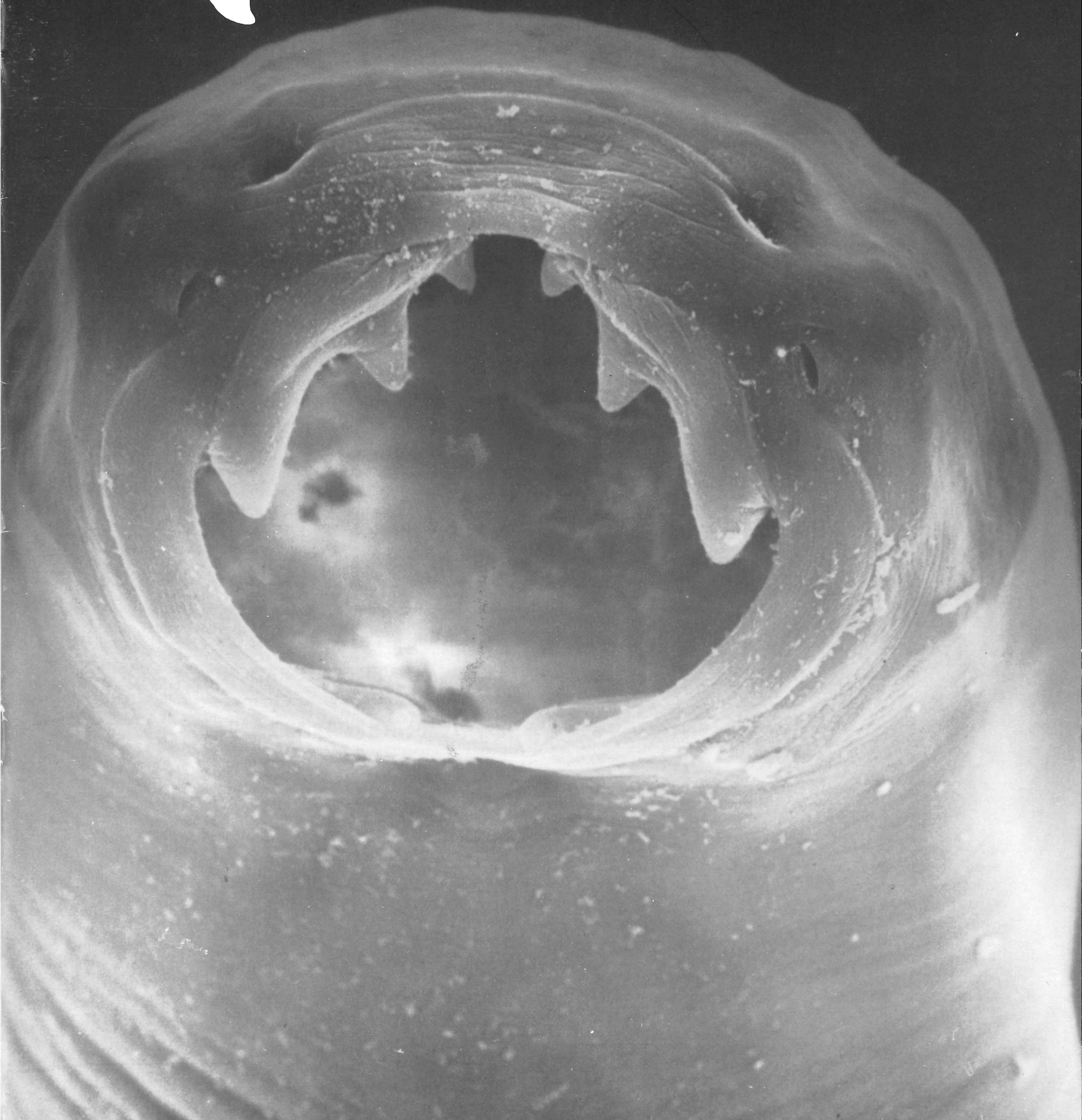
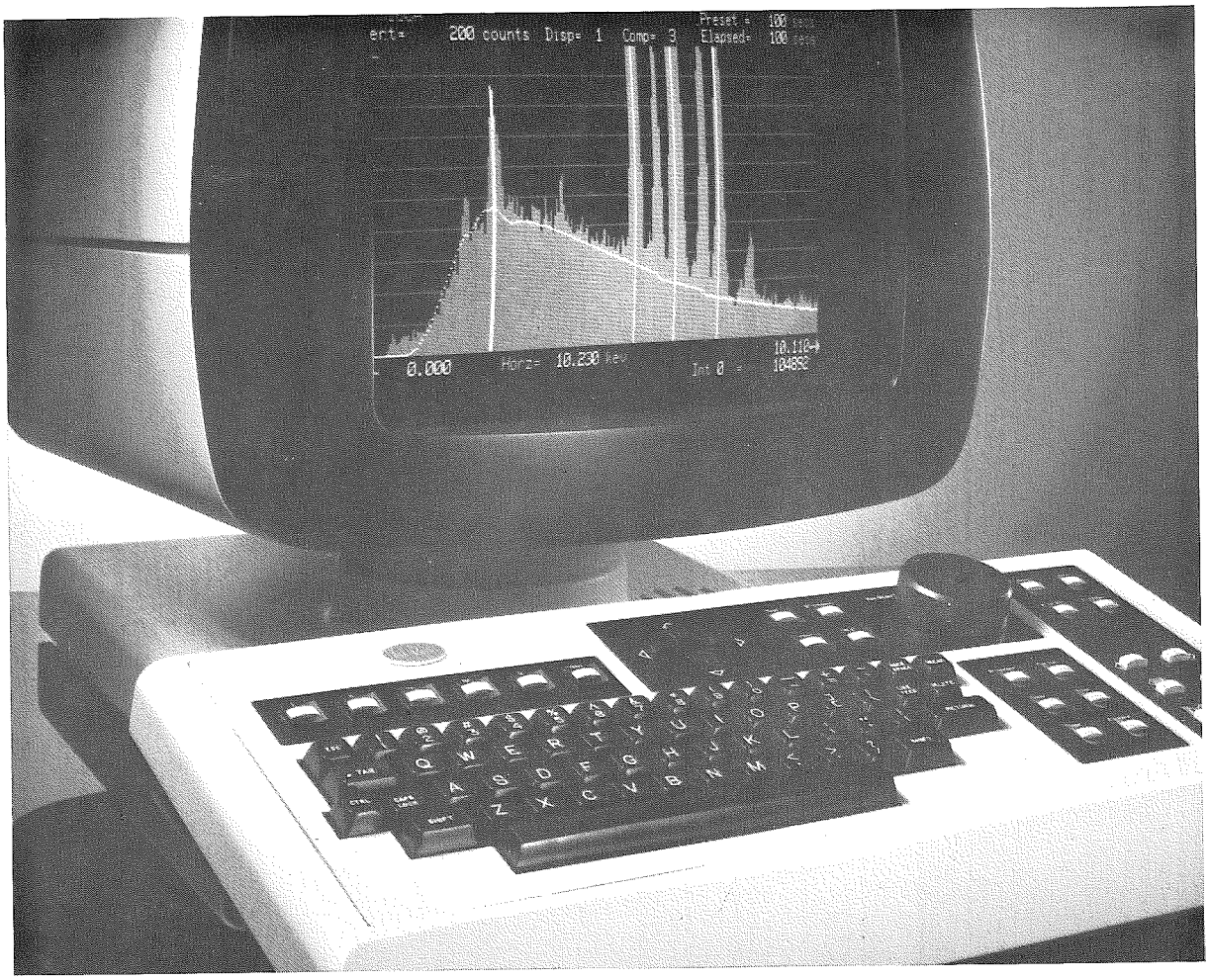
TSEM
Texas Society for Electron Microscopy

JOURNAL
VOLUME 15, NUMBER 2, 1984





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TEXAS SOCIETY FOR ELECTRON MICROSCOPY JOURNAL
VOLUME 15, NUMBER 2, 1984
ISSN 0196-5662

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

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

ON THE COVER

Ancylostoma caninum, 900X

A scanning electron micrograph showing the anterior end of the hookworm **Ancylostoma caninum.** The name **Ancylostoma** is derived from the Greek words for hook (ancylos) and mouth (stoma). The anterior end of this parasite is curved into a prominent hook.

Hookworms are classified as nematodes and are intestinal parasites of carnivores and omnivores. Specific hosts include dogs, cats, man, elephants, rhinoceroses and others. They are blood suckers and attach themselves to the wall of the small intestine. The teeth are probably used to help anchor the parasite and to abrade pieces of the mucosal lining. **Ancylostoma** infection is a potentially fatal disease that can cause severe anemia, especially in young pups.

Submitted by Ronald W. Davis, Department of Anatomy, Texas A&M University College of Medicine, College Station, Texas 77843-1114.

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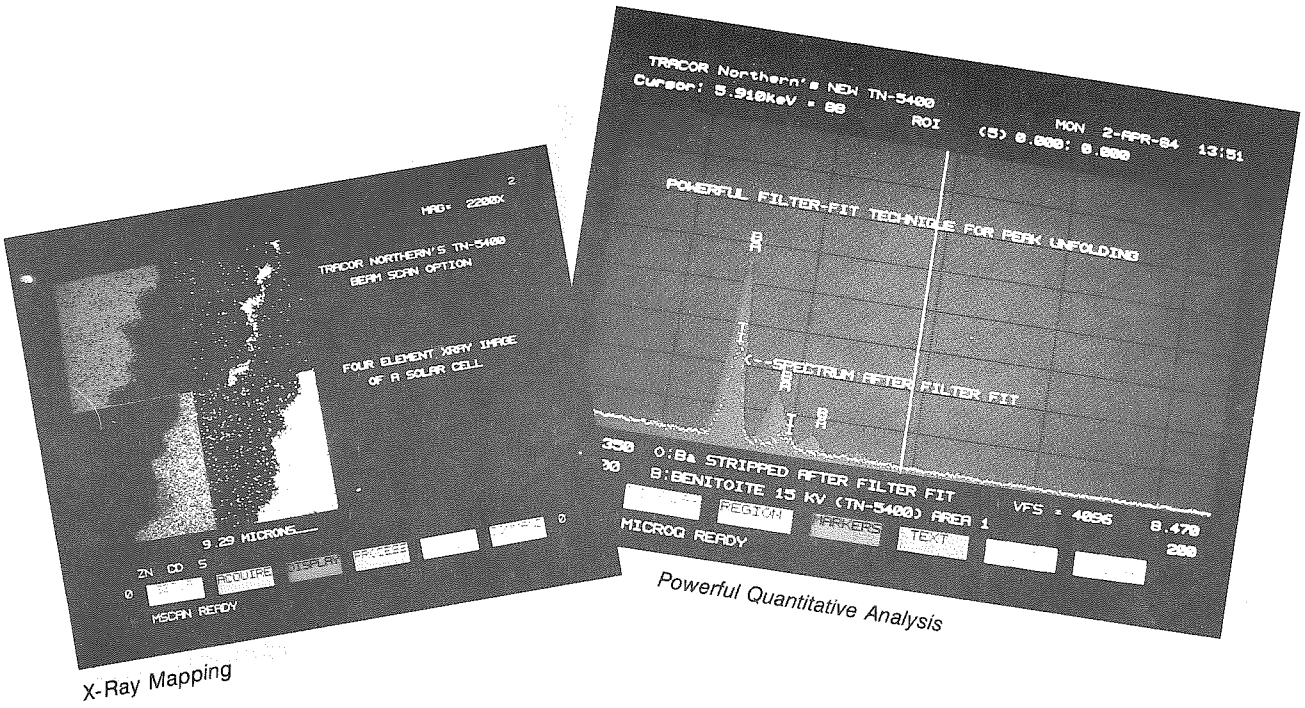

