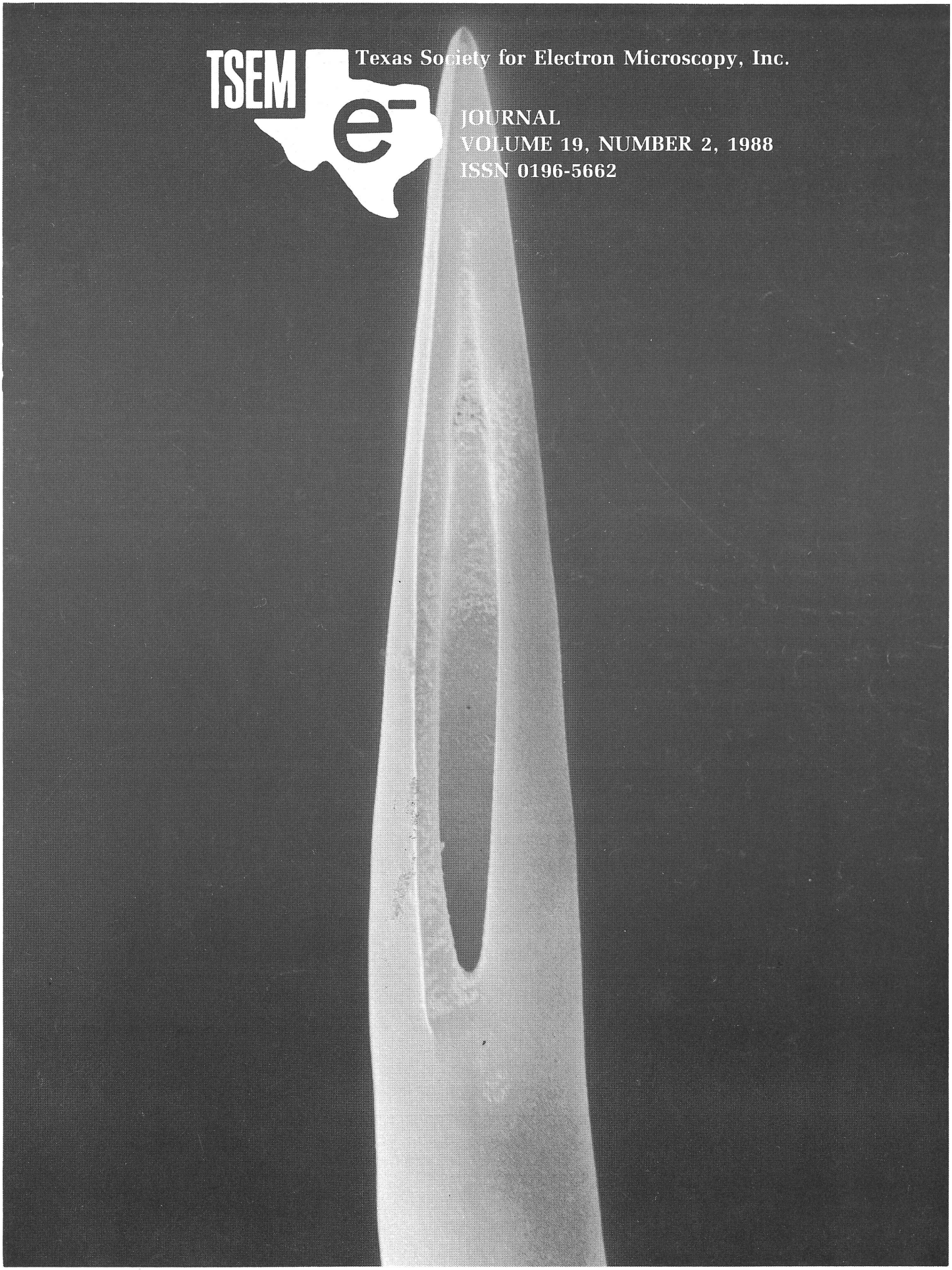

Texas Society for Electron Microscopy, Inc.

JOURNAL
VOLUME 19, NUMBER 2, 1988
ISSN 0196-5662

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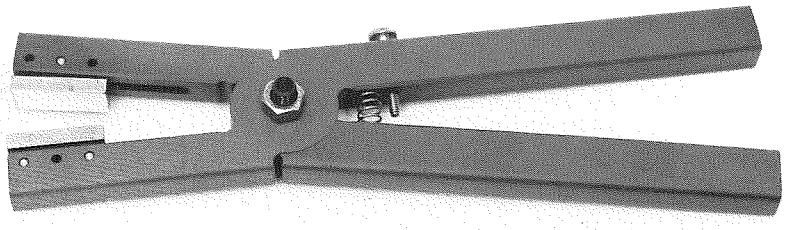
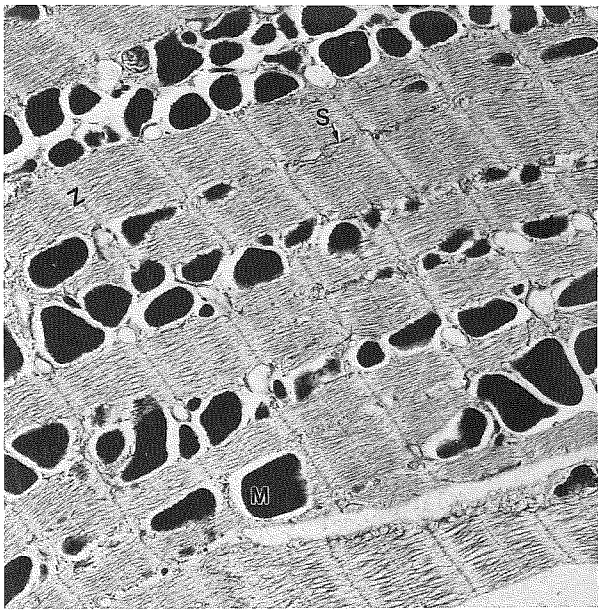
- **Peldri II: tissue drying for S.E.M.**
- **ALKAR Glass: the hardest, for ultrastructure and histology preparations**



39800 Pelco® CryoPliers based on a design by Dr. Linda J. McGuffee, Department of Pharmacology, University of New Mexico School of Medicine, Albuquerque, NM.



Rat Ventricle muscle cells shown in longitudinal section. Tissue was quick-frozen with the CryoPliers, freeze-dried, vapor fixed with osmium tetroxide, and embedded in Spurr resin. There is less ice damage in the sarcomeres at the top of the figure than at the bottom. Sarcomere structure is well preserved. Z = z-line, S = sarcoplasmic reticulum, M = mitochondria. Magnification = 25000X. With permission: Dr. A.O. Jorgensen, Department of Anatomy, Univ. of Toronto, Toronto, Ontario, Canada. (Note: original print reduced 57% as shown above)

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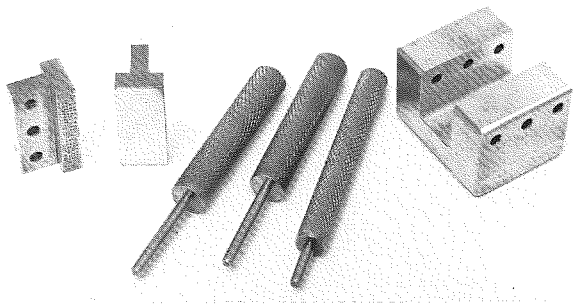

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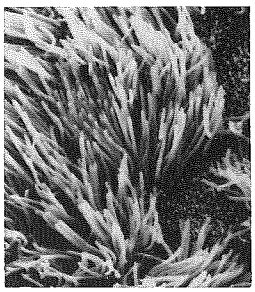

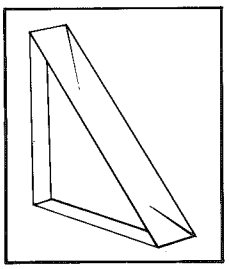

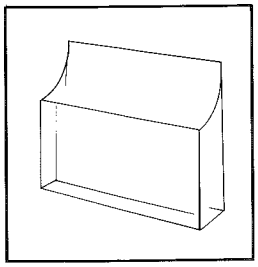

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TEXAS SOCIETY FOR ELECTRON MICROSCOPY
JOURNAL
VOLUME 19, NUMBER 2, 1988
ISSN 0196-5662

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

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Scanning electron micrograph by Ronald W. Davis, Department of Medical Anatomy, Texas A&M University, College Station, Texas, of a rattlesnake fang. Note that it has a hollow core through which the venom in ejected. (Approximate magnification 50x).

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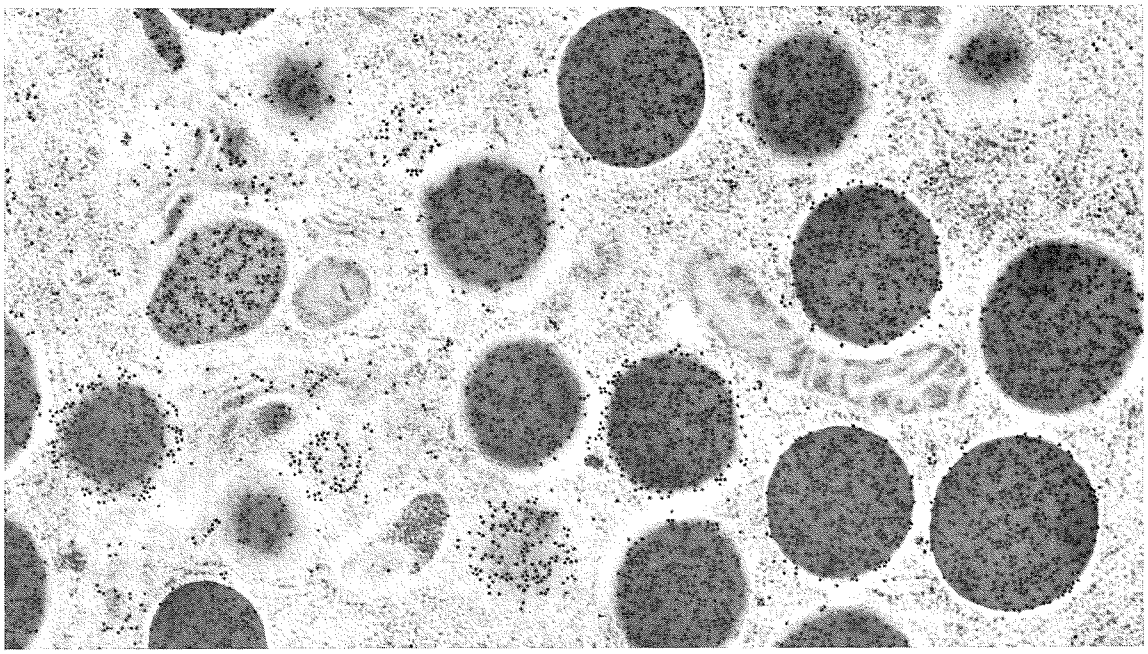

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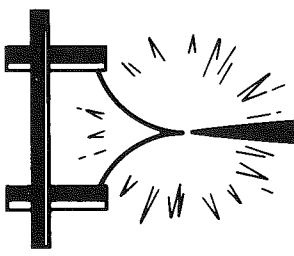

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TSEM, Inc., has started the process of finalizing tax exemption with the Internal Revenue Service. The process requires financial records for the past three years and proposed budgets for the next two. We are currently waiting for documents to complete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

Sincerely,

Bob Droleskey

Bob Droleskey
Treasurer, TSEM, Inc.

