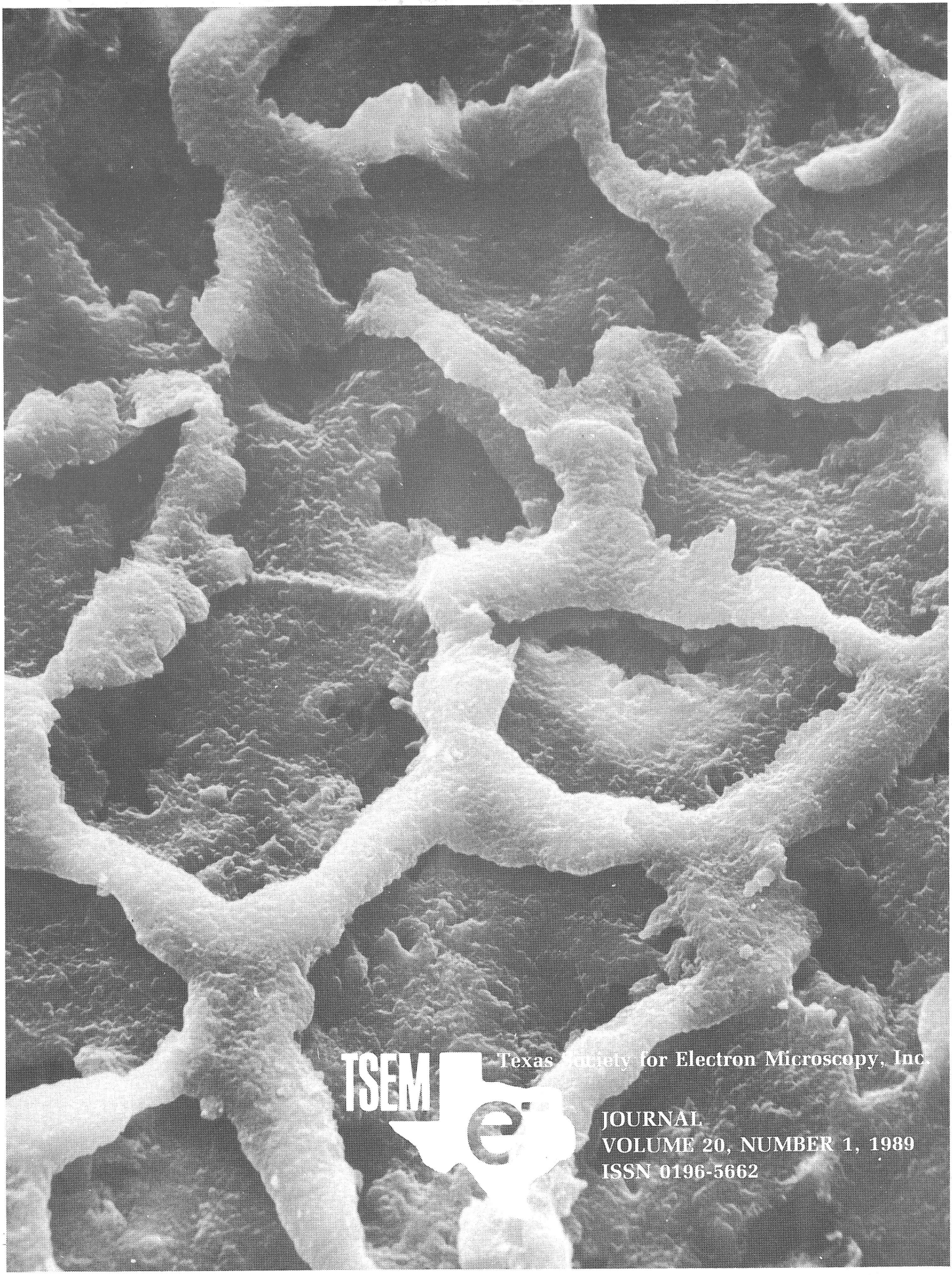

TSEM

Texas Society for Electron Microscopy, Inc.