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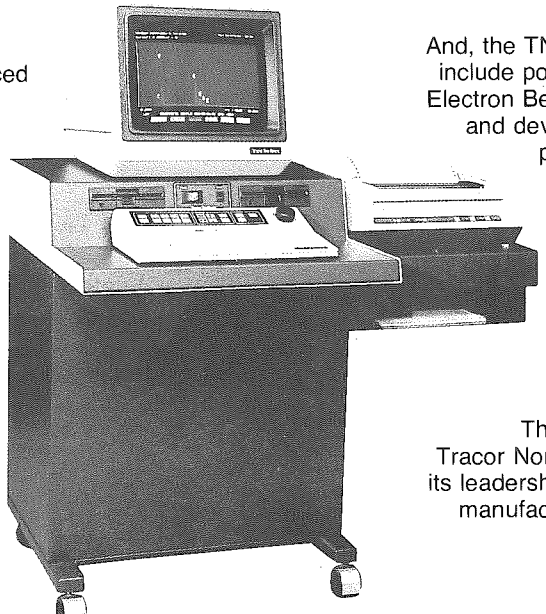

And, the TN-5400 can be expanded to include powerful Digital Control of the Electron Beam. This feature pioneered and developed by Tracor Northern, provides for the collection of digital linescans and the collection and processing of digital x-ray maps. Never before has there been an x-ray micro-analysis system from any manufacturer which, feature for feature, can compare to the TN-5400.

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President's Message

It is my pleasure and honor to have the unique opportunity to serve as President of TSEM for another year. This has resulted from the fact that Allen Shannon was unable to complete his term as President Elect because of career changes he is making. Since we therefore had no one to assume the office of President for 1984-85, the Executive Council asked me to serve another term since it was the feeling of the Council that it would be unfair to ask our new President Elect Hilton Mollenhauer to immediately become President. Although I am saddened that Allen will not be able to serve as our President, I will do my best to fill in for him. I will, however, need the support of the entire membership if we are to have a productive year. In particular, I am counting on the assistance of members of the Executive Council during this period. For your information, the names of the individuals comprising the Executive Council for 1984-85 are listed on page 3 of this issue of the Journal.

Those of you who were unable to attend the Spring Meeting in College Station missed a good meeting. The contributed papers were excellent and we had two outstanding invited presentations by Herb Hagler and Charles Fiori. Bruce Mackay also gave the type of "first rate" Presidential Lecture we have come to expect at each year's Spring Meeting. The meeting was also highlighted by a social at the home of Hilton and Barbara Mollenhauer as well as a banquet high in Rudder Tower overlooking the campus of Texas A&M. Thanks go to many people for making the meeting a success. In particular I'd like to single out the Mollenhauers and Wayne Sampson as well as the administration of the College of Medicine at Texas A&M.

While on the topic of meetings I want to note that the sites of our next three meetings are now set. This year's Fall Meeting will be October 25-27 at the Rodeway Inn in Arlington, Texas, next to Six Flags. Additional information on this meeting and the first call for papers will be mailed early this summer. The 1985 Spring Meeting will be April 11-13 at the Menger Hotel in San Antonio. This will be the Twentieth Anniversary Meeting of our Society so please make plans to attend. In the Fall of 1985 we will meet jointly with the Louisiana Society for Electron Microscopy in Beaumont. The exact dates of this meeting have not yet been determined.

In checking our membership rolls we have found the names of quite a few poeple who haven't paid dues for a number of years. We are currently in the process of removing the names of these individuals from our mailing lists. As a result, this may be the last issue of the Journal that some of you will receive unless you renew your TSEM membership. If you are unsure about your status as a member please contact Randy Moore. We certainly don't want to lose anyone.

In closing I'd like to remind those of you in the material sciences that we are planning some special sessions for you at the Arlington meeting. John Lang is helping to organize things and has been appointed as an **ad hoc** member of the Executive Council to represent the material sciences. John can be reached at 512-928-6054 if you would like to volunteer to help him. Hopefully, from now on TSEM will have more to offer to those of you in the material sciences.

I look forward to seeing you in Arlington this Fall.

Sincerely,

Charles W. Mims
President

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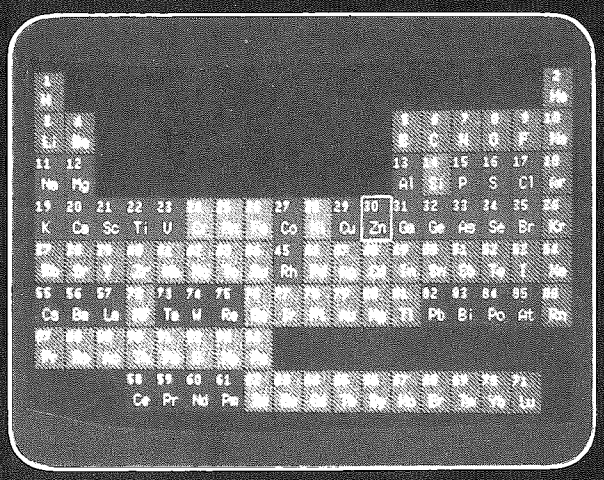
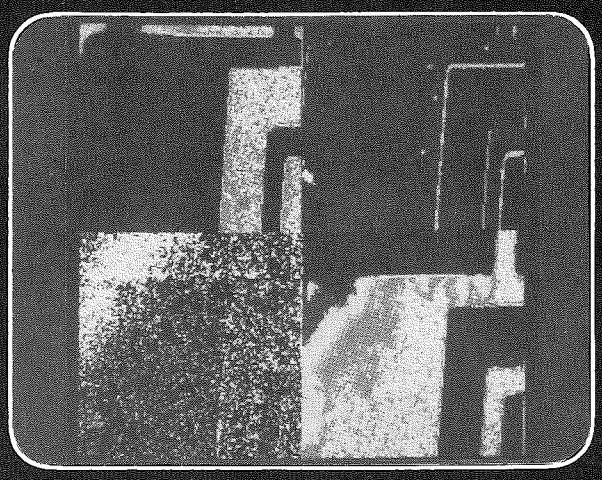


Automatic elemental analysis.



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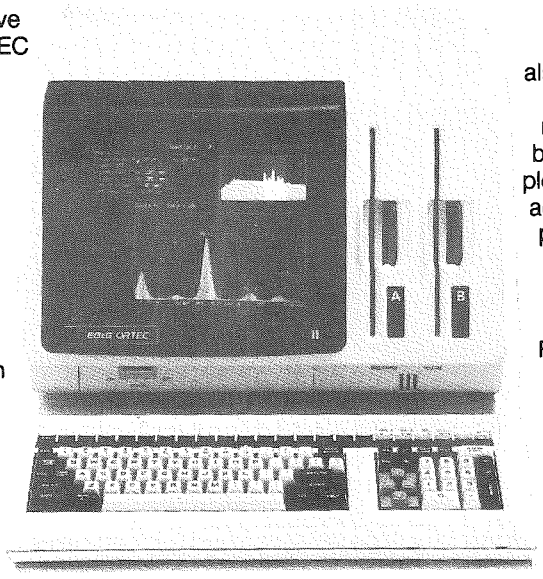

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ASSETS ON OCTOBER 13, 1983:		
Certificate of Deposit No. 91099, Univ. Natl. Bank, Galveston[1]	$ 2,000.00	
Certificate of Deposit No. 10-141345, Houston First Savings[1]	2,851.73	
Certificate of Deposit No. 240-0064030, Republic Bank of Waco	2,033.57	
Checking Account No. 7914-448-1, Republic Bank[1]	3,897.82	$ 10,783.12
RECEIPTS:		
Membership Dues	$ 1,903.00	
Tyler Meeting[4] Registration and Contributions[5]	3,070.00	
Interest		
Certificate of Deposit No. 91099[1]	96.34	
Certificate of Deposit No. 240-0064030	119.69	
Certificate of Deposit No. 10-141345[2]	229.35	
Checking Account No. 7914-448-1	113.89	
TSEM Journal		
Advertising Revenue	$ 2,400.00	
Subscriptions and Back Issues	80.00	$ 8,012.27
EXPENSES:		
Secretarial Expenses	$ 300.00	
TSEM Journal		
Printing Costs for Vol. 14, No. 3	1,480.00	
Printing Costs for Vol. 15, No. 1	2,395.00	
Tyler Meeting[4]		
Speakers (Trelease)[6]	626.70	
Electrical Work for Display Area	96.88	
Student Travel[7]	482.50	
Treasurer's Expenses (Name Tags, Xeroxing, etc)	28.87	
Sheraton Inn	2,162.99	
Returned Check	10.00	
TSEM Journal Editor's Expenses	240.52	$ 7,823.46
ASSETS ON APRIL 26, 1984:		
Certificate of Deposit No. 64766, Univ. Natl. Bank, Galveston[1]	$ 2,000.00	
Certificate of Deposit No. 10-0475417, United Savings of Texas[2]	3,081.08	
Certificate of Deposit No. 240-0064030, Republic Bank of Waco	2,153.26	
Checking Account No. 7914-448-1, Republic Bank of Waco	3,737.59	$ 10,971.93

[1]Certificate of Deposit No. 91099 matured in February of 1984. These funds were re-invested in Certificate of Deposit No. 64766 at Univ. Natl. Bank of Galveston.

[2]Certificate of Deposit No. 10-0141345 matured in December of 1983. These funds were re-invested in Certificate of Deposit No. 10-0475417. Prior to maturation of Certificate of Deposit No. 10-0141345, Houston First Savings was renamed United Savings of Texas.

[3]Includes $100.00 for Paul Enos Memorial Fund and $100.00 for Student Travel Fund.

[4]Net loss on Tyler meeting = $327.94.

[5]Contributors: Bausch and Lomb, Micro-Tech, JEOL, SPI Supplies, Ted Pella, Princeton Gamma Tech, AMRay, American Optical, Micro Engineering Inc., E.I. DuPont de Nemours, Inc., Electron Microscopy Sciences, Philips Electronics, Tracor-Northern, Ernest Fullam, Polaron.

[6]Includes $100.00 from Paul Enos Memorial Fund.

[7]Includes $100.00 from Student Travel Fund.