1988 TREASURER'S REPORT

For Period Ending 15 August, 1988

ASSETS ON 1 JANUARY, 1988:		
Certificate of Deposit No. 10-7199995	$ 2,267.03	
Certificate of Deposit No. 10-8829764	4,536.73	
Certificate of Deposit No. 111-849-6	3,015.92	
Checking Account No. 015210-01	5,793.30	$15,612.98
RECEIPTS:		
Registration & Exhibitor Fees - Dallas Meeting	$ 3,280.00	
Individual and Corporate Dues	2,111.00	
Journal Ad Revenue 19:1	1,800.00	
Donations	95.00	
Checking Account Interest	142.51	
Interest Earned on Certificates of Deposit	282.47	$ 7,710.98
EXPENSES:		
Dallas Meeting Expenses	$ 4,488.83	
Galveston Meeting Expenses	118.43	
Student Travel	100.00	
Meeting Announcement/Program Printing and Mailing	1,750.00	
Journal Printing & Postage	3,124.63	
Professional Fees	1,000.00	
Journal Subscription Refunds	60.00	
Treasurer's Expenses	126.81	$10,768.70
ASSETS AS OF 15 AUGUST, 1988:		
Certificate of Deposit No. 10-7199995	$ 2,267.03	
Certificate of Deposit No. 10-8829764	3,982.50	
Certificate of Deposit No. 111-849-6	3,152.62	
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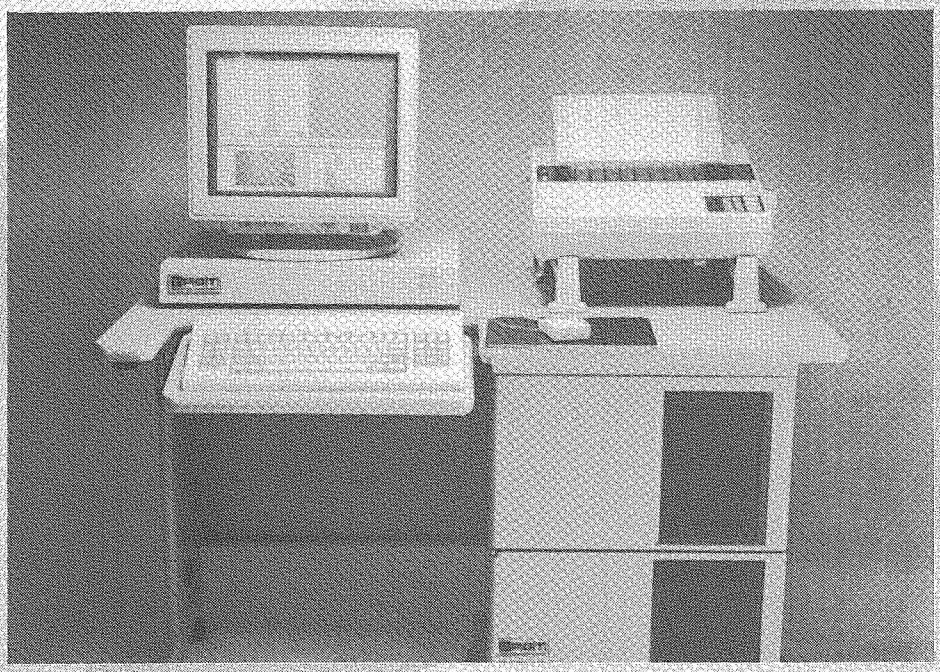

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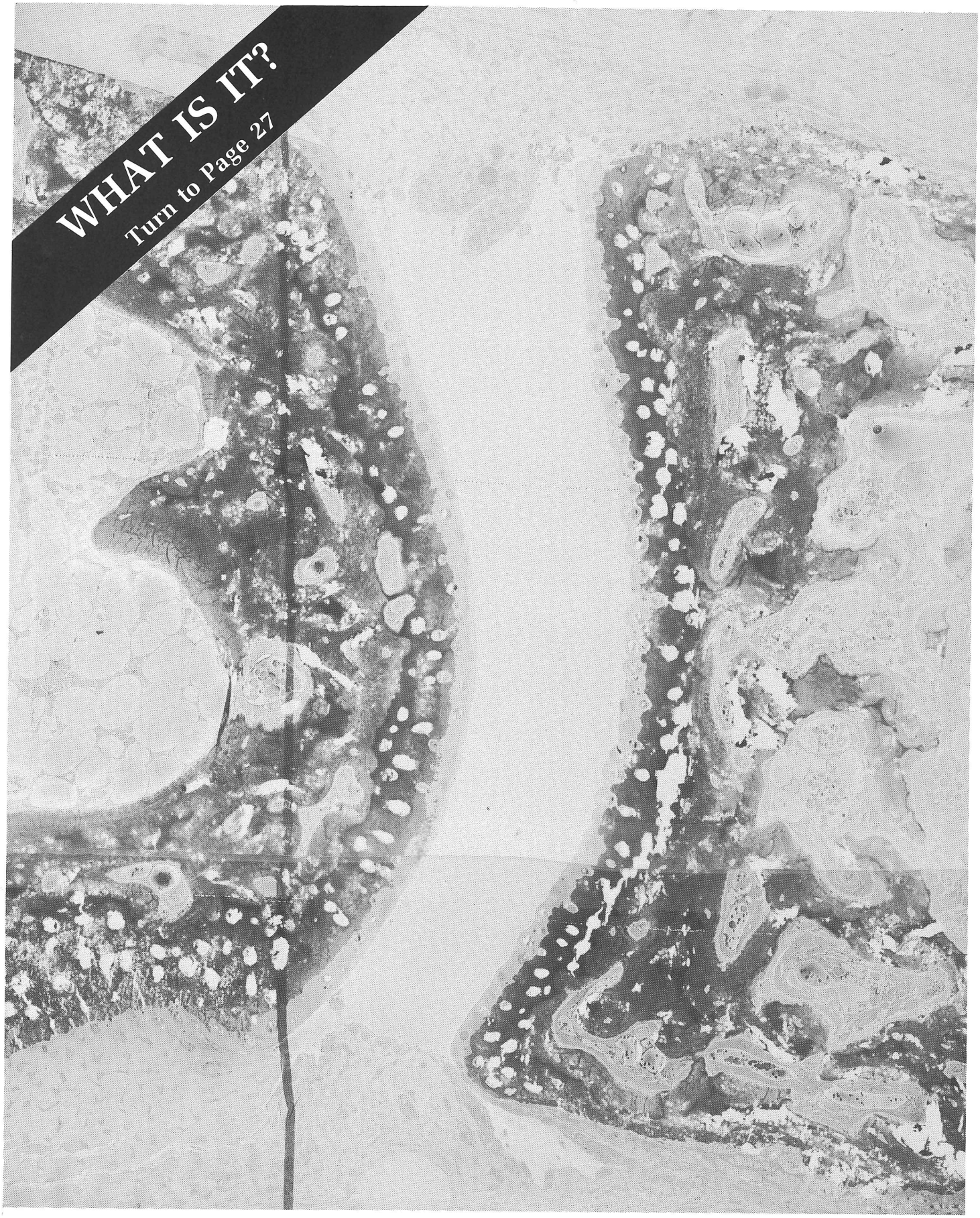
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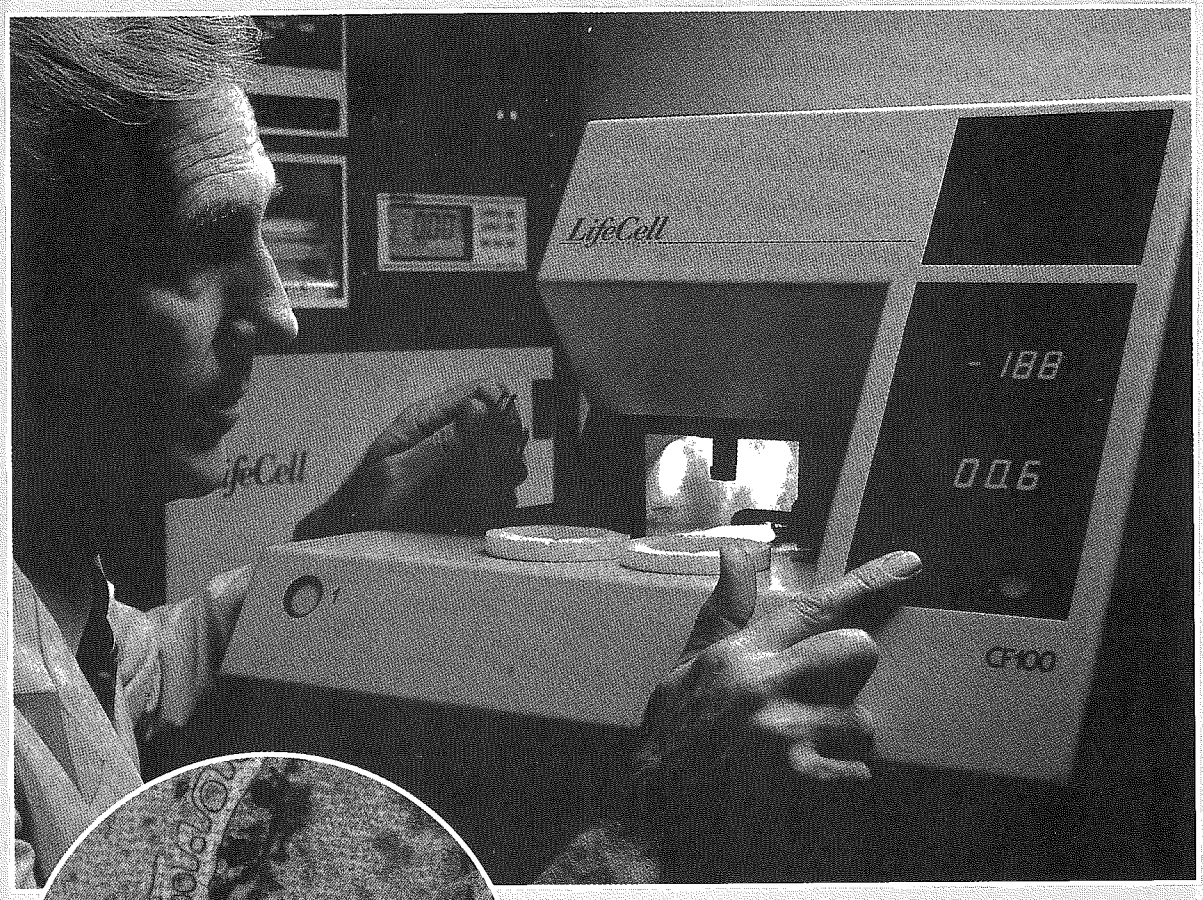

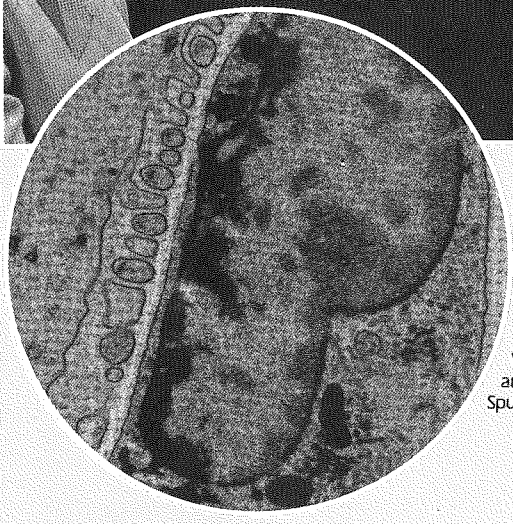

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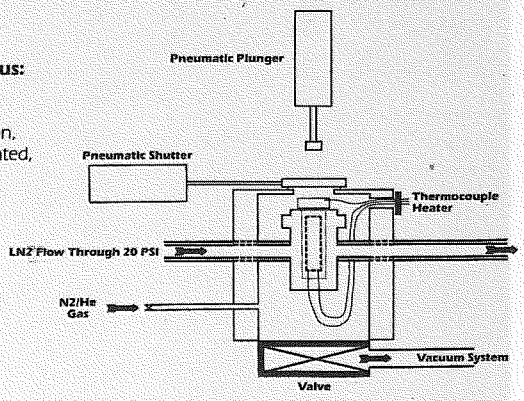

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COMPUTER-GENERATED BLOCK LABELS FOR TRANSMISSION ELECTRON MICROSCOPY

By

M. Steglich, M.S.

Department of Pathology
Box 85
The University of Texas
M.D. Anderson Hospital and Tumor Institute
1515 Holcombe Blvd.
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(713) 792-3310

INTRODUCTION

The labeling of tissue blocks is a critical step in the processing of tissue for transmission electron microscopy. In this paper, a method is described for preparing block labels rapidly and reliably with the use of a computer program written in BASIC.