JOURNAL
VOLUME 20, NUMBER 1, 1989
ISSN 0196-5662

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TEXAS SOCIETY FOR ELECTRON MICROSCOPY
JOURNAL
VOLUME 20, NUMBER 1, 1989
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."

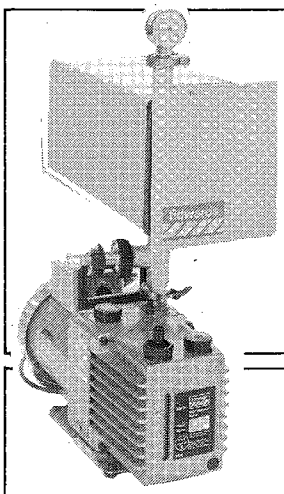
ON THE COVER

Scanning electron micrograph of the inside surface of an isolated cuticle from a tomato fruit. The cuticle is formed from an epoxy-like material called cutin. It forms a protective layer on the outside surfaces of plant organs. For this micrograph the underlying tissue was chemically removed leaving only a cutin mold of the epidermal cells.

The cuticle and an overlying layer of wax helps protect plants from desiccation and infection by pathogens.

Micrograph by R.W. Davis, Department of Medical Anatomy, Texas A&M University, College Station, Texas. (Magnification approximately 2500X.)

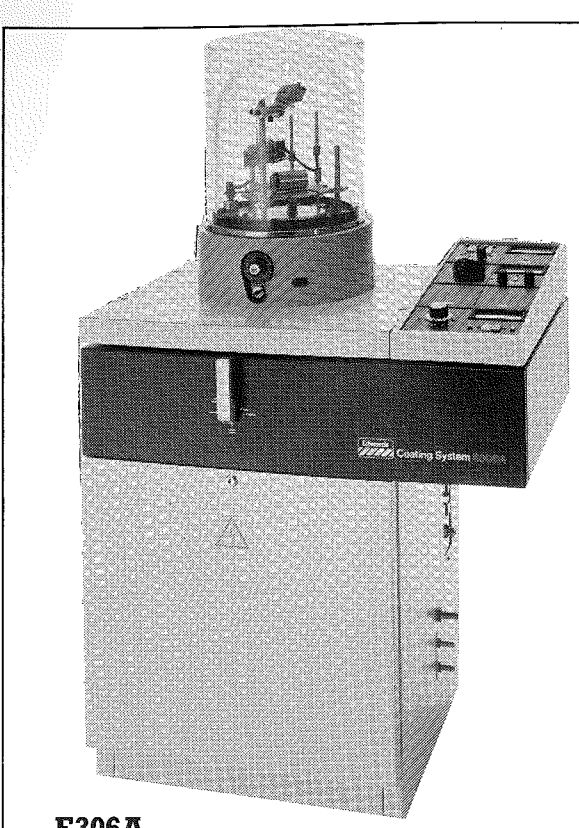
Buyers Guide to EM specimen preparation



PD3

Plate Degasser

For degassing photographic films and plates prior to EM use. Chamber can be mounted horizontally or vertically above vacuum pump. More than one chamber can be used for increased capacity.



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ETD4

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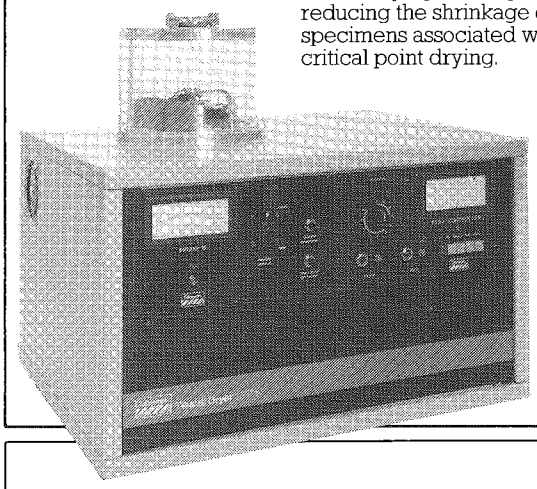