Respectfully submitted,

Randy Moore, Treasurer
Texas Society For Electron Microscopy

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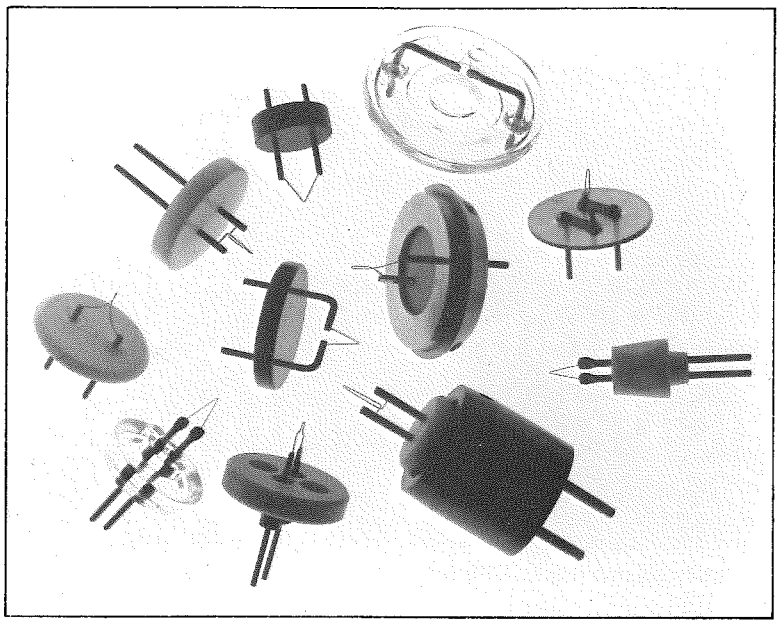

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A Computer Program to Facilitate Morphometric Analyses of Cellular Ultrastructure

By

Darrell S. Vodopich and Randy Moore

Department of Biology
Baylor University
Waco, Texas 76798

ABSTRACT

In this paper we describe a computer program to facilitate morphometric analyses of cellular ultrastructure. Raw data for the program are dot-counts obtained by superimposing a test grid on micrographs. The output lists relative volumes of organelles and includes statistical comparisons. The program is written in FORTRAN IV and is available free of charge from the authors.

INTRODUCTION

For years biologists have published qualitative studies of cellular ultrastructure. However, current morphometric techniques allow quantitative descriptions of ultrastructural changes in cells exposed to various treatments. Unfortunately, quantification of organellar volumes is tedious and must be followed by statistical analyses. We have developed a computer program, MORPHO, to relieve much of this tedium.

PROGRAM DESIGN AND INPUT

MORPHO is designed for projects with multiple tissue treatments and replicate samples from each treatment. Random, unbiased micrographs are taken for each sample, and the relative area of each organelle is estimated by counting dots on a test grid superimposed on each micrograph. These "dot-counts" for each organelle constitute the raw data for the project and for MORPHO. Extensive discussion of morphometric techniques (e.g., sampling procedures, design of test grids, etc.) are presented elsewhere (1-3).

The number of dots overlying each organelle on a micrograph is proportional to the relative volume of that organelle. After these dot-counts are entered into MORPHO, the program then calculates the relative volume of each organelle in micrographs of a sample (e.g., as a percent of the protoplasm, cytoplasm, or any combination of organelles of your choice). The percent relative volumes (PRV's) for each organelle in samples within a treatment are then arcsin transformed and compared using a t-test. This comparison of PRV's between treatments is automatically done for each organelle. Relative volumes also are conveniently listed for use in any other statistical test of your choice.

The t-test compares the mean PRV for an organelle in one treatment with the mean PRV for that organelle in another treatment. This test compares means for unequal sample sizes and involves two formulas. (4, 5).

Formula 1:
$$s^2 = \frac{\Sigma X_1^2 + \Sigma X_2^2}{(n_1 - 1) + (n_2 - 1)}$$

where s^2 is the pooled variance, ΣX_1^2 and ΣX_2^2 are the sums of the squared deviations of the PRV's from the mean PRV for the samples within treatments one and two, and n_1 and n_2 are the number of samples in treatments one and two, respectively.

Formula 2:
$$\text{Student's } t = \frac{|\overline{X}_1 - \overline{X}_2|}{\sqrt{s^2(n_1 + n_2) / (n_1 n_2)}}$$

where $\overline{X}_1$ and $\overline{X}_2$ are the mean PRV's for treatments one and two, respectively.

Data input and program operation are interactive and designed for an inexperienced user. Organellar names and dot-counts are typed in response to prompts, and a simple interactive editor is included for minor corrections. MORPHO creates all files automatically, and permanently stores the raw data in a sequential file for subsequent analysis.

OUTPUT

MORPHO includes options for output of four tables: 1) raw data, 2) PRV's for organelles within each sample (Table 1), 3) arcsin transformed PRV's for analysis by ANOVA programs supplied by the user, and 4) t-test comparisons of PRV's for organelles subjected to different treatments (Table 2). The listing of raw data allows for easy comparison with laboratory data sheets to detect typing errors. The tables of PRV's and t-values are generated automatically for all organelles.

LIMITATIONS AND AVAILABILITY

Although this 500-line program was developed on a VAX 11/780, only standard FORTRAN IV commands were used. Therefore, compatibility with other machines is good. MORPHO is dimensioned for 25 organelles, from 25 replicate micrographs for each sample. However, a programmer can easily alter these dimensions. The number of samples and treatments within a project is unlimited.

MORPHO is available free of charge from Darrell S. Vodopich, Biology Department, Baylor University, Waco, TX, 76798, as a printed copy of the program. The package includes test data, a sample output, a user's guide, and documentation.

ACKNOWLEDGEMENTS

We thank the Computation Center of Baylor University for providing the services necessary for this project. We also thank Wayne Fagerberg and Don Hay for critically reviewing the manuscript.

REFERENCES

(1.) M.W. Steer. Understanding Cell Structure. (Cambridge Univ. Press, Cambridge. 1981).

(2.) R. Toth. An introduction to morphometric cytology and its application to botanical research. Amer. J. Bot. 69 (1982) 1694-1706.

(3.) E.R. Weibel. Stereological Methods. Vol. 1. Practical Methods for Biological Morphometry. (Academic Press, London. 1979).

(4.) H. Orr, J.C. Marshall, T.L. Isenhour, and P.C. Jurs. Introduction to Computer Programing for Biological Scientists. (Allyn and Bacon, Inc., Boston. 1973).

(5.) M.W. Snedecor and W.G. Cochran. Statistical Methods. (Iowa State Univ. Press, Ames. 1967).

Table 1. A sample table produced by **MORPHO** after calculation of mean percent relative volumes of organelles in random micrographs from a sample.

RELATIVE VOLUMES OF ORGANELLES EXPRESSED AS A PERCENTAGE OF: PROTOPLASM

TREATMENT: LOW TEMPERATURE **SAMPLE: 1**

ORGANELLE	MICROGRAPHS 1	2	3	... 25	MEAN
Nucleus	4.85	4.85	8.06		5.9
Vacuole	4.85	4.58	8.06		5.9
Chloroplast	9.71	9.71	16.13		11.8
Dictyosome	1.94	1.94	2.42		2.1
Mitochondria	5.83	5.83	4.84		5.5
Hyaloplasm	72.82	72.82	60.48		68.7

Table 2. A sample table produced by **MORPHO** after a t-test comparison of the mean percent realtive volumes across multiple samples within a treatment.

TREATMENTS: WITH NULCEUS VS WITHOUT

TREATMENT: WITH AUXIN RELATIVE VOLUMES ARE BASED ON: PROTOPLASM

PRVs: 5.92 12.39 2.52

MEAN = 6.94 STD. DEV. = 5.01 STD. ERR. = 2.89

TREATMENT: WITHOUT AUXIN RELATIVE VOLUMES ARE BASED ON: PROTOPLASM

PRVs: 10.87 5.48 13.75

MEAN = 10.03 STD. DEV. = 4.20 STD. ERR. = 2.42

T VALUE = 1.589 DEGREES OF FREEDOM = 4

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One year's dues in the form of a check or money order should be sent with the application for Membership form. (Regular $10.00. Student $2.00. Corporate $75.00).

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Signature of TSEM Regular Member making the Nomination

_____
Date 19

This application for Membership in the Society or this application for transfer from the grade of Student to Regular or Regular to Student Member should be sent to the TSEM Secretary. The form will be presented at the next meeting of the Executive Council for their approval (majority vote). The nominees will then be presented by the council to the membership at the next general business meeting for their approval (majority vote). Nominees will be added to the membership rolls at that time.

Presented to the Council at _____ meeting. Date _____

Action _____

Send Application to:

Joiner Cartwright, Jr.
Department of Pathology
Baylor College of Medicine
Houston, Texas 77030

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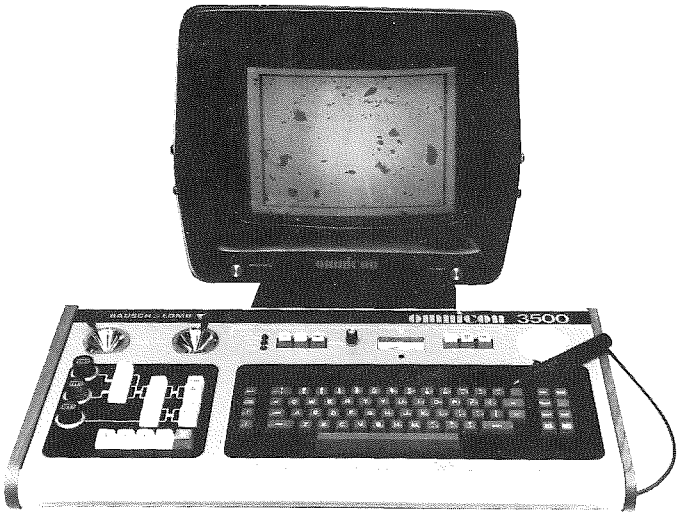

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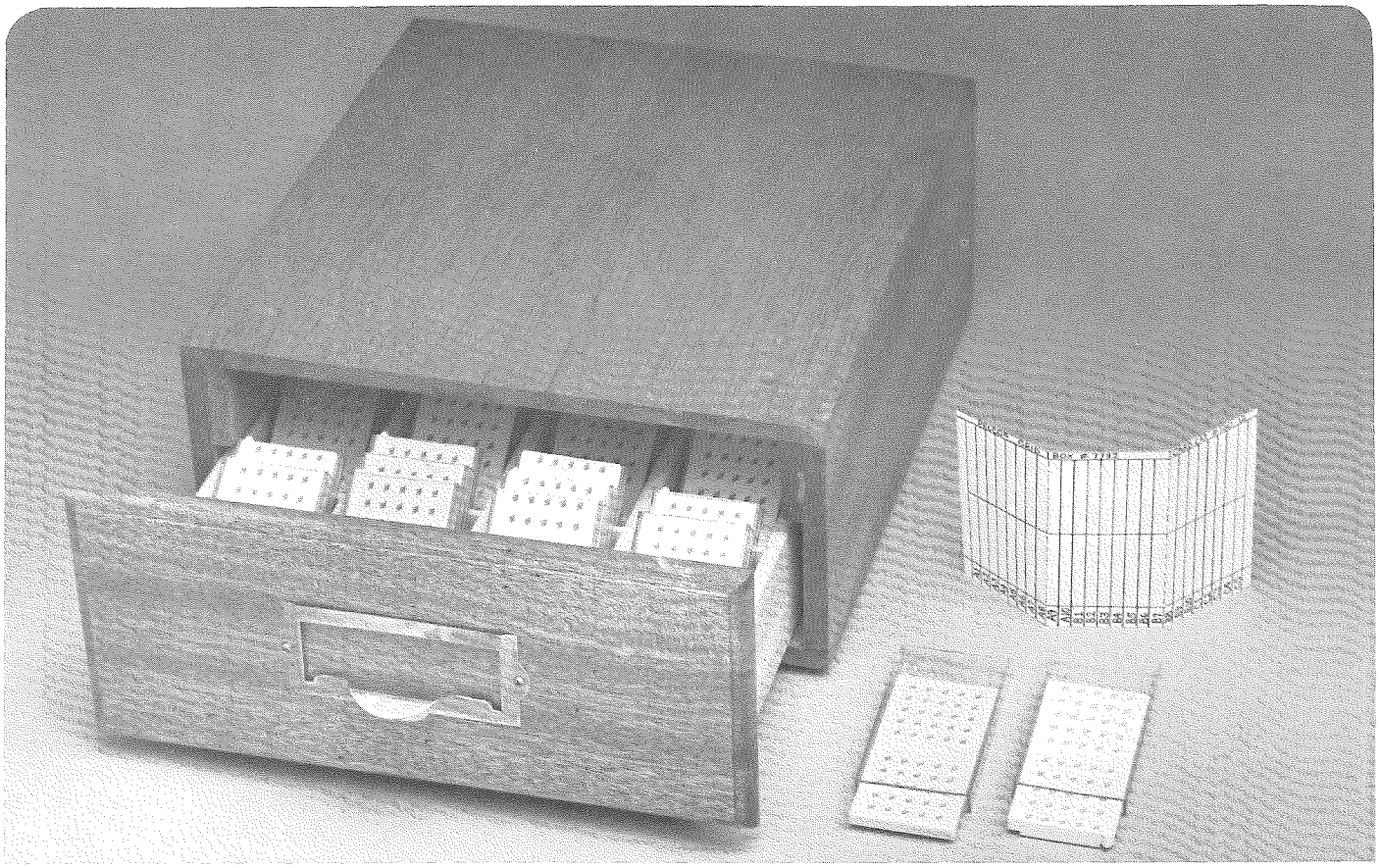