In the Pathology EM laboratory at the University of Texas System Cancer Center, specimens submitted for electron microscopy are given sequential numbers that are cross-referenced in a computer database with the pathology department's accession number and the patient's hospital number. For each specimen, a minimum of five pieces of tissue are embedded in BEEM capsules, and a reserve supply is embedded in a single disk. In the case of solid human tumors, the EM specimen has been visually selected by a pathologist, and it is therefore likely that at least some of the blocks will be representative and of suitable quality for ultrastructural evaluation. In the case of needle biopsies, it is often necessary to section a larger number of blocks before one containing tumor cells is found, and our practice is to embed all the tissue from aspirates and the entire core of a cutting needle biopsy. Entire endomyocardial biopsies are also embedded in capsules.

Individual tissue blocks must be legibly labeled, and it is time-consuming and often unreliable to print them by hand using a pencil. Until recently, our practice was to type the labels with a IBM Selectric III typewriter equipped with a correctable film ribbon. The labels were consistently readable, but their preparation required a moderate amount of time, and typographic errors were not uncommon. I have therefore devised a computer program to eliminate these disadvantages. It is simple to use, and can be run by a technician who is not familiar with details of computer operation, by following a few simple steps.

The program allows the preparation of labels for an unlimited number of specimens commencing with any selected specimen or experiment number. Each can be subdivided, if desired, into as many as 26 subgroups. The subgroups can in turn have an unlimited number of labels, but all the subgroups will have the same number of labels. The labels are printed on standard typewriter paper. They are cut out, and in our laboratory, the first label consisting only of the specimen number, is placed in a disk with the reserve supply of tissue. The remaining labels, which are consecutively numbered, are trimmed and placed inside the BEEM capsules.

The program is written in the BASIC programming language for an IBM PC-AT computer coupled to a Quietwriter printer. To copy the program onto your computer the BASIC software must be on the program disk (either hard disk or floppy). Load BASIC (or BASICA if you are using advanced BASIC), and when the prompt appears, type in the program as written. Finish by typing SAVE "EMNUMBERS" (or whatever name you select for the program). The program will be stored on the disk with your BASIC files and can be accessed with the following commands:

1. BASIC (or BASICA if you use advanced BASIC)
2. Load "EMNUMBERS"
3. Run "EMNUMBERS"
4. Follow program prompts

PROGRAM

```
10  LPRINT CHR$(27);CHR$(73);CHR$(1);
20  PRINT "ENTER FIRST EM NUMBER": INPUT E
    : PRINT E "Correct ? (Y/N)"
30  G$ = INPUT$(1)
40  IF G$ <> "Y" THEN IF G$ <> "y" THEN
    PRINT CHR$(7):GOTO 20
50  PRINT "ENTER LAST EM NUMBER" : INPUT L
    : PRINT L "Correct ? (Y/N)"
60  H$ = INPUT$(1)
```

```
70  IF H$ <> "Y" THEN IF H$ <> "y" THEN
    PRINT CHR$(7):GOTO 50
80  PRINT : PRINT "EM NUMBER" E
90  PRINT "ENTER NUMBER OF SPECIMENS FOR
    THIS NUMBER (1 = A,2 = B,3 = C,4 = D,5 = E . . .)"
    : INPUT S : PRINT
100 IF S = 0 THEN GOTO 90
110 IF S>26 THEN PRINT "I AM NOT
    PROGRAMMED TO PRINT MORE THAN 26
    SPECIMENS"
120 IF S>26 THEN PRINT CHR$(7)
130 PRINT
140 IF S>26 THEN GOTO 90
150 PRINT "ENTER THE NUMBER OF BLOCKS" :
    INPUT B : PRINT
160 IF B = 0 THEN GOTO 150
170 IF S>1 THEN GOTO 240
180 LET N = 0: LPRINT E : GOTO 210
190 LPRINT E CHR$(8) "-" CHR$(8) N
200 IF N = B THEN GOTO 220
210 LET N = N + 1 : GOTO 190
220 IF E = L THEN GOTO 320
230 LET E = E + 1: PRINT "NEXT EM NUMBER" E :
    GOTO 90
240 Z = 0
245 LET Z = Z + 1
250 LET X = 65
260 LET N = 0: LPRINT E CHR$(X) : GOTO 290
270 LPRINT E CHR$(X) "-" CHR$(8) N
280 IF N = B THEN GOTO 300
290 LET N = N + 1: GOTO 270
300 IF S = Z THEN GOTO 220
310 LET Z = Z + 1 : LET X = X + 1 : GOTO 260
320 PRINT "DO YOU WANT TO MAKE ANY MORE
    LABELS ? (Y/N)"
330 Z$ = INPUT$(1)
340 IF Z$ <> "Y" THEN IF Z$ <> "y" GOTO
    360
350 GOTO 20
360 LPRINT CHR$(27); CHR$(73); CHR$(0);
    CHR$(12);
370 REM THE FOLLOWING PRINTER CONTROL
    CODES ARE FOR THE IBM QUIETWRITER
380 REM LINE 10 - CHR$(27); CHR$(73); CHR$(1); -
    CHANGE FONT TO HOLDER *
390 REM LINE 360 - CHR$(27); CHR$(73); CHR$(0);
    - CHANGE TO TO HOLDER A
400 REM LINES 40, 70, AND 120 - CHR$(7); - BELL
410 REM LINES 190, 260 AND 270 - CHR$(8); -
    BACKSPACE
420 REM LINE 360 - CHR$(12); - FORM FEED
430 SYSTEM
```

MODIFYING THE PROGRAM

Lines 370 through 420 are REM statements. They have no function other than to serve as reminder statements and have no effect on running the program. If using a printer other than the Quietwriter, refer to these statements to determine what Quietwriter printer code was used in which line for what purpose (i.e. line 380 tells you that in line 10 of the program, the printer code to change to font holder * is "CHR$(27); CHR$(73); CHR$(1);"). Every printer manual will have a listing of all the control codes for the printer. Check all of the REM statements to determine if the printer codes in the referenced line of the program need to be modified for the printer which will be used.

This program was written to conform with our standard procedure of having a Courier 10 PC font typestyle in holder "A" (which is the standard holder) of our Quietwriter and a Prestige 15 PC font typestyle in holder "*" (which is the alternate holder). The program is written to print the numbers with the font typestyle in holder "*". To print with the font typestyle in holder "A", delete line 10 and change line 360 to read "LPRINT CHR$(12);".

If using a printer other than the Quietwriter, such as the Proprinter, lines 10 and 360 will need to modified. To print with the standard print of the Proprinter, delete line 10 and "CHR$(27); CHR$(73); CHR$(0);" in line 360. To have the Proprinter print in Condensed Print, look up the printer codes in the Proprint manual. You will find that the printer code for Condensed Print is "LPRINT CHR$(15)". This will replace "LPRINT CHR$(27); CHR$(73); CHR$(1);" in line 10. Likewise, to return the printer to its normal printing mode, find the printer code to cancel the Condensed Print mode. The printer code for canceling the Condensed Print mode is "LPRINT CHR$(18)". This will replace "LPRINT CHR$(27); CHR$(73); CHR$(0);" in line 360. Any other changes for the printer will be done in a similar manner. If a prefix is needed in your EM number, then the following lines will need to be added or changed:

```
15  PRINT "ENTER PREFIX" : INPUT P$
230 LET E = E + 1: PRINT "NEXT EM NUMBER"E
231 PRINT "DO YOU WANT THE SAME PREFIX
    FOR" E"? (Y/N)"
232 J$ = INPUT$(1)
233 IF J$ <> "N" THEN IF J$ <> "n" THEN
    PRINT P$ E : GOTO 90
```

```
235  PRINT "ENTER NEW PREFIX"
237  INPUT P$ : GOTO 90
260  LET N = 0: LPRINT P$ CHR$(8)E CHR$(X) :
     GOTO 290
270  LPRINT P$ CHR$(8)E CHR$(X) "-" CHR$(8) N
```

This will enable any combination of characters to be used as a prefix for the EM number.

Acknowledgment

The author would like to thank Madonna M. Yancey for typing this manuscript.

```
26798
26798-1
26798-2
26798-3
26798-4
26798-5
26799A
26799A-1
26799A-2
26799A-3
26799A-4
26799A-5
26799B
26799B-1
26799B-2
26799B-3
26799B-4
26799B-5
```

FIGURE 1: EM numbers from the program for the IBM PC-AT and the Quietwriter Printer, using a Prestige 15 PC font typestyle.

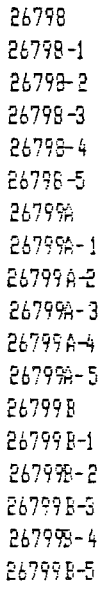

FIGURE 2: EM numbers from the program modified for the IBM PC-AT and the Proprinter using condensed print.

```
EM88-26798
EM88-26798-1
EM88-26798-2
EM88-26798-3
EM88-26798-4
EM88-26798-5
EM88-26799A
EM88-26799A-1
EM88-26799A-2
EM88-26799A-3
EM88-26799A-4
EM88-26799A-5
EM88-26799B
EM88-26799B-1
EM88-26799B-2
EM88-26799B-3
EM88-26799B-4
EM88-26799B-5
```

FIGURE 3: EM numbers from the program for the IBM PC-AT and Quietwriter printer modified for a prefix.