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President's Message

It always has been a pleasure to associate with the members of TSEM, but the last year has been especially interesting because I have been able to see many sides of our Society. Certainly, as in all societies, there are problems (the tax status, agitation for Bandera as a permanent meeting site, etc.), but TSEM still stacks up as the "best little EM Society in the USA." Over the years, our membership has maintained itself: unfortunately, as we gain new members, we seem to lose presidents to other states (Ward Kischer, Randy Moore, Charles Mims, Jerry Berlin, to mention a few). Twice a year our members organize meetings in which national *quality* presentations are given. During these meetings, our members have the opportunity to exchange views, techniques, talk science, and have a good time. Additionally, the Society publishes a journal and all for dues on only $10.00 per year. Quite a bargain!

Now, in all seriousness, I would like to discuss the journal, our meetings, and the membership.

The *Texas Society for Electron Microscopy Journal* deserves our support. As presently constituted, it publishes fully refereed articles, notes on techniques; funny, different or unusual EM pictures; data on the Society (membership list, financial status of Society, etc.); and advertisements. For most members there are, therefore, outlets for three types of contributions (articles, notes, micrographs). Unfortunately, most of us are not taking advantage of these possibilities. I encourage you to increase your submissions. The editor cannot produce a quality journal without your help. There are people who think a journal with a regional title cannot make it, but I believe we can prove them wrong. Look at the *New England Journal of Medicine!*

TSEM meetings. There has never been a society in which the members are more sympathetic toward presentations. Whenever you make a presentation, the members are encouraging, helpful, and eager to share your knowledge. It is not necessarily so in all meetings. There are meetings where the participants would rather tear you up than learn what you have to say. A sympathetic audience makes an outstanding opportunity for graduate students and technicians as well as professors to learn and practice the art of scientific presentations. TSEM members have been aware of this for some time, and they have developed a means to encourage student participation by providing travel and lodging support, prizes, etc. In the long run, however, it is senior researchers to whom the Society must encourage to bring their students to our meetings. In my experience the quality of the TSEM presentations are equal to or better on the average than those presented at most national meetings. We may be a local society, but our standards, as they should be, are at a national level.

