slide of the thick section of the case and given to the pathologist for interpretation.

Another application of diagnostic EM is rapid viral diagnosis. The simplest technique for identification is negative staining. This method enables one to see viral particles in clinical specimens in less than three hours.

In conclusion, successful and continuous, high quality EM results depend on constant attention to the complete process from the patient to the prints. If any one of the links in the chain of events goes wrong, then the results of the final micrographs are no doubt adversely affected.

Included at the end of this article are six electron micrographs depicting some typical pathology cases done in our laboratory.

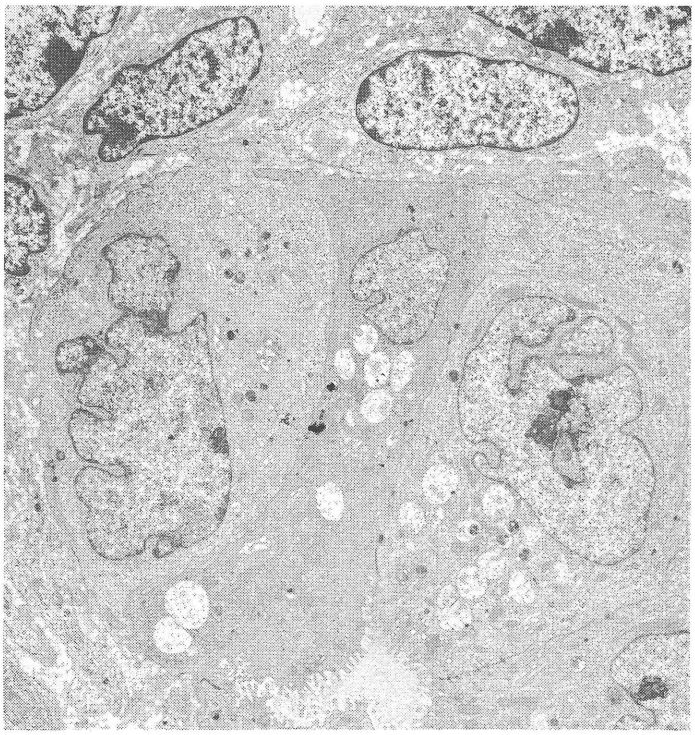

Figure 2. Endometrial curetting showing ultrastructural characteristics of tumor cells of adenocarcinoma probably arising in the gastrointestinal tract. × 6000

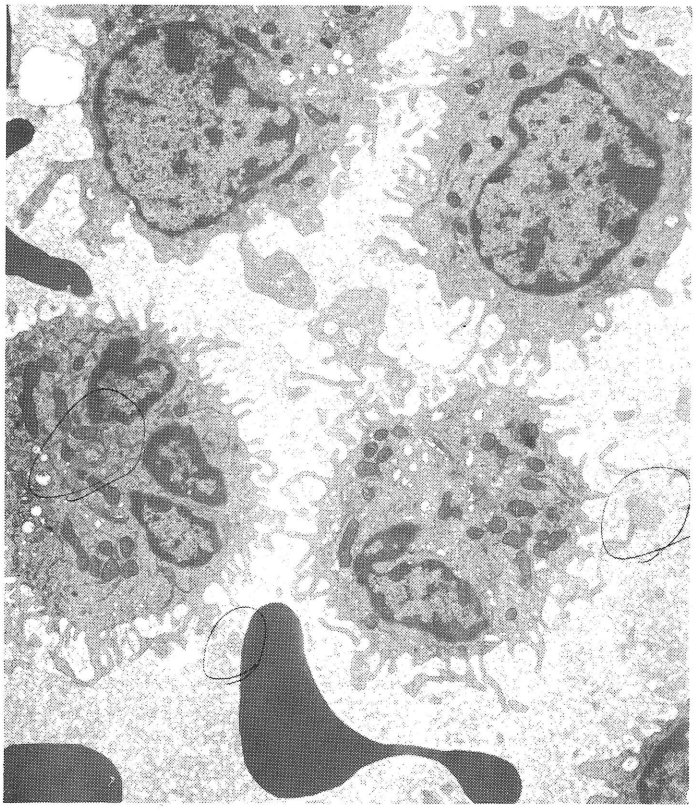

Figure 3. Buffy coat of blood showing classic case of Hairy Cell Leukemia. × 1500

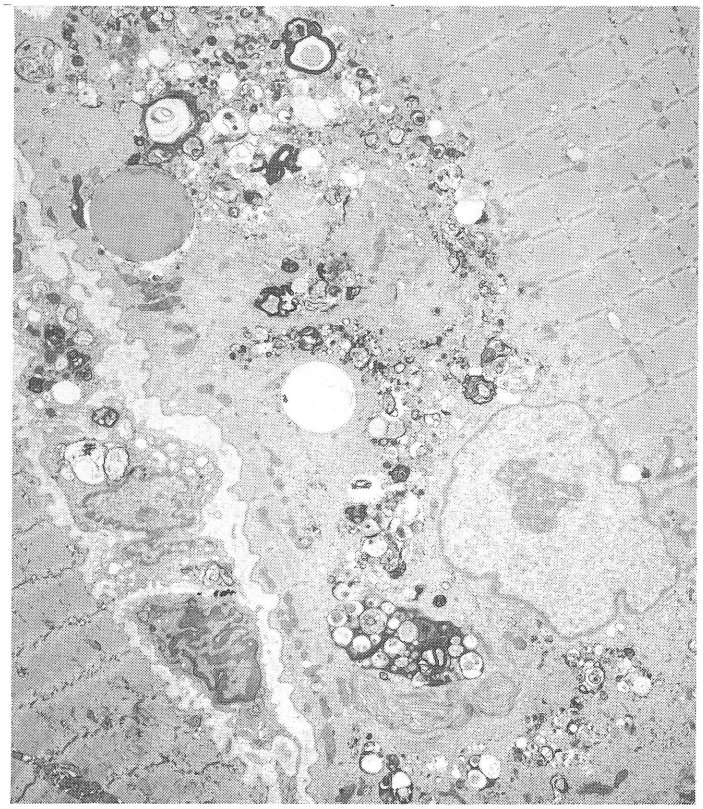

Figure 4. Muscle biopsy showing inflammatory myopathy consistent with inclusion body myositis. × 6000

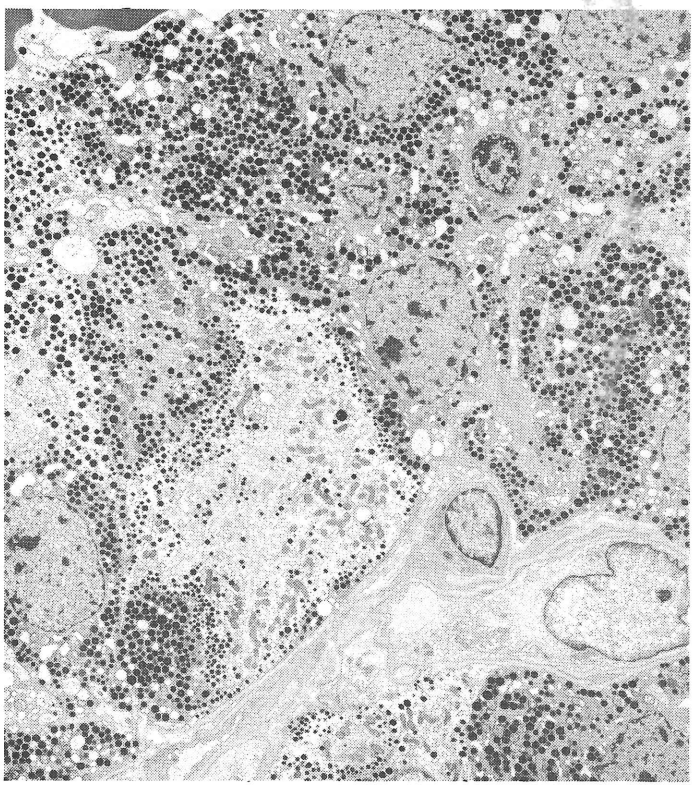

Figure 5. Pituitary tumor showing secretory granules ranging in diameter from 230-500 nanometers which is unusual for corticotroph cell adenomas. This tumor was diagnosed as pituitary adenoma, diffuse type, producing adreno cortico tropic hormone. × 45000

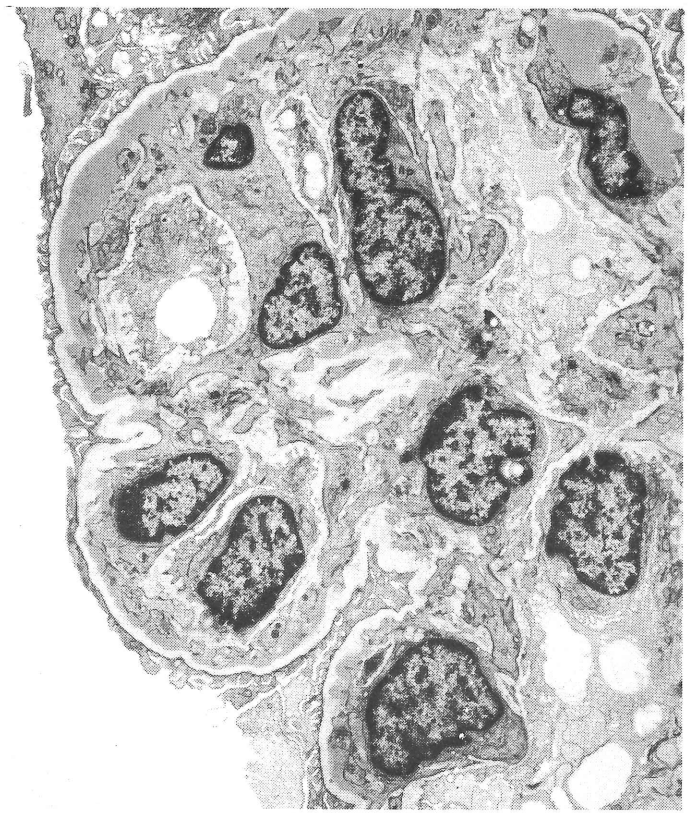

Figure 6. Kidney biopsy showing striking subendothelial and intramembranous electron-dense deposits. Consequently diagnosed as diffuse proliferative lupus nephritis. × 6000

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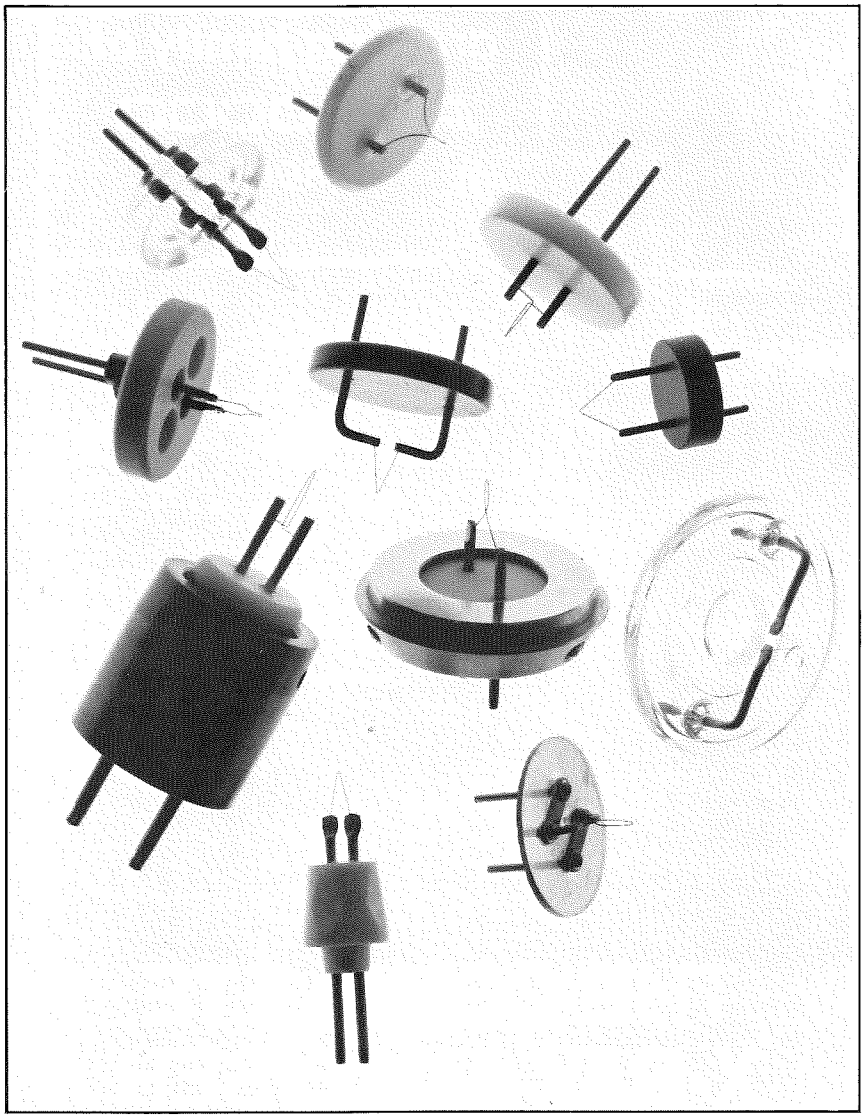