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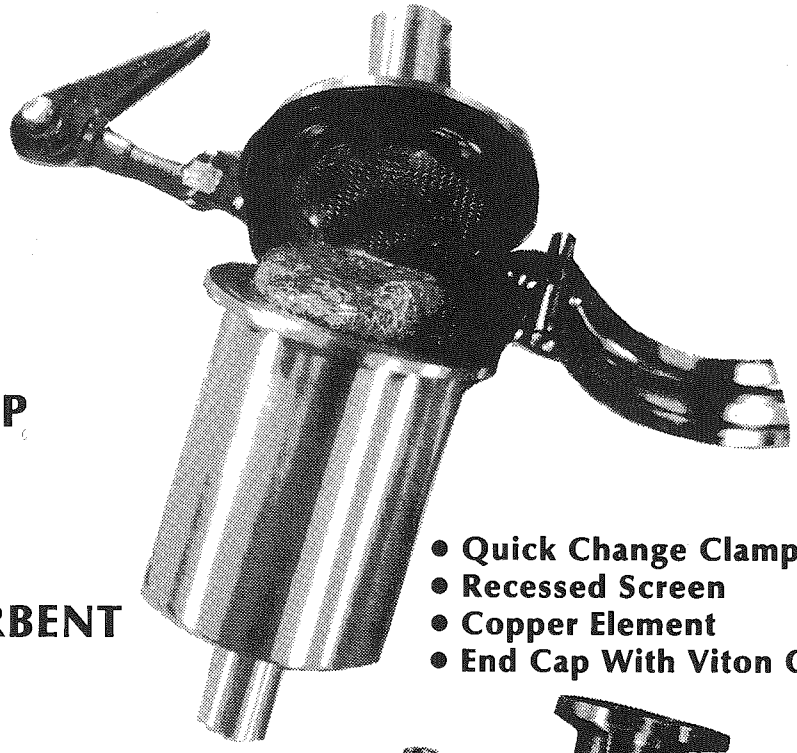

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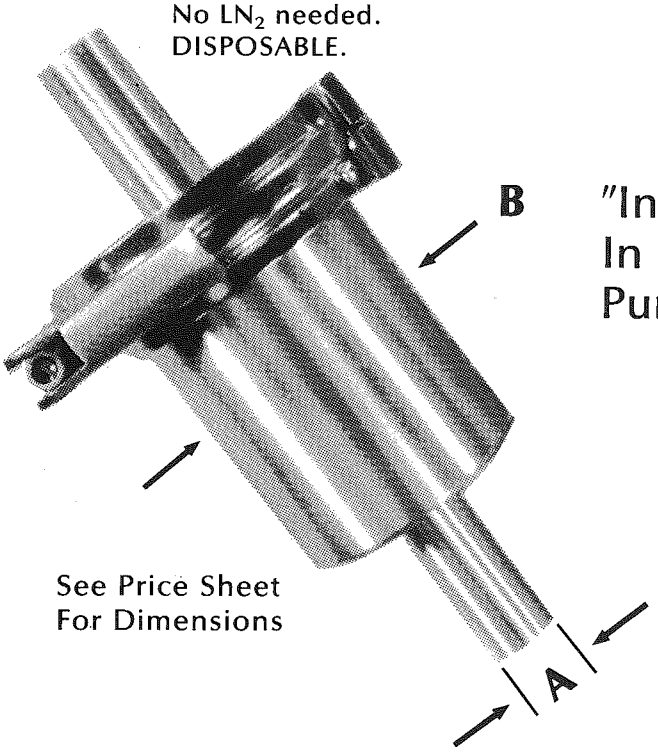

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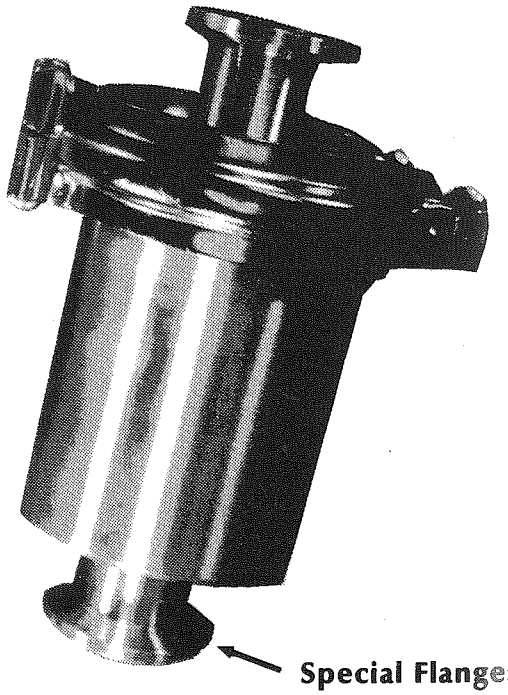

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IN SHORT

CRITICAL POINT DRYING

By

David Cowell

WHY DRY?

The advent of Scanning Electron Microscopy (SEM) in the study of surface morphology in biological applications made it imperative that the surface detail of specimen was preserved. Air (evaporation) drying of specimens can cause deformation and collapse of structures. The primary cause of such damage being the effects of surface tension. The specimen is subjected to forces which are present at the phase boundary as the liquid passes into a gas phase and evaporates. The most common specimen medium, water has a high surface tension, while that for acetone is several times lower. The surface tension could be reduced by substitution of a liquid with a lower surface tension, with expectations of reduced damage during drying.

WHAT IS A CRITICAL POINT?

Initial experiments were conducted, using CO_2 to find what volume changes occured to a fixed mass of gas, when the temperature was held constant and the pressure varied. This was done for a series of fixed temperatures. The results are best understood by considering the graph obtained from plotting pressure (P) against volume (V) for the series (Fig. 1); the curves obtained are termed isothermals.

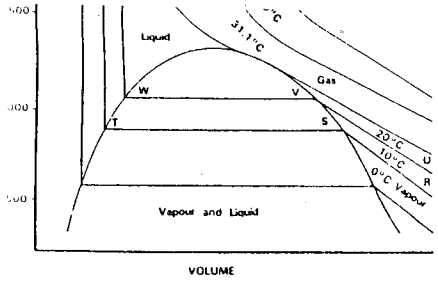

FIGURE 1: Isothermals for CO_2.

(wet specimen)
H_2O
↓
Acetone 30%*
↓
100%
↓
CO_2**
↓
CPD
(dry specimen)

* 50/60/70/80/90 Typically 10 min each.
** Flush typically 3 times.

In the simplest terms, the graph can be interpreted in this way: There are six isothermal plots. Three show very vertical and horizontal portions (0°, 10°, 20° C), and three are more regular curves (31.1°, 40°, 50° C). The vertical portions for the three lower temperatures demonstrate no volume change with increasing pressure and signify an incompressible liquid. The horizontal portions show an abrupt change from the liquid state (vertical line) to the down sloping gaseous state. These three graphs are essentially the same except that the specific transition points are different due to variations in temperature. Effectively, what this demonstrates is that within this temperature range the gas is compressible and will abruptly change from the liquid to the gas state at a certain pressure. In terms of specimen preparation, this can result in drying artifacts.

The three curved plots for the higher temperatures do not show horizontal portions and therefore do not possess an abrupt phase change. The temperature at which this begins to happen is called the Critical Temperature. This critical temperature has an associated critical pressure and density and for any particular mass of gas, a critical volume. In terms of specimen preparation, what this means is that if we heat the liquid (CO_2) in a closed system it will reach a point where there is no difference between the liquid and vapor phases. Furthermore, the graph shows that if the liquid is kept at or above the critical temperature, it will be in and remain in the vapor state (it can not form a liquid at the critical temperature) until the pressure is reduced to zero.

It is apparent that we can utilize the critical point phenomenon as a drying technique, as it achieves a phase change from liquid to dry gas without the effects of surface tension and is therefore suitable for delicate biological specimens.

CO_2 remains the most common, but not the only, medium for which to apply the CPD procedure, and is termed the "transitional fluid". It is not miscible with water and we have to replace the water in the specimen with another fluid which is compatible with CO_2. This is ideally the intermediate fluid which can also be used as a dehydrating fluid. These two descriptions are sometimes interchanged depending on the portion of the procedure being discussed. Prior to any of these stages the specimen is typically preserved with aldehyde and osmium fixatives.

The specimen is usually processed through varying concentrations of dehydration fluid, culminating in complete replacement of the water with the

intermediate fluid. Because it has a low surface tension the specimen is less likely to experience damage due to evaporation while quickly transferring it to the CPD chamber. Being miscible with CO_2, the intermediate fluid ensures satisfactory conditions for exchange with the transition fluid. This is done by flushing or purging the CPD chamber with several changes of CO_2.

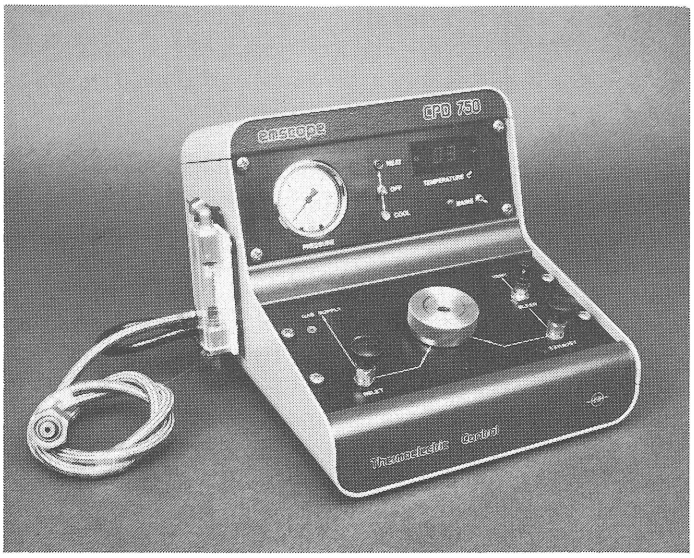

FIGURE 2: A Critical Point Dryer.

"TRUE" CRITICAL POINT

Ideally, we wish to have a situation where the liquid fills the specimen chamber while still only accounting for approximately 50% of the total volume available. This is to ensure that the specimens are not uncovered during initial flushing stages, and in addition, this should enable the Critical Constants of temperature, pressure, and density to be achieved relatively simultaneously without the occurrence of either excessive pressure or evaporation.

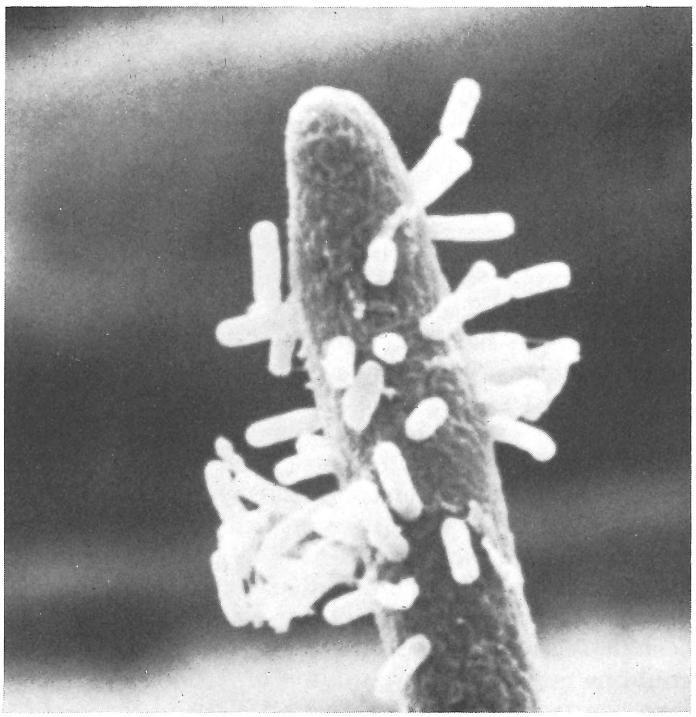

FIGURE 3: Scanning electron micrograph of critically point dried specimen, showing bacteria adhering to the top of a fungus *(Erysiphe graminis)* which itself was infecting a barley leaf. (Magnification x8000).

ACKNOWLEDGEMENTS

Fig. 1 and 2 — Emscope Labs Ltd - Ashford.
Fig. 3 — Dr. Alan Beckett, University of Bristol.

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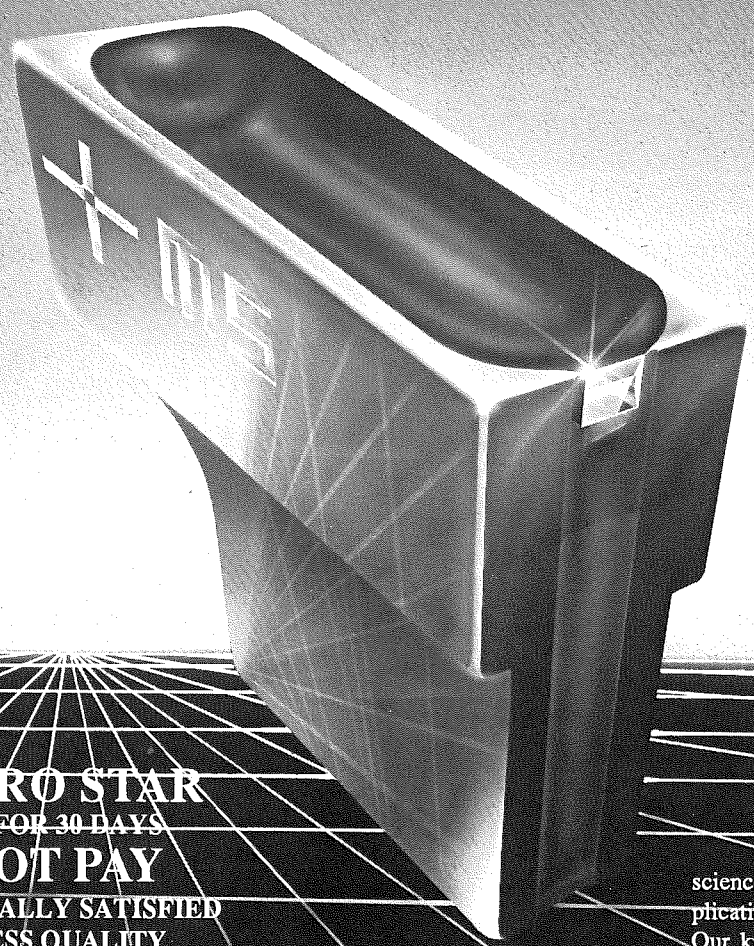

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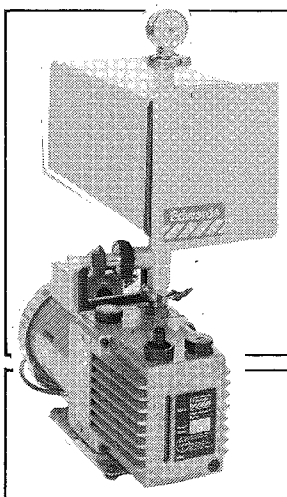

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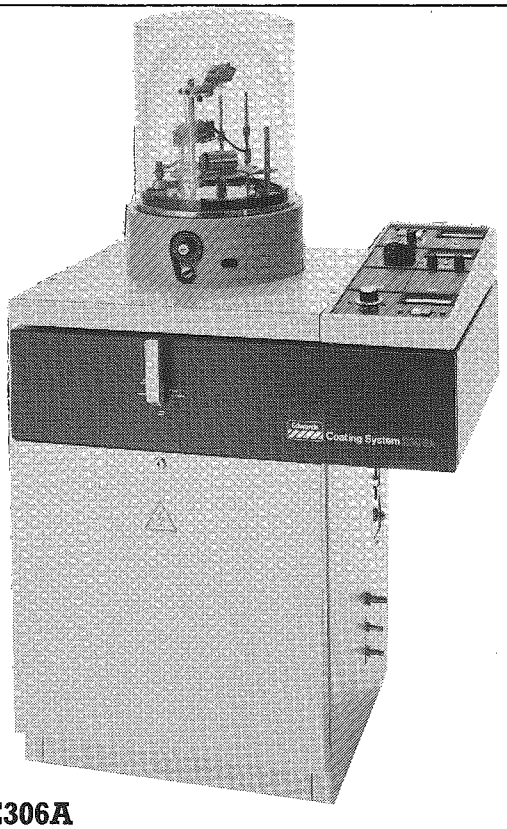

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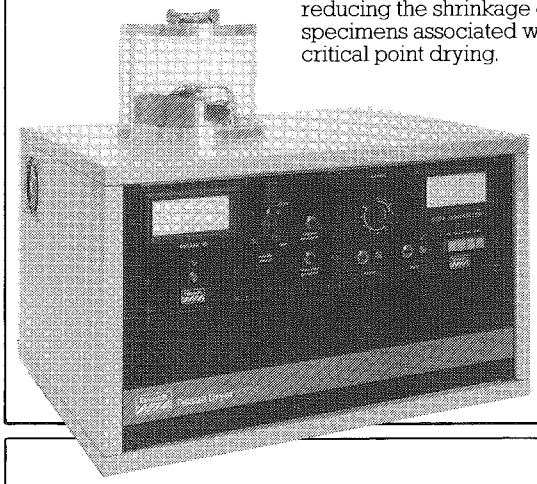

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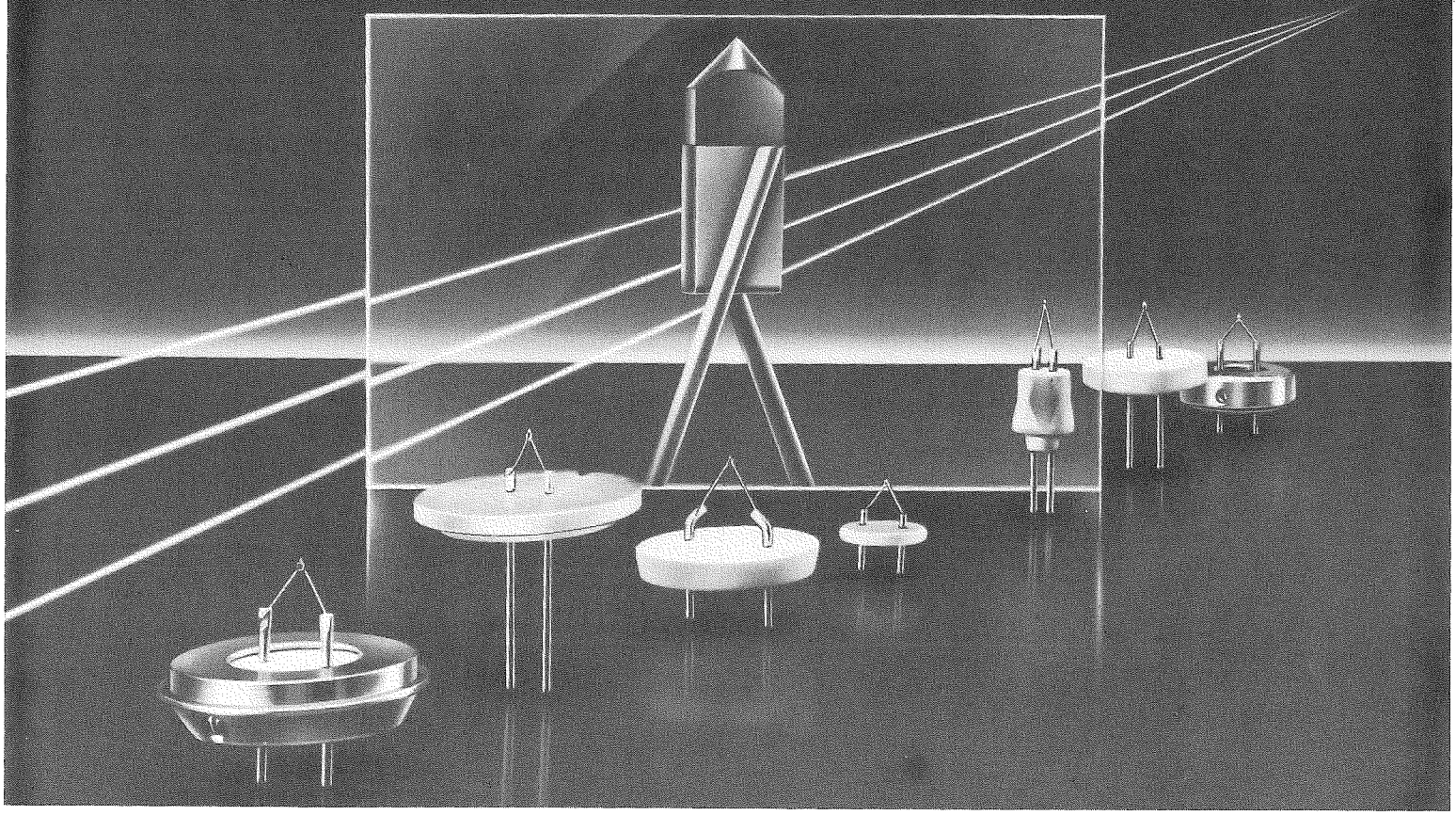

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Abstracts

BIOLOGICAL SCIENCES

PLATFORM PRESENTATION — FALL 1988

THE EFFECTS OF ISOMETAMIDIUM ON ERYTHROCYTES AND ERYTHROCYTE CARRIER CELLS. E.G. MOORE, J.R. DeLOACH, USDA, ARS, Veterinary Toxicology and Entomology Research Laboratory, P.O. Drawer GE, College Station TX 77841 and G.G. WAGNER, Department of Veterinary Microbiology and Parasitology, Texas A&M University, College Station TX 77843.

The drug, isometamidium, is the most widely used anti-trypanosomal agent against African trypanosomiasis. However, the drug causes severe toxic side effects. The drug could possibly be used more efficaciously without toxic side effects in cattle if it can be administered in an encapsulated form such as in carrier erythrocytes. Thus, we have determined the extent of interaction of isometamidium with bovine cells. Bovine erythrocyte exposure to the drug caused decreased osmotic fragility and morphological changes. Normal bovine erythrocytes tolerated up to 1 mg/ml drug with no effects. Carrier bovine erythrocytes were highly susceptible to the drug with increased osmotic fragility and decreased encapsulation potential. Scanning electron micrographs of carrier erythrocytes exposed to the drug revealed the formation of enkephalocytes and other abnormal morphology. Control bovine erythrocytes showed greater tolerance of the drug. Apparently, access of the drug to the interior of the bovine erythrocyte membrane allows the drug to be more interactive with the membrane.

ELECTRON MICROSCOPY OF SINDBIS VIRUS INFECTION IN VERTEBRATE AND INVERTEBRATE CELL CULTURE. M.L. Miller & D.T. Brown, Cell Research Institute & Dept. Microbiology, University of Texas, Austin, TX 78713.