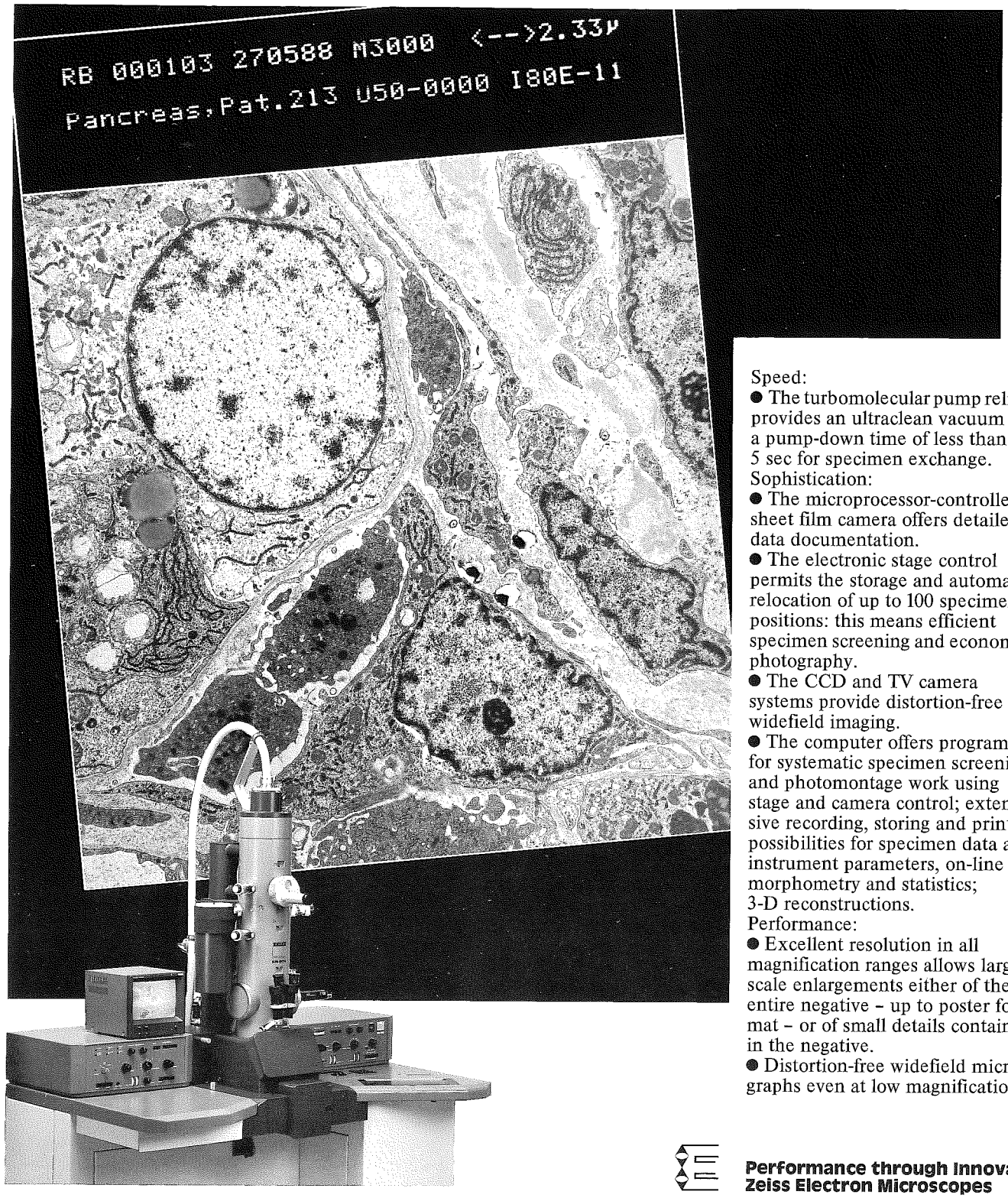
The relatively small size of our meetings has some advantages that I think we could consider using more effectively. "Small" means we can much more easily experiment. For example, we can run experimental presentations, presentations not patterned after the usual platform or poster presentations of other societies. We could organize micro-symposia, micro-review papers, round-table discussions, technique demonstrations, analysis demonstrations, etc. By expanding the concept of our presentations, TSEM meetings potentially can be of much more value to the participants. Let me give a few examples. "User-organized micro-symposium": this means two or three people get together and organize themselves and others to give a "micro-symposium" on a particular subject of mutual interest. The micro-symposium might only contain three or four papers involving both original and review data, but it would serve to pull together research ties among our members. In another example, a person might ask three colleagues to develop a round-table discussion on the questions of "how to determine where Ca^{2+} and/or NA^{+} ions actually are located in a certain kind of cell or tissue." With audience participation, they could brainstorm the problems and outline both the difficulties and rewards of a particular avenue of research. Meetings should not just report on research — they should encourage *future* research. In a technique-rich field like electron microscopy, just discovering how to perform a specific technique may offer tremendous rewards. All of us cannot travel to other laboratories, but authors could present papers or paper/demonstrations devoted to understanding techniques. Two or three labs can participate in showing how they differ or agree on specific techniques. For example, "rotary shadowing" — some work seems better than others. We need to use our imaginations in thinking about alternate ways to present our materials. Because we are a relatively small organization, we can respond quickly; if one method of presentation does not work, we can try another. The future program officers will have more work using this approach, but the Society stands to gain substantially from such activity.

Finally, I would like to speak about the TSEM membership. We estimate that there are two to three times as many people using electron microscopy in Texas than are in our Society. One glaring weakness in the Society is the lack of participation by people from the physical and materials science aspects of EