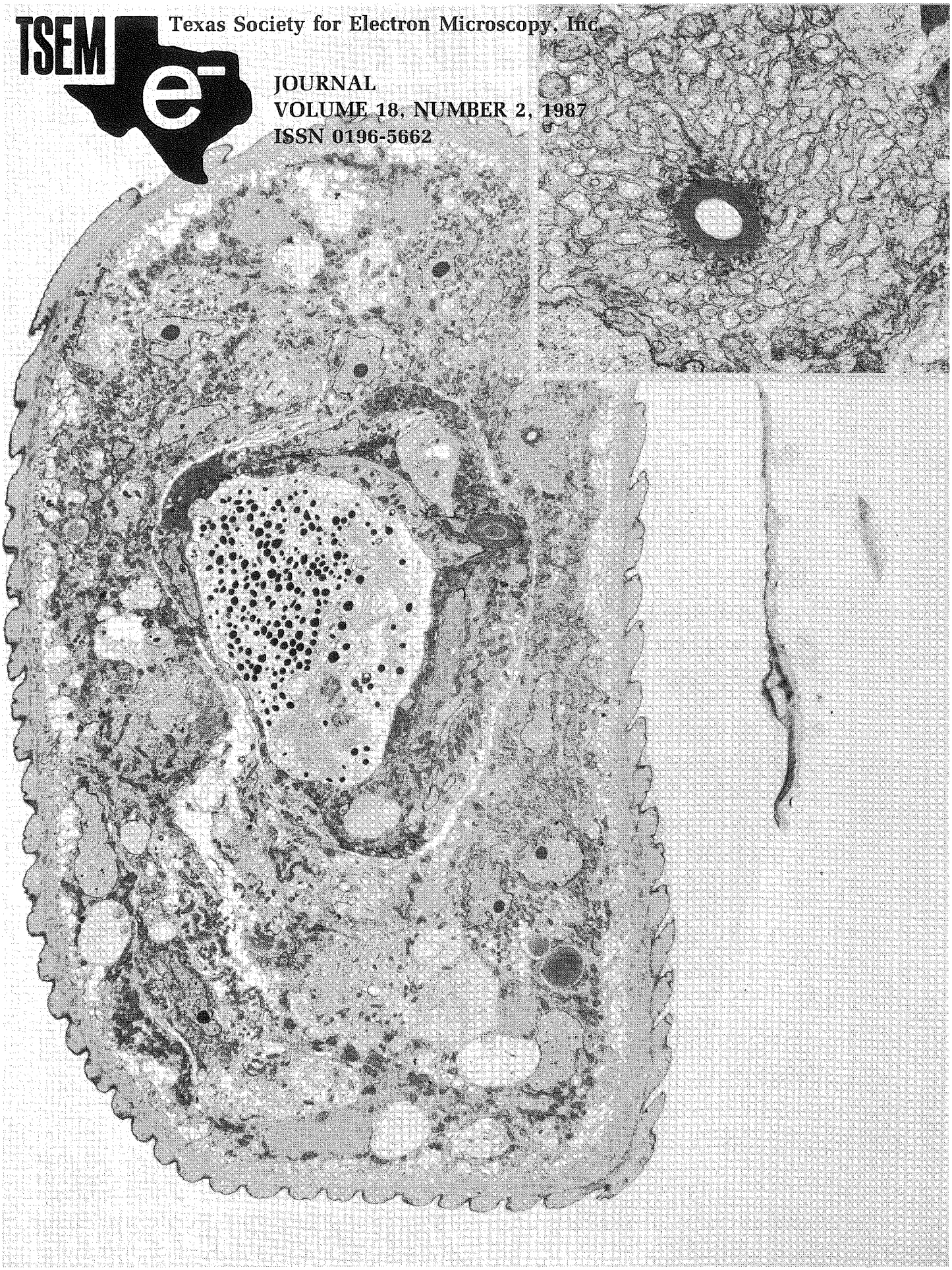
TSEM
Texas Society for Electron Microscopy, Inc.

JOURNAL
VOLUME 18, NUMBER 2, 1987
ISSN 0196-5662



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ISI SEMs: TO SEE THE WHOLE TRUTH

Contents

TEXAS SOCIETY FOR ELECTRON MICROSCOPY, INC.
JOURNAL
VOLUME 18, NUMBER 2, 1987
ISSN 0196-5662

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

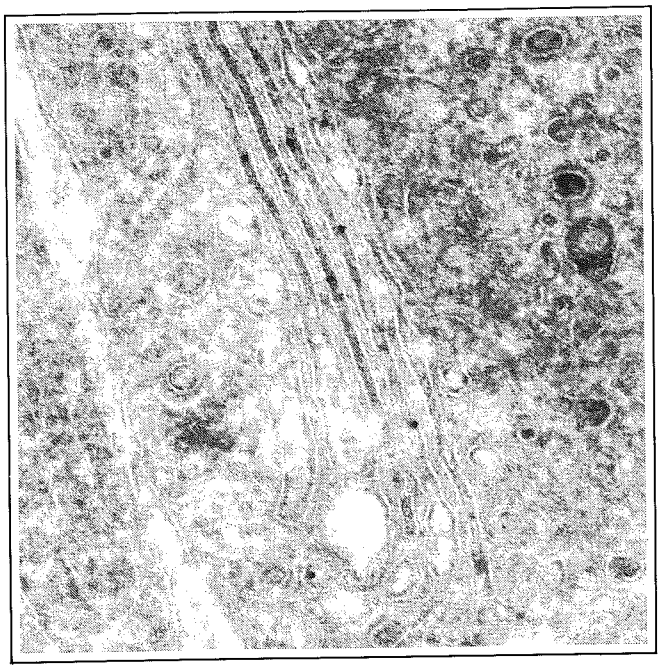
Texas Society for Electron Microscopy

"For the purpose of dissemination of research with the electron microscope."

ON THE COVER

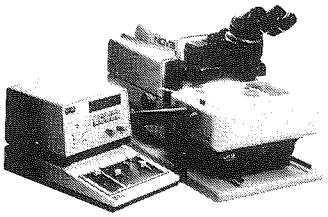
Cross section through the thoracic region of the adult, tomato root knot nematode, *Meloidogyne icognita*, (x4,400). Conventional fixation techniques did not adequately preserve the nematode's ultrastructure. As a result, 100μ sections of the nematode were obtained by using a Vibratome and collecting the sections directly in a bath of fixative. The inset is a higher magnification illustration of the terminal excretory duct located near the stylet, (x22,400). Secretions from this duct are thought to be responsible for the degeneration of host tissue, causing root knot formation. Micrographs by Bob Droleskey, Veterinary Toxicology and Entomology Research Laboratory, College Station, Texas.

For results you can publish



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

IMMUNOCYTOCHEMISTRY



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President's Message

With the past two meetings (Houston and Waco), during Randy Moore's tenure as President, we have begun a trend of alternating meeting sites between large cities and smaller towns which, I think, will be very effective. This trend will be continued during my term in office when we meet at the Mayan Ranch in the small western town of Bandera in the Fall of 1987 and then in Dallas in the Spring of 1988. Randy and Bob Blystone did much to make the Houston and Waco meetings very successful. Don Hay (our Program Chairman), Bob Turner (who initiated arrangements for the Bandera meeting), and Bill Gonyea, who is handling local arrangements in Dallas, are doing much now to make these two meetings equally successful.

The Bandera meeting is going to be a lot of fun! Dr. Jean-Paul Revel is a very well respected electron microscopist, President-elect of the Electron Microscopy Society of America, and an excellent speaker! There'll be bar-b-quin', two steppin', trail ridin'. . . and who can resist the opportunity of seeing Randy Moore on a horse?? As the song says: "Don't call him a cowboy 'til you see him ride!"

At the Dallas meeting we are planning to have a couple of speakers. We are going to have someone who will talk to us about cryo techniques for specimen preparation and someone who will talk on immunocytochemical techniques at the ultrastructural level. This meeting will be held at the Executive Inn and Conference Center near Love Field. Bill Gonyea and Don Hay have already put a lot of work into this meeting and it promises to be a good one.

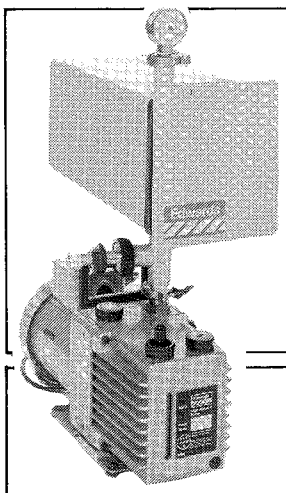
I would like to call your attention to the outstanding job Ron Davis is doing with the *TSEM Journal*. The latest issue (Vol. 18, No. 1) features an address list of members, the first since Vol. 15, No. 1, 1984. The membership roster is a very important tool that The Society depends upon, and Wayne Sampson has put a lot of work into keeping it up to date. Other interesting information on The Society can be found in the article "A Survey of TSEM Assets" and "A Survey of TSEM Abstracts". Bob Turner's article on "Suggested Procedures and Guidelines for an Electron Microscope Laboratory in a Medical Facility" is also very good. In short, there is good reading in *The Journal* and Ron has done yeoman's work in putting it together. **But he needs our help!** I know I am just as lax as the next person. However, he is correct in saying that without more scientific articles, *The Journal* will become nothing more than an ad sheet and news letter. Our Corporate Members are supporting it very well. More input is needed from the rest of us. *The Jounal* affords an excellent opportunity for graduate students and technicians who are just starting to write scientific papers, just as the TSEM meetings are a good forum for first-time speakers. Faculty members should encourage their participation.

Sincerely,

Joiner Cartwright, Jr., President
Texas Society for Electron Microscopy
August 21, 1987

ADVERTISERS INDEX

Buyers Guide to EM specimen preparation



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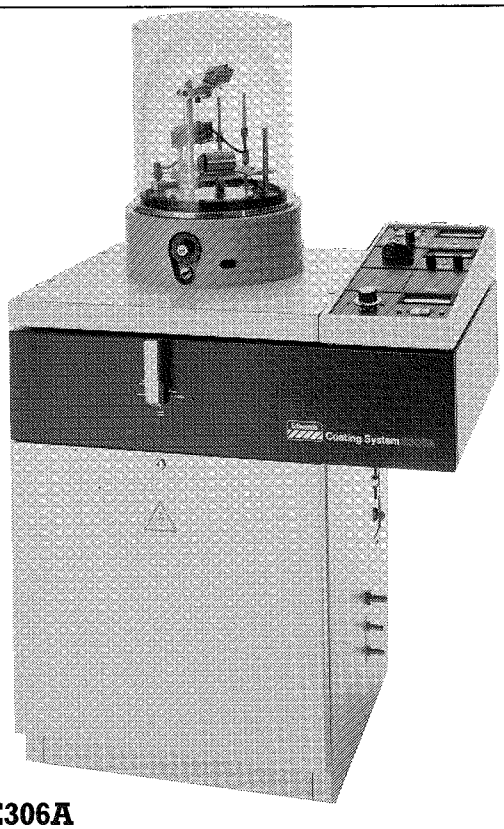

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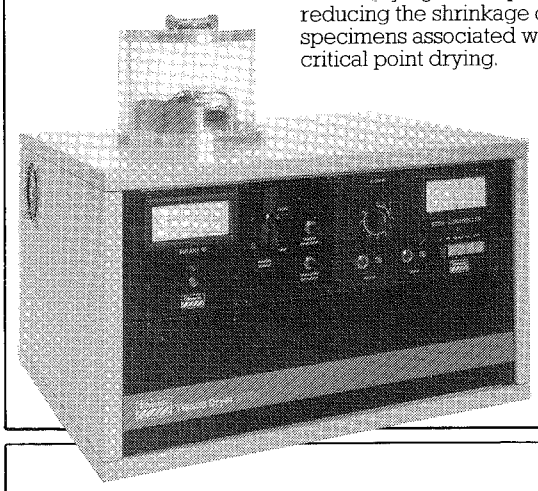

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Biological Photographers	$1,236.41	
Waco Dues and Registration	1,323.00	
EMSA Grant	500.00	
Checking Account Interest	64.73	
Corporate and Individual Dues	973.00	
Journal Ad Revenue 18:1	2,250.00	
Journal Ad Revenue 18:2	1,200.00	
Interest Earned on Certificates of Deposit	353.43	7,900.57
EXPENSES:		
Waco Meeting Expenses	1,894.50	
Travel and Honorarium for Dr. Mims	585.00	
Journal Printing	2,288.00	
Mailing Expenses	84.37	
Checking Account Charge	40.19	
Waco Student Travel	112.00	$ 5,004.06
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*Certificate of Deposit #66177, University National Bank of Galveston, reached maturity on 14-3-87. The money from that certificate was redeposited in Certificate of Deposit #097-212-5, Lamar Savings of Bryan.