Sindbis virus is cycled in nature through insect and animal hosts. Infection in either cell type in laboratory culture yields equivalent progeny virus. Host protein and RNA synthesis shuts down in infected vertebrate cells within 3 hr. post-infection (PI) with mortality occurring within 24 hr. Host functions in infected invertebrate cells do not shut down; these cells become persistently infected with no change in growth rate or metabolism. To correlate these differences with ultrastructural changes, monolayers of hamster kidney (BHK) and mosquito cells were infected with Sindbis virus and processed for electron microscopy at designated time points. Virus is first seen budding from the plasma membrane of BHK cells at 4 hr. PI and cytopathic vacuoles appear by 6 hr. PI. These structures become more numerous as larger amounts of virus can be observed budding from the cell surface. By 15 hr. PI, cells show obvious cytopathic effect as they begin to die. Breakdown of the nucleus and cytoplasm is apparent and most cells have disintegrated by 36 hr. PI. In contrast, some budding of virus from the plasma membrane of mosquito cells is first visible at 6 hr. PI. Virions begin to accumulate in vacuoles present in these cells at 10 to 12 hr. PI and virus-packed vesicles are found by 20 to 25 hr. PI. Release of virus from the cell appears to result through exocytosis of these vesicles. These observations suggest that this type of compartmentalization of virus multiplication in insect cells protects them against the cytopathic effects observed in animal cells.

INTERVERTEBRAL DISK MINERALIZATION IN THE PROGRESSIVE ANKYLOSIS MOUSE: AN ULTRASTRUCTURE STUDY. H. Wayne Sampson, Department of Anatomy, Texas A&M University College of Medicine, College Station, TX 77843

Intervertebral disks taken from progressive ankylosis mice from 4 to 18 weeks of age were studied by techniques using KOH digestion and alizarin red staining, as well as light microscopic, electron microscopic, and electron energy dispersive x-ray microanalysis techniques.

Gross morphological KOH-alizarin red studies reveal what appears to be a progression from syndesmophyte formation through joint bridging to total fusion. The syndesmophyte formation and bridging appears to encircle the disk, but ankylosis of the vertebral body seems to be localized to the anterior surface of the disk. Light microscopic techniques of 4 week animals demonstrate the presence of small, irregular, eosinophilic, acellular foci of necrosis in the fibrocartilage disks that stain positive for calcium with alizarin red stain and Von Kossa. This is followed at 6 weeks by a proliferation of hyaline cartilage at the periphery at the end plate; the cartilage spans the disk and becomes necrotic. The adjacent vertebrae lay down new bone on their ventral surfaces which occasionally advances across, but does not completely span the intervertebral disk. Electron microscopic techniques reveal the necrotic foci seen in light microscope studies to be massive accumulations of mineral deposits within the extracellula matrix. Chondrocytes of older animals demonstrated postmortem changes and contained numerous large vacuoles. These chondrocyte changes are probably due to loss of nutrients resulting from the increased extracellular mineral deposition.

ULTRASTRUCTURAL AND IMMUNOHISTOCHEMICAL EVIDENCE FOR TRANSCAPILLARY MOVEMENT OF SUPEROXIDE DISMUTASE AND CATALASE INTO MYOCYTES. L.L. Chudej, N. Christodoulides, J.R. Koke, and *N. Bittar, Southwest Texas State Univ., San Marcos, and *Univ. of Wisconsin, Madison.

Exogenous superoxide dismutase (SOD) and catalase (CAT) can reduce reperfusion injury. We infused SOD, CAT, and SOD+CAT into the coronary circulation of dog hearts (in situ) and rat hearts (Langendorff perfused) prior to and during a 15 minute episode of ischemia. Reperfusion (0 to 120 minutes) was followed by intracoronary fixation. Cryotome sections of hearts were exposed to rabbit antibody prepared against the exogenous SOD and CAT added to the coronary circulation. Bound rabbit antibody was detected by FITC conjugated goat-antirabbit IgE antibody. Using TEM, we observed concentrations of electron dense material in vesicles of capillary endothelia, between capillaries and myocytes, and in vesicles within myocytes. As this material was only observed in heart treated with SOD and/or CAT, and since these enzymes contain metal and would appear electron dense if sufficiently concentrated, these results suggest concentration and transport of CAT and SOD occurred by vesicular transport across the capillary endothelium and into the myocyte. This interpretation was supported by immunohistochemistry. The interior of myocytes from rat hearts which had been exposed to SOD and CAT in the coronary circulation showed a strong reaction to antibodies prepared against these enzymes, whereas myocytes from rat hearts not exposed to exogenous SOD and CAT only bound the CAT antibodies. This indicates the anti-SOD we prepared is specific for the exogenous SOD, and also indicates exogenous SOD is transported efficiently from the coronary circulation into the cytoplasm of myocytes. Gold conjugated antibodies will be used to locate exogenous SOD at the ultrastructural level.

DEMONSTRATION OF POLYMORPHONUCLEAR LEUKOCYTE (PMN) GRANULE HETEROGENEITY USING IMMUNOLOCALIZATION AND ENERGY DISPERSIVE SPECTROSCOPY (EDS). E.S. BUESCHER, S.A. LIVESEY, J.G. LINNER, S.M. McILHERAN, D.S. HARRISON, G. KRANNIG. Cryobiology Research Center, University of Texas Health Science Center at Houston; Houston, TX 77030

As the acute inflammatory cell of the body, the PMN plays crucial roles in defense against infection and in acute inflammation. In each role, the PMN utilizes both lysosomal (azurophil) and secretory (specific) granules, the content and composition of which are heterogeneous. Using colloidal gold immunolocalization and EDS of cryofixed and molecular distillation dried PMN/PMN fractions embedded in either osmicated Spurr's resin or Lowicryl K4M, we have begun to examine the content and composition of PMN granules. Using whole PMN embedded in Spurr's resin, lactoferrin (Lf) and CD15 antigens were immunolocalized to subpopulations of intracellular granules using rabbit anti-Lf and a murine monoclonal IgM directed against 3-fucosyllactose respectively. Double labeling experiments showed that multiple types of PMN granules exist. Using sucrose density gradient purified granule fractions embedded in Lowicryl, at least 8 different elemental patterns were noted by EDS. Five of the 8 patterns were also seen in an azurophil granule fraction purified on PVP-silica; 3 of the 8 patterns were seen in a specific granule fraction purified by the same method; 4 of the 8 patterns were seen in PMN cell fragments produced by sonication. These results confirm that human PMN granule heterogeneity exists and is extensive. They show a specific granule location for CD15 antigen and that EDS analysis is a useful method for granule categorization. They suggest that combining immunolocalization and EDS may allow further definition of both PMN granule heterogeneity and the function of the individual granule types.

ULTRASTRUCTURAL LOCALIZATION OF INTERNALIZED HYALURONIC ACID IN CULTURED RAT LIVER ENDOTHELIAL CELLS USING A NOVEL STREPTAVIDIN GOLD PROBE by M.D. CHRISTENSEN, C.T. McGARY AND P.H. WEIGEL, Department of Human Biological Chemistry and Genetics, Univ. of Texas Medical Branch, Galveston, TX 77550

We have previously measured the specific receptor mediated endocytosis of hyaluronic acid (HA) in liver endothelial cells (LEC) using ^{125}I-HA. The aim of this study was to extend the biochemical results by the visualization of endocytosed HA at the EM level. Two approaches were taken. First, HA was adsorbed on colloidal gold particles by the same method used to adsorb protein. This has not been reported for charged polysaccharides. Surprisingly, this approach appeared to work. In the second approach, a unique amine-containing derivative of HA was biotinylated. The biotin-HA was then incubated with 5 nm streptavidin (SA) gold for 2 hr at 4°C. This complex was then quenched by addition of 1 mM biotin. The sample was centrifuged twice to remove unbound HA and free biotin. LEC were incubated with the HA-biotin-SA-gold for 20 min at 37°C, washed with PBS and fixed with 2% glutaraldehyde for 16 hr at 4°C. A competition control with excess free HA was conducted to assess the nonspecific uptake of the gold complex. A further control for the second probe was to use SA-gold saturated with biotin. The HA-gold showed a 50% specific internalization. The HA-biotin-SA-gold showed ~70% specific uptake, which is in better agreement with the specific internalization of the ^{125}I-HA. We observed an essentially identical accumulation of both gold complexes in the lumen of intracellular compartments, in small vesicles, in larger vacuoles and in type III endosomes (multivesicular bodies). The high sensitivity and specificity of the HA-biotin-SA-gold probe will be useful for further studies of the endocytosis and metabolism of HA (supported by NIH grant GM 35978).

TWO STRUCTURAL STATES OF Z BANDS IN CARDIAC MUSCLE. M.A. GOLDSTEIN, L.H. MICHAEL, J.P. SCHROETER AND R.L. SASS, Section Cardiovascular Sciences, Dept. Medicine, Baylor College of Medicine, Houston TX 77030.

We have recently tested the hypothesis that the two structural states of the Z band lattice prevously shown in skeletal muscle also exist in cardiac muscle and are likewise related to the contractile state of the muscle. We have compared the form and dimensions of the Z band lattice in rat papillary muscle fixed at rest with and without EGTA using electron microscopy and optical diffraction. In unstimulated muscle, the Z band lattice form called basketweave predominated and the Z spacing (defined as the repeat distance of a tetragonal array of cross-cut thin filaments from the same sarcomere) was 23.93nm ± 0.37nm. Muscles exposed to EGTA exhibited the small square lattice form and the Z spacing was 20.50nm ± 0.19nm. The Z spacings in the two lattice forms were similar in cardiac and skeletal muscles such that the decrease in Z spacing in the transition from basketweave to small square in this study was similar to the increase in Z spacing previously demonstrated in skeletal muscle in the transition from small square to basketweave. The Z lattice form and dimensions in unstimulated cardiac muscle resembled those in tetanized skeletal muscle. These findings are consistent with the higher resting tension in cardiac muscle and suggest that Ca^{++} may be important for the maintenance of the expanded Z lattice form.

PRESERVATION OF THE Z BAND LATTICE IN SKELETAL MUSCLE BY A SLAM FREEZE-FREEZE SUBSTITUTION METHOD. D.L. MURPHY, R.J. EDWARDS, AND M.A. GOLDSTEIN, Section of Cardiovascular Sciences, Dept. Medicine, Baylor College of Medicine, Houston TX 77030.

In glutaraldehyde-fixed skeletal muscle, the Z band is a tetragonal array of 10nm axial filaments connected by Z filaments. We have examined Z bands and A bands in muscle prepared by the slam-freeze - freeze-substitution method.

Each strip of soleus muscle 1 x 3mm was mounted for slam-freezing against a pre-cooled copper block. Muscles were transferred to an osmium-acetone slurry maintained at liquid N_2 temperature. Freeze substitution was carried out at -80°C for 24hrs. Muscles were allowed to warm to room temperature, dehydrated in ethanol, and embedded in LX112 resin. The best examples from 3 experiments show a gradation in preservation from surface to interior. Microcrystal ice damage appears 15-20um from cell surface. Subtle changes in the Z band lattice are sometimes observed at 10um or less from the cell surface when all other structures look well preserved. These changes are distinguished from orientation effects on the lattice appearance by examination of cross section, longitudinal sections and tilted specimens. Important factors in optimal preservation are 1) caution in tissue handling; 2) timing of tissue removal with copper block cooling; 3) blotting of excess liquid from mounted muscle; 4) prolonged times in osmium slurry at -80°C; 5) nature of tissue surface such as collagen content. Repeatability is still a major concern but regions immediately subjacent to the sarcolemma are consistent in form and dimension with glutaraldehyde fixed material and living muscle.