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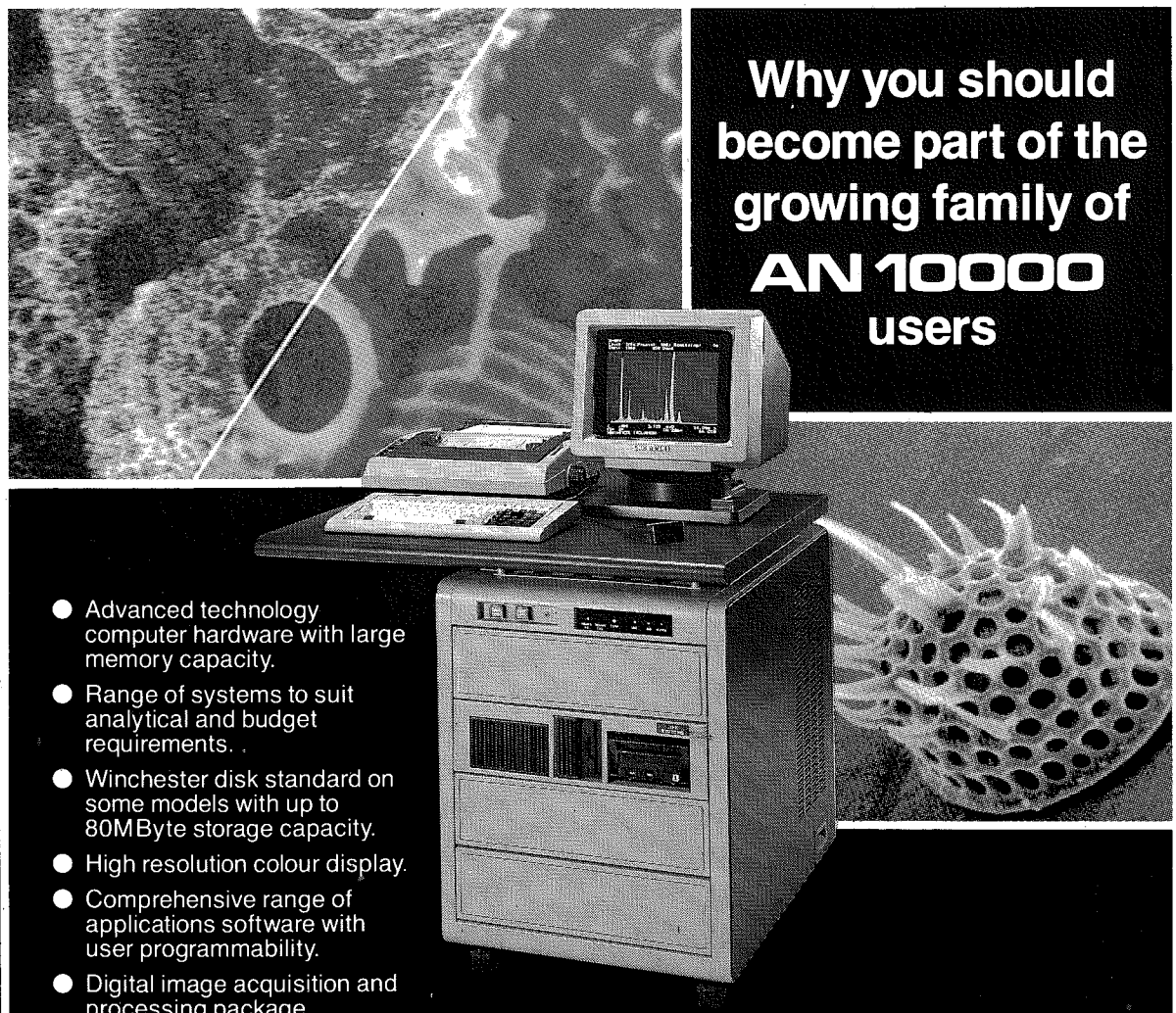

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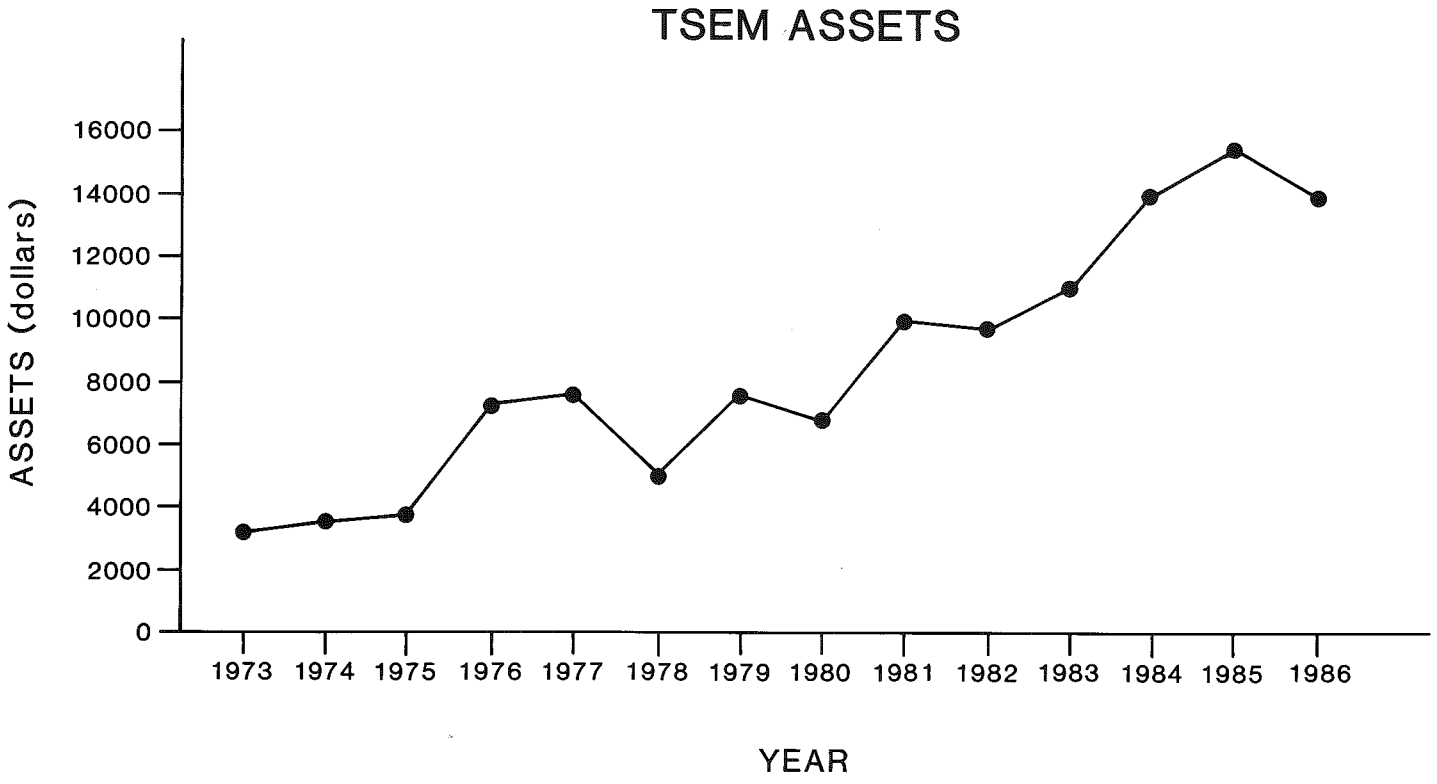
A SURVEY OF TSEM ASSETS

A survey of TSEM assets, as published in past issues of the journal, reveals a steady strengthening of the society's financial position. This has allowed the executive council to propose that certain incentives be initiated to encourage student and technologist participation in the society and its meetings. The council has recognized that one of the greatest assets of the Texas Society for Electron Microscopy is an increased and continued participation by these members.



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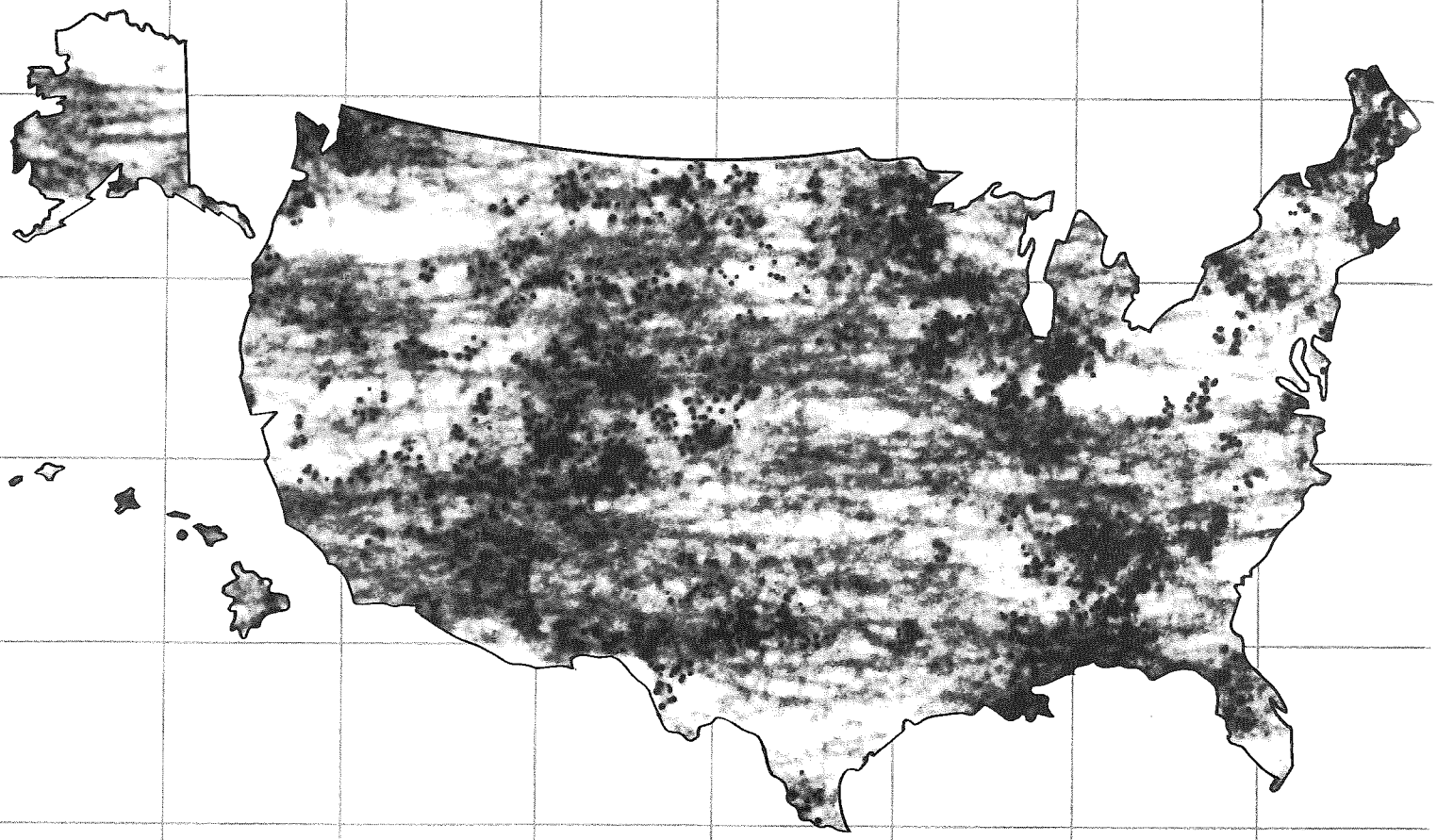

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the micrograph depicted within the USA map
Double immunogold staining of alpha-actinin and myosin in a chick heart fibroblast: myosin, labeled with 5-nm gold is confined to regions between alpha-actinin(10-nm gold).

(*Reference available on request ;* "The molecular organization of myosin in stress fibers of cultured cells", Gabriele Langanger, Marc Moeremans, Guy Daneels, Apolinary Sobieszek, Marc de Brabander and Jan de Mey)

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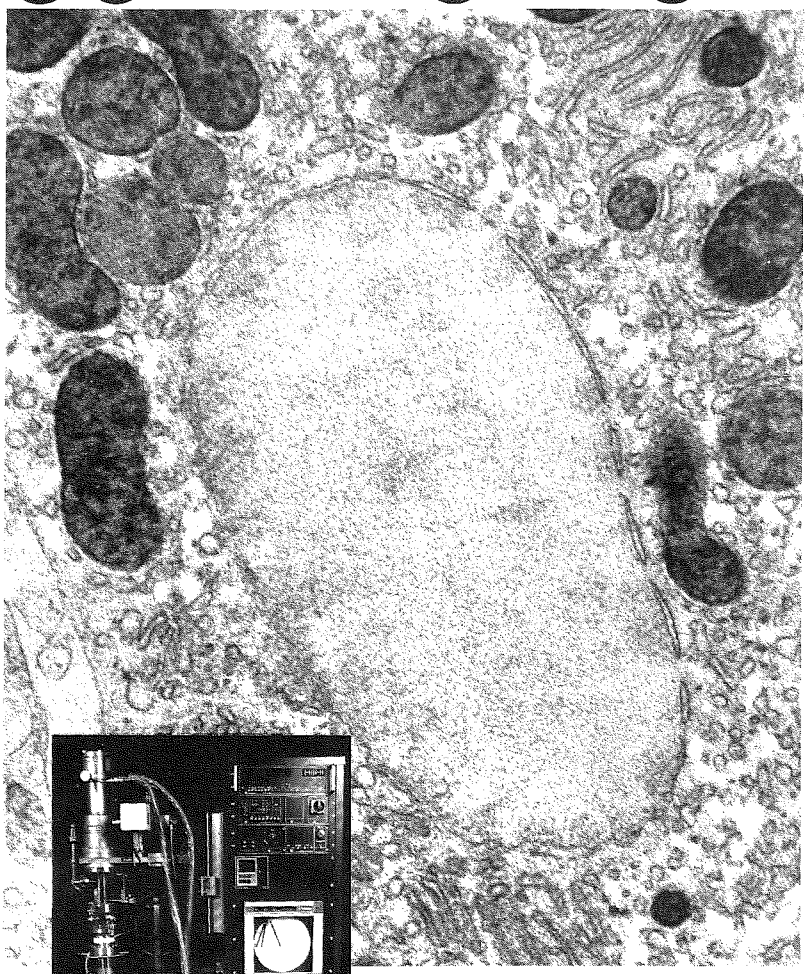

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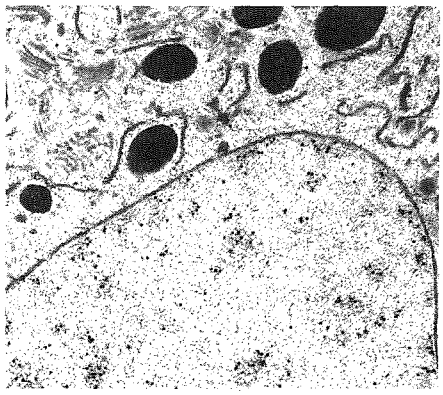

Colloidal gold labeling with antinuclear antibody (ANA) positive serum. Localization specifically to heterochromatin of the nucleus. (MCF-7 culture cell).

A SIMPLE METHOD FOR CONVERTING BETWEEN TOTAL MAGNIFICATION AND INTERNAL DISTANCE SCALES IN MICROGRAPHS

by Ronald W. Davis
Electron Miscroscope Center
University of Idaho
Moscow, Idaho 83843

After producing good quality light or electron micrographs, students and other workers sometimes find it difficult to determine the length of scale bars used to represent distances in the original specimen. One method that can be used takes advantage of the rule that for every 1000 magnifications, a line 1mm long represents 1 μm in the original specimen (**Shillaber,** 1944; **Postek,** 1980). This works well for low to medium power electron micrographs but does not address the problems of how to convert to units other than micrometers or how to convert a distance scale to total magnification. Another method involves the use of slide-rule-like calculators sold commercially by electron microscopy supply houses. These devices are adequate in many instances but their price makes them unattractive to many investigators.

I would like to offer the following method for converting total micrograph magnification to internal specimen distance (a scale bar) and internal specimen distance (scale bar length) to total magnification. This procedure is quick, simple, requires very litle or no computation for converting to or from any of the commonly used units of distance and costs only as much as a plastic millimeter rule.

Specifically, the method involves only a shift of a decimal point to the left when converting from total magnification to a scale and to the right when converting from a scale to total magnification. The number of places the decimal point is shifted varies for each unit of measurement and a table (Table 1) can easily be constructed to allow quick reference.

Consider the example where we have a micrograph of 200X and wish to place a distance scale on it. The length of the scale for any unit can be determined by consulting Table 1 to find how many places to the left the decimal point in the total magnification must be shifted. If we want to represent units of micrometers, the decimal points must be shifted three places to the left. This will result in the number .2 and this will be the number of millimeters equal to 1μm in the specimen. This would result in a scale bar .2mm long and far too short to be useful. By consulting the table, we find that if the decimal is shifted one place to the left, a 20mm scale will equal 100μm or if it is shifted two places, a 2mm scale will equal 10μm. Both of these may be acceptable bar lengths and the more convenient one can be chosen.

Consider now a situation opposite that described above where one is studying a micrograph in the literature that has a scale bar which is labeled 10nm and wishes to know the total magnification of the print. Here we only need to 1) measure the length of the scale in millimeters and 2) move the decimal point five places to the right (as determined from the table). For example, we measure the scale as 10mm long. We then move the decimal point five places to the right and determine that the total magnification of the micrograph is 1,000,000X.

In practice, this procedure is very simple to apply. I have been using it in microscopy courses for several years and have found that students understand and use it quickly. Because most workers in the scientific community are used to thinking in metric units of measurement, they will often find it very easy to accurately estimate the length of a scale on a micrograph. This method allows a very rapid, direct, and simple procedure for determining distances within a micrograph when only the total magnification is known or for determining total magnification when only a scale bar is given.

TABLE 1

Unit of Measurement	Number of Places to Shift Decimal Point
1.0 millimeter	0
0.1 mm = 100μm	1
0.01 mm = 10 μm	2
1.0 μm	3
0.1 μm = 100 nm	4
0.01 μm = 10 nm	5
1.0 nm	6
0.1 nm = 1 Å	7

REFERENCES

1. Shillaber, Charles Patten, 1944, Photomicrography in Theory and Practice, John Wiley and Sons, Inc., N.Y.

2. Postek, M.T., K. Howard A. Johnson, and K. McMichael, 1980, Scanning Electron Microscopy, A Student's Handbook, Ladd Research Industries.

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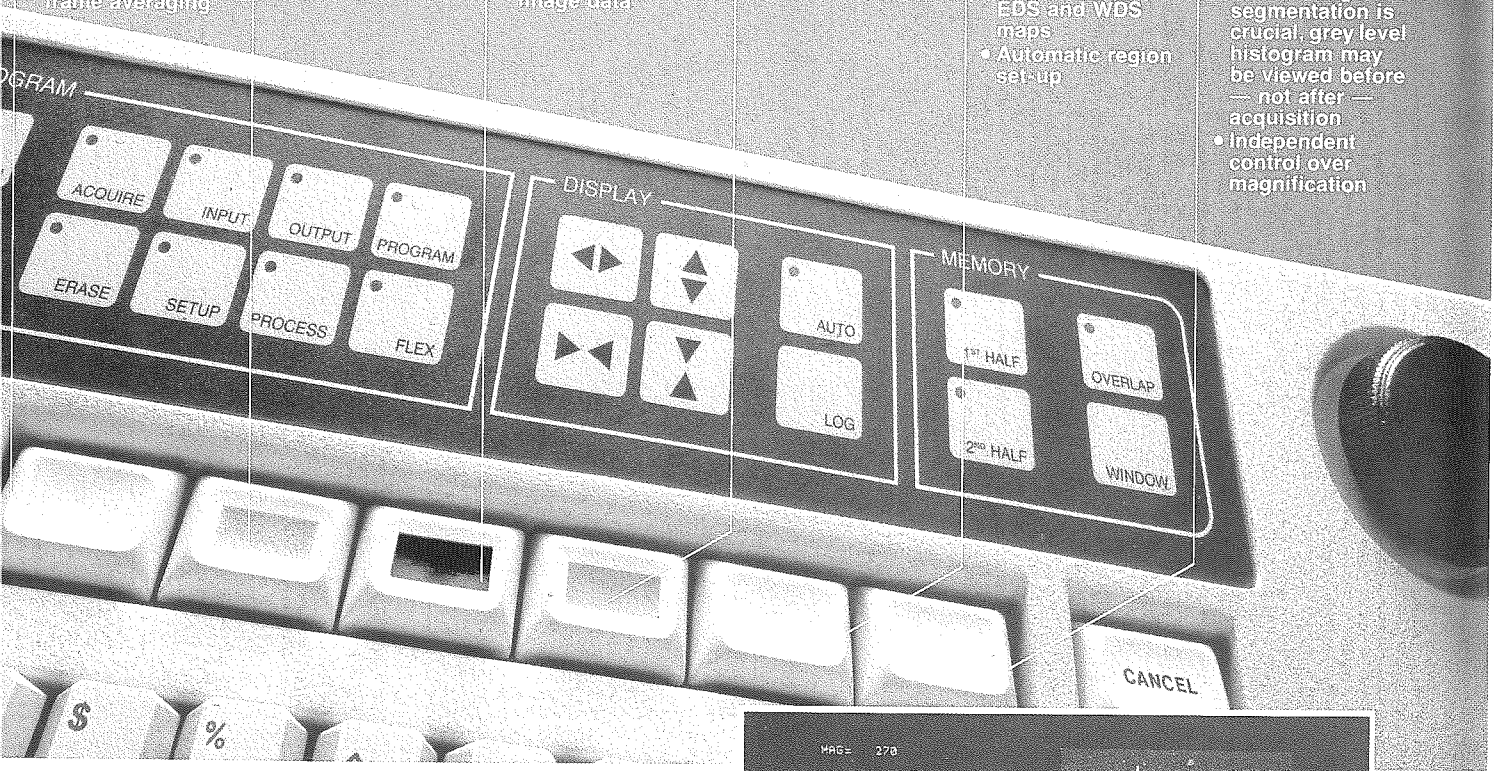

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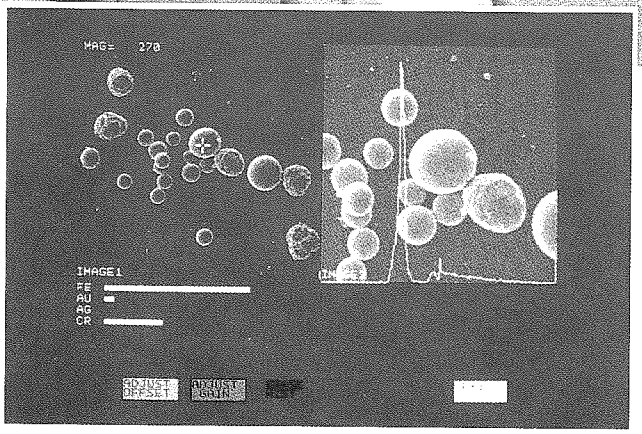

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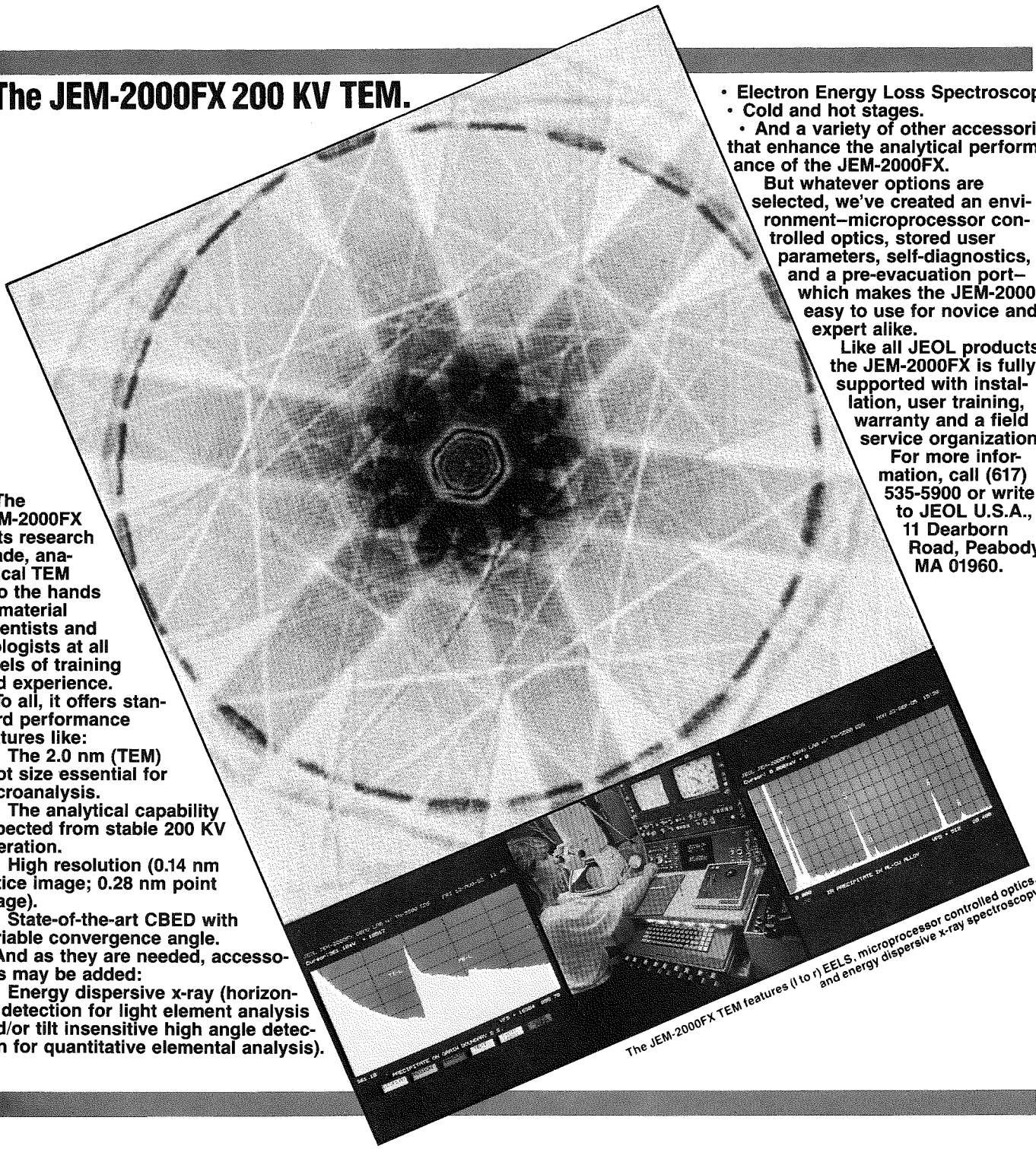

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GUIDELINES FOR SEEKING EMPLOYMENT

by Timothy Mislan
University of Oklahoma
Department of Botany and Microbiology
Norman, Oklahoma 73069

The purpose of this article is to provide a person searching for a job in the field of electron microscopy with some tips that have proven to be successful.

A concise yet informative resume is very important. It is from this resume that a potential employer will form his intitial opinion of you and your qualifications. The following should be included.

Name
Address
Telephone Number
Career objective(s)
Education
Work Experience
EMSA certification (if applicable)
Instrumentation (include all microscopes and auxilliary equipment with which you are familiar).

The resume should be confined to one page, and should be of quality print on high grade paper. A cover letter typed on the same stationery should accompany your resume. The cover letter should be of a personal nature (i.e., no form letters) and provide specific information such as various E.M. techniques mastered, exact date available, and willingness to relocate.

Once a good resume and cover letter have been written, a variety of approaches can be used to contact potential employers. Start be registering with the Statistical Office of EMSA. This can be done by contacting Dr. John H.L. Watson, EMSA Stastitical Officer, 654 Hupp Cross, Birmingham, Michigan 48010. You will be asked to complete and return a short questionnaire listing your qualifications, which will be forwarded to employers who contact the Statistical Office. The Statistical Office provides the potential employee with a listing of those employers seeking individuals with E.M. experience. Updated addenda are mailed at regular intervals. These openings are also listed in the "Positions Open" section of the *EMSA Bulletin*.

Another very successful approach is to contact the secretaries of each EMSA Local Affiliate Society, whose names and addresses are also published in the *EMSA Bulletin* (LAS News). In a cover letter, explain why you are contacting them and include your resume and a brief, single spaced advertisement which can be included in their newsletter. As long as you are willing to relocate, these officers are often aware of E.M. openings in their area, and they can put you in touch with the appropriate people.

It is of considerable help to contact friends in the field and alert them to the fact that you are looking for a new E.M. position. Often these people will know of openings via the grapevine, and they can make the initial contact for you. This holds true for sales representatives of E.M. equipment.

Letters of reference are very important once the initial contact with a potential employer has been made. Ask at least three people who are familiar with your E.M. experience to write letters which can be forwarded upon request to a potential employer.

As a final note, once you have applied for an opening by mail, follow-up phone calls are often beneficial. A phone call to a potential employer demonstrates your sincere interest, and it will give him or her a chance to become familiar with your communication skills.

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EMSA Bulletin 15(2)
1985, page 52, 54

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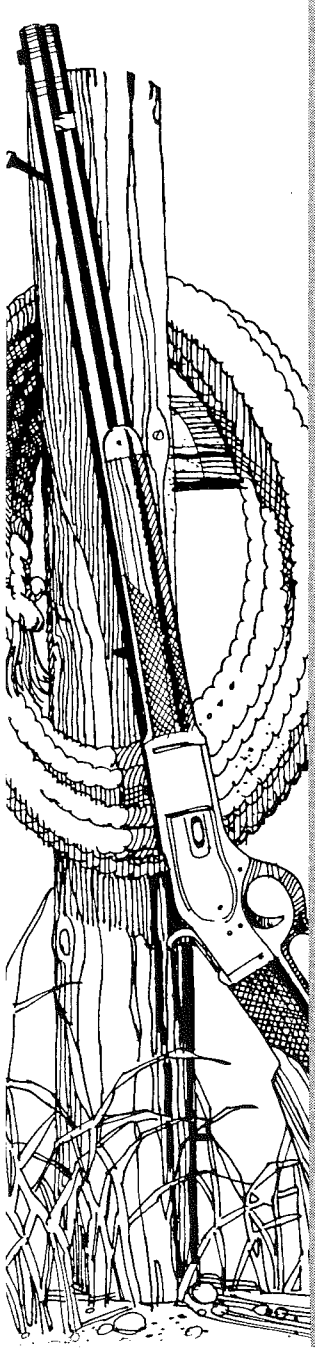

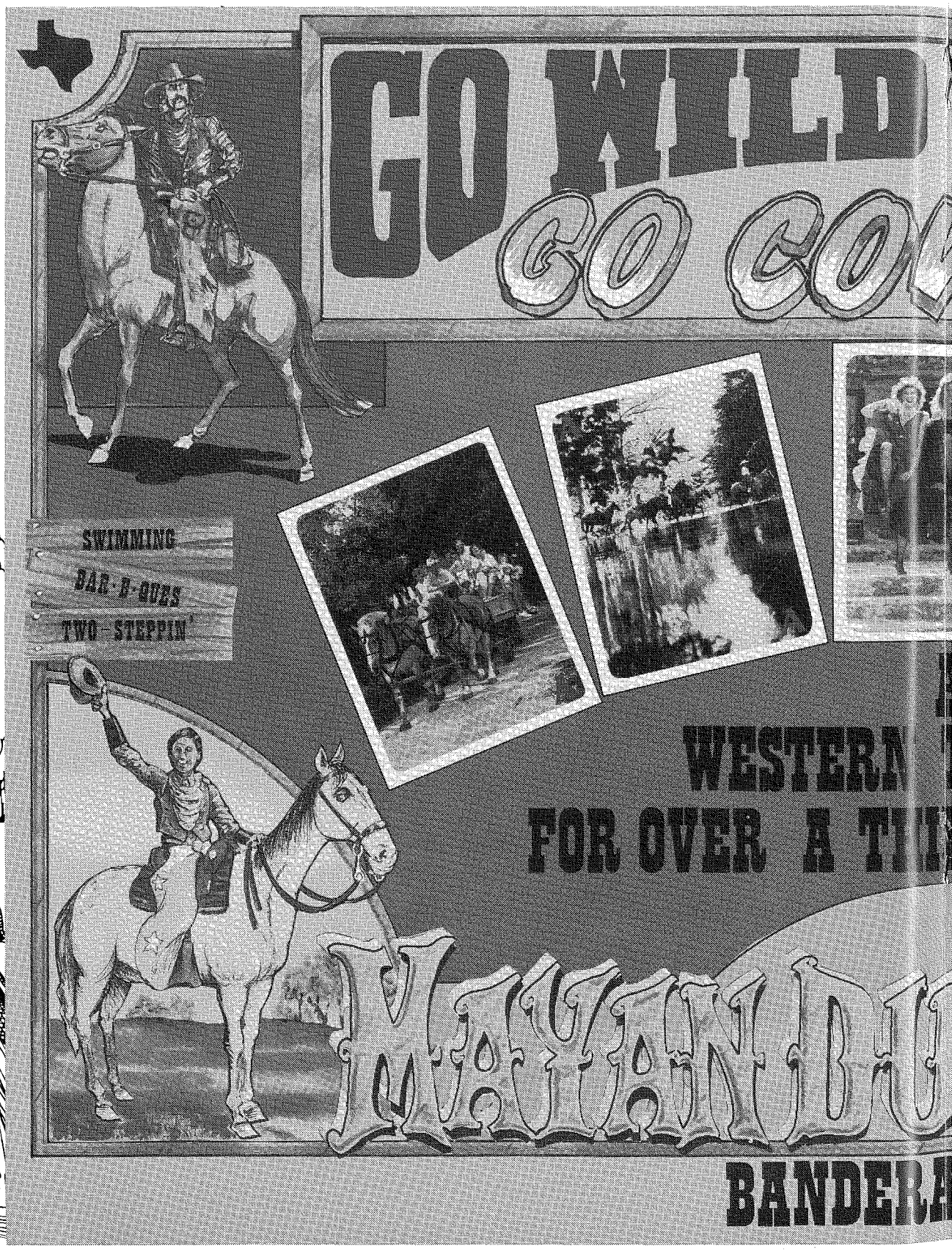

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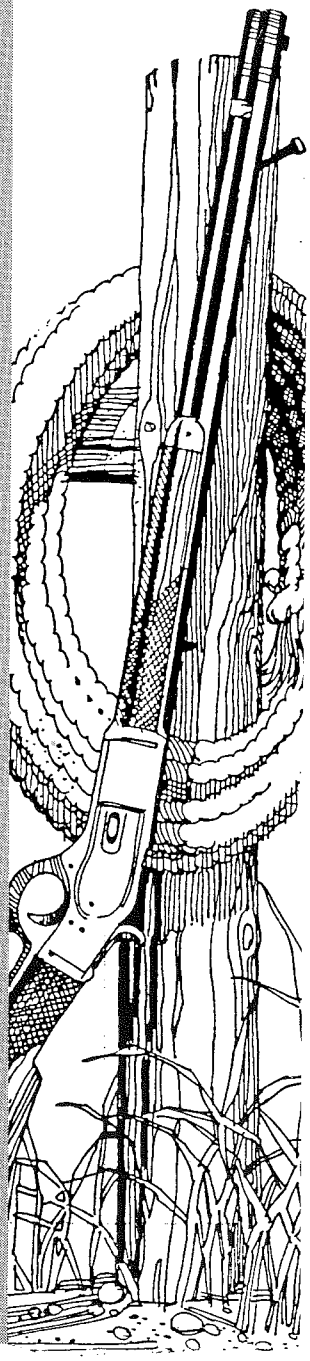