Respectfully Submitted,

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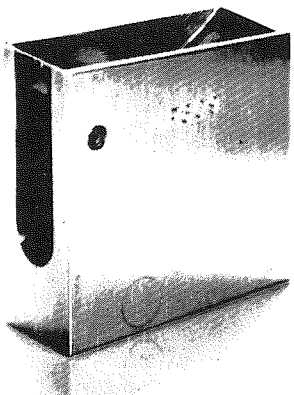

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Editor's Message

Well, here it is, time to send another TSEM Journal off to the printer. As with anything, the Journal is not the effort of just one person and I suppose it is well past due to acknowledge some of the people that have helped along the way. They are: Cee Cee Lytle and Janet Natowitz who type much of the material, including manuscripts and article revisions and put up with what they must sometimes view as my capricious personality; Angelique Graves who printed all the Jerry Berlins and did the cutting and pasting for the picture on the back cover; Wayne Sampson who listens to me complain about being editor; and finally the folks at American Printing in Temple who do a lot of work with a minimum of instruction from me. That is not really final, there are the advertisers, the ad agencies, the people that send in manuscripts. . .oh well, I imagine you get the idea.

I have been informed that my appointment as Editor is just about up and that I, or someone else, will have to decide if the scepter is to be passed. I have mixed feelings.

Some of the stuff I do as editor is fun, for example, working with the people that prepare micrographs for the front cover, coming up with ideas for good-bye photos (e.g., Drs. Mims and Berlin) and receiving an occasional compliment.

Some of the stuff I do as editor is not fun. For example, worrying — about everything. But the worst, the absolute worst thing about being editor is getting the idea that nobody cares.

What gives me the idea that nobody cares about the TSEMJ? Well, I'm glad you asked. The main reason is the paucity of papers that are presented for publication in our preeminent periodical. Preeminent is a carefully chosen descriptive word in this case. The TSEMJ has had the reputation of being the best local EM Society publication out of the 29 EMSA local affiliate societies. In spite of this, people do not send enough manuscripts, and the editor (this has been an historic problem in TSEM) starts panicking about not having anything to print.

What is really bad, is that if every member of TSEM sent in just one paper and we printed 3 in each edition, there would be enough to last 150 years! The Journal is a perfect place for students and technicians to start getting some publications of their own. As for the senior researchers, who doesn't have, stuck away in some drawer, a small project that did not turn out important enough to publish in a major journal and you've been saying, "I really should do something with that." Well, this is your chance.

So folks, whether I continue as editor or someone else accepts the scepter of office, the problem will continue unless **you** do something about it.

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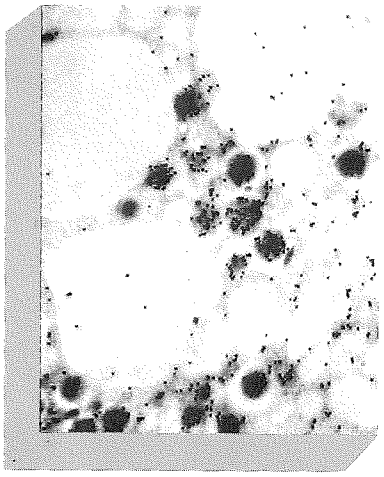
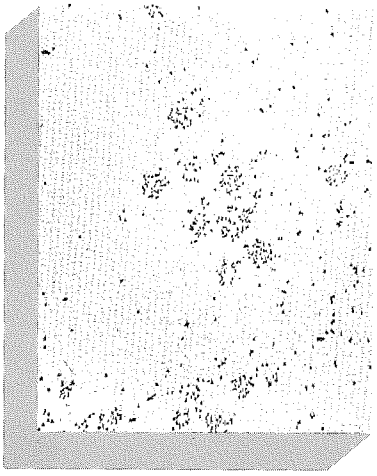
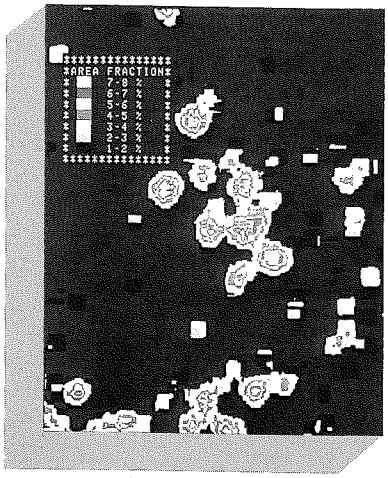


(1) The unprocessed digital image from a TEM micrograph of an unstained, thin section of the human pancreas with the beta granules gold tagged.*



(2) Derivative processing of (1), with gray level transformation, isolates the Au tagged anti-insulin tracer (yellow).



(3) Processing (2) with a digital image density filter produces a quantitative mapping of the Au particulate density per unit area.

**Micrograph courtesy of R. Smith, DIABETES AND ENDOCRINE RESEARCH CENTER, University of Pennsylvania School of Medicine.*

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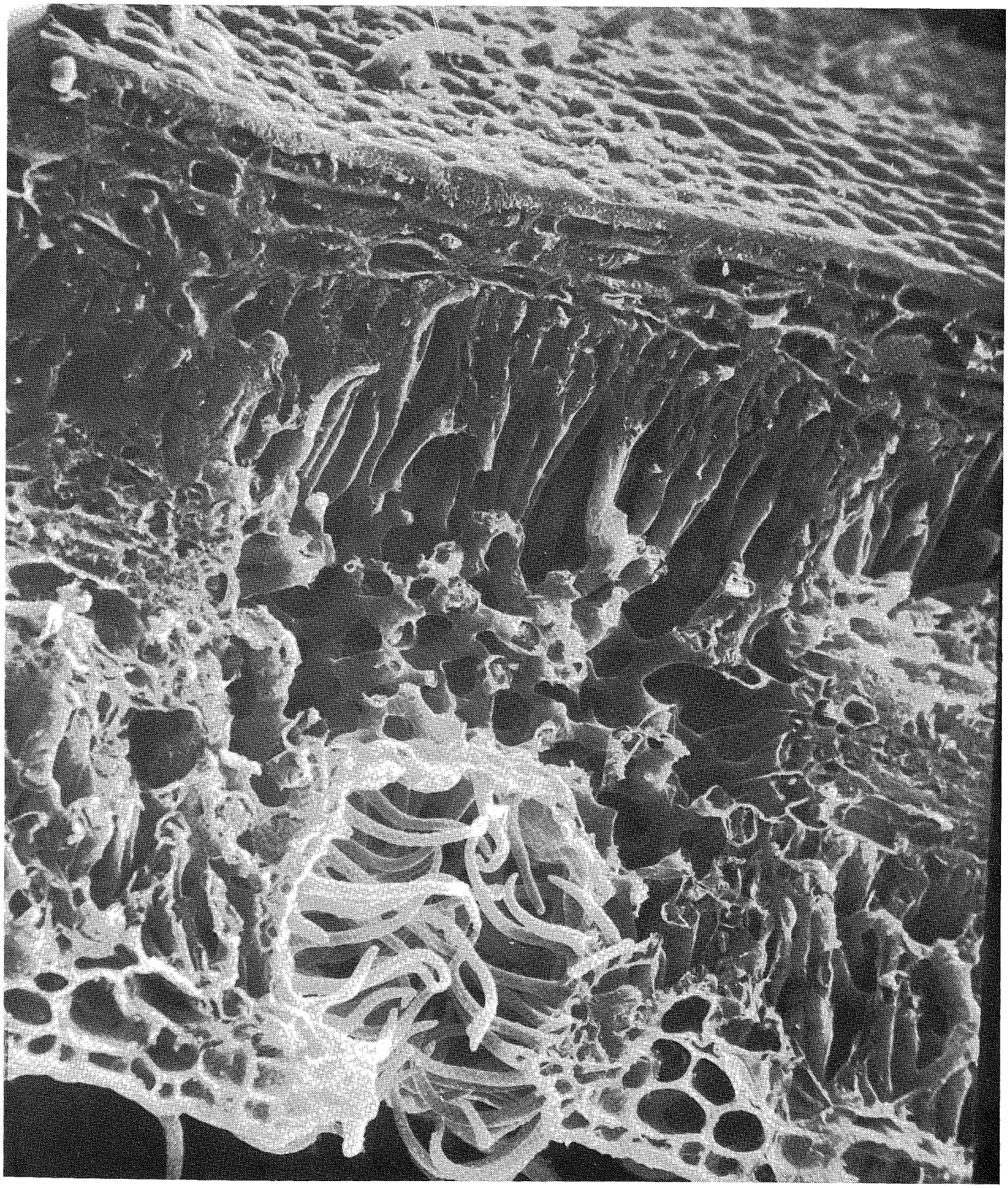


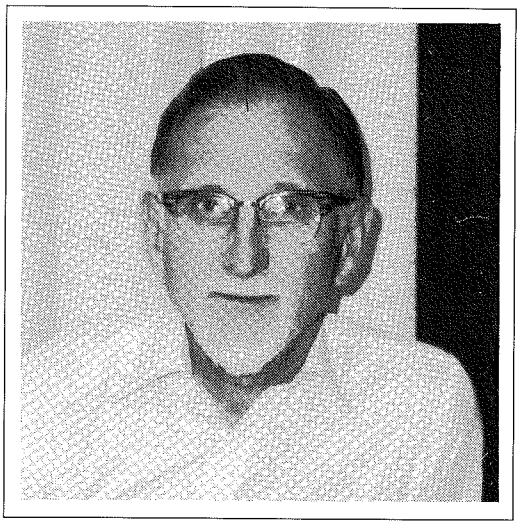
Interested? . . . turn to page 33

TSEM SALUTES DR. HILTON H. MOLLENHAUER

From Current Contents,® July 6, 1987

This Week's Citation Classic®

Mollenhauer, H.H. Plastic embedding mixtures for use in electron microscopy.
Stain Technol. 39:111-4, 1964
(Electron Microscope Laboratory, University of Texas, Austin, TX)



Hilton H. Mollenhauer

This paper describes an epoxy embedding resin for electron microscopy that provides good tissue preservation and is easy to section with both glass and diamond knives. (The SCI indicates that this paper has been cited in over 1,875 publications.)

Hilton H. Mollenhauer
Veterinary Toxicology and Entomology
Research Laboratory
Agricultural Research Service, USDA
College Station, TX 77840
and
Department of Pathology and Laboratory Medicine
College of Medicine, Texas A&M University
College Station, TX 77843

February 2, 1987

In 1964 I was at the University of Texas in Austin working in the Cell Research Institute on characterization of plant Golgi apparatus, using mostly maize root tips as the model system. Although this tissue was excellent from the standpoint of ulstrastructural topography and consistency, it was not always easy to embed or section for electron microscopy. As in many plant systems, the primary problems were poor penetration of resin and lack of resin binding to the cell walls that often resulted in separation of tissue from resin during sectioning. In this particular instance, these factors were exaggerated since this was a highly student-oriented laboratory where most of the students worked with plants, algae, or fungi and faced the difficult prospect of sectioning with glass knives.