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Abstracts

EFFECT OF PARTIAL NEPHRECTOMY AND OCHRATOXIN A ON RENAL STRUCTURE: A LIGHT AND ELECTRON MICROSCOPE STUDY. S. Geerling, H.H. Mollenhauer, A. Stein, and T. Phillips, Dept. of Pathology and Lab. Med., College of Med., and Dept. of Vet. Public Health, College of Vet. Med., Texas A&M Univ., College Station, TX 77843.

Ochratoxin A (OA) is a food-borne mycotoxin produced by Aspergillus ochraceus. It has been implicated in Balkan nephropathy in humans, and has been reported to be the most prevalent mycotoxin in human foods in the United Kingdom. This study was done to evaluate the effects of OA on renal function and morphology of rats, and to assess whether a prior partial nephrectomy enhances the mycotoxic effects. When administered to rats, OA produced focal necrosis of renal tubular epithelial cells, confined mostly to the inner cortex and extending into the subcortical zone at the cortico-medullary junction. The cellular changes included redistribution of organelles, loss of endoplasmic reticulum integrity, and reduction in the amount of basal infoldings of plasma membranes. In addition, there was a unique aberration consisting of tightly-packed and interdigitating spherical aggregates of smooth endoplasmic reticulum in numerous proximal tubules. Partially nephrectomized rats showed similar, but substantially enhanced, responses to OA. Thus, partial nephrectomy appears to be a useful procedure for accentuating the effects of nephrotoxic agents and facilitating evaluation and diagnostic procedures. Additionally, these data suggest that impairment of renal function significantly enhances the health hazards of mycotoxins such as OA.

FEEDING BEHAVIOR IN THE PROTOSTELIDS. K. D. Whitney and W. E. Bennett, Department of Biology, University of Texas at Arlington, Arlington, TX 76019, and Department of Biology, University of North Carolina, Chapel Hill, NC 27514.

Ultrastructural observations on the feeding techniques of three protostelid species (in the genera Ceratiomyxella, Nematostelium, and Schizoplasmodium) are presented. These species all have a multinucleate, amoeboid trophic phase which alternates with a stipitate sporocarp phase. In agar culture these amoebae are provided with bacteria and yeast as a food source. Actively feeding amoebae engulf bacterial cells and incorporate them in typical food vacuoles. When a feeding amoeba encounters a yeast cell, however, a pseudopodial process penetrates the yeast cell wall and enters the cell lumen. The cytoplasm of the yeast cell is incorporated into one or more food vacuoles, and these vacuoles are drawn out of the yeast cell through the perforation in the wall. When all of the yeast cytoplasm has been removed the pseudopodial process of the amoebae withdraws, leaving the cell wall of the yeast intact, except at the point of penetration. The feeding behavior of non-fruiting vampyrellid amoebae (Vampyrellidae, Protozoa) is quite similar to that of the protostelids examined in this study. The use of feeding techniques to assess mycetozoan phylogeny is discussed.

SECRETORY ACTIVITY OF GOBLET CELLS ASSOCIATED WITH SURGICAL TRAUMA AND SYMPATHETIC INTERRUPTION: STEREOLOGICAL ANALYSIS J. Leon McGraw, Jr., Dept. Biology, Lamar Univ., Beaumont, Tx. 77710

Groups of three male rabbits provided three study populations: Group I - Normal; Group II - abdominal surgery exposed but did not injure sympathetic ganglia; Group III - abdominal surgery removed coeliac and mesenteric ganglia and a portion of the sympathetic trunk. All groups were given water adlibidum but no food two days prior to biopsies and were anesthesized with nembutol during biopsies and surgery. Biopsies on groups II and III were done four days after surgery. The duodenum of all groups was biopsied one inch below the stomach and prepared for TEM with a cacadylate buffered glutaraldehyde, osmium, ethanol, Spurr method. Stereological analysis was applied to the golgi-ER complex and lysosomes of the goblet cells from all groups.

Surgical trauma, a type of sympathetic stimulation, produced no significant changes in the relative volumes (Vv) of the golgi-ER or lysosomes and no significant changes in the membrane surface densities (SD) of lysosomes. Thus, no apparent change in secretory activity results after sympathetic stimulation by surgical trauma.

Interruption of sympathetic innervation to the duodenum resulted in significant decreases in the Vv of the golgi-ER and lysosomes of goblet cells but did not affect the SD of the lysosomes. This indicates a decrease in secretory activity results from interruption of the sympathetic nerve supply to these cells.

HUMAN FETAL PANCREAS. RSimonsen, I.Dawidson, D.Stoops, Dept. Pathology and Surgery, Univ. of Texas, Dallas, Texas 75235 Intact fresh human fetal pancreases of gestational age 11-18 weeks were obtained from aborted fetuses under sterile conditions, and placed in cold Hanks solution containing antibiotics, cleaned from connective tissue and major blood vessels, and either chopped or digested with collagenase into 0.1-1.0mm fragments. The fragments were placed in hydrophobic tissue culture wells with cell culture media (RPMI 1640 with 10% fetal calf serum) and incubated at 37^{0}C in either 95% O_2 and 5% CO_2 or in air. Tissue prior to culture revealed by both light and electron microscopy small islets appearing to bud from ductal structures. These budding islets contained insulin and glucagon demonstrated with peroxidase-anti-peroxidase technique, and ultrastructurally contained A, B, and a few D cells. After approximately one week in culture epitheliod appearing cells by light microscopy became the predominant cell type, and immunoperoxidase staining for insulin and glucagon disappeared. Ultrastructurally these cells were characterized by large nuclei containing prominent nucleoli, cytoplasm with abundant rough endoplasmic reticulum and no secretory granules, plasma membranes with microvilli and small intercellular ductal lumina. Therefore, the "epitheliod" cells noted by light microscopy were ultrastructurally identified as "ductal" type cells. It is commonly accepted that during normal embryogenesis islets bud from small ducts. Furthermore, Laffery has demonstrated using light microscopy and immunoperoxidase technique with fetal mouse pancreas that "epitheliod" appearing cells produce insulin following transplantation to other mice. We believe that the epitheloid cells that predominate in tissue culture are primarily ductal in origin, and may therefore have the potential to mature or differentiate into B cells following transplantation.

X-RAY MICROANALYSIS OF THE ELEMENTAL CONTENT OF RAT PULMONARY MACROPHAGES CHALLENGED BY PSEUDOMONAS AERUGINOSA. Nancy K.R. Smith, R. Lee Boyd*, Andrzej K. Lewinski, and John A. Mangos*, Dept. Cellular and Structural Biology and *Dept. Pediatrics, University of Texas Health Science Center, San Antonio TX 78284.

Electron probe X-ray microanalysis of freeze-dried ultrathin sections provides the capability of measuring intracellular elemental content in individual cells. This methodology was used to investigate the stimulus-permeability coupling responses associated with phagocytosis of Pseudomonas aeruginosa by cultured rat pulmonary alveolar macrophages (PAMs). PAMs were challenged with Pseudomonas suspended in Geys buffer at a bacteria-to-PAM ratio of 50:1 for 1 hour at 37°C. Pellets (1 mm^3) of the unchallenged control PAMs, challenged PAMs and Pseudomonas alone were frozen in propane and 0.1 µm cryosections were cut at -100°C. X-ray spectra were collected for nucleus and cytoplasm of 39 control PAMs and 36 challenged PAMs and for 40 Pseudomonas. Concentration values (mmole/kg dry weight) were obtained for Na, Cl, K, Ca, Mg, P, S for each cell by comparison with salt-PVP standards. There were no differences in elemental content between nucleus and cytoplasm. In the control PAMs, the content was similar to other mammalian cells. In the challenged PAMs, Na concentration was 4 times that of control PAMs ($p< 0.001$) whereas Cl was double ($p< 0.001$), K was 29% lower ($p< 0.001$) and Ca was 4 times higher ($p< 0.05$). These results demonstrate elemental content changes in cultured PAMs challenged with Pseudomonas, indicative of a membrane stimulus-permeability response associated with the phagocytic process.

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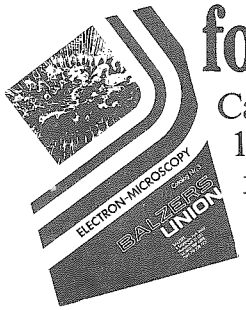

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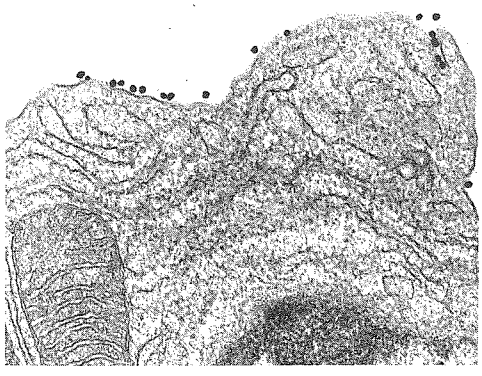

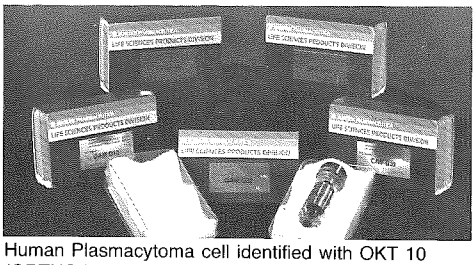

Human Plasmacytoma cell identified with OKT 10 (ORTHO-MUNE™) monoclonal antibody and GAM G30, according to De Waele et al. J. Histochem. Cytochem. 1983, 31, 376. Micrograph (X 82.250) by courtesy of Dr. M. De Waele, Dept. Hematology, University Hospital of the Free University of Brussels, (A. Z.–VUB), 1090 Brussels, Belgium.