ULTRASTRUCTURAL FEATURES OF UNCOMMON LUNG TUMORS. Bruce Mackay, Department of Pathology, The University of Texas M.D. Anderson Cancer Center, Houston.

The great majority of primary tumors of the lung are carcinomas, but occasionally neoplasms with an unusual appearance by light and electron microscopy are encountered. Study of the fine structure of the cells provides insight into their nature but does not always determine the histogenesis of the tumor. A sclerosing hemangioma is not, as its name implies, a vascular tumor, whereas the so-called intravascular bronchiolo-alveolar tumor does appear to be an endothelial cell neoplasm. The cell type of one peculiar tumor of vacuolated cells that we have studied is still an enigma. Primary soft tissue tumors of the lung are uncommon and must be distinguished from sarcomatoid carcinomas or carcinosarcomas. Mesothelium should be considered when seeking the cell of origin of rare lung neoplasms since sub-mesothelial mesenchymal cells are capable of differentiating to form epithelial or spindle cells or both. The possibility of a metastasis must also kept in mind when assessing an unusual lung tumor: a rare malignant glomus tumor presenting as a coin lesion is an example.

KAPOSI'S SARCOMA. Mannie Steglich, Nelson G. Ordonez, Bruce Mackay, Department of Pathology, The University of Texas M.D. Anderson Cancer Center, Houston.

Moritz Kaposi first described this lesion in 1872 as multiple idiopathic pigmented sarcoma of the skin. It has received considerable attention in recent years because of the frequency with which it occurs in patients with the acquired immunodeficiency syndrome (AIDS), and it is recognized that lymph node and visceral involvement is common in these patients. We have studied 20 cases of Kaposi's sarcoma with the electron microscope. The material was obtained from biopsies of cutaneous lesions, and the light microscopy showed the typical spectrum seen in early to late involvement, with a variable admixture of spindle cells and vascular channels. The spindle cells have mesenchymal features at the ultrastructural level, and resemble primitive fibroblasts, but they display a tendency to phagocytose erythrocytes. The spindle cells aggregate and form crudely constructed vessels with poorly developed cell junctions and fragmented basal lamina. Gaps between the cells are common, and erythrocytes escape through these spaces into the surrounding stroma. The endothelial potential of the spindle cells is confirmed by immunocytochemical studies which show positive staining with endothelial cell markers.

EFFECTS OF MONENSIN ON THE CONVERGENCE OF ENDOCYTIC AND LYSOSOMAL PATHWAYS IN SOYBEAN PROTOPLASTS. R.D. RECORD and L.R. GRIFFING, Dept. of Biology, Texas A&M University, College Station, TX 77843

Convergence of the endocytic and lysosomal pathways is revealed ultrastructurally by collocalization of cationized ferritin (CF, an electron dense marker) and the histo-chemical reaction product of acid phosphatases (AcPase) in soybean protoplasts (from suspension culture SB1, Glycine max (L.) Merr.). Organelles found to be double-labelled include the Golgi complex and multivesicular bodies (MVB). Monensin does not inhibit initial CF uptake and subsequent delivery to the MVB. However, following long-term (3h) treatment in monensin, AcPase reaction product is no longer found in MVB. Monensin also causes swollen cisternae and/or vesicles associated with the Golgi complex. Monensin may inhibit transport of active AcPase from the Golgi to the MVB without inhibiting the transport of CF from the plasma membrane to the MVB. These observations support the hypothesis that the Golgi complex mediates the transport of lytic enzymes but not endocytic markers to the MVB in plants. Supported by NSF Grant DMB 86-07788.

SILICON-CONTAINING SPHERES ON THE ENDO- AND EXCOPERIDIUM OF GEASTRUM. H. J. Arnott and K. D. Whitney, Dept. Biology, University of Texas at Arlington, Arlington, TX 76019-0498.

Examination of the surfaces of the endo- and exoperidium of Geastrum pectinatum Pers. (University of California-Berkeley, Herbarium specimen no. 976146) revealed numerous spherical bodies. These spheres range in diameter from one to over 20 µm. They were found on the surface, in indentations, or partly imbedded in the peridial surfaces. The exoperidial surface is relatively flat and is composed of a mat of elongated hyphae which may partly cover the spheres. On the endoperidial surface the hyphae are less clearly defined and the surface is rougher with many shallow depressions. Spheres are often found in these depressions where they are closely associated with calcium oxalate crystals which are abundant on the surface. Examination of the spheres with energy dispersive x-ray analysis revealed, in addition to the background, only one major component, namely, silicon. Similar examination of the calcium oxalate crystals showed only calcium. When isolated spheres were examined with light microscopy they appeared as transparent round bodies. Careful manipulation of the diaphram and/or the condenser shows that the spheres act like small lenses. At present, the exact chemical nature and origin of these spheres are not determined.

ELECTRON MICROSCOPY OF THE LIPID-RICH CORE REGION OF HUMAN ATHEROSCLEROTIC PLAQUES, UTILIZING OSMIUM-TANNIC ACID-PARAPHENYLENEDIAMINE (OTAP) AND OSMIUM-THIOCARBOHYDRAZIDE-OSMIUM (OTO) TECHNIQUES.
Keith F. Klemp and John R. Guyton, Depts of Medicine and Cell Biology, Baylor College of Medicine, Houston, TX 77030

Intense extracellular lipid deposition is associated with cell necrosis in the core region of atherosclerotic plaques. Routine EM fails to distinguish extracellular lipid droplets from vesicles, due to extraction of lipid, and thus ultrastructure of core region has remained unknown. Tannic acid acts as a mordant in osmicated tissue to improve greatly the visualization of membranes. Retention and visualization of neutral lipid is obtained by applying 1% para-phenylenediamine in 70% ethanol to the same tissue, or by using the OTO sequence. OTAP is preferred, as it clearly distinguishes neutral lipid droplets from vesicles with aqueous centers. Small lipid droplets (30 to 400 nm profile diameters) and vesicles occupied more than 90% of area occupied by lipid-rich structures in the core region. Cholesterol crystals, lipid droplets of size similar to those in foam cells (0.4 to 6 u), and larger neutral lipid deposits (>6 u) occupied less than 10% of this area. Cells and cellular lipid were absent. Abundant vesicles were associated with the nearby presence of cholesterol crystals, while small lipid droplets were predominant in areas without crystals. Many droplets had surface defects in the form of pits and vesicular blebs. These findings provide evidence for extracellular processes of lipid deposition and transformation in atherosclerosis.

3D RECONSTRUCTION OF CELL MORPHOLOGY FROM ELECTRON MICROGRAPHS USING INTERACTIVE COMPUTER GRAPHICS. Julian P. Heath, Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030

The three dimensional architecture of the cytoskeleton and membrane systems of migratory cells in vivo presents a particular challenge to the electron microscopist. Conventional serial thin sectioning and reconstruction is hampered by the random orientation of cellular processes and the extracellular fibrillar substratum on which the cells move.

We are studying the morphology of fibroblasts in collagen gels by 400 kV intermediate voltage electron microscopy of thick plastic sections that enclose whole cells. Selective staining of membranes, filaments and microtubules is achieved by using antibodies and gold particles. A tilt series of images is used to reconstruct cell shape with the aid of an interactive computer graphics system. The gold particles provide precise three dimensional coordinates for cellular structures which are reconstructed as wire frame or surface shaded models. These computer models of the cells can be rotated to yield new views not obtainable in the electron microscope.

MORPHOLOGY AND DEVELOPMENT OF DAMSELFLY CAUDAL GILLS (FAMILY: COENAGRIONIDAE)• MELISA L• MOORMAN AND JAMES V. ROBINSON, Dept. of Biology, University of Texas at Arlington, Arlington, Texas 76019.

The larvae of the Zygoptera (damselflies) can be found in a variety of aquatic habitats. The larvae are hydropneustic using oxygen dissolved in the water for respiration. Thin-walled regions of the larval body undergo cutaneous respiration. In addition the damselfly abdomen ends in three caudal lamellae or gills which greatly increase the surface area available for respiration. These caudal gills are also utilized in swimming and defensive behaviors. Caudal gills may be autotomized during interspecific or intraspecific aggressive encounters and occasionally during molting. The regenerated gills differ from the original gills both in size and morphology.

The aim of this study was to utilize scanning electron microscopy to describe the morphology of the original and regenerated gills of members of the family Coenagrionidae. Anatomical and morphological changes can be detected in the larvae after gill removal. MacNeill described the development of caudal gills as protrusive growth with duplex gills being formed. Scanning electron micrographs indicate the presence of the chevron bands on the gills which MacNeill suggests are successive stages of protrusive growth. Abdominal changes in the larvae and the development of both the original and regenerated caudal gills are presented.

PULMONARY RESPONSE FOLLOWING A SECOND ASBESTOS EXPOSURE. J.O. FORD AND R.F. DODSON, Dept. Cell Biology and Environmental Sciences, University of Texas Health Center at Tyler, Tyler TX 75710.

Previous studies of asbestos-induced diseases employing animal models have usually been limited to the investigation of chronic events. However, short-lived neutrophils, which are capable of damaging tissue through the release of super-oxide radicals and elastase, have been shown to constitute a significant component of the acute response to asbestos in our animal model. Animals receiving two exposures to amosite asbestos exhibited both "established" lesions of closely-packed, fiber-laden macrophages, as well as "new" lesions consisting of a mixed cell inflammatory response. A repeated infiltration of neutrophils to the site of renewed lung injury following a second exposure to asbestos was further supported by evaluation of lung lavage fluid. Cell counts and morphological data will be presented for several acute time frames (up to 28 days) between asbestos exposures.

STRUCTURE AND DEVELOPMENT OF STINGING EMERGENCES OF CNIDOSCOLUS TEXANUS. Tammy L. Hancock, Dept.of Biology, University of Texas at Arlington, Arlington, Texas 76019

Cnidoscolus texanus is one of a group of plant species that posess subepidermal emergences that cause a stinging reaction upon contact with the skin. This reaction has previously been shown to be both chemical and mechanical in nature. Fresh samples of material were chemically fixed and observed to determine the anatomical properties as well as the developmental sequences involved in the maturation of the stinging emergences. Structural evidence as to what is occurring when tip breakage occurs as well as external wall features are presented. Uniform patterning of wall deposits are present on the external surface of all emergences. Two distinct types of tips were observed to be present on the emergences. One type of tip having a characteristic bulbous shape that fractures below the base upon contact. The second type of tip observed had no bulbous appearance. Both types of emergences have a pedestal base from which the stinging cell emerges subepidermally. Stinging emergences occurred on all above ground plant structures, being most numerous on stems and petioles. Leaf surfaces, both abaxial and adaxial contained stinging emergences along the vascular network. Flower parts, including peduncle, calyx and corolla are also densely covered with stinging emergences.

BIOLOGICAL SCIENCES

POSTER PRESENTATION — FALL 1988

FREEZE PRESERVATION OF BACTERIA AND PLANT CELLS. Hilton H. Mollenhauer and Robert E. Droleskey, Veterinary Toxicology and Entomology Research Laboratory, ARS-USDA, College Station, TX 77841.