Araldite epoxy resins were introduced into the laboratory shortly after the report of A.M. Glauert and coworkers.[1]. Similarly, Epon resins were used and

evaluated shortly after their introduction by J.H Luft.[2]. Epon resins had some advantages over Araldite, most notably lower viscosity and simple formulation changes that allowed adjustment of block hardness. However, Epon resins were not as easy to section with glass knives as were Araldite resins, and they had a considerable tendency to chatter and form rippled sections.

The Epon-Araldite mixture that is the subject of this reminiscence was the result of a very simple question: If two resins are mixed together will the resulting resin block exhibit some characteristics from both components and will these characteristics be the ones that will most benefit the problem? A scientific basis for resin formulation and cleavage of sections was not available at that time nor, in fact, is it available today, although much progress has been made and some commitment to improve embedding resins is now visible. In any event, the Epon-Araldite resin mixture proved useful and became the standard for the laboratory for many years. The mixture was easier to section than either Epon or Araldite alone, especially with glass knives, yet provided good tissue preservation and minimal separation of resin from tissue. Its stability in the electron beam was not as great as had been hoped but was no worse than that of Araldite. However, the deciding factor was ease of sectioning. Except for the use of lecithin-doped Spurr resin,[3] the Epon-Araldite mixture is, perhaps, still the resin of choice if both good tissue preservation and ease of sectioning are required.

REFERENCES

1. **Glauert A M, Rogers G E & Glauer R H.** A new embedding medium for electron microscopy. *Nature* 178:803, 1956. (Cited 180 times.)

2. **Luft J H.** Improvements in epoxy resin embedding methods. *J. Biophys, Biochem. Cytol.* 9:409-14, 1961. (Cited 10,500 times) [See also: **Luft J H.** Citation Classic. (Barrett J T. ed.) *Contemporary classics in the life sciences. Volume 1: cell biology.* Philadelphia: ISI Press, 1986, p. 3.]

3. **Mollenhauer H H.** Surtactants as resin modifiers and their effect on sectioning. *J. Electron Microsc. Tech.* 3:217-22, 1986.

EDITORIAL POLICY

LETTERS TO THE EDITOR

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

ELECTRON MICROGRAPHS AND COVER PHOTOS

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

REGIONAL NEWS

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

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

TECHNICAL SECTION

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

PUBLICATION PRIVILEGES

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

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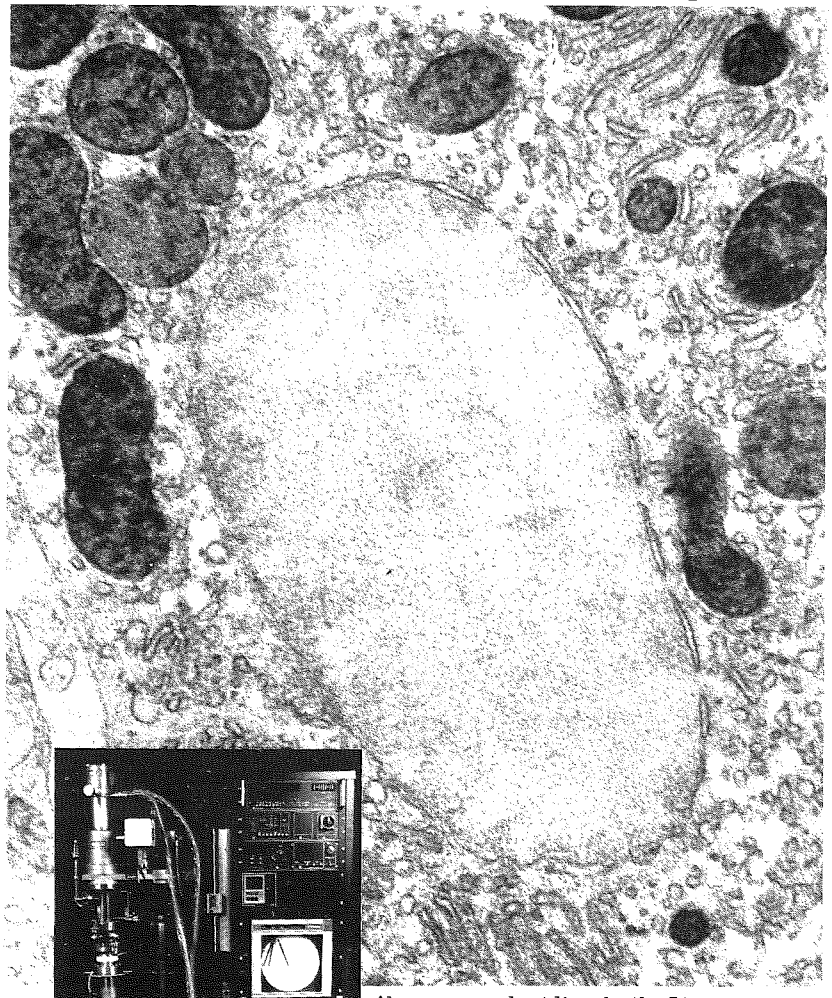

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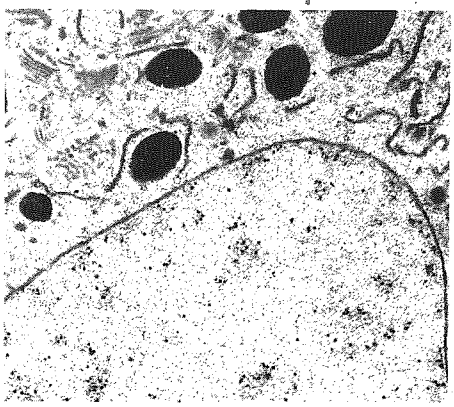

Colloidal gold labeling with antinuclear antibody (ANA) positive serum. Localization specifically to heterochromatin of the nucleus. (MCF-7 cultured cell).

A NEW DEVICE FOR STAINING THIN SECTIONS

By

Glenn A. Hoskins
Texas Tech University
Department of Biology
Lubbock, Texas 79409

A NEW STAINING DEVICE

Biological thin sections are not inherently electron opaque which makes heavy metal staining essential in order to obtain adequate contrast in the transmission electron microscope. Routine staining procedures usually include uranyl acetate followed by lead citrate. Problems such as contamination, tearing, or complete removal of sections are common to traditional staining techniques. By "traditional staining techniques" it is meant staining in a drop of uranyl acetate, washing with distilled water, staining in a drop of lead citrate, and washing in distilled water.

Most of the problems in staining thin sections are directly attributable to the atmosphere surrounding the grids being stained. Since most EM labs are not equipped with special air filtration systems, it seems natural to assume that airborn particulates are present. These particulates can find their way onto sections when we are transporting the grid from water to uranyl acetate, uranyl acetate to water, water to lead citrate, lead citrate to water, and in the final drying step. This also assumes that the sections are clean before staining.

Sections which are allowed to dry before staining seem to adhere to the grid better than sections which are taken from the microtome and stained while the grid is still wet. For this reason, in our lab, we always allow our grids to dry prior to staining.

It is essential to use the least amount of washing in order to minimize section damage. This many times leads to the introduction of another common artifact, lead carbonate precipitate. All of the lead citrate stain must be washed from the grid or the lead citrate will combine with carbon dioxide in the air to form lead carbonate precipitate. This leads to the dilemma of quantitating the minimum amount of washing necessary to wash off all of the lead citrate without overwashing, which may lead to section damage or removal.

Washing the grid after staining is usually accomplished by either running a steady stream of water over the grid, or by dipping the grid into several baths. In either case the possibility of tearing or removing sections from the grid are considerable.

The need for consistently good quality staining necessitated development of a new type of staining technique. A system which is closed to the atmosphere eliminates contamination due to airborn particulates as well as eliminating carbon dioxide which could cause lead percipitate on sections. Another consideration is the flow rate of solutions over the sections. The flow rate must be slow to minimize section damage and removal.

The end result of much experimentation and several prototypes is seen in the following diagram.

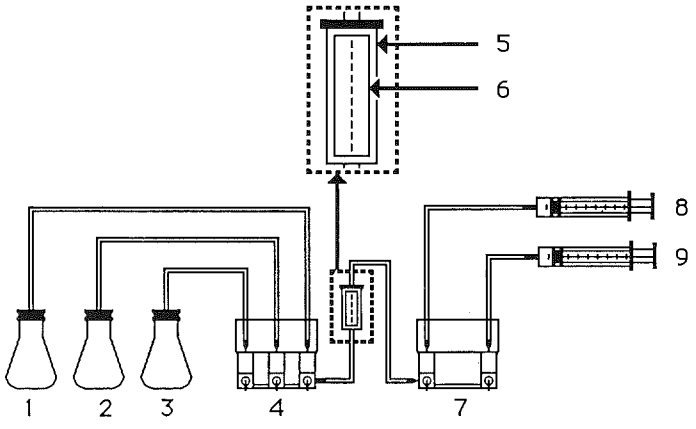

Figure 1. Diagram of the TEM GRID STAINER.

In Figure 1, numbers 1, 2, and 3 are flasks containing stock solutions of uranyl acetate, lead

citrate, and double distilled water respectively. We use unfiltered stains made from double distilled water in our system. The flasks are stoppered and Tygon tubing runs down into each flask through a hole which can be bored into the stopper, or stoppers are available which already contain holes. A piece of filter paper is taped over a second hole in each stopper to filter air as it is pulled into the flask to take the place of the fluid leaving the flask.

Numbers 4 and 7 are two and three way gang valves which can be obtained at most pet stores with aquarium supplies.

Number 5 is a vial which once contained grids. Any plastic vial which has a pop off lid and can be made air tight will work. The length and diameter of the vial is important as these dimensions will affect the number of grids which can be stained and how much stain will be used as well as the amount of waste stain which will be created. It is best to try to find a vial with a diameter just slightly greater than that of the Tygon tubing which enters and exits the vial.

Tygon tubing is preferred because it is inert to the stains used. We use tubing with an inside diameter of 0.125″. A cork bore equal to or just slightly smaller in diameter than the Tygon tubing is used to create the holes in the lid and bottom of the vial. Once the Tygon tubing is inserted into the lid and bottom of the vial, a little Super Glue will seal the tubing. The Super Glue can come loose if put under too much stress this is why a pop off cap on the grid holder vial works better than a screw top. The stresses caused by screwing and unscrewing the cap causes the glue to come loose.

Number 6, the grid holder, is a piece of Tygon tubing which has been cut in half and small slits are cut into it running parallel to the length of the tubing. The slits must be smaller than the grids, about 1mm, or the grids will fall into the slits when they are being placed into them. To place the grids into the slits you merely press opposing sides of the tubing which opens up the slit allowing the grids to be inserted. The number of grids which can be stained at one time is dependent upon the length of the grid holder vial, and the grid holder.

Numbers 8 and 9 are syringes. These syringes should be of a volume which will accommodate one complete run. We use a small diameter grid holder vial, and very little excess tubing to reduce the amount of stain needed and stain waste created. This makes it possible for us to use 60cc syringes. With our system, we use 7cc of each stain each run and create 40cc of waste stain each run. Extra waste stain is created because the stain swirles around in the grid holder vial while washing and it takes extra volumes of water to fully remove the stain from the system. It is desirable to obtain a vial with a diameter just slightly larger than that of the tubing, as mentioned previously, because as the difference between the two diameters grow, so does the amount of washing it will take to eliminate the stain from the grid holder vial.

In Figure 1 we show only one waste stain syringe, but if it is desirable to keep the waste uranyl acetate separated from the lead citrate a three way gang valve could be substituted for the two way gang valve and an extra syringe could be added.