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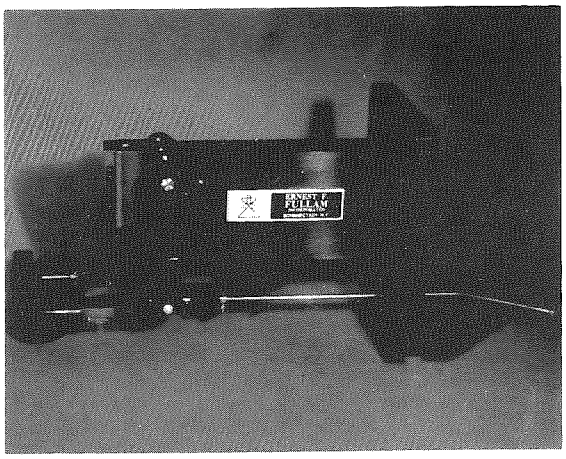

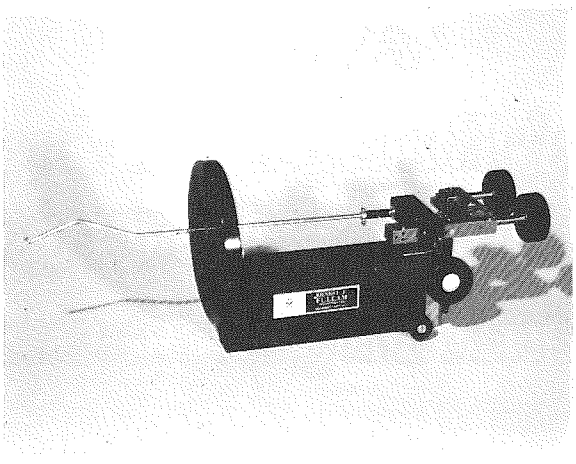

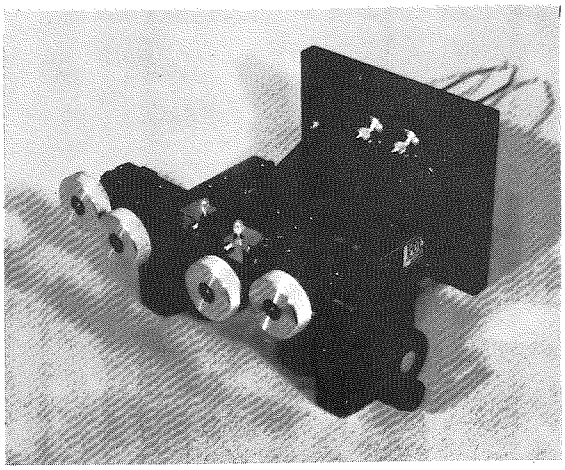

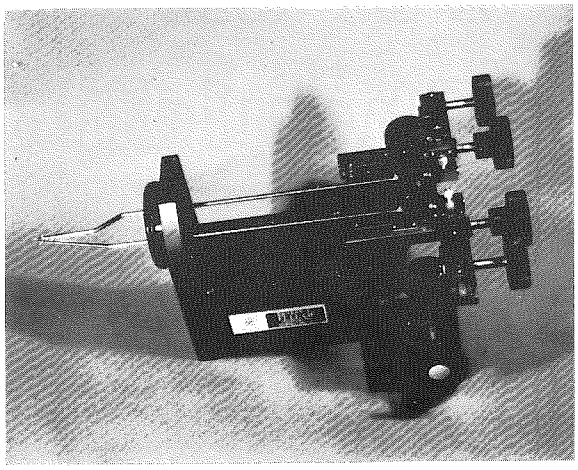

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EMBEDDING THICK-WALLED PLANT AND FUNGAL SPECIMENS FOR TRANSMISSION ELECTRON MICROSCOPY

By

Charles W. Mims

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Thick-walled plant and fungal specimens are often very difficult to prepare successfully for examination with transmission electron microscopy (TEM). As a result, many worthwhile projects are often abandoned because of problems encountered with tissue preparation. The purpose of this paper is to describe a fixation and embedding protocol that has worked well for me on various difficult specimens. While none of the procedures I employ are really unique, it is hoped that this article will be helpful to beginning electron microscopists as well as to more experienced workers who are attempting to examine thick-walled specimens for the first time.

The only fixatives I use are glutaraldehyde and OsO_4. I normally fix material overnight[1] with either 3% or 5% glutaraldehyde in 0.05M sodium phosphate

[1]As used here the term ''overnight'' can be from 12-24 hours.

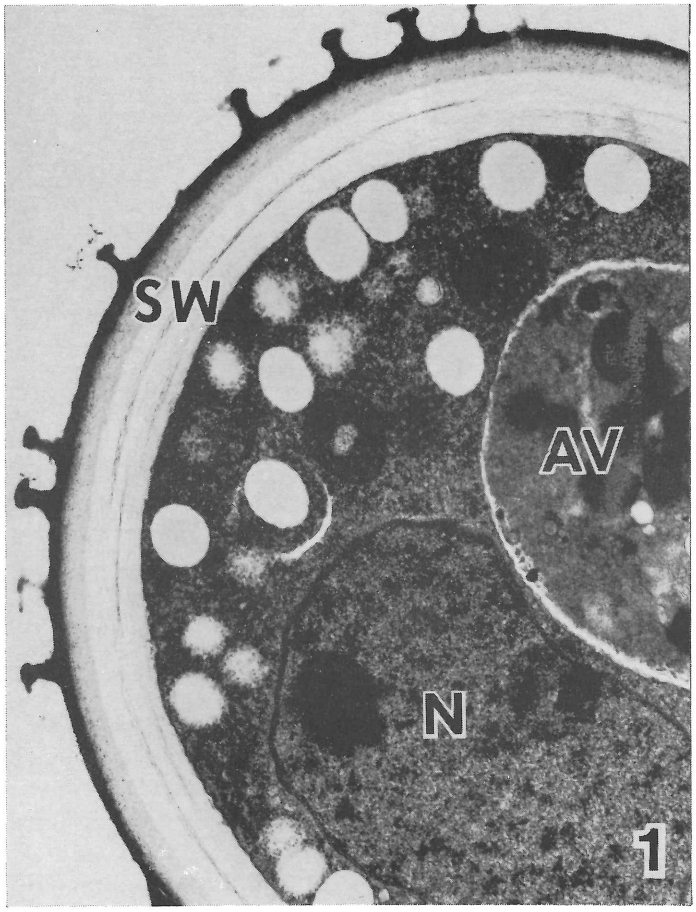

FIGURE 1. Portion of a mature, 24 hour old spore of the myxomycete **Didymium iridis**. A prominent nucleus (N), autophagic vacuole (AV) and multilayered spore wall (SW) are evident. X20,000.

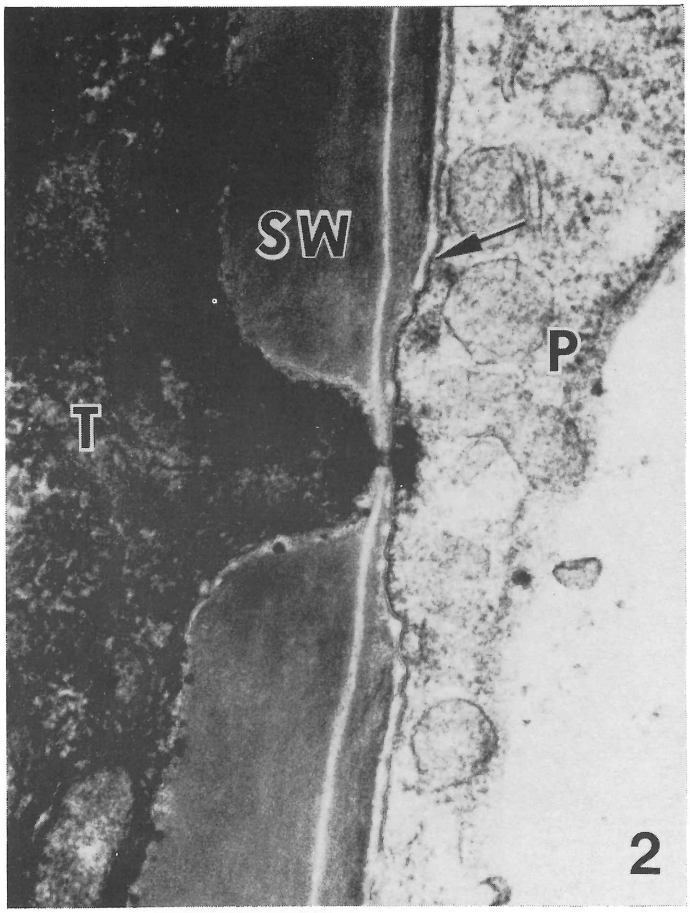

FIGURE 2. Base of a mature teliospore of the rust fungus **Puccinia podophylli.** A small septal pore is evident between the very dense teliospore (T) and its tail or pedicel (P). Note the thick, electron-opaque spore wall (SW). Unit membrane resolution is evident in the cell membrane (arrow) of the pedicel. X100,000.

buffer, pH 7.2. Equal parts of the refrigerated stock solutions of glutaraldehyde (6% or 10%) and buffer (0.1M) are mixed immediately before use in a glass specimen vial and the resulting solution is allowed to warm to room temperature before the specimens (small pieces of material 1 mm or less in diameter) are added. If the specimens float on the fixation solution a small amount of a wetting agent such as Kodak Photo-Flo 200 is added using a wooden applicator stick. At no time is this entire protocol are the specimens placed in a vacuum.

After 15 minutes at room temperature the specimen vials are placed in a refrigerator (3°C) and left overnight. The following morning the original fixation solution is removed and the specimens are washed with 2 or 3 changes of buffer for a total of about 30 minutes and then post-fixed for 2 hours in a refrigerator at 3°C with 2% OsO_4 mixed 1:1 with buffer. The buffered OsO_4 solution is then removed and the specimens are washed with 3 changes of distilled water for a total of 30 minutes and then bulk stained overnight in a refrigerator with 0.5% aqueous uranyl acetate.

If pressed for time I often use a somewhat different procedure than the one described thus far. This approach involves first fixing the specimens for 15 minutes in phosphate buffered glutaraldehyde as noted above. This solution is then removed and replaced with fresh glutaraldehyde and buffer. To the resulting solution an equal volume of cold OsO_4 is added. The material is then processed to uranyl acetate as described above.

After bulk staining in uranyl acetate specimens are rinsed in distilled water for 10 minutes and dehydrated at 10-15 minute intervals in a graded ethanol series to 100% ethanol. Following 30 minutes in two rinses of 100% ethanol the specimens are treated for 30 minutes in two changes of 100% acetone and finally embedded in Spurr's plastic (1).

When using Spurr's plastic it should be emphasized that is is imperative that all the water be removed from the specimens prior to placing them in plastic. It is for this reason that I routinely use two changes of both 100% ethanol and 100% acetone during polymerization. I routinely use the "hard" mixture of Spurr's plastic (1) and usually make up only 1/4 to 1/2 a recipe at a time. In addition to always using freshly mixed plastic, I also infiltrate the specimens

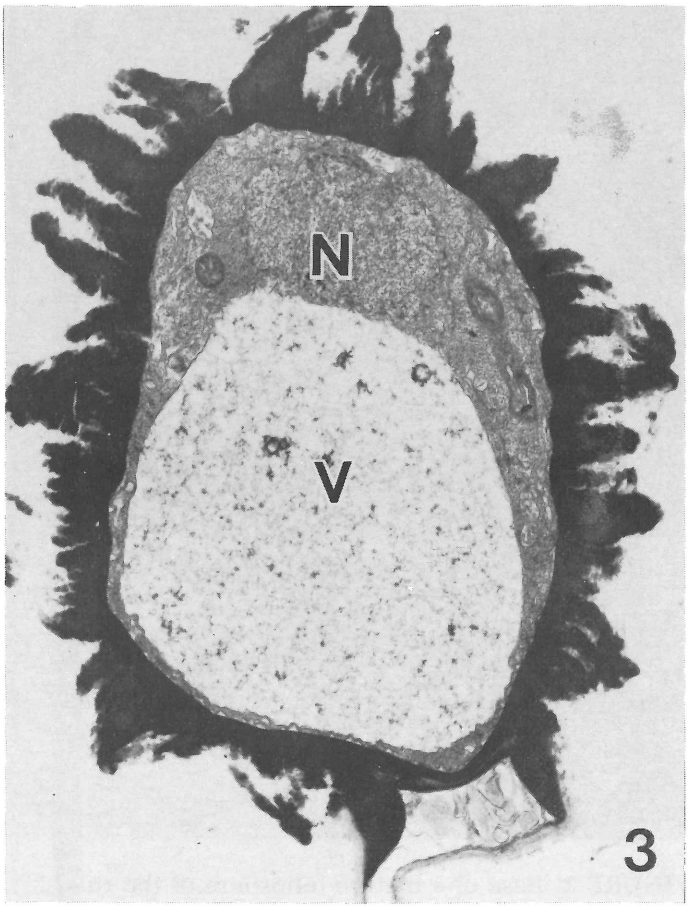

FIGURE 3. Immature basidiospore of the fungus **Pisolithus tinctorius.** Note the thick, spiny, electron-opaque spore wall. The spore nucleus (N) and a large vacuole (V) are evident within the spore. X10,000.

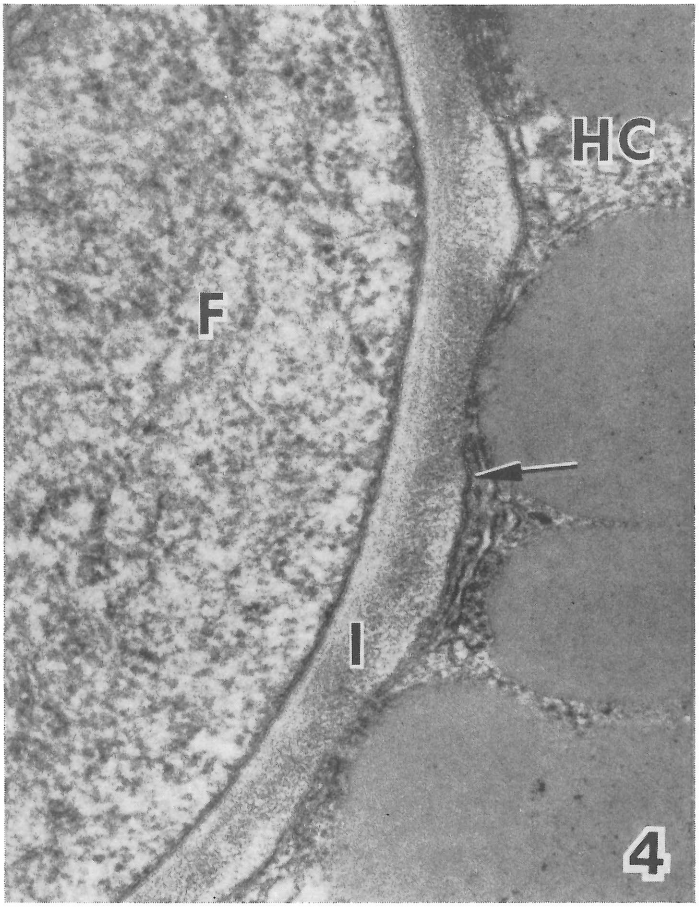

FIGURE 4. Portion of a haustorium of the rust fungus **Gymnosporangium juniperi-virginianae** within a thick-walled cell of its host **Juniperus virginiana.** The fungal cytoplasm is visible at (F) while a portion of the host cell is visible at HC. Although not well defined, the fungal wall is present in region I. Note the unit membrane resolution of the host cell plasma membrane. X65,000.

over an extended period of time according to the following schedule:

2 parts 100% acetone: 1 part plastic (overnight)
1 part 100% acetone: 2 parts plastic (overnight)
100% plastic (overnight)[2]

Specimens are finally placed in embedding molds containing freshly mixed plastic, allowed to sit at room temperature for 1-2 hours and placed in a 70°C oven for 12-24 hours.

Examples of some thick walled specimens prepared using the protocol described above are shown in Figures 1-6. Although it is sometimes possible to section such material with glass knives, the chances of obtaining acceptable sections are much greater with a diamond knife. Even then, however, success is not always guaranteed, and it appears that some specimens simply will not yield usable sections.

[2]When dealing with particularly thick walled specimens multiple changes of 100% plastic are often used for a 12 to 24 hour period.

In closing, I want to emphasize that there is still much important TEM work to be done on thick-walled plant and fungal specimens. In my opinion, the protocol described here will give you a good chance of success with different specimens. Each type of material will, however, present its own particular problems to the researcher. Don't be afraid to experiment with different approaches, but try to avoid simultaneously introducing so many variables to your protocol that you will be unable to identify factors that really contribute to better fixation and infiltration of your specimens.

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(1.) A.R. Spurr. A low viscosity embedding medium for electron microscopy. J.Ultrastruct. Res. 26: 31-43. 1969.

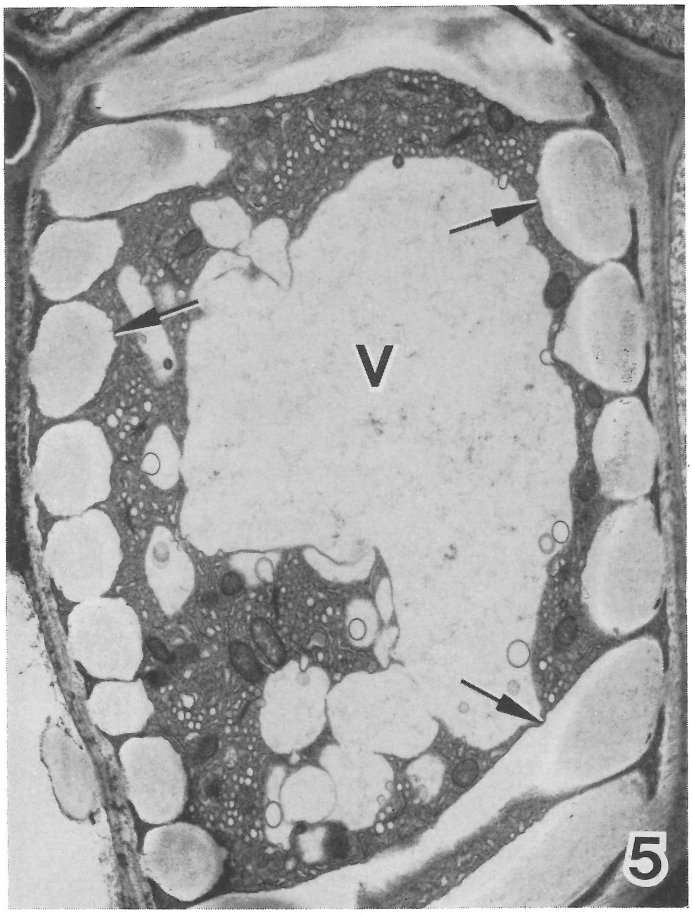

FIGURE 5. Developing xylem cell from a young leaf of **Camellia sasanqua.** Note the large central vacuole (V) and the thickenings of the cell wall (arrows). X7,500.

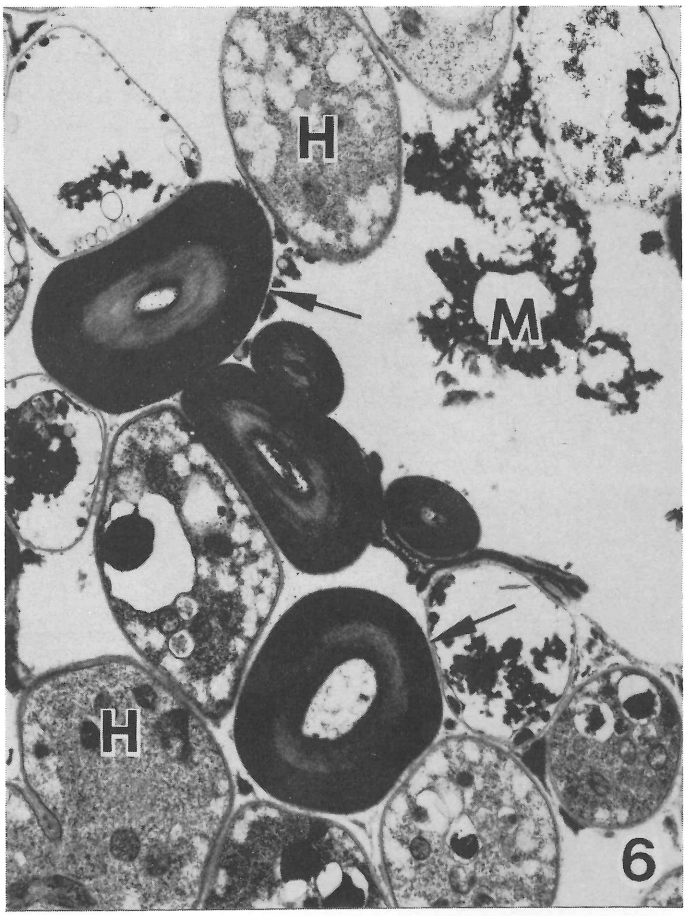

FIGURE 6. Portion of a basidiocarp of the fungus **Ganoderma lucidum.** Visible are cross sections of thin-walled, generative hyphae (H), thick-walled specialized hyphae (arrows) as well as masses of extra-cellular matrix material (M). X12,000.

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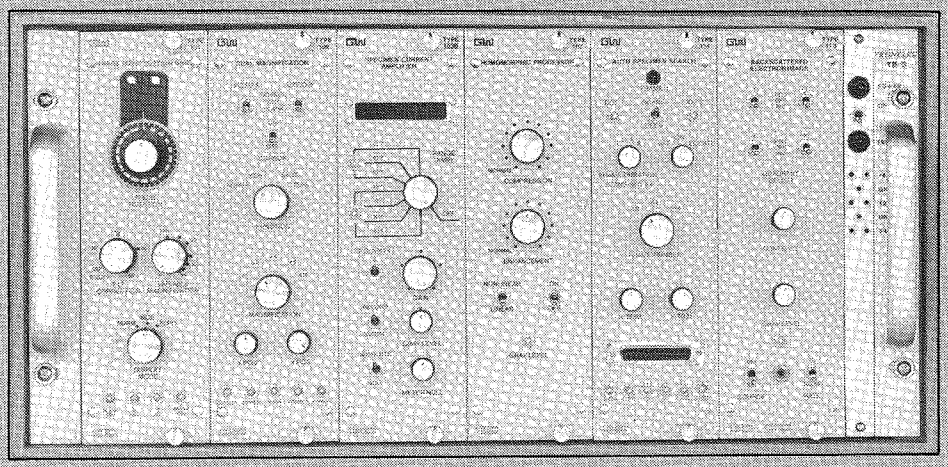

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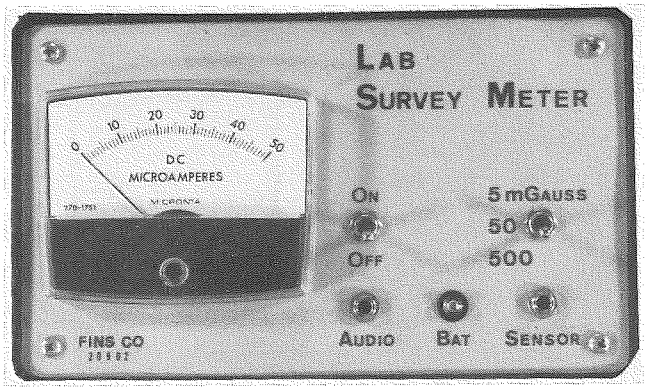

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For further information contact:

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(Reprinted from EMSA Bulletin, Vol. 14, No. 1, Spring 1984)

EDITORIAL POLICY

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Letters to the editor are printed as they are received in the order of their arrival. These letters reflect the opinion of the individual TSEM member and do not necessarily reflect the opinions of the editor or the society. The content of the letters should be concerned with the philosophical or operational aspects of the TSEM, the Journal and its contents, academic or national policies as they apply to TSEM and/or its members and electron microscopy in general. Editorial privilege may be evoked to insure that the LETTERS SECTION will neither be used as a political forum nor violate the memberships' trust.

ELECTRON MICROGRAPHS AND COVER PHOTOS

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

REGIONAL NEWS

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

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

TECHNICAL SECTION

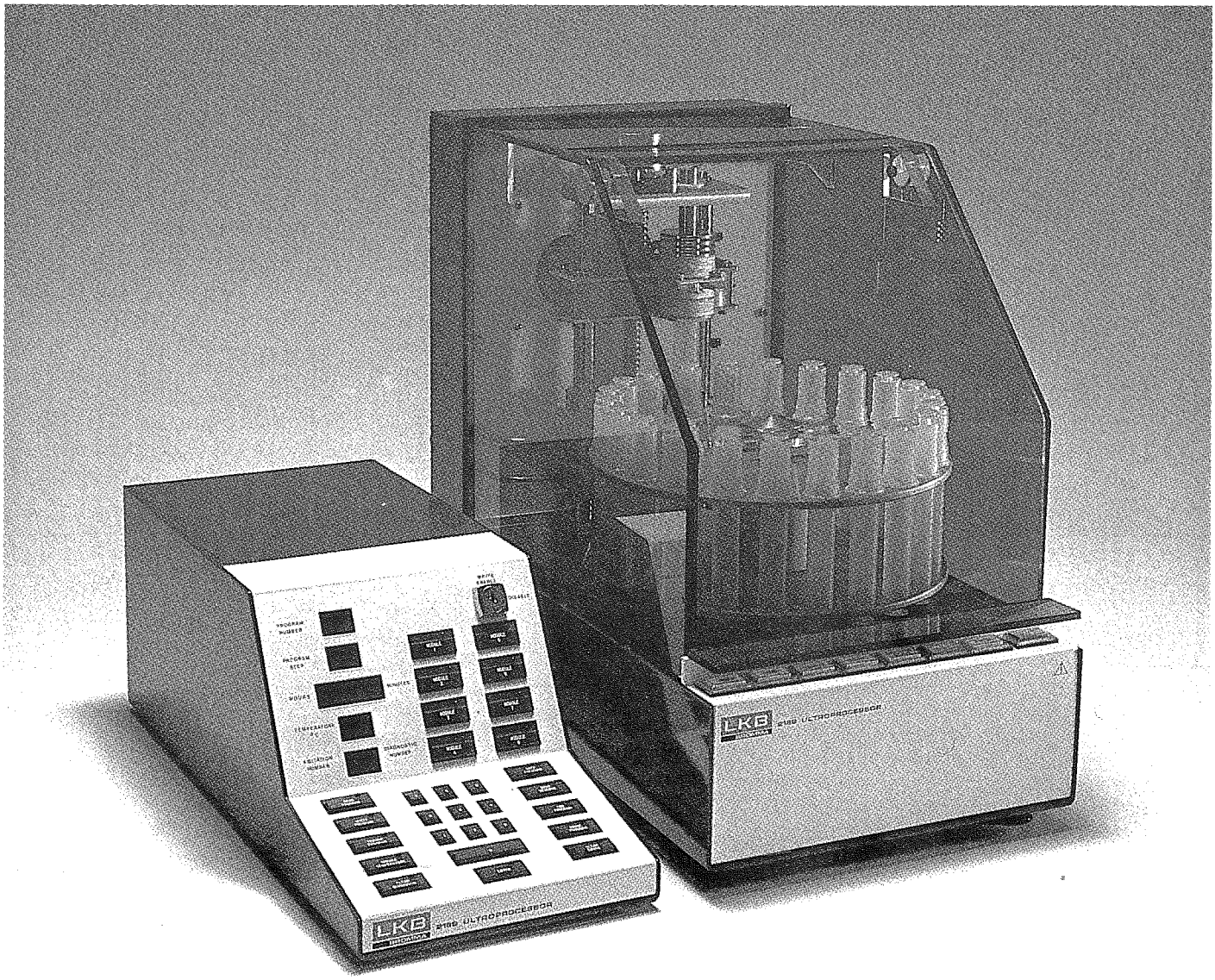
The Technical Section will publish TECHNIQUES PAPERS, HELPFUL HINTS, and JOB OPPORTUNITIES. The TECHNIQUES PAPERS will describe new or improved methods for existing techniques and give examples of the results obtained with methods. The format of the Technique Papers will be the same as that used for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

PUBLICATION PRIVILEGES

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

Introducing the UltroProcessor

A new concept in automated tissue processing for electron microscopy



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For full facts on the UltroProcessor: Circle Reader Service Card No. 13

SHORT COURSES AND WORKSHOPS

THIRD ASIA PACIFIC ELECTRON MICROSCOPY WORKSHOP
August 24-28, 1984
Singapore
For further information contact Dr. J.J. Cockayne, Electron Microscope Unit, University of Sydney, NSW 2006, Australia 2-6922351.

SCANNING ELECTRON MICROSCOPY AND X-RAY MICROANALYSIS: THEORY AND PRACTICE - MATERIALS SCIENCE
October 15-19, 1984
Lake Mohonk, New Paltz, NY
For further information contact Dr. Angelos V. Patsis, Department of Chemistry, CSB 209, State University of New York, New Paltz, NY 12561, phone (914) 257-2175.

ADVANCED SCANNING ELECTRON MICROSCOPY AND X-RAY MICROANALYSIS: THEORY AND PRACTICE-MATERIALS SCIENCE
October 22-26, 1984
Lake Mohonk, New Paltz, NY
For further information contact: Dr. Angelos V. Patsis, Department of Chemistry, CSB 209, State University of New York, New Paltz, NY 12561, phone (914) 257-2175.

SCANNING ELECTRON MICROSCOPY AND X-RAY MICROANALYSIS: THEORY AND PRACTICE-BIOLOGY AND MEDICINE
October 22-26, 1984
Lake Mohon, NY 12561
For further information contact: Dr. Angelos V. Patsis, Department of Chemistry, CSB 209, State University of New York, New Paltz, NY 12561, phone (914) 257-2175.

SCANNING ELECTRON MICROSCOPY: SPECIALIZED COURSE FOR BIOLOGISTS AND MATERIALS SCIENTISTS
September 17-21, 1984
Cambridge University, England
For further information contact the Administrator, Royal Microscopical Society, 37/38 St. Clements, Oxford OX4 1AJ, phone (0865) 248768/721081

ELECTRON MICROSCOPY IN PATHOLOGICAL DIAGNOSIS
September 17-21, 1984
Glasgow, Scotland
For further information contact the Administrator, Royal Microscopical Society, 37/38 St. Clements, Oxford OX4 1AJ, phone (0865) 248768/721081

ANNUAL ELECTRON BEAM TESTING COURSE
September 30 — October 5, 1984
Santa Clara, CA
For further information contact George Lukianoff, Electron Beam Testing Course, P.O. Box 2, Hopewell Jct., NY 12533

MONITORING & MAINTAINING THE ELECTRON MICROSCOPE
December 10-14, 1984
Warwick University, England
For further information contact the Administrator, Royal Microscopical Society, 37/38 St. Clements, Oxford OX4 1AJ, phone (0865) 248768/721081

ADVANCED ELECTRON IMAGING TECHNIQUES FOR BIOLOGISTS
December 17-21, 1984
National Institute for Medical Research, London, England
For further information contact the Administrator, Royal Microscopical Society, 37/38 St. Clements, Oxford OX4 1AJ, phone (0865) 248768/721081

CALENDAR OF MEETINGS

35th ANNUAL MEETING OF THE AMERICAN INSTITUTE OF BIOLOGICAL SCIENCES (AIBS)
August 5-9, 1984
Colorado State University, Ft. Collins, CO
For further information contact: Dr. Ralph R. Baker, Dept. of Botany and Plant Pathology, Colorado State University, Ft. Collins, Co 80523, phone (303) 491-6944.

EMSA/MSC JOINT ANNUAL MEETING
August 12-17, 1984
Detroit, MI
For further information contact: Linda Sicko Goad, Great Lakes Research Division, The University of Michigan, 2200 Bonlsteel Blvd., Ann Arbor, MI 48109, phone (313) 763-5393.

3rd ASIA PACIFIC ELECTRON MICROSCOPY CONFERENCE
August 29-September 1, 1984
Singapore
For further information contact: Dr. Ny Cheng Siong, ARC, 303 Tanglin Road, Singapore 1024 Telex UN1ARC RS 38806.

CENTENNIAL SYMPOSIUM ON HIGH RESOLUTION ELECTRON MICROSCOPY
January 7-11, 1985
Arizona State University, Tempe, AZ
For further information contact: Dr. P. R. Buseck, Department of Geology, Arizona State University, Temple, AZ 85287.