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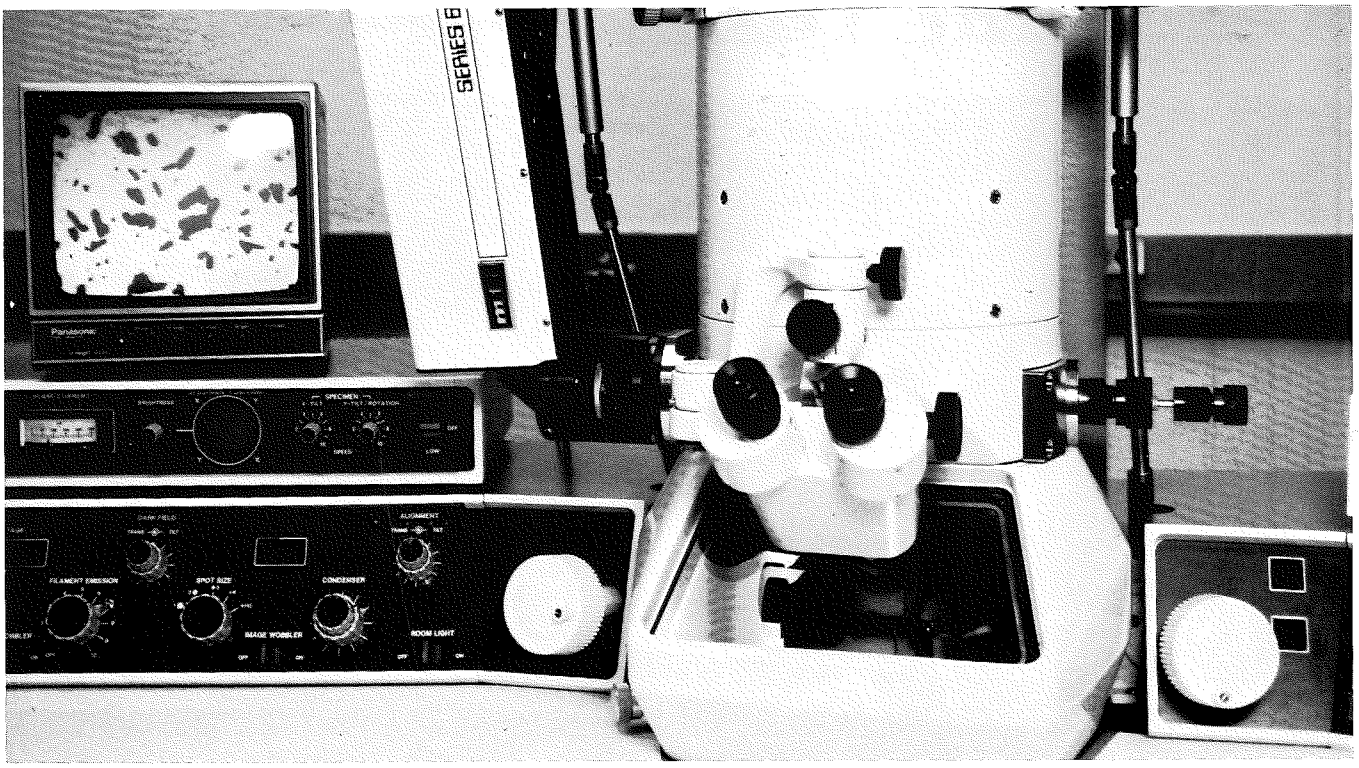

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A SURVEY OF TSEM ABSTRACTS

A survey was made of abstracts published in the TSEMJ of papers given at past meetings. I did not have a complete set of journals, but most of the issues back to 1973 were available. The abstracts were counted and correlated with the institution of origin. In all cases the institution of the first author of the paper was considered the place of origin.

As would be expected, because of their sizes, the largest number of papers came from the University of Texas (228) and Texas A&M University (123). Sixty-six percent of the total number of abstracts (total = 777) came from Texas universities and colleges. The rest came from institutions in other states and from places like the U.S. Air Force School of Aerospace Medicine, hospitals, and private industry.

What came as a surprise was the number of papers originating from out-of-state (232), notably, the University of California as a whole (56), Louisiana State University (44), and Tulane (15). These three accounted for about half of the out-of-state papers. Figure 1 shows a breakdown of the number of papers coming from various institutions.

Fig. 1
Number of abstracts, from various institutions, published in the TSEMJ since 1973.

University of Texas	
Arlington	31
Austin	30
Dallas	45
El Paso	1
Galveston	25
Houston	59
San Antonio	37
total	228
Baylor	50
Lamar	7
Rice	6
SMU	9
Stephen F. Austin	37
Texas A&M University	123
Texas Tech U	24
Texas Womens U	7
University of Austin	13
total	306
University of California (all campuses)	56
Louisiana State University	44
USDA	19
Tulane	15
total	134

Total number of abstracts counted, including those from institutions not listed = 777.

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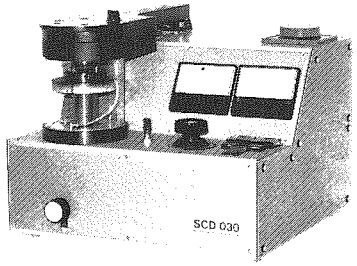

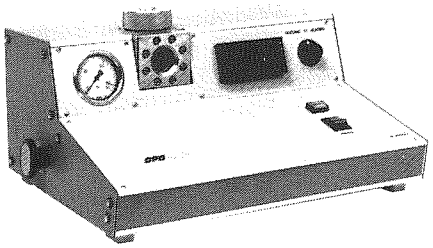

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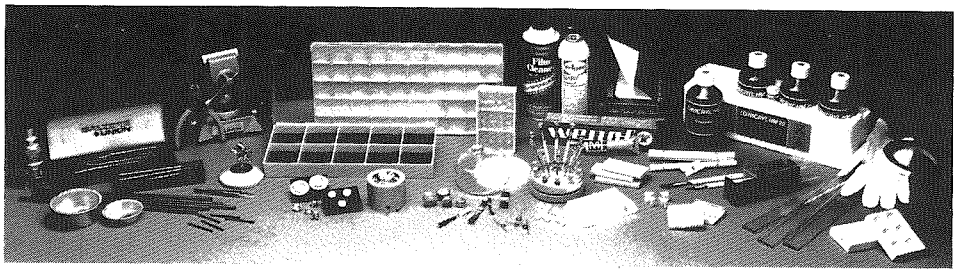

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Abstracts

BIOLOGICAL SCIENCES

PLATFORM PRESENTATION — SPRING 1987

EFFECT OF FIXATIVE ON GOLGI APPARATUS SWELLING FOLLOWING TREATMENT WITH MONENSIN. Hilton H. Mollenhauer and D. James Morre, Veterinary Toxicology and Entomology Research Laboratory, ARS-USDA, College Station, TX 77841 and Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, IN 47907.

Swelling of Golgi apparatus cisternae following monensin treatment occurs in a wide range of plant and animal species and is thought to be a universal response to monensin poisoning. The effect is most pronounced in trans cisternae. However, in plants, and to a lesser extent animals, swelling is influenced by the fixative used to preserve the cells. Specifically, swelling is less (in animal Golgi apparatus) and nonexistent (in plant Golgi apparatus) when the tissues are fixed in potassium permanganate as compared to fixation in glutaraldehyde/osmium tetroxide. These observations bear both on the mechanism of action of monensin as well as on potentially important differences between plant and animal Golgi apparatus. The approach followed was to compare images of Golgi apparatus preserved by various chemical fixatives as well as by freezing and low temperature substitution in acetone and osmium tetroxide. The differences in swelling response appear to reflect differences between plant and animal Golgi apparatus. The results of this study indicate that swelling of Golgi apparatus cisternae occurs when cells are exposed to monensin and that the image of non-swollen Golgi apparatus cisternae in potassium permanganate-fixed tissues may be an artifact. However, the mechanisms for these effects remain obscure and, therefore, further studies are planned using video-enhanced light microscopy to observe the effect of monensin on Golgi apparatus in living cells.

THE POST-PARTUM DEVELOPMENT OF THE MONODELPHIS DOMESTICA LUNG M.E. Gardner and R.V. Blystone, Department of Biology, Trinity University, San Antonio, TX. 78284

The post-partum development of the Monodelphis domestica lung was followed through the first ten days after birth. The lungs from 23 of these genetically inbred marsupials ranging from 0 to 10 days post-partum were excised and prepared for transmission electron microscopy. Of special interest was the transition of Alveolar type II cells to Alveolar type I cells. The position and size of multilammelar bodies (MLB) was also noted. At day 0, the ratio of type I to type II cells was 1:10. By day ten , this ratio was 1:2, much closer to the ratio of 1:1 reported in adult rat tissue. The ratio shift seemed to be associated with a mass expulsion of MLB's from the type II cells into the lumen of the airspaces. This expulsion took place approximately two days post-partum. With further study, this system could illustrate the timing, mechanism, and possibly the inductive processes associated with the transition of type II cells into type I cells.

(Thanks are extended to the Southwest Foundation of San Antonio and the Genetics Laboratory of Trinity University for access to the newborn Monodelphis.)

MORPHOGENESIS AT THE SHOOT APEX OF THE MOSS PHYSCOMITRIUM PYRIFORME: A QUANTITATIVE ANALYSIS OF APICAL CELL POLARITY AND MEROPHYTE DEVELOPMENT. R. Fulginiti, J.D. Mauseth, Dept. of Botany, University of Texas, Austin, TX 78713.

The single apical cell of the leafy gametophyte of the moss Physcomitrium pyriforme is an inverted pyramid with three cutting faces. It undergoes an asymmetric division which results in a derivative cell and a continuation of the apical cell. The derivative will develop into a merophyte composed of stem and leaf tissues; the apical cell will divide again asymmetrically along another cutting face, producing another derivative. The relative volumes of organelles within the apical cell are: nucleus=12.4%, plastids=5.8%, vacuoles=15.0%, mitochondria=4.0% and hyaloplasm=74.7%. We have been unable to detect lateral asymmetry: the site of the next division of the apical cell is not detectably different from the rest of the cell. However, the organelles are vertically distributed with greater amounts of some in the base of the cell (base: N=6.5%, P=7.8%, V=20.6%, M=4.5% and H=66.7%; apex: N=11.5%, P=4.5%, V=11.0%, M=3.4% and H=80.6%. The vertical polarity of the apical cell causes the derivative to be enriched in vacuoles and plastids. When the derivative divides transversely, it results in an upper, outer cell that is distinct cytologically from a lower, inner cell. As the upper cell gives rise to leaf lamina, some organelles decrease in relative volume (values at plastochron 3 are: N=16.7%, P=4.5%, V=11.6%) while others increase (H=80.0%). At plastochron 4, the costa becomes distinct as its cells become more vacuolate (V=9.3%), whereas lamina remains cytoplasmic (vacuole decreases to 5.0%) and meristematic until plastochron 6 at which point there is an increase in vacuole relative volume.

THE INFLUENCE OF MICROGRAVITY ON THE STRUCTURE OF CHLORENCHYMA CELLS IN LEAVES OF ZEA MAYS. Randy Moore, Eddie McClelen, and Mark Fondren, Department of Biology, Baylor University, Waco, TX 76798

We used the microgravity of outer space to determine the influence of gravity on the structure of chlorenchyma cells of leaves of Zea mays. Microgravity induced significant changes in the structure of the endoplasmic reticulum. We frequently observed concentric ER in flight-grown seedlings --- such alterations in the ER were not present in Earth-grown seedlings. Microgravity also significantly decreased the number of dictyosomes in chlorenchyma cells. Plastids of flight-grown seedlings possessed more and larger inclusions than those of plants grown on Earth. These results 1) indicate that microgravity significantly alters the structure of chlorenchyma cells in Zea mays, and 2) will be discussed relative to corresponding studies of other cell types. This research was supported by a grant from the National Aeronautics & Space Administration (NASA).

COMPARISON OF TESTA CHARACTERISTICS FOR CASSIA SPECIES FROM TEXAS AND HAWAII. Louis H. Bragg and Rebecca S. Westover, Dept. Biology, The University of Texas at Arlington, Arlington, 76019

Seeds of different species of Cassia from Texas and Hawaii were examined with SEM for differences in characteristics that would further establish species distinctness. Differences were observed in surface patterns and in presence or absence of pitting and pit locations. Transectional observations revealed differences in the internal features between the examined species. The distinctive testa characteristics between these limited species warrant examination of the seeds of the remaining Cassia species for taxonomic purposes.

COMPARISON OF SEED COAT MORPHOLOGY OF SEVEN SPECIES OF OROBANCHE (OROBANCHACEAE) USING SEM. Barbara C. Reuter, Dept. Biology, The University of Texas at Arlington, Arlington,76019

This study investigates the usefulness of seed coat morphology as a taxonomic descriptor for seven species of Orobanche (Orobanchaceae). Scanning electron microscopy (SEM) was used to facilitate measurements on the seeds of this genus. The seeds of all species studied appear similar under visual inspection. They are minute, have a reticulate testa and are dark brown-black in color. There are significant differences between the length and width of the seeds as well as the number of cells across the length and width of the seeds but the degree of overlap is so great that the degree of reliability for using any of these traits for taxonomic purposes is very low. It is suggested that developmental and anatomical studies may yield more useful information.

THE USE OF ELECTRON MICROSCOPY AS A RAPID MEANS OF IDENTIFYING A CASE OF INFLUENZA CAUSING DEATH IN AN ADULT MALE. Cameron E. McCoy, William B. McCombs, III, JoAnn Culpepper, and Maria Manriquez, Scott and White Memorial Hospital, Scott and White Clinic, Texas A&M University College of Medicine, Temple, Texas 76508.

In February 1986, a 46 year old man died less than 2 days after admission to the hospital from complications following a febrile type illness. Specimens taken at autopsy were negative for common and rare bacteria including Legionella and Rickettsia. Viral cultures were done on spleen, liver and lung.

A Hemabsorption positive virus was isolated from the lung and liver using the MDCK cell line. One of these cultures was processed for electron microscopy because pathologists were anxious to determine a cause of death. Electron microscopy confirmed an influenza-like virus in the membranes of infected cells as well as attached to absorbed RBC's. The State Health Department identified the virus as Influenza B.

Death from Influenza is rare in apparently healthy people in this age group. Two cases were reported in 1985, where death of young people was due to a toxic shock syndrome-type disease caused by Influenza A. This patient had a rash and rapid cardiovascular collapse, fitting a pattern of toxic shock syndrome following Influenza B infection. To date, the same syndrome has not been reported due to Influenza B.

ENZYME DIGESTION STUDIES OF THE EMBRYONIC CHICK HEART: LOCALIZATION OF SULFATED COMPLEX CARBOHYDRATES. J.T. Ellard and D.A. Hay, Dept. of Biology, Stephen F. Austin State University, Nacogdoches, TX 75962.

The myocardium and endothelium from stage 14 atrioventricular (AV) explants were examined for the presence and distribution of sulfated complex carbohydrates (SCC) as revealed by the ruthenium red (RR) and high iron diamine-silver protein (HID-SP) techniques. Atrioventricular regions of chick hearts were grown for 12 and 36 hours on three-dimensional rat-tail collagen gels. Following fixation in glutaraldehyde, the explants were digested by chondroitinase ABC, heparitinase, Streptomyces hyaluronidase, and a combination of chondroitinase ABC followed by heparitinase. All explants were routinely processed for transmission electron microscopy. Initial results using RR in controls (no enzyme treatment) revealed on electron-dense line along the matrical surfaces of both myocardium and endothelium as well as labeled granules within the gel extracellular matrix. High iron diamine-silver protein markers appeared as a distinct layer of larger dots along both myocardial and endothelial surfaces and as scattered dots within the gel matrix. Enzyme digestion demonstrated the presence of chondroitin sulfate and heparin sulfate proteoglycans and hyaluronic acid when used in conjunction with HID-SP and RR staining. Results utilizing the in vitro model (3-D collagen gels) closely parallel those obtained in vivo, thus contributing to the validity of the model.

ULTRASTRUCTURAL IMMUNOCYTOCHEMISTRY OF PLANT CELLS AND PROTOPLASTS. L.R. Griffing, N.F. McGee, and T. Dreher, Biology Department, Texas A&M University, College Station, Texas 77843.

Immunocytochemistry of plant cells can provide information about the subcellular location of any of a variety of suitably antigenic macromolecules. However, routine protocols for plant tissue processing, fixation, and embedding-matrix infiltration for this technique are not yet established. In this study, brome mosaic virus-infected protoplasts are used as a model system to assess the available protocols. The protoplasts are infected with viral RNA. The presence of viral-associated proteins, e.g., coat protein, is assessed with a specific antibody. Background labelling can be checked using uninfected protoplasts and/or pre-immune sera. The ability of a given protocol to detect proteins of low abundance can be determined by looking at different stages (times) of infection. The application of the derived protocol to a variety of endogenous plant cell proteins will be described.

MYOCARDIAL SECRETIONS IN EMBRYONIC CHICK HEARTS: THE AUTORADIOGRAPHIC LOCALIZATION OF EXTRACELLULAR MACROMOLECULES. J.R. Turner and D.A. Hay, Dept. of Biology, Stephen F. Austin State University, Nacogdoches, TX 75962.

Certain macromolecules (such as glycoproteins, chondroitin sulfate- and heparin sulfate-proteoglycans) have been identified in vivo which are thought to be involved in the developmental events that result in the formation of endocardial cushions in the embryonic heart. The effects of these macromolecules are difficult to isolate in vivo, so an in vitro model utilizing atrioventricular (AV) canals explanted onto collagen gels has been developed. A comparison of the presence and distribution of these macromolecules in vitro and in vivo is necessary before further work can be done using this system. Radioactive isotopes (tritiated amino acids, tritiated fucose, $^{35}SO_4$) were used to label the macromolecules in vitro. Explants from two day chick embryos were placed on collagen gels and incubated for 12 and 36 hours, then fixed in 2% glutaraldehyde and prepared for autoradiography. Label was present throughout the myocardium and endothelium. It also extended in a gradient from the myocardial/gel interface decreasing in density farther away from the myocardium. Less label was present in those explants which had the myocardium removed after 12 hours. The in vitro labeling was comparable to in vivo labeling. This similarity in the morphogenetic event of secretion provides supporting evidence for further use of the AV explant/3-D gel system to study embryonic heart development.

The vast majority of muscle biopsy cases can be diagnosed by light microscopy using snap frozen skeletal muscle stained with enzyme histochemical methods. In many instances the clinical symptoms and other laboratory findings will suggest some form of inflammatory myopathy. Without electron microscopic examination one recently described form of inflammatory myopathy with vastly different clinical implications will be almost uniformly missed.

The entity called "inclusion body myositis" was first reported as a clinical-pathologic phenomenon by Yunis and Samaha. The clinical manifestations are frequently confusing and suggested a neuropathic process in many instances. The disease occurs more commonly in middle aged or elderly males, and electrophysiologic studies often suggest neurogenic atropy in addition to findings of myopathy.

We present the light microscopy, enzyme histochemistry and ultrastructural findings in three cases of inclusion body myositis diagnosed in our laboratory. We emphasize the peculiar ultrastructural changes, including filaments, vacuoles and membranous whorls characteristic of this disease. Implications for this diagnosis are quite significant indicating that the patient will be refractory to steroid therapy (unlike other forms of inflammatory myopathy).

ULTRASTRUCTURE OF IN VITRO COTTON FIBERS. J.D. Berlin, J. Tatum, G. Hoskins, N. Trolinder, and J.R. Goodin. Dept. Biological Sciences, Texas Tech University, Lubbock, TX 79409.

We have recently developed a technique for growing in vitro cotton fibers from pieces of ovules in liquid media. Light and electron microscopy was used to examine the cotton ovules, ovule calli, and cells sloughing off the calli that elongate to become in vitro cotton fibers. The cells will be compared to fibers that develop on the plant. The outer epidermal layer of the ovular pieces appears to be the source for fiber primordia; however, the possibility exists that other cells within the ovule are involved in callus formation. Callus cells resemble in vivo outer epidermal cells in that they are small, dense cells that often have an enlarged nucleolus. Two elongation regions have been observed on some fibers. Starch-containing bodies are present in the very young cells, but the starch disappears with age. The elongating fibers have numerous dictyosomes, mitochondria, and individual cisternae of RER. This work was supported in part by Cotton Incorporated.

EPITHELIOID SARCOMA J.M. Meis, B. Mackay and N.G. Ordonez Department of Pathology, The University of Texas System Cancer Center, Houston

Electron microscopy has provided a great deal of information about the fine structure of the cells of human tumors, and has in some instances revealed the nature of the proliferating cell when it could not be identified by light microscopy alone. There are, however, instances in which the histogenesis of a tumor remains undetermined even following intensive ultrastructural study. An example is the neoplasm known as epithelioid sarcoma. This uncommon tumor usually develops on the distal upper or lower extremity, and it can mimic a granuloma or certain malignant tumors by light microscopy with the result that it is readily misdiagnosed. It is, however, malignant, and has a high incidence of local recurrence and metastasis, often after intervals of many years. By combining electron microscopy and immunocytochemistry with the routine light microscopy, it is usually possible to identify an epithelioid sarcoma, but the findings from these techniques have failed to reveal the cell of origin. Despite consistently positive immunoreactivity for cytokeratin, and some epithelial features by electron microscopy, a mesenchymal derivation is favored. In a study of 16 cases, we found that 11 exhibited relatively consistent features, while the remaining 5 were more variable in their clinical behavior and histopathology.