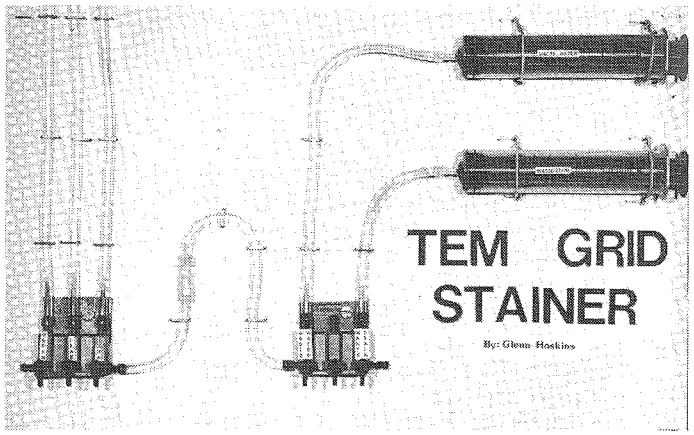

Figure 2. The TEM GRID STAINER

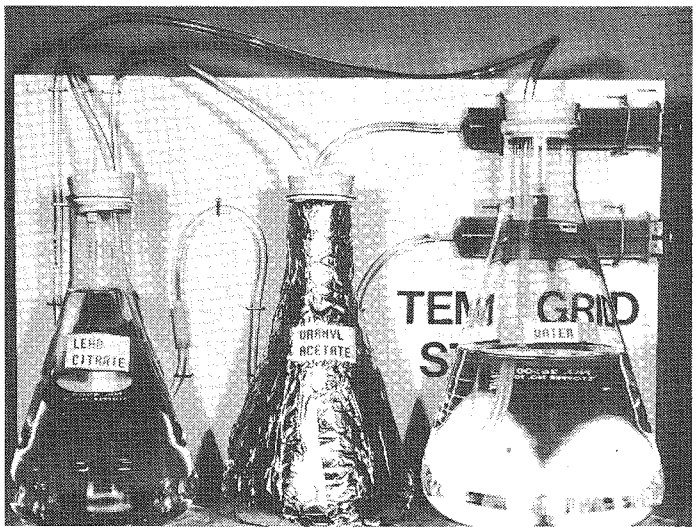

Figure 3. A view of the flasks used with the system

The following is a list of instructions we use with our staining system:

1. Open the water valve and the waste water valve and pull 60cc of fluid into the waste water syringe. Empty the water out of the waste water syringe. This will wash out the system of any contamination before placing any grids into it.
2. Place the grid(s) into the grid holder and the grid holder into the grid holder vial.
3. Open the uranyl acetate (UA) valve and the waste water valve and pull stain through the system with the waste water syringe until it reaches the waste stain/waste water valves. In our system this is 2cc. **Note: Be sure to pull all**

fluids through the system very slowly (about 1cc/3 seconds).

4. Close the waste water valve and open the waste stain valve. Pull 3-5cc of fluid into the waste stain syringe to ensure that the stain in the grid holder vial is not diluted.
5. Close the waste stain valve and the UA valve.
6. Set a timer for the desired staining time and let the system set for this time.
7. Open the water valve and the waste stain valve and pull water into the system with the waste stain syringe until there is no more yellow color to the fluid in the grid holder vial. This is 15cc in our system. Be sure to note this amount because when you wash the lead citrate from the system there will be no color to assist in telling when the stain is washed from the system.
8. Close the waste stain valve and open the waste water valve. Pull another 10cc of water through the system with the waste water syringe to ensure the system is flushed of all UA.
9. Close the water valve and open the lead citrate valve. Pull the same amount of lead citrate through the system as you did in step 3 with the UA.
10. Close the waste water valve and open the waste stain valve. Pull 3-5cc of lead citrate into the waste stain syringe to ensure that the stain in the grid holder is not diluted.
11. Close the waste water valve and the lead citrate valve.
12. Set a timer for the desired staining time and let the system set for this time.
13. Open the water valve and the waste stain valve and pull water into the system with the waste stain syringe. Pull the same volume of water into the system as you did in step number 7.
14. Close the waste stain valve and open the waste water valve. Pull another 20cc of water through the system with the waste water syringe to ensure the system is flushed of all lead citrate. We use 20cc instead of 10cc here because it is critical that all of the lead citrate be washed from the system.
15. Remove the grid holder from the grid holder vial. Remove the grid(s) from the grid holder.
16. Remove the waste water syringe and the waste stain syringe from the system. Dispose of the waste stain into a waste stain container and wash the waste water down the fume hood rain.

In conclusion, this new type of staining device saves time and aggravation over conventional techniques. It produces consistently good quality staining with little effort. Figures 4-7 are examples of the staining quality obtained from this device.

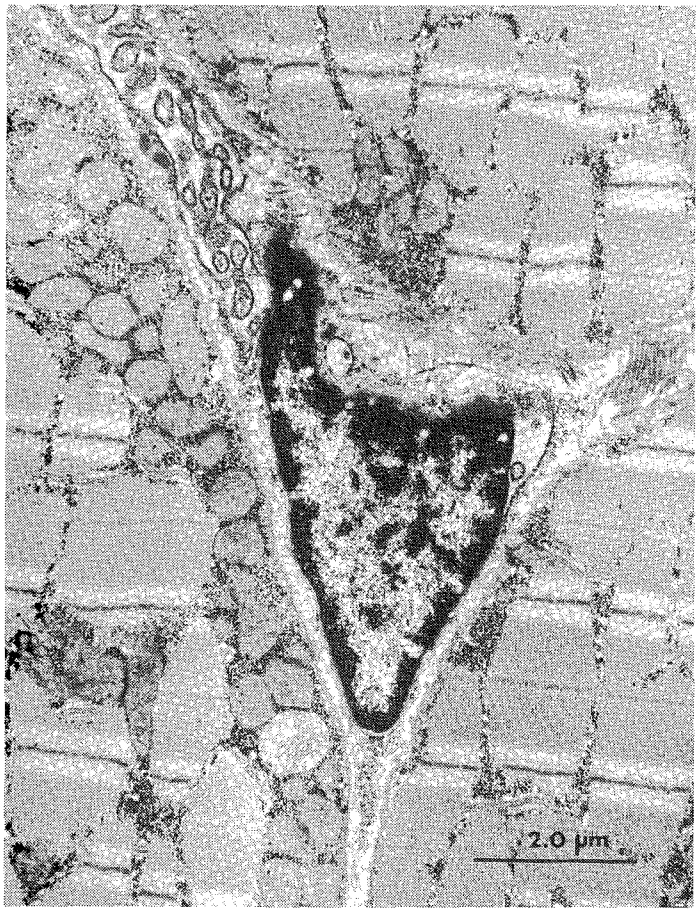

Figure 4. Rat skeletal muscle.

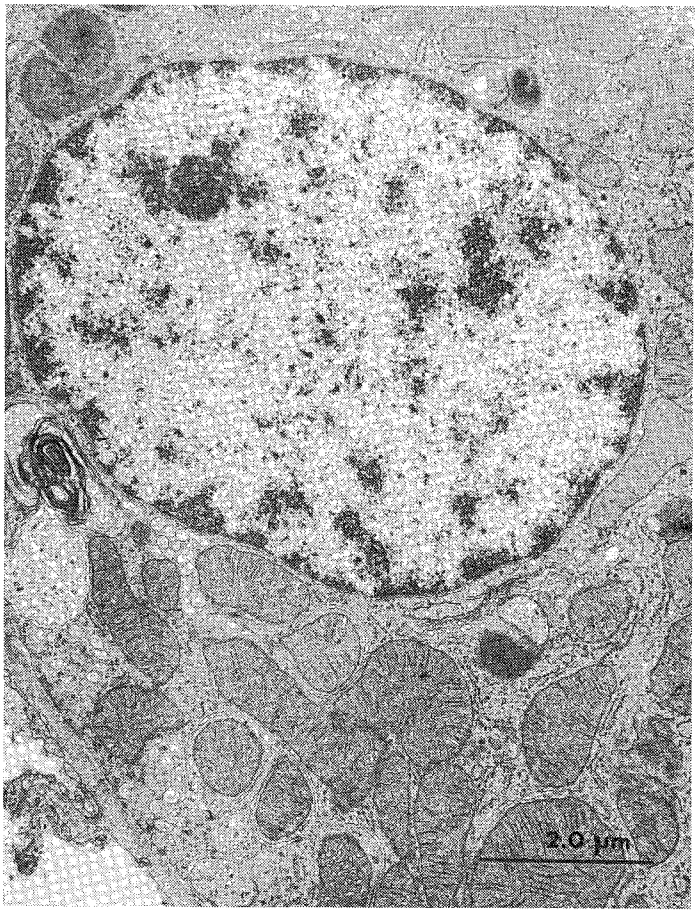

Figure 5. Rat kidney.

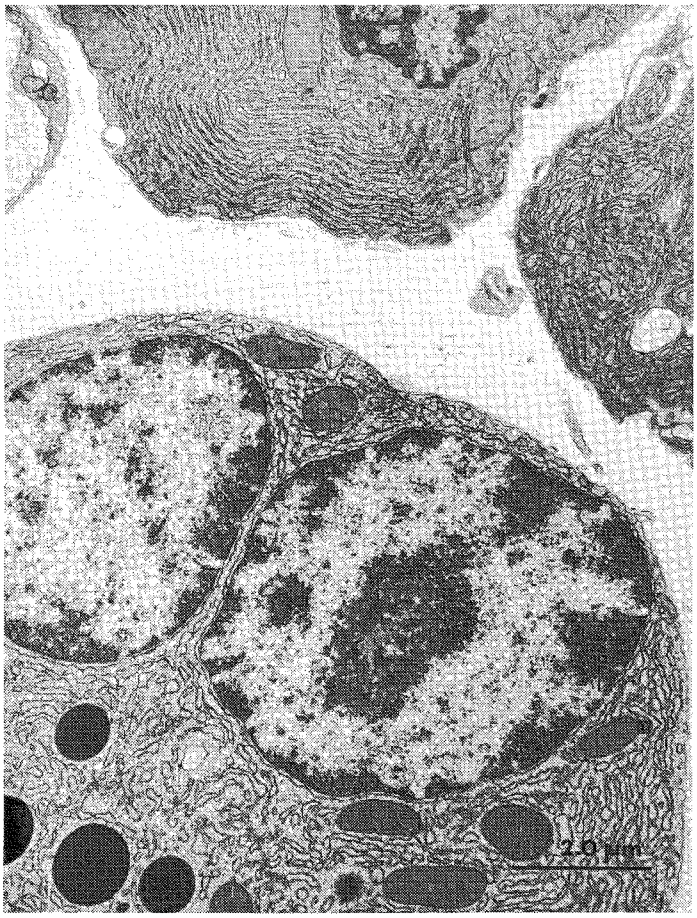

Figure 6. Rat pancreas.