FALL, 1984 MEETING OF THE TSEM
October 25-27, 1984
Arlington, Texas

20TH ANNIVERSARY MEETING OF THE TSEM
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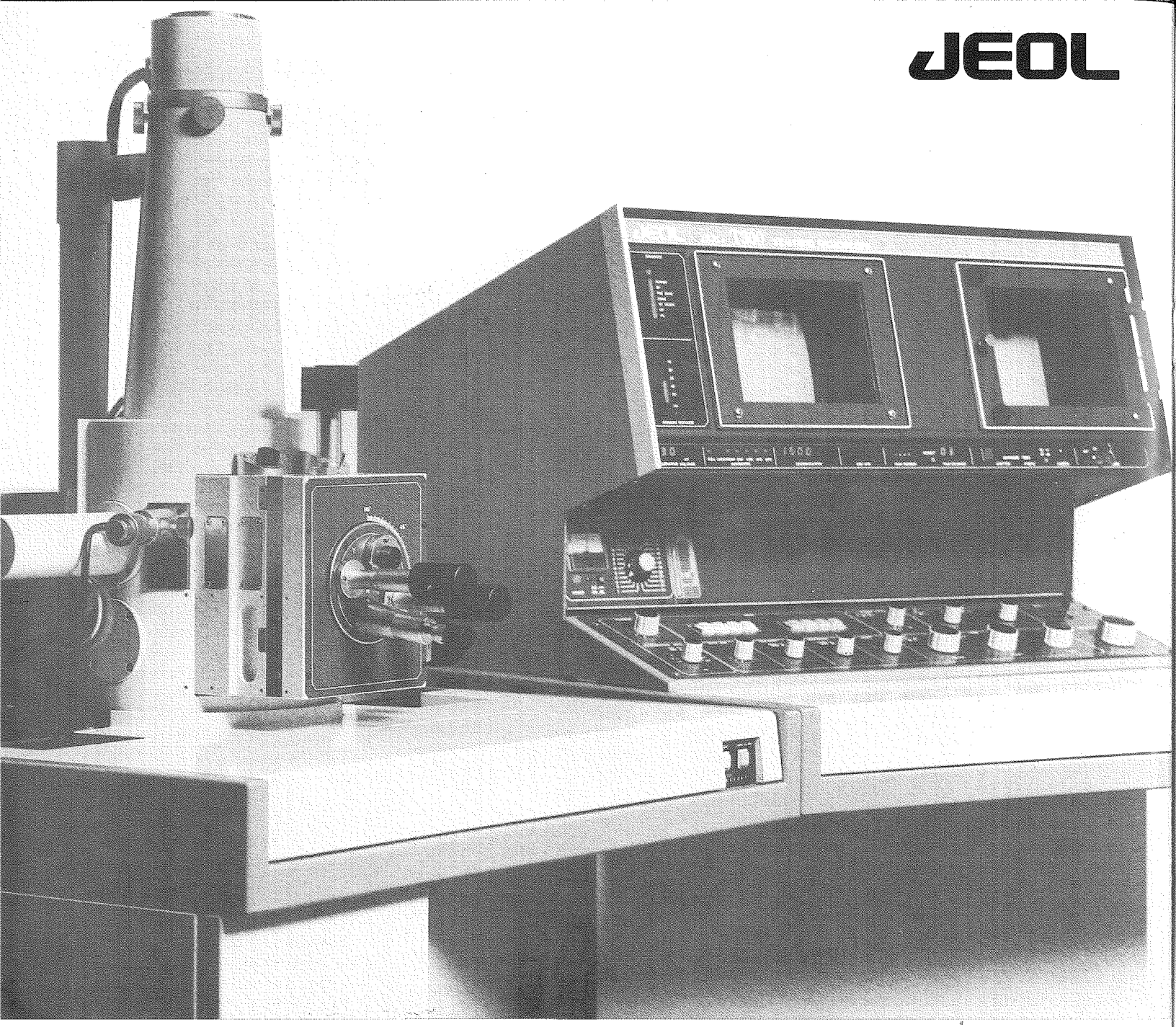

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GUIDELINES: Manuscripts written in English will be considered for publication in the form of original articles, historical and current reviews, case reports and descriptions of new and innovative EM techniques. It is understood that the submitted papers 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.

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

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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.

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

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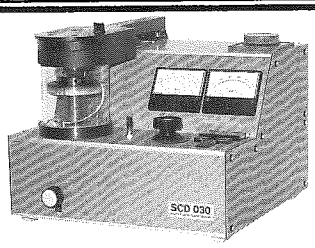

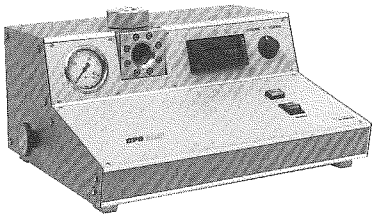

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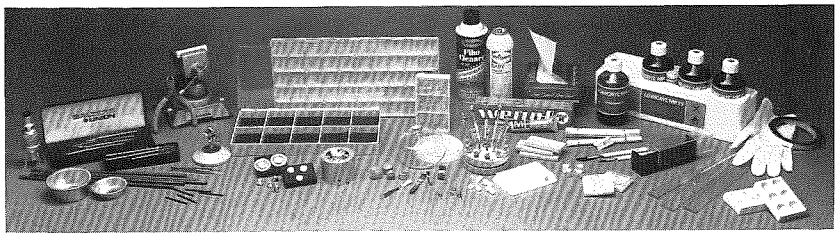

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Non-Academic Careers for Electron Microscopists: The Future in View

This is a summary of a symposium presented to the Joint Meeting of the Western Societies of Electron Microscopy, Asimolar, CA, (May 12, 19893). There were five panel members: Ted Pella, founder and owner of Ted Pella, Inc., purveyor of electron microscope supplies; Helen Thomas, an electron microscopist trained for biological research and currently working for Hewlett Packard as a materials science specialist; Dr. Bart Yatchmenoff, a Ph.D. in biological electron microscopy from Northeastern University and currently Western Field Representative for Amray Inc.; Dr. Gloria Yu (M.D.), trained as a pathologist and electron microscopist at Albert Einstein and currently Chief Diagnostic EM Pathologist at the Veterans Administration Hospital in Martinez, CA; Dr. John C. Belton, director of the EM lab at California State University, Hayward.

For the purposes of discussion, five levels of electron microscopy were defined in terms of the amount of formal training and experience. The discussants evaluated the employment opportunities for these different technical levels. The participants also categorized the current employment outlook in the different industrial sectors. A small portion of the dialogue follows:

Helen Thomas — "There are jobs for those who know how to apply themselves. If you have a quick mind and are interested in learning the semiconductor industry, this may be your employment chance. The microchips are getting smaller and smaller, and a scanning microscope is a necessity for those to be produced. So, I can offer you encouragement!"

Ted Pella — "When I was asked to participate in this panel, I had difficulty in relating to the topic because it's so hard to categorize microscopy. You're speaking in a large percentage of cases to biomedical people, yet most of the jobs at this point don't seem to be in the biological field; they seem to be in the industrial field . . ."

The following conclusions were drawn from the proceedings:

1. Electron microscopy is used in a diversity of industrial and nonacademic agencies. Most of the current demand is in scanning electron microscopy or microprobe analysis.
2. At the current time there are more employment prospects in materials science than there are in biomedical research. The most rapid increases in employment may develop in the microchip industry.
3. For technicians wishing to work on biomedical material, the best prospects seem to be in medical pathology. This work, often associated with metropolitan hospitals, requires some special training not provided in most university laboratories.
4. All panel members agreed that EM trainees should first learn the techniques of transmission EM and later learn special procedures or SEM. A breadth of experience with different samples and different procedures should be encouraged.
5. Employment prospects can be much greater if the person is willing to consider living in any geographical area.

Any person wishing to obtain a full transcript of these proceedings should send a large self-addresses envelope to:

Dr. Collin Murphy
Dept. of Ophthalmology
S-315
UCSF School of Medicine
San Francisco, CA 94143

(Reprinted with permission, from EMSA Bulletin, Vol. 14, No. 1, Spring 1984)

Registry of Educational Programs

The *Registry of Educational Programs* in *Electron Microscopy for North America* is off the press and can be obtained from Bill Redmond, Electron Microscopy Facility, Department of Biology, State University of New York, New Platz, NY 12561. The cost, which is to help defray publication and mailing costs, is $3, payable to EMSA. In order to keep the *Registry* and EMSA's records as current as possible, please send Bill any updated information about your courses — this is especially valuable for short courses. If you have brochures or fliers concerning your short course, make sure Bill gets a copy. If you find mistakes in the *Registry's* listings, please send the corrections directly to Bill Redmond so be can correct EMSA's master list. Please type all information sent to Bill concerning corrections. This is particularly important with regard to individuals' names which may have multiple spellings. Illegible handwriting is frequently responsible for incorrect entries.

EMSA Proceedings 1983

San Francisco Press, Inc. (Box 6800, San Francisco, CA 94101-6800) has taken over publication of the *Proceedings of the Annual EMSA Meeting*, beginning with the 41st (Phoenix) 1983 meeting. Copies are $45; EMSA and MAS members *prepaying by personal check* get 20% off and pay only $36. (California purchasers add sales tax.) The same prepayment prices are available for the Proceedings of the 1984 Annual EMSA/MSC meeting. Shipment after August 1, 1984.

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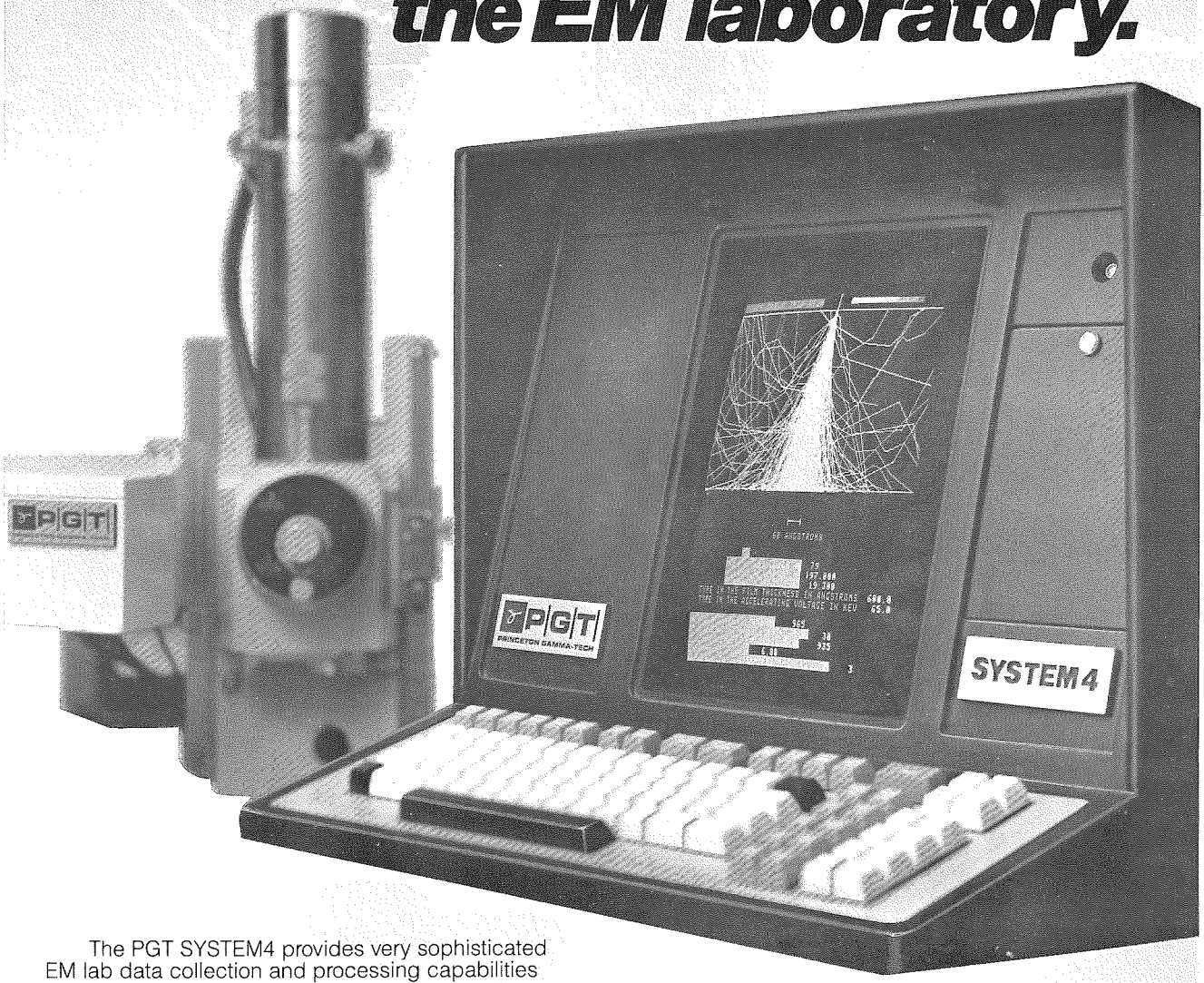

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