SARCOMAS OF THE GASTRO-INTESTINAL TRACT B. Mackay, J.Y. Ro, C. Floyd and N.G. Ordonez, Department of Pathology, The University of Texas System Cancer Center, Houston

Tumors composed of spindle cells that arise from the stomach or small or large intestine are almost all soft tissue neoplasms, and they are generally assumed to be of smooth muscle derivation unless it can be proved that the cells are some other type. We have studied a series of 50 cases, most of them malignant, using a battery of immunocytochemical staining procedures in addition to electron microscopy. Smooth muscle characteristics were identified in some cells of some of the tumors at the ultrastructural level, but the majority of the sarcomas displayed little or no evidence of smooth muscle differentiation. Positive immunostaining for desmin correlated with the presence of abundant smooth muscle myofilaments by electron microscopy. Vimentin was positive in most of the tumors and was consequently of limited specificity. Seven tumors stained for S-100 protein, and three of these showed evidence of schwann cell differentiation by electron microscopy. Epithelioid transformation by light microscopy correlated with loss of smooth muscle features. The study demonstrates that only a minority of sarcomas of the gastro-intestinal tract possess fine structural evidence of smooth muscle derivation, though most may nevertheless be composed of mesenchymal cells with latent potential for manifestation of smooth muscle features.

POST EMBEDDING IMMUNOLABELING USING ULTRARAPID FREEZING AND MOLECULAR DISTILLATION DRYING. J.G. Linner and S.A. Livesey. Univ. Texas Health Science Center at Houston.

Ultrarapid freezing followed by molecular distillation drying and resin embedding offers several advantages as a technique for post-embedding ultrastructural immunocytochemistry. The aim of this study has been to refine components of this technique and apply immunolabeling using streptavidin colloidal gold for the localization of water soluble enzymes in the cyclic AMP response system and antigens of known cellular location. Ultrarapid freezing using the metal mirror technique has been assessed for tissue and cultured cell samples using a variety of methods of sample application. Sample holder design, precooling artifact and a comparison of liquid helium versus liquid nitrogen cooling of the mirror finished copper bar will be discussed. Molecular distillation drying is a technique for the removal of cellular water from ultrarapidly frozen samples without recrystallization. This technique will be discussed in terms of improvement in equipment design and the development of a small scale device. To date, the technique has been limited to osmicated, Spurr's resin embedded tissue. Vapor phase osmication, while stabilizing membrane components, has limited antigen-antibody interaction with some but not all antigens. Recent progress using low temperature embedding at -30°c with Lowicryl K4M and -60°c with Lowicryl HM20 as a method of structural preservation without osmication will be reviewed. Immunolabeling using an indirect technique with a secondary biotinylated antibody and streptavidin colloidal gold as the electron dense marker has been applied to components of the cyclic AMP response system (catalytic subunit, RII and InhibitorI) and antigens of known cellular location, eg. DNA. Sensitivity and specificity of labeling will be correlated to the method of sample preparation.

BIOLOGICAL SCIENCES

POSTER PRESENTATION — SPRING 1987

ULTRASTRUCTURE OF SCHISTOSOMA MANSONI EGG. J.H. Smith, P.J.G. Neill, W.M. Kemp. Dept. Pathology and Laboratory Medicine, College of Medicine, Texas A&M University, College Station, TX. 77843-1114.

Previous work on Schistosoma mansoni eggs has been done on the outer shell and newly hatched miracidium as preservation of whole eggs for transmission electron microscopy by fixatives, dehydrants and embedding media has been poor. Freeze-substitution and Spurrs embedding media preserve the internal ultrastructure of this helminth egg shell, developing miracidium and periembryonic envelope. The mature miracidia are identical to those described after eggs hatch. The egg shell consists of 3 previously-described layers perforated by cribriform pores. Underlying the egg shell, a layer of densely-packed branching filaments (Reynold's layer) increases as maturation proceeds. A single layer of squamous cells, von Lichtenberg's layer, closely adheres to the Reynold's layer; their nuclei being located at the poles of the egg. Study of developing miracidia indicate that epidermal plates develop from cytons separate from and superficial to those of the epidermal ridges. A periembryonic space between von Lichtenberg's layer and the developing miracidium is initially filled with electron lucent fluid, but subsequently becomes filled with irregularly granuloflocular material.

CRYSTALS IN HUMAN TUMORS M.C. Steglich, J.Y. Ro, N.G. Ordonez and B.Mackay, Department of Pathology, The University of Texas System Cancer Center, Houston

An occasional finding within a tumor is the presence of crystals, either within the neoplastic cells or in ducts of a glandular lesion. They may merely be an interesting curiosity, but in some instances, identification of the crystals is helpful in establishing the diagnosis. If the crystals are large or sufficiently numerous, they can be seen in light microscopic sections, but when they are small or sparse, electron microscopy is needed for their detection. Uncommon tumors that contain intracytoplasmic crystals are alveolar soft parts sarcoma and juxtaglomerular cell tumor. More common neoplasms in which the cells occasionally contain crystals are islet cell tumor and myeloma. Extracellular crystals may form in the lumen of acini in salivary and prostate adenocarcinomas, where they presumably arise through condensation of secretion since they are not present in the surrounding acinar cells.

T-2 EFFECTS ON CELLULAR ULTRASTRUCTURE IN THE PANCREAS, LIVER, KIDNEY, AND INTESTINE OF MICE. Hilton H. Mollenhauer, Donald E. Corrier, and Robert E. Droleskey, Veterinary Toxicology and Entomology Research Laboratory, ARS-USDA, College Station, TX 77841.

Tissues from mice given daily doses of T-2 (2.0 mg/kg body weight) by stomach tube were evaluated by light and electron microscopy after 1, 2, 7, 9, and 12 days of treatment. Clinical signs of toxicosis, other than softening of fecal pellets, were not observed in the treated mice. No changes in cellular ultrastructure were observed in either pancreas or kidney. Brush border changes and some cellular necroses were observed in the intestinal epithelium. The most significant changes, however, were observed in liver. These included changes in lipid stores (i.e., quantity and size of lipid droplets), patterns of endoplasmic reticulum distribution, and Golgi apparatus. Small vesicles, usually containing only one VLDL particle, were common in hepatocytes after 1-2 days of treatment. The pattern of ultrastructural change was similar to that observed previously in rats exposed to aflatoxin B-2. The liver changes were transitory in that they maximized at 1-2 days posttreatment and then declined so that by 7 days posttreatment the liver cells appeared almost normal.

ON THE BACK COVER

What Is It?

This SEM shows several podocyte cell bodies and numerous cell processes, called foot processes or pedicles, wrapped around glomerular capillaries in a mouse kidney. The pedicles interdigitate and form a filtration complex that allows liquid to pass through into the glomerular space but retards the movement of larger blood constituents. This is part of the mechanism by which the kidney clears the blood of metabolic waste products.

Pieces of kidney were fixed, dehydrated, critical point dried and coated by standard methods. Faces that had been previously exposed by cutting with a fresh, sharp, clean razor blade were examined. Micrograph by R.W. Davis, Department of Medical Anatomy, Texas A&M University.

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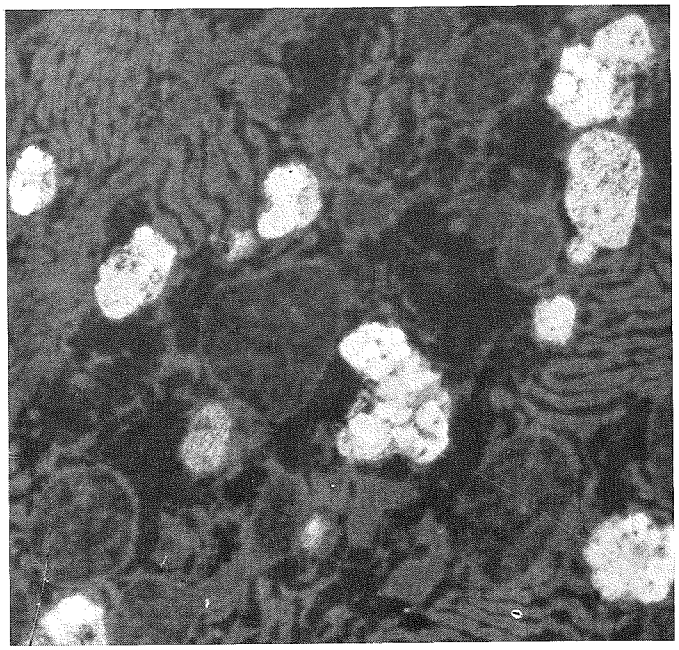

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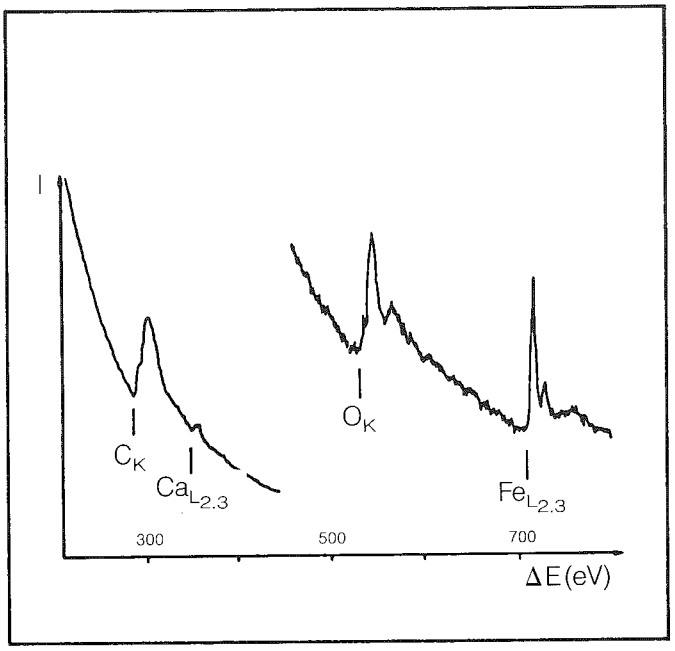

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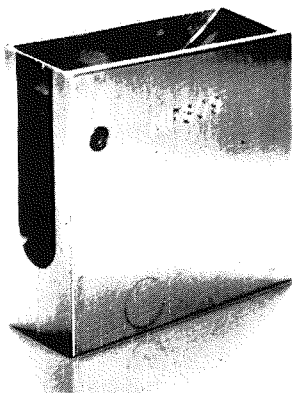

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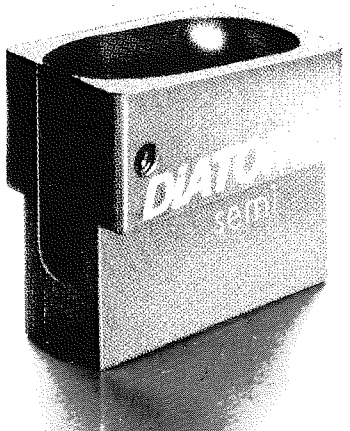

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5. Diamond knives
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7. SEM specimen preparation
   a. Critical point drying
   b. Freeze drying
   c. Thin film coating
   d. Conductive staining
8. Freeze etching
9. Freeze substitution
10. Replication techniques, shadow casting
11. Rapid freezing
12. Negative staining
13. Oxygen plasma etching
14. EM autoradiography
15. Cryultramicrotomy
16. EM immunocytochemistry and labelling
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20. Standards for x-ray: biological
21. Standards for x-ray: materials
22. Other: specify _____

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Other Techniques And Procedures

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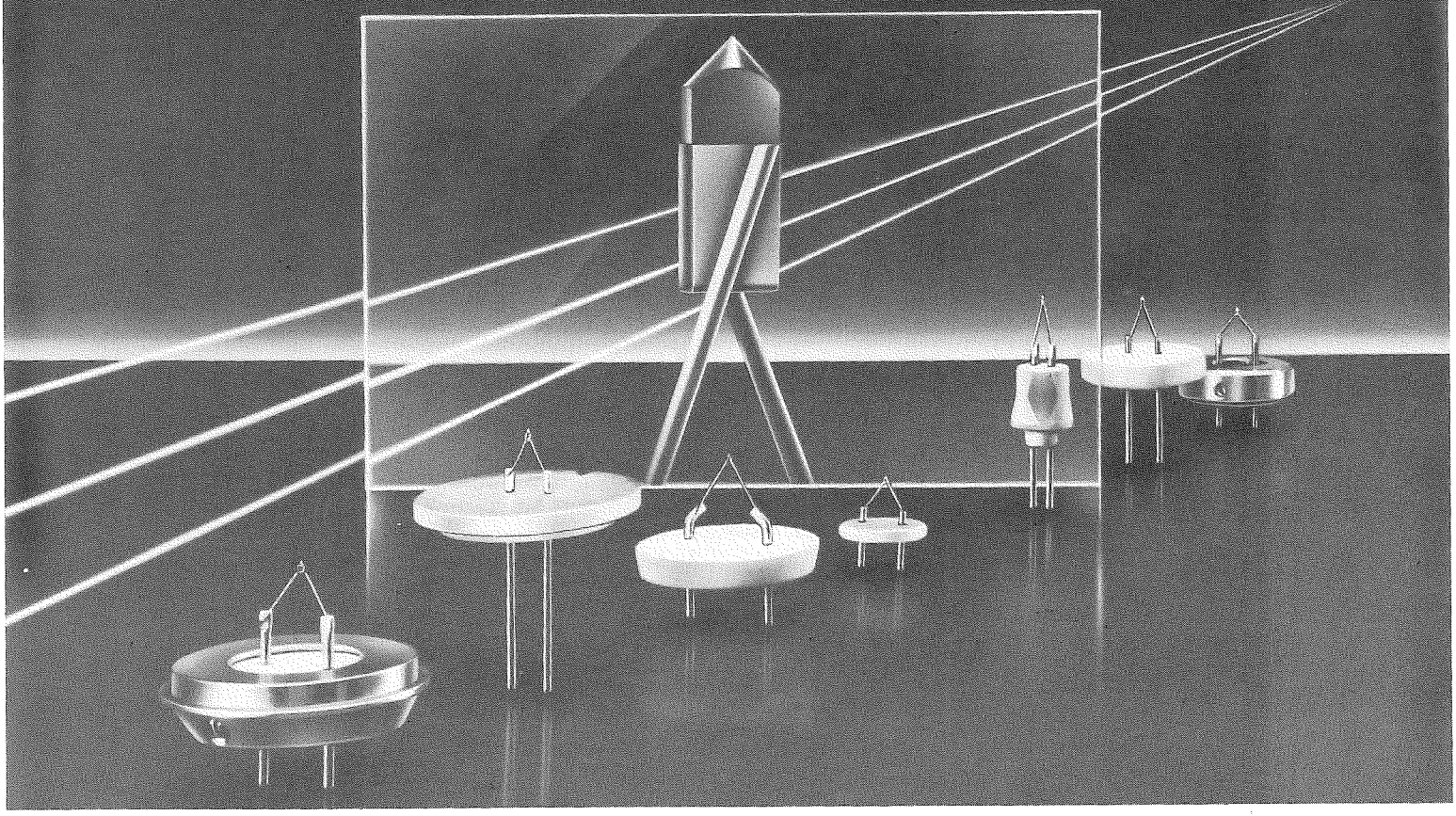

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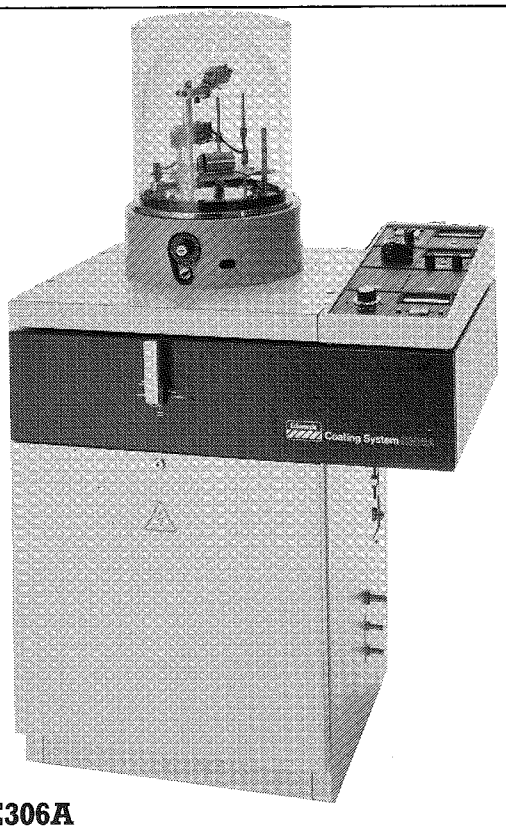

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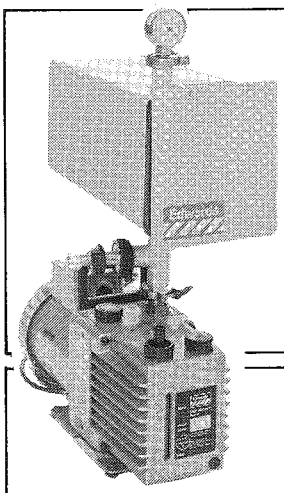

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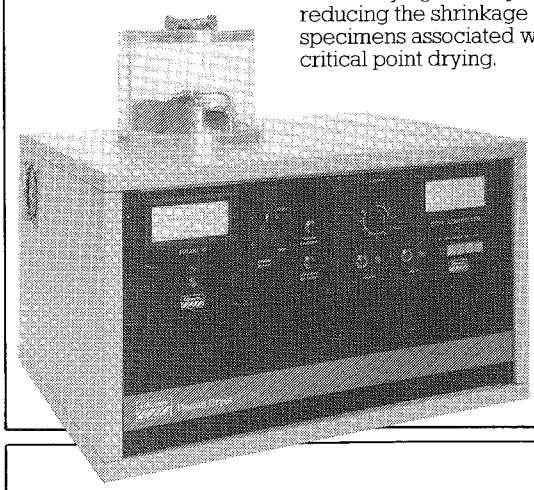

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Announcement
For All Members

The Education Committee of EMSA is starting a library of researcher-written instructions for operating various instruments related to electron microscopy.

Once a sufficient data base has been established, member may obtain copies for use in their own labs.

We ask that you send one good copy of your directions (not the manufacturer's) for operating such instruments as: electron microscopes, ultramicrotomes, vacuum equipment, freezing apparatus, analytical instrumentation, etc.

Please include your name, address, phone number, instrument model and date when directions were written.

Send to:

Instrument Directions
c/o Dr. John Bozzola, Director
Center for Electron Microscopy
Southern Illinois University
Carbondale, IL 62901

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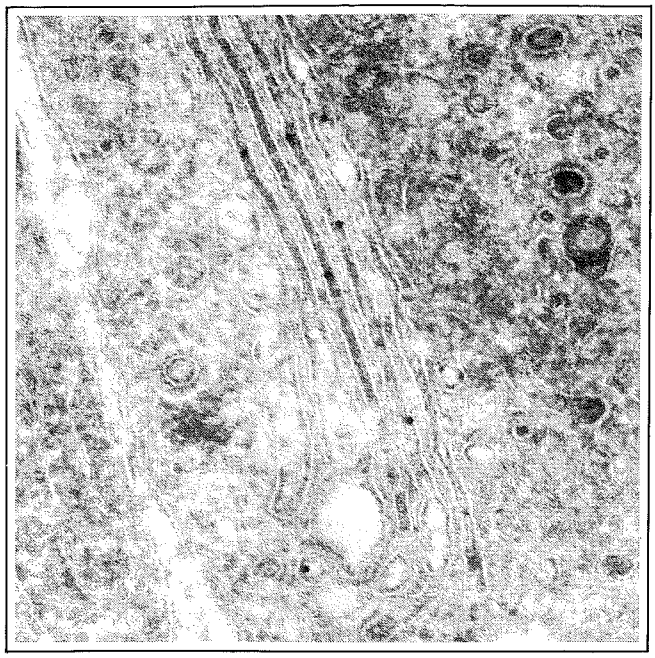

Precise and sensitive localization of Immunoglobulin A receptors to the exoplasmic side of Golgi membranes and associated vesicles in an intestinal epithelial cell, using cryoultramicrotomy and gold labelling. Dr. Jan Slot, University of Utrecht, and Dr. Lahja Sevéus, LKB.

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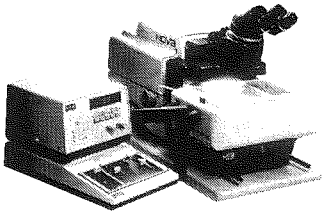

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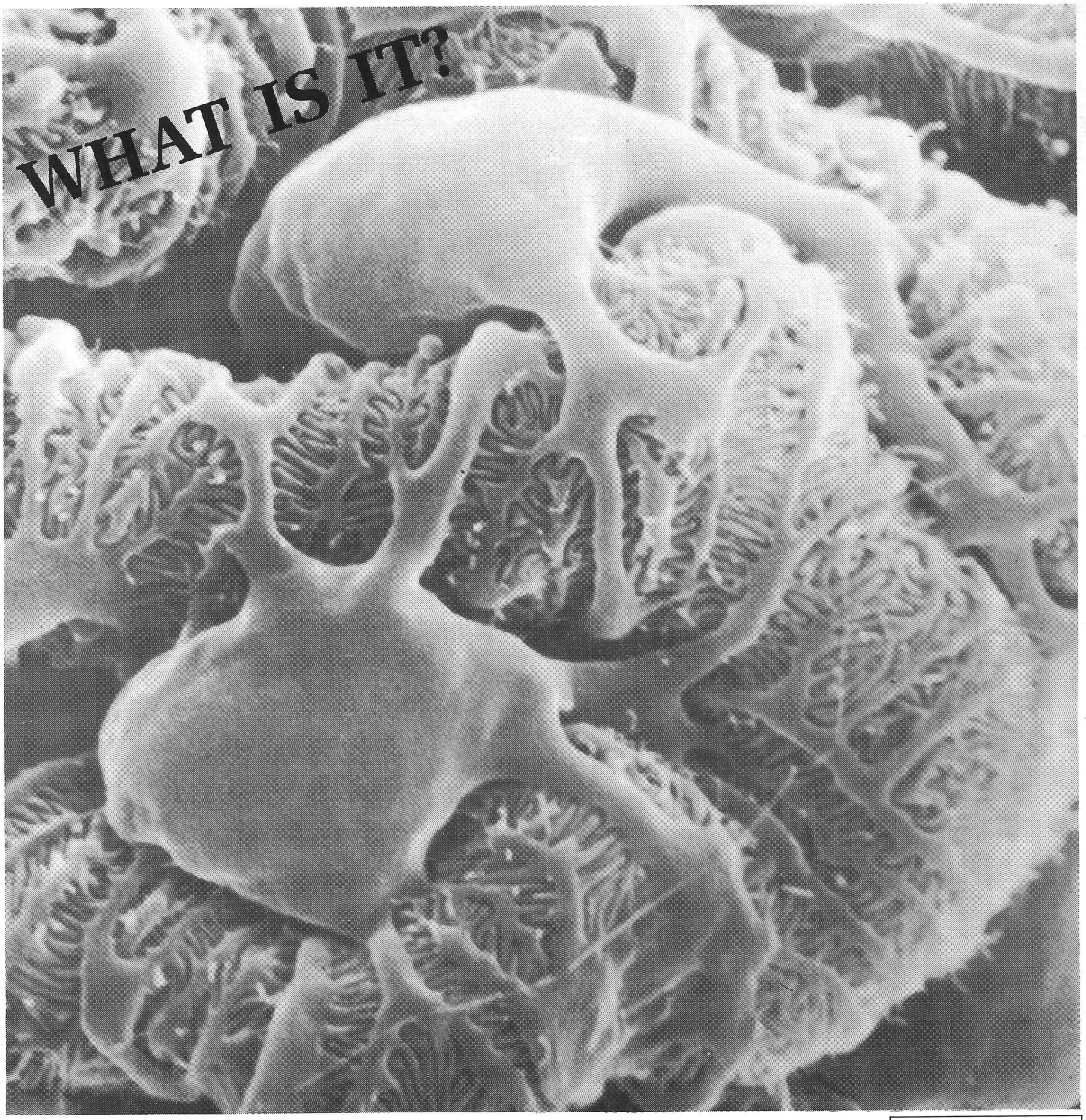

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