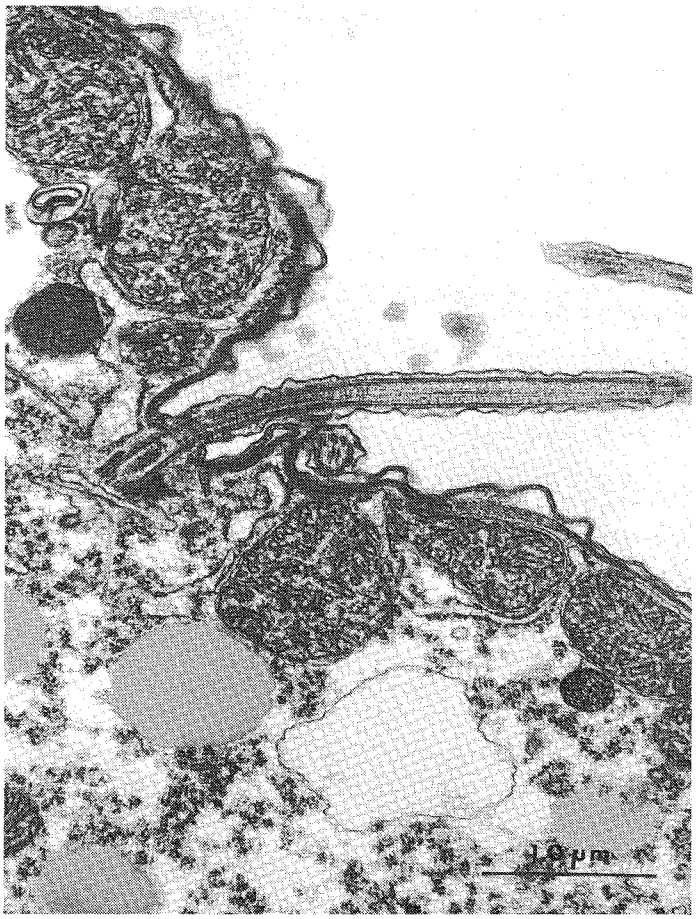

Figure 7. Tetrahymena Pyriformis.

Editor's Note:

The preceding paper by Glen Hoskins was submitted very close to the time when I had to send the Journal to the printer. Although it did go through the usual review process and was okayed for publication, some of the suggested revisions could not be made. Most notable were references. The following were considered pertinent by the reviewers:

Avery, S.W. and Ellis, E.A. 1978. Methods for removing uranyl acetate precipitate from ultrathin sections. Stain Technology. 53(3):137-40.

Kuo, J. 1980. A simple method for removing stain precipitate from biological sections for transmission electron microscopy. J. Microscopy. 120(2):221-4.

Mollenhauer, H.H. 1974. Poststaining sections for electron microscopy. Stain Technology. 49(5):305-8.

Mollenhauer, H.H., Droleskey, R.E. 1985. Some characteristics of Epoxy embedding resins and how they affect contrast, cell organelle size, and block shrinkage. Journal of E.M. Technique. 2:557-62.

Mollenhauer, H.H. 1986. Secrets of successful embedding, sectioning and imaging. In: Ultrastructure Techniques for Microorganisms. Aldrich, H.C. and William, J.T. edd. Plenum Press, N.Y.

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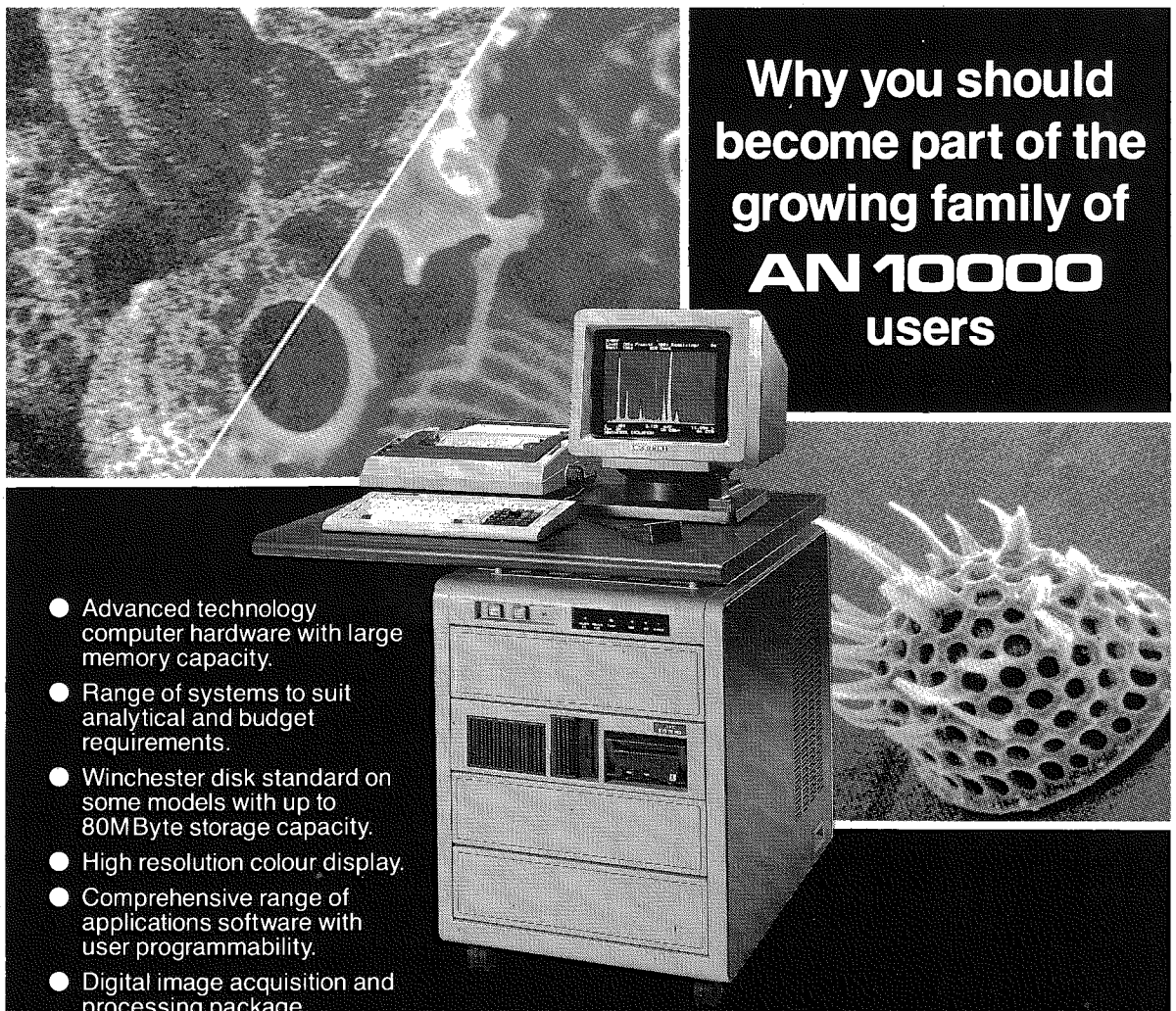

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Abstracts

BIOLOGICAL SCIENCES

PLATFORM PRESENTATION — FALL 1987

VIRUS-LIKE PARTICLES IN THE MANDIBULAR ORGAN OF THE SPIDER CRAB, LIBINIA EMARGINATA Ernest F. Couch, Dept. Biology, Texas Christian University, Fort Worth, TX 76129

Viral-like particles have been observed in the mandibular organ of the spider crab. These particles were found in all animals examined. The viral-like particles were usually confined to a cytoplasmic vacuole which might represent a form of autophagic vacuole. Although the particles appeared in every gland examined and in nearly every cell within the gland most cells did not show any pathogenic effects from their presence. However, some of the cells were heavily infected and appeared to suffer damage. These cells exhibited mitochondria with swollen cristae and other cytoplasmic alterations. In some cases the vacuole containing the viruses was electron lucent and presumably was in an early stage of development. Other vacuoles were very electron dense and may represent a more mature stage of development. Some of the particles appeared to be hollow while others had dense cores. This may represent different stages in the development of the virus. Included within the presumed autophagic vacuole were filamentous structures presumably derived from cytomembranes trapped by the vacuole. Also, frequently one or more lipid droplets would be present. Since the nucleus has never been found to be involved in the development of these particles it is presumed that they must represent an RNA virus. Similar viral particles have been found in other crustaceans. In some they have been found to be pathological. However, it has not yet been determined if these virus-like particles cause harm to the spider crab.

MONOCLONAL ANTIBODIES AND THE OTOLITHIC MEMBRANE OF THE CHICK EMBRYO. C.D. FERMIN, A.E. LOVETT, M. IGARASHI and K. DUNNER, Dept. of Otorhinolaryngology and Communicative Sciences, Baylor College of Medicine. Houston, Texas 77030.

In an effort to characterize the otolithic membrane of the chick (Gallus domesticus), we used monoclonal antibodies known to react with glycoproteins, because the organic matrix of the membrane resembles extracellular coats. Embryos and hatchling chicks were used. Immunoperoxidase and fluorescence were used to show fibronectin, laminin, collagen IV, and calmodulin in the membrane. Modifications to standard published techniques were used. Fibronectin-like immunoreactivity was found surrounding the statoconia in lower layer of the membrane, to a lesser degree in the subcupular zone and a scattered reaction around the statoconia in the middle and the upper layers of the membrane. Laminin was found mainly in the subcupular area and to a lesser degree around the statoconia of the lower layer. Collagen IV was found as a strong precipitate around the apical cytoplasm of hair cells and supporting cells and as a weak precipitate in the subcupular zone. Calmodulin was found mainly in the subcupular zone of young embryos. We have run one and two dimensional gels in which glycoproteins and several protein bands considered inherent to the organic phase of the membrane were identified. The data collected thus far allowed us to speculate that the otolithic membrane shares ultrastructural immunocytochemical and biochemical properties with extracellular coats, glycoproteins and glycosaminoglycans. We are now trying to determine which components change in an attempt to understand critical periods of development. (Supported by NINCDS grants 22604, 10940, NASA grant NAG2- 342 and DRF).

TERMINAL MITOSIS IN THE SENSORY EPITHELIA OF THE CHICK INNER EAR. C.D. FERMIN, A. ENGE, M. IGARASHI, and J.CRAWFORD. Dept. of Otorhinolaryngology and Communicative Sciences, Baylor College of Medicine, Houston, Texas 77030.

Cytodifferentiation and synaptogesis of the inner ear sensory epithelia follow terminal mitosis, which was demonstrated by tritiated amino acids. Long and short term incubation of the isotope were used. We prefer short term incubation because it yields less background in relation to strong activity in the dividing nuclei. Determining which area of the epithelia develops first tells us indirectly which group of cells will mature first. In the chick cochlea it has been shown that incorporation of ^{3}H-thymidine lasts longer in the distal and proximal areas of the cochlea respectively than in the central area. The differences were significant ($p \leq 0.005$). The results indicated that the cochlea developed bidirectionally from a mid-basal location and helped to explain the changes (shift) of frequency during the onset of audition which for a long time were considered to be contradictory. In the vestibule we have analyzed the gradients of the utricle and saccule and determined that there were as many or more mitosing cells in the saccule than in the utricle of the same embryo despite the fact that the saccule is about three times smaller than the utricle. As in the mouse (determined by others), the saccule may constitute an active growing zone between the auditory and the vestibular organ demarking the original point where the pars superior and pars inferior of the early otocyst originally grew from. On the other hand, the saccule may mature precociously and thus, mitose first. (Supported by NINCDS grant 22604, 10940, NASA NAG2-347

A COMPARISON OF HEXADECANE UPTAKE BY ACINETOBACTER ClW USING CONVENTIONAL AND CRYOSUBSTITUTION FIXATIONS. D.W. MARTIN AND J.R. STEWART, Dept. Biology, University of Texas at Tyler, Tyler, Texas 75701.

Acinetobacter spp. are nonmobile, gram-negative coccobacilli found commonly in both soil and water. Acinetobacters are obligate aerobes and they metabolize the hydrocarbons of crude-oil as their sole carbon and energy source. Hexadecane-grown cells of ClW, a new strain, were shown previously to take up hexadecane unaltered and to store it in ultramembrane- (monolayer membrane) bounded bodies. Using conventional fixation procedures, extensive outer membrane evagination and cell membrane invagination were involved in the internalization process; mesosomes were clearly shown to enlarge and become engorged with hydrocarbon. Using cryosubstitution, hexadecane was visible in the periplasm and as ultramembrane-bounded cytoplasmic enclusion; mesosomes were not observed, however, in hexadecane-grown cells or control cells. Pleomorphism was observed commonly in hexadecane-grown cells regardless of the fixation procedure.

THE ULTRASTRUCTURE OF A CHLORELLA-LIKE ALGA. L.T. MOBLEY. Dept. Biology, University of Texas at Tyler, Tyler, Texas 75701

An algal contaminant was isolated from a basal salts medium that had been autoclaved. Based on pigment analysis, plastid organization, and stored photosynthate, it is a member of the division Chlorophycophyta. Because it is a nonmotile, unicellular organism with a cup-shaped chloroplast, it is a member of the genus Chlorella, which species is yet to be determined. The average cell length is 8.7 µm. Cell walls are cellulosic based on histochemical staining (I_2KI) and oil reserves are visible at the light and electron microscopic levels.

CYTODIFFERENTIATION OF THE CHICK VESTIBULAR EPITHELIA AND HISTOCHEMISTRY OF THE OTOLITHIC MEMBRANE. C.D. FERMIN, K. DUNNER, M. IGARASHI and A.E. LOVETT. Dept. of Otorhinolaryngology and Communicative Sciences, Baylor College of Medicine, Houston, Texas 77030.

We are determining the developmental gradients of the maculae and ampullae (angular and gravitational detectors) of the inner ear with TEM histochemistry in chick embryos and hatchlings. We showed last year that the epithelia of the utricle and saccule transformed from a homogenous layer of cells at about stage 15 to a pseudostratified epithelia by stage 25. We now show that cytodifferentiation and synaptogenesis of hair cells and may occur later than reported before. We will be corroborating these observations with monoclonal antibodies and colloidal gold to proteins in the walls of the synaptic vesicles. We also want to determine if a correspondence between maturation of the utricular and saccular maculae and the otolithic membrane (OM) exists. To accomplish this we have utilized immunocytochemical and biochemical techniques (accompanying abstract) and histochemical staining of the OM with anionic and cationic stains reacted with different structural components of glycosaminoglycans. Alcian blue yielded a smooth and even precipitate over the statoconia and the organic substance (OS) cementing them together. Tannic acid stained the central core, the subcupular zone and the OS between statoconia. Ruthenium red stained well the OS between and in statoconia but only faintly in the central core. These patterns indicated that at least three moieties existed in the organic matrix. Biochemical assays of the membrane have yielded at least six components. Statoconia genesis may precede synaptogenesis, but statoconia maturation may not. (Supported by NINCDS grants 22604, 10940, NASA NAG2-342 and DRF).

A SCANNING ELECTRON MICROSCOPE STUDY OF PNEUMONYSSUS SIMICOLA. G. B. FRANK, W. I. BUTCHER, H. DAVIS. Veterinary Pathology Branch, USAF School of Aerospace Medicine, Brooks AFB Texas 78235-5301

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

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

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

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

PHYLOGENETIC TRENDS IN SEED COAT CHARACTERS OF DESMANTHUS SPECIES. LOUIS H. BRAGG, Dept. of Biology, University of Texas at Arlington, Arlington, TX 76019, and MELISSA LUCKOW, Dept. of Botany, University of Texas at Austin, Austin, TX 78712.

Twenty seven populations of Desmanthus, representing nineteen species and three varieties, were examined with SEM for differences in characteristics that might reveal relationships between and among species. Differences in pleurogram shapes, seed coat topographies, and hypodermal features were found.

THE URTICATING HAIRS OF TWO SPECIES OF JUMPING SPIDERS. John P. Nelson, Jr., Daniel R. Formanowicz, Jr. and Edmund D. Brodie, Jr., Department of Biology, The University of Texas at Arlington, Arlington, TX 76019.

We examined two species of jumping spider (Phidippus apacheanus and P. audax; Salticidae) from the infraorder of spiders Araneomorphae for the presence of urticating hairs. Urticating hairs were found on the dorsal abdomen of both sexes as well as both species of these spiders. These hairs correspond to Type II urticating hairs previously described only in tarantulas (Mygalomorphae: Theraphosidae). The urticating hairs of P. apacheanus and P. audax were numerous along the periphery of the abdomen, decreased in number towards the center, and appeared to be attached directly to the exoskelton by a well defined socket.

STRUCTURAL STATES IN THE Z BAND CORRELATE WITH STATES OF ACTIVE AND PASSIVE TENSION IN SKELETAL MUSCLE. M.A. GOLDSTEIN, L.H. MICHAEL, J.P. SCHROETER, and R.L. SASS. Dept. Medicine, Section of Cardiovascular Sciences, Baylor College of Medicine, Houston, TX 77030.

The Z band in skeletal muscle has two distinct structural states-a relaxed (small square or ss) form and a maximally activated (basket weave or bw) form. Changes in two sets of Z band filaments are responsible for these two structural states. Z bands and A bands in relaxed, tetanized, stretched, and stretched-and-tetanized rat soleus muscles have been examined by electron microscopy and optical diffraction. In relaxed muscle, the A band spacing decreases with increasing load and sarcomere length, but the Z lattice remains in the ss form and the Z spacing changes slightly. In tetanized muscle at sarcomere lengths up to 2.7μm, the Z lattice assumes the larger bw form. Active, but not passive, tension is sufficient to cause this Z lattice change.

COMPARISON OF ACTIVE AND PASSIVE IRIDOPHORES IN SQUID. KAY M. Cooper and Roger T. Hanlon, Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77550.

The dermis of the bay squid Lolliguncula brevis contains both physiologically active iridophores and physiologically passive iridophores. Active iridophores in the dermis of the dorsal mantle change reversibly from non-iridescent to iridescent. This change is induced in vitro by 10^{-7} -10^{-6} M acetylcholine (ACh), with a measurable increase in reflectivity upon treatment of the active cells with the putative neurotransmitter. The change in optical properties is correlated with a striking change in the ultrastructure of platelets within the cells. Active iridophores in a non-iridescent state have platelets that contain material having flocculent to ribbon-like ultrastructure, while iridophores in an iridescent state have platelets with material of uniform ultrastructure (Cooper & Hanlon, J.exp.Biol. 121:451, 1986). Conversely, passive iridophores located in the dermis of the ventral mantle have an overall pearl-like appearance that does not vary in vivo. Application of ACh to passive iridophores does not result in a change in optical properties of the cells nor in a measurable increase in reflectivity. There is little change in the ultrastructure of the platelet material of passive iridophores upon ACh treatment. The majority of material within the platelets is uniform ultrastructure, both before and after immersion in ACh. The difference in inducible ultrastructural change between active and passive iridophores supports the hypothesis that the basis of activity in physiologically active squid iridophores is the lability of the material within the active iridophore platelets.

SYNAPTONEMAL COMPLEX RECONSTRUCTION FOR KARYOTYPING ACHLYA RECURVA. H.R. Williamson and J.T. Ellzey. Department of Biological Sciences, University of Texas at El Paso, El Paso, Texas 79968.

The karyotype for Achlya recurva was established by Win-Tin and Dick (1975), using light microscopy, and the haploid chromosome number for Achlya recurva is said to be n=6. Since the light microscopic methods for karyotyping have been questioned for organisms with small nuclei, electron microscopy and synaptonemal complex reconstruction are necessary to accurately count all the chromosomes in Achlya spp.. Cultures of Achlya recurva have been synchronized and fixed for electron microscopy. Serial sections have been taken and photographed on the Zeiss EM-10A. The synaptonemal complexes in these photographs were traced and analyzed using an HP Vectra with a digitizer and the "Metamorphosis" program to create a three dimensional image of meiotic nuclei with synaptonemal complexes. The reconstruction is being utilized to determine the correct karyotype for the fungus. Further investigations concerning the synaptonemal complex within the Oomycetes are in progress.

SATELLITE CELL INVOLVEMENT IN EXERCISE-INDUCED INCREASES IN MUSCLE MASS IN CATS. C.J. Giddings and W.J. Gonyea, University of Texas Health Science Center, Dallas, TX 75235.

Exercise-induced increases in muscle mass result from enlargement of existing muscle fibers (hypertrophy) and from increases in muscle fiber number (hyperplasia). The mechanisms involved in these processes are not known. The role of satellite cells in exercise-induced increases in muscle mass was investigated in seven adult cats, which performed weight-lifting exercise for an average of 194 wks. Trained and control wrist flexor muscles were perfused with glutaraldehyde. Muscle fiber bundles were teased from each muscle and embedded in Epon. Fiber areas were measured using a Summagraphics digitizing tablet. All myonuclei and satellite cell nuclei were counted in cross-sections using electron microscopy. Satellite cell frequency and myonuclei per unit area were calculated. Three cats received tritiated thymidine intravenously before sacrifice for autoradiographic studies. Training resulted in a mean 16.8% increase in muscle mass (range 0-67%). Trained muscles possessed both larger and smaller fibers than were found in control muscles. Myonuclei/unit area and satellite cell frequency were not different from control muscles. Labeled satellite cells were observed in autoradiograms from trained muscle. Weight-lifting exercise induces satellite cell activation and division. Satellite cells may fuse with muscle fibers undergoing hypertrophy providing a source of additional myonuclei; in addition, satellite cells may leave the muscle fiber basal lamina and participate in new fiber formation.

CRYPTOSPORIDIOSIS IN A CALIFORNIA KINGSNAKE (LAMPROPELTIS GETULUS CALIFORNIAE). L. V. STRIBLING, W. I. BUTCHER, H. DAVIS. Veterinary Pathology Branch, USAF School of Aerospace Medicine, Brooks AFB Texas 78235-5301

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

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

ELECTRON MICROSCOPY OF LIPID DEPOSITION IN CYCLOSPORINE-TREATED MICE BEARING CARDIAC ALLOGRAFTS. K. SHAFFER, H. Higley, B. Mathews and J. Trentin. Baylor College of Medicine, Houston, Texas 77030.

Cyclosporine-A (CYA) resistant chronic cardiac allograft rejection takes the form of an accelerated atherosclerotic process. Whether the pathologic mechanism results from a continuing low level inflammatory process that is atherogenic or involves an alteration of lipid metabolism by the immunosuppressive agent itself is unknown. Subcutaneous cardiac allografts in the high plasma cholesterol (HPC) mouse strain, maintained by daily CYA treatment, were examined for lipid deposition at both the light and electron microscopic level and compared to results obtained from allografted normal strains with low plasma cholesterol levels. Successful immunosuppression was monitored by visual examination of heart pulsitility. Frozen sections of allografts from CYA treated HPC mice contained an increased amount of oil red O positive material during the first and second weeks of engraftment. Rises were also seen in serum cholesterol after two weeks of CYA treatment. Glutaraldehyde-fixed samples were stained by the osmium-thiocarbohydrazide-osmium (OTO) method for lipid and examined by electron microscopy. Increased amounts of electron dense lipid vacuoles were seen in both intra- and extra- cellular compartments, i.e., within mononuclear cells, fibroblasts and cardiac myocytes as well as on interstitial and perivascular connective tissue fibers. CYA-A treated normal mice bearing pulsatile allografts and Vehicle-treated HPCs with rejecting allografts exhibited fewer of these morphologic changes. Supported by PHS Grant No. HL34186.

QUANTITATIVE DIGITAL X-RAY IMAGING APPLIED TO FROZEN HYDRATED TISSUE SECTIONS

A.J. SAUBERMANN, AND R.V. HEYMAN
Microprobe Center, University of Texas Health Science Center, Houston, Texas 77030

Using our algorithm for x-ray analysis of frozen hydrated then dried cryosection, we have developed a method for quantitative digital x-ray imaging of biologically relevant elements as both dry weight and wet weight concentrations as well as images of cell water. Images are acquired using an AMRay 1400T SEM with a Tracor Northern 2000 EDS system attached to a PDP 11/23, at 1.4 nA probe current, 20 keV, and 4 sec dwell time. Data is transferred to an IBM AT/PC for image display and data retrieval. Each pixel is fully background corrected and has a precision and accuracy which is equivalent to conventional dwell times. Images (64x64 pixels) provide a combination of information on elemental location and amount, in essence extending the use of x-ray analysis to provide unbiased quantitative elemental anatomic information. Because each pixel is quantitative, data retrieval and visual elemental comparisons are possible. Such combinations of morphological and analytical information provide an intuitively understandable conceptualization of elemental concentrations as they relate to each other and to morphological structure.

ENERGY-DISPERSIVE ANALYSES OF TISSUES PREPARED BY CRYOFIXATION AND MOLECULAR DISTILLATION DRYING. IRENE PISCOPO, Philips Electronic Instruments, Mahwah, NJ; DRS. STEPHEN LIVESEY AND JOHN LINNER, Cryobiology Laboratory, University of Texas Health Science Center at Houston, The Woodlands, TX

Parotid and liver tissues were processed by cryofixation and molecular distillation drying. Following drying, the tissues were processed by two different methods: 1) the dry sample was exposed to osmium vapors and vacuum infiltrated with pure Spurr resin at room temperature and polymerized at 60°C; 2) the dry tissue was held at -30°C in vacuum, infiltrated with pure K_4M resin and polymerized with ultraviolet light at -30°C.

The tissues were cut wet and dry. Energy-dispersive analyses were done to see if embedding media or cutting conditions have an effect on retention of elements. Distinct differences were found with different resin and sectioning procedures.

MATERIALS SCIENCES

POSTER PRESENTATION — FALL 1987

INNOVATIVE MICROSCOPY WITH VIDEO RECORDING. J.G. Bigham and T.G. Morris, Electronics Quality Assurance Failure Verification Laboratory, General Dynamics/Fort Worth Division, Fort Worth, Tx. 76101.

Failure analysis documentation has been greatly improved through the use of video recording. In an electronics/metallurgical analysis requiring extensive detailed images, a montage of 4x5 Polaroids^{tm} has been used, a very expensive, tedious, and time consuming process. A video tape is a more graphic, easily presentable, and less costly method. It allows zooming in for higher magnification without the loss of orientation. The video cassette recorder is easily attached to the supplemental video output connection of the Scanning Electron Microscope. Audio capabilities can be added for narration. The price of film compared to the cost of a tape shows considerable savings. In addition, a tape is reusable if first efforts are unsuitable or not needed for permanent records. A video cassette recorder is a valuable asset to the Scanning Electron Microscope laboratory.

BIOLOGICAL SCIENCES

POSTER PRESENTATION — FALL 1987

CORRELATION OF GLUCOSE CONCENTRATION AND SPORE VOLUME IN BACILLUS THURINGIENSIS VAR. KURSTAKI. MICHAEL C. PACE, ALAN J. NEUMANN, AND GARY R. WILSON, Dept. of Biology, Texas A&M University, College Station TX 77843

Bacillus thuringiensis var. kurstaki is a gram positive rod that forms an insecticidal protein crystal during sporulation. Previous studies indicate crystal volume can be modified by altering the glucose content of the growth medium. Growth medium modifications can also change spore heat resistance and other spore properties. This study probes the correlation between the amount of glucose included in nutrient growth medium and average spore volume. Spores were grown in media containing 0,1,5 and ten grams of glucose per liter (w/v) of nutrient agar respectively. Samples of cleaned spore and crystal preparations were stained with uranyl acetate, applied to Formvar^{R} coated grids and allowed to air dry. T.E.M. studies were performed to obtain accurate measurements which were used to calculate mean spore volumes for samples obtained from each type of medium.

INTERACTIONS OF EPITHELIAL AND STROMAL CELLS IN VAGINAL ADENOSIS. DK Roberts, NJ Walker, TH Parmley, Dept. OB/GYN, Wesley Med Ctr, Univ KS Sch Med-Wichita, KS 67214.

Epitheliam-stromal cell interactions are known to be important in epithelial cell morphogenesis in the embryo. Vaginal Adenosis can be viewed as a circumstance in which embryonic epithelial differentiation takes place in the adult. Therefore, we sought ultrastructural evidence of epithelial-stromal cell interactions in vaginal adenosis. Two main types of ultrastructural interactions that might facilitate communication between epithelial and stromal cells were observed. In the first type, epithelial cells extended cytoplasmic processes through the lamina densa and made direct contact with cells in the stromal compartment. In the second type, cells with the cytoplasmic granules characteristic of mast cells appeared to act as intermediaries between the epithelial and stromal compartment.
We conclude from this that as vaginal adenosis undergoes squamous metaplasia and is eventually transformed into a stratified squamous epithelium, epithelial-stromal interactions are involved.

OVERLAPPING LYTIC AND ENDOCYTIC PATHWAYS IN PLANTS. R.D. RECORD and L.R. GRIFFING, Dept. Biology, Texas A&M University, College Station, TX 77842.

A histochemical study of acid phosphatases (AcPase) in protoplasts (from soybean suspension culture SB1, Glycine max L.) reveals AcPase activity in ER, Golgi cisternae, multivesicular endosomes (MVE), and some vacuoles, indicating the involvement of these organelles in the lytic pathway. Using cationized ferritin (CF) as a marker for endocytosis, overlap between the endocytic and lytic pathways is determined. This is made particularly clear by using cerium "trapping" of the AcPase reaction product. The use of cerium is an improvement over conventional lead-based visualization of AcPase in plants since very little reaction product diffuses out of the organelles into the cytoplasmic matrix, i.e. the reaction product is "trapped". All four possible combinations of CF label and AcPase activity are seen in MVE, i.e. both CF and AcPase, CF alone, AcPase alone, and no CF or AcPase. CF and AcPase activity infrequently coexist in the same region of Golgi cisternae. Large central vacuoles contain vesicles with morphology and labeling (AcPase and CF) similar to the intralumenal vesicles of MVE, (M.A. Tanchak and L.C. Fowke, 1987, Protoplasma, in press). It is implied that the MVE fuse with vacuoles and deposit their intralumenal vesicles in the vacuoles. These observations support the notion that mature plant vacuoles are analogous to animal lysosomes. Analysis of the development of the different labeling patterns will require further study of the dynamics of the endocytic and the lytic pathways.

PROTEIN A-GOLD LOCALIZATION OF TYPE IV COLLAGEN IN GASTRIC MUCOSA. Kathryn B. Smith and Karmen L. Schmidt, Dept. Path., University of Texas Health Science Center, Houston, TX 77030.

Localization of type IV collagen in the gastric mucosa by the protein A-gold technique was best obtained with mild phosphate-buffered paraformaldehyde fixation and low-temperature embedding in Lowicryl. Rats were sacrificed by aortal perfusion, following which the gastric lumen was injected with either phosphate-buffered 1% paraformaldehyde or half-strength Karnovsky's fixative (Karnovsky, M.J., 1965, J. Cell Biol. 27: 137A-138A) with 0.02% picric acid in phosphate or cacodylate buffers. Stomach tissue was processed for EM and then embedded in LRWhite or Lowicryl K4M. The LR White was polymerized either at 45°C or at room temperature under ultraviolet light, and the Lowicryl at -25°C under longwave UV light. Thin sections were placed on butvar-coated nickel grids, and incubated with anti-type IV collagen (courtesy of Dr. Hynda Kleinman, NIH) followed by protein-A gold (EY laboratories).

Although the ultrastructural preservation was not optimal, antigenic specificity was best demonstrated with the 1% paraformaldehyde fixation. Tissue embedded in Lowicryl produced more specific binding to type IV collagen than tissue prepared in LRWhite. For LRWhite, polymerization by ultraviolet light preserved antigenicity better than heat polymerization and phosphate buffer was preferable to cacodylate buffer. These data further confirm previous reports using other tissues that the antigenicity of collagen proteins is extremely sensitive to fixation and embedding procedures (Laurie, G.W., et al., 1980, J. Histochem. Cytochem. 28: 1267-1274; Stephens, H., et al., 1982, 44:81-84). (Supported by NIH Grant AA 06887 to Dr. K. Schmidt.)

RAPID METHOD OF DETECTING SPERM ANTIBODIES BY ELECTRON MICROSCOPY. Cameron E. McCoy, JoAnn Culpepper and William B. McCombs, Scott & White Memorial Hospital, Scott & White Clinic, Texas A&M University College of Medicine, Temple, Texas 76508.

Colloidal gold labelled antibodies to human IgG and IgA were used to detect sperm antibodies in infertility patients using a rapid negative staining technique. Washed sperm were reacted with the labelled antibody in solution, allowed to dry on formvar coated grids, and stained with phosphotungstic acid. Ultrastructural localization of antibodies to surface sites of the acrosome and tail was compared with other antibody detection methods.

Two different mechanisms may be involved in the immunopathology of infertility: (a) an immunoglobulin deposit induced locally in the testis, (b) a systemically induced immunoglobulin directed against spermatazoal antigens by the female or the male (auto-antibodies). Most tests have been developed to detect the second type.

An immunobead assay for sperm antibodies has found acceptance in many clinical laboratories as a reproducible test. Bio-Rad beads coated with antiserum to human immunoglobulin form rosettes around sperm with attached surface antibody. This is a semi-quantitative though somewhat subjective test. By this method, bound IgG or IgA on sperm has correlated with other parameters of infertility.

We suggest the use of colloidal gold techniques in addition to immunobeads to further study the sperm surface sites affected by antibody. Preliminary work indicates some specificity and sensitivity because antibodies have not been found on normal sperm and have been detected on only a select population of infertility patients.

MORPHOLOGIC CHARACTERISTICS OF NEONATAL AND DEVELOPING RABBIT COLON. A. B. O'Connor, L. T. Garretson, G.D. Potter, K.L. Schmidt. University of Texas Medical School, Houston, TX 77030.

We have shown recently that exposure of neonatal rabbit distal colon to the bile acid, taurodeoxycholate (TDCA), fails to elicit a secretory effect although the adult response is profound (Amer. J. Physiol., in press). The basis for this difference was sought using light and electron microscopy. Distal colons from 1, 5, 8, 11 and 15 day post-partum and adult New Zealand white rabbits were sampled and prepared for microscopy At day one, mucosa, submucosa, muscularis externa and serosa were identified. Villi were absent. Average mucosal height was 125 μm. Short, sparse crypts were embedded in the lamina propria. Undifferentiated columnar, vacuolated and goblet cells lined the crypts and goblet cells and colonocytes formed the surface epithelium. All cell types were mature except colonocytes, which were short and united by shallow junctional complexes. No changes were noted in 5 day colons except colonocyte microvilli were taller. By 8 days, the colonocytes were more elongated and bore taller microvilli. At 11 days, all mucosal layers were well developed. Crypts were longer and more tubular. Goblet cells were more numerous. The colonocyte glycocalyx was more dense and the junctional complex appeared fully mature. By 15 days, the mean mucosal height had increased to 175 μm. Crypts were deeper and exhibited branches. Using special stains, semiquantitation of mucosal mast cells was performed. Few or no mast cells were identified until 15 days post-partum. These data indicate that the difference in secretory response to TDCA between neonatal and adult colon may lie in morphological characteristics of the paracellular pathway. (supported by NIH Grant DK37260)

BINDING OF KLEBSIELLA TO TRACHEAL EPITHELIAL CELLS: SPECIFICITY OF PILIATED BACTERIA BINDING TO CILIATED CELLS IN VITRO. Robert E. Fader, Robert A. Cox, Virginia A. Pellegrini, Peter C. Moller, and David G. Ritchie. Shriners Burns Institute and Univ. of Texas Medical Branch, Galveston, Texas 77550.

Enzymatically dissociated hamster tracheal epitheal cells, when seeded onto collagen coated Millicel filters, will differentiate into functional ciliated and secretory cells within 5 days. These cultures consist of columnar ciliated and mucus cells which are closely associated with squamous basal epithelial cells. We report here on the use of these cultured HTE cells to demonstrate both the specificity of Klebsiella binding to tracheal epithelial cells and the importance of pili to this process. Pili are bacterial surface appendages that have been reported to promote bacterial adherence to mucosal epithelial cells. Piliated and nonpiliated phase bacteria ($1x10^8$ bacteria/ml) were incubated with HTE cells for 1 hour at 37°C. The wells were rinsed 5X with PBS to remove nonadherent organisms, and the tissue was fixed and prepared for SEM viewing. Twenty ciliated and nonciliated fields were examined at 3.67 KX magnification and the organisms adhering to the HTE cells were quantitated. Results indicated that piliated phase organisms adhered preferentially to ciliated cells. Nonpiliated organisms were essentially nonadherent. The results of this study suggest that pili serve as the adhesin for Klebsiella colonizing the trachea and that pili-cilia interaction is a necessary prerequisite for colonization. Supported by grant #15876 from Shriners of North America.

LOCALIZATION OF LIPOSOMES IN LIVER, LUNG, AND KIDNEY BY FREEZE FRACTURE (FF) AND TRANSMISSION ELECTRON MICROSCOPY (TEM). P.A. Mitchell, K.L. Schmidt, J. Lautersztain, A.R. Khokhar, R. Perez-Soler. The University of Texas Medical School, M.D. Anderson Hospital, Houston, TX 77030.

Liposomes or multilamellar vesicles (MLV) have shown promise for the treatment of some forms of cancer as carriers of lipophilic chemotherapeutic agents, including cis-bis- neodecanoate 1,2 R,R diaminocyclohexane (NDDP). While mice bearing gross liver metastases of M5076 reticulosarcoma have been reported to survive up to 40 days longer when treated with MLV plus NDDP as compared to NDDP alone, the mechanism of action is unclear. The cellular localization of MLV in mouse liver, lungs, and kidneys was studied by FF and TEM to delineate possible mechanisms. MLV were produced from a 7:3 molar ratio of dimyristoylphosphatidyl choline and dimyristoylphosphatidyl glycerol, with or without NDDP, and injected into normal CD1 Swiss mice or M5076 reticulosarcoma-bearing C57BL 6 mice. No differences in cellular distribution were noted between MLV with or without NDDP. At 5 min, MLV were observed within hepatocytes, Kupffer cells, endothelial cells, and the sinusoids of both normal and tumor-bearing liver, as well as the tumor cells of the latter. The distribution at 2 hrs after injection was similar to that at 5 min, with possible signs of MLV decomposition. Uptake appeared to be by endocytosis. The FF micrographs of the kidney indicate that MLV are able to cross the basal lamina surrounding the capillaries and enter the tubular cells. In the lungs, MLV were not observed within types I or II pneumocytes at either time period, although they were present in the endothelial cells and lumen of the capillaries. These results suggest that MLV may directly interact with the parenchyma of liver and kidney, and that the fenestrated endothelium may allow this access. Direct observation of MLV transfer, however, remains elusive. (Supported by Public Health Service Grants RR-5511-23 and PHS CA- 41581)

TERMINAL NERVE PROFILES AND THEIR DISTRIBUTION IN THE CORNEAL EPITHELIUM. Y.S. Blocker & J.P.G. Bergmanson, University of Houston, Institute for Contact Lens Research, Houston, Tx 77004

Previous ultrastructural studies of the innervation of the cornea have provided conflicting evidence as to whether nerves penetrate the anterior limiting lamina (Bowman's membrane) and if fibers project more superficially than the basal cells.[1,2] The present study was conducted to confirm or disprove the existence of the rami perforans to the epithelium. In addition we examined how close to the epithelial surface axons reach and further explored the pattern of the nerve fibers in the central cornea of the rabbit, cat and primate. In all species examined, it is found that the rami perforans is a very infrequent feature of the central cornea and that most of the fibers of the richly innervated epithelium gain access to this layer more peripherally to travel to the corneal apex. The great majority of epithelial nerve fibers terminate parallel and close to the epithelial basement membrane, and only sporadically a single axon will reach the more superficial layers of the epithelium.

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ULTRASTRUCTURAL LOCALIZATION OF THE NITRATE-SENSITIVE H^+-ATPASE. NANCY F. MCGEE[1], STEPHEN K. RANDALL[2], HEVEN SZE[2], AND LAWRENCE R. GRIFFING[1], Dept. of Biology, Texas A&M University, College Station, TX 77843; Dept. of Botany, Univ. Maryland, College Park, MD 20742.

The nitrate-sensitive ATPase has been localized in young oat roots using immunocytochemistry. Polyclonal antibodies to the holoenzyme and the 72 kDa (catalytic) subunit were used for on-grid immunolocalization using protein A-colloidal gold. The predominant location of the gold particles varied depending on the distance of the cells from the root tip. In a single young cell (within 1 mm from the tip), only a few of the many vacuoles showed labelling. Label at the wall/plasma membrane interface was present but low. In slightly older cells within the first mm, vacuole label became more pronounced, but again restricted to a subpopulation of the vacuoles. Label at the plasma membrane/wall interface was pronounced at cell corners, being more frequent on the longitudinal walls than on the transverse walls. Older cells had more uniform vacuolar label which was frequently associated with internal membranes. Less label was found associated with vesicles in close proximity to the Golgi apparatus. The specificity of the label and implications for vacuole ontogeny will be discussed.

THE DISTRIBUTION OF PEROXISOMES IN NORMAL AND ALCOHOLIC RAT LIVERS. DELFINA C. DOMINGUEZ AND JOANNE T. ELLZEY. Dept. of Biological Sciences The University of Texas at El Paso, El Paso, Tx. 79968.

Peroxisomes were reported for the first time at the electron microscope level in mouse kidney (Rhodin, 1954). In 1966, De Duve and coworkers, demonstrated that these organelles were rich in catalase and hydrogen peroxide-producing oxidases in rat liver. Peroxisomes are ubiquitous organelles in eukaryotic cells. They show similar morphology and enzymatic composition in all tissues. Despite extensive research on peroxisomes and their enzymatic composition, little is known about their role in metabolic processes in animal cells. Recent experimental work suggests an important involvement in lipid metabolism, and changes in number and enzymatic content are characteristic of certain diseases. In most pathologic processes affecting hepatocytes, the morphology or structure of peroxisomes is altered, therefore, if a metabolic or pathologic process affects the liver a possible involvement of peroxisomes must be considered. Morphometric analysis of peroxisomes identified by the cytochemical localization of catalase with 3,3'-diaminobenzidine are in progress.

RENAL AND LIVER LESIONS IN CHICKENS FED AFLATOXIN. H.H. Mollenhauer, D.E. Corrier, W.E. Huff, L.F. Kubena, R.B. Harvey, R.E. Droleskey. Veterinary Toxicology and Entomology Research Laboratory, USDA-ARS, P.O. Drawer GE, College Station, TX 77841.

Male broiler chicks were given feed containing 0, 1.25, 2.5, and 5.0 ppm aflatoxin/g feed. The chicks were killed, and samples of liver and kidney were taken for electron microscopy on days 3, 6, 9, 17, and 21. No changes were observed at 1.25 ppm aflatoxin in either liver or kidney. Changes were observed in kidney at 2.5 ppm and in liver and kidney at 5.0 ppm aflatoxin. In liver, the primary changes were accumulation of lipid vesicles in hepatocytes, enlargement of bile canaliculi, reduction in mitochondrial size, mild lymphocytic infiltration and mild hepatocellular degeneration. In kidney, the most consistent lesions were thickening of glomerular basement membranes, loss of some epithelial cell foot processes, and loss of pseudopodia from the surfaces of endothelial cells that border the basement membranes. No fusion of foot processes was observed and no fibrous material was present in the basement membranes. Additionally, variable degenerative changes were observed in cells of the proximal convoluted tubules. Both liver and kidney changes increased progressively through the sampling period of 21 days. The responses that we observed in liver are consistent with those of other investigators. Aflatoxin is primarily a liver toxin, and no reports of ultrastructural renal damage in chicks are available. However, the glomerular changes we observed are consistent with the reduction of glomerular filtration rates reported in mammals.

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What Is It?

Answer from Page 13

This micrograph shows the cut edge of an oleander (Nerium oleander) leaf. An interesting feature of this plant is the presence of "stomatal crypts" in the lower surfaces of the leaves. The stomata are found within the crypts which are in turn filled with epidermal hairs. These appear to be adaptations to increase the humidity of the microenvironment around the stomata and decrease water loss due to transpiration.

Oleander is a large, poisonous shrub native to the Mediterranean region and often grown in the United States as an ornamental. It is a member of the family Apocynaceae which includes Periwinkle, Dogbane, and Frangipani, a fragrant flower used to make Hawaiian leis.

Micrograph by R.W. Davis, Dept. of Medical Anatomy, Texas A&M University, College Station, Texas.

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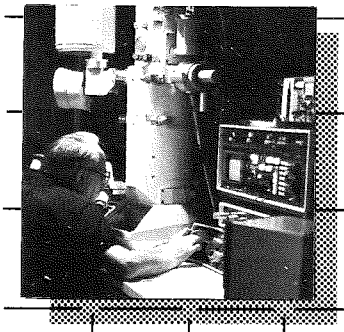

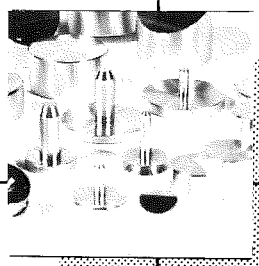

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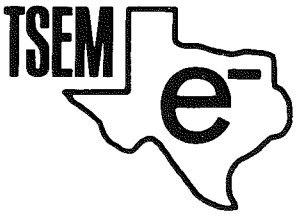

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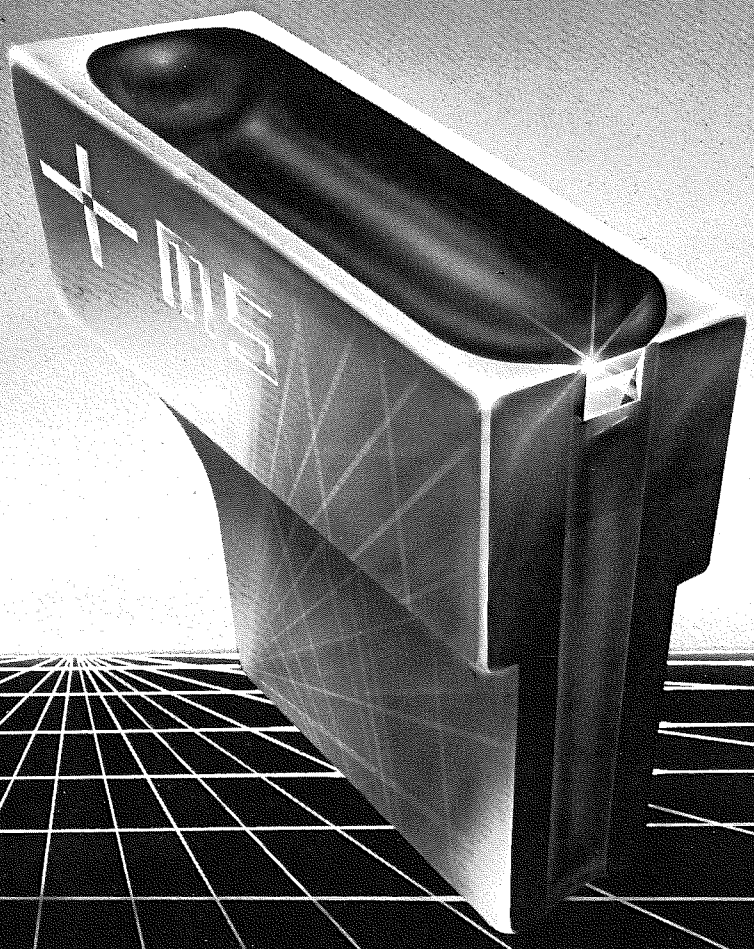

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