A simple freeze preservation device was evaluated using *Salmonella typhimurium* and maize rootcap cells. The device was manufactured by the authors and consisted of a specimen clamp mounted on the end of a spring-loaded arm which propelled the tissue at high speed into liquid propane. The root tips were split 2X lengthwise and the center section plunged into liquid propane. *Salmonella* grown on agar-impregnated filter paper were plunged into liquid propane as for the root tips above. The substitution fluid consisted of 2% osmium tetroxide in acetone. Good preservation was limited to the outer 1-2 layers of root tip cells, and for about 10-15 cells inward from the surface of the *Salmonella* culture. A notable feature of either tissue was the smooth (i.e., nonundulating) aspect of the cell surface and the high contrast of the bounding membranes. Moreover, there was little or no shrinkage of cytoplasm from the cell surfaces. As noted in other freeze-substituted bacterial cells, there was no region with clearly-delineated DNA strands. In the plant cells, staining was most satisfactory in cell wall constituents, plasma membrane, and Golgi apparatus secretory vesicles (both membrane and product). Endoplasmic reticulum, Golgi apparatus cisternae, multivesicular bodies, and vacuoles were not well contrasted although they appeared well-preserved. Coated vesicles along the cell surface were well-preserved and contrasted. Although the device was exceedingly simple, it functioned as well as most commercial units, at least in respect to the preservation of the tissues sampled.

ISOLATED CRYPT CELLS FROM RAT INTESTINAL MUCOSA STUDIED BY TEM AND SEM. DONNA S. TURNER, KATHRYN S. MCKEE, MARGARET PUTMAN, and BUFORD L. NICHOLS, Department of Cell Biology and USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, Texas 77030.

Crypt cells from rat intestine have been used in a bioassay to detect factors in the diet which alter the rates of cell proliferation in mucosa. The present study was conducted in order to characterize the morphology of the cells used in this bioassay system. The mucosal epithelial cells were fractionated by sequential changes in 37°C calcium-free bathing media using the method of Harrison and Webster as modified by Bronstein et al. High frequency vibration was used at low amplitude to shake off the enterocytes. The crypt cells were collected between 12 and 18 minutes of vibration. They were washed in Dulbecco's PBS solution containing ionized calcium and centrifuged to form a pellet. The fractions were then processed routinely for TEM or microcentrifuged directly on to a coverslip and processed for SEM. This preparation method enables us to demonstrate that the crypt cells are morphologically different from the villar enterocytes and that they resemble immature crypt cells in situ. The identity of the crypt cells was further confirmed by the demonstration of thymidine incorporation into DNA, a function specific for intestinal crypt cells.

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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.

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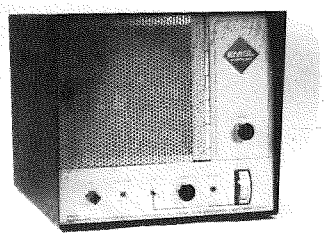

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Answer from Page 7

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Micrograph by H. Wayne Sampson,
Department of Medical Anatomy, Texas A&M University, College Station, Texas.

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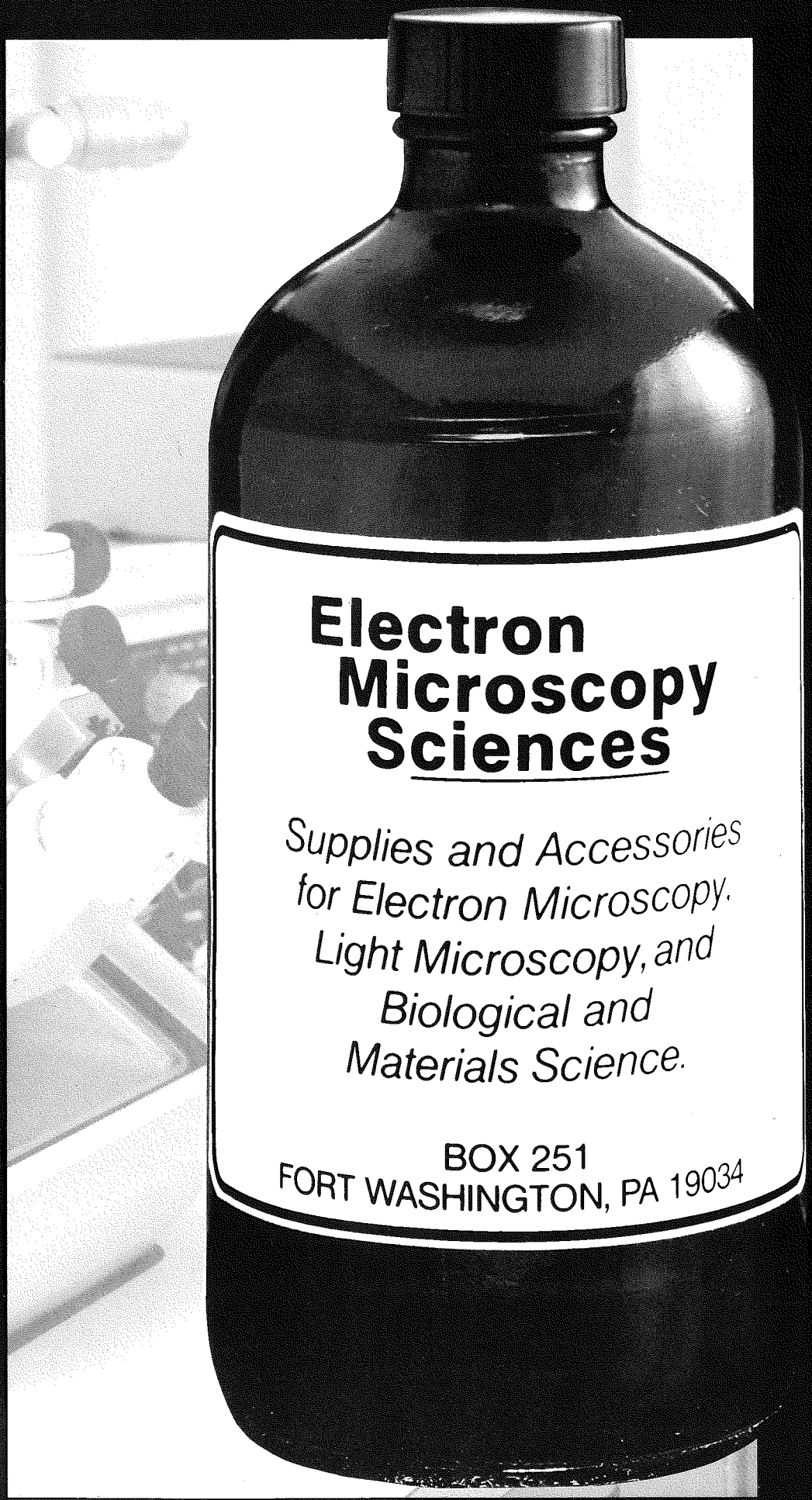

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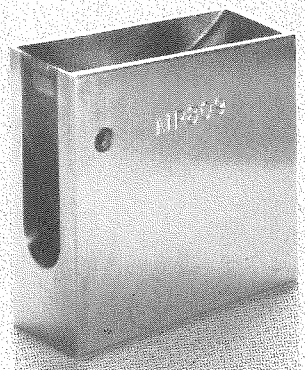

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Goodbye
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Good Luck
to
Dr. Randy Moore
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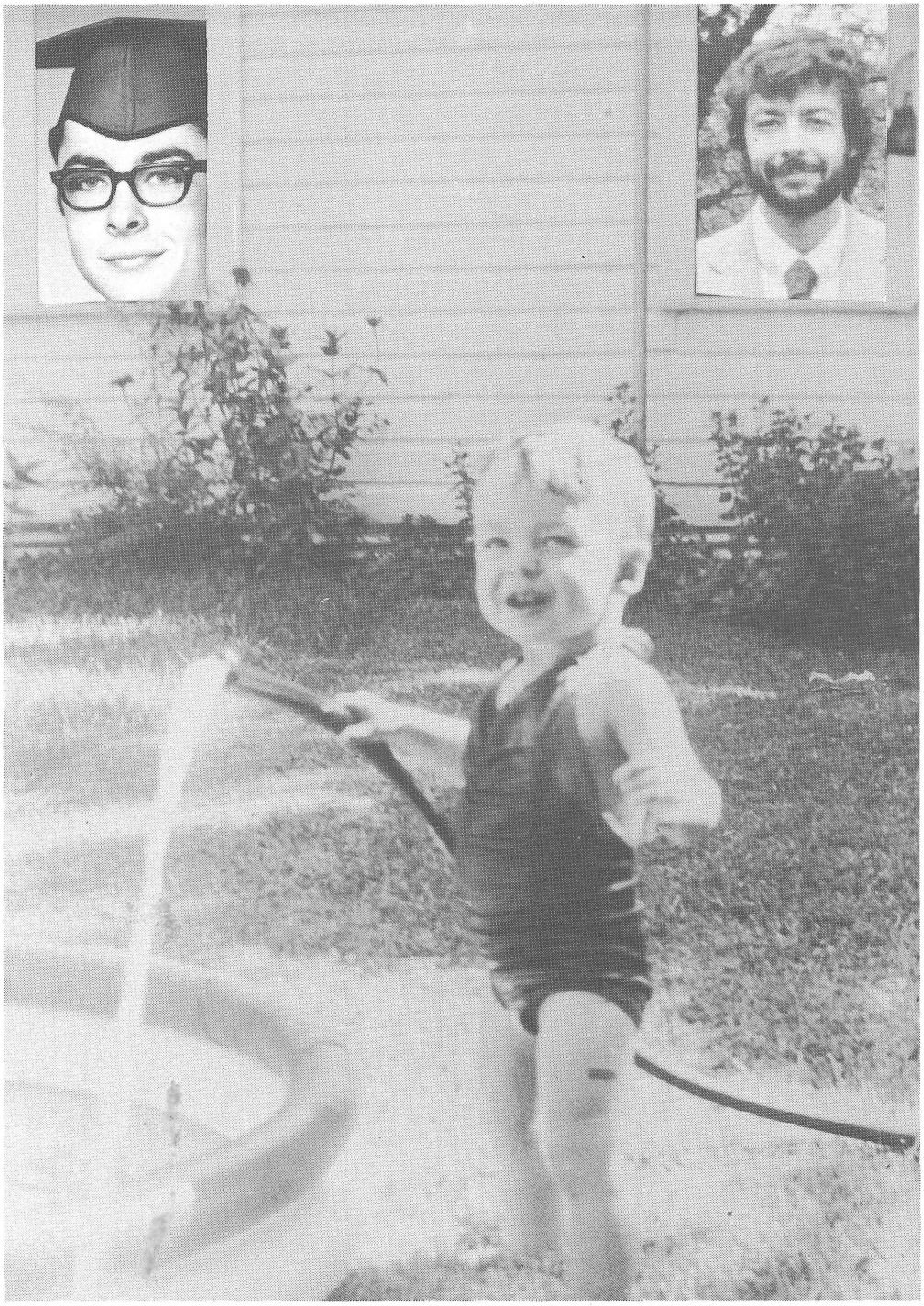

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Texas Society for Electron Microscopy, Inc.
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College of Medicine
Texas A&M University
College Station, Texas 77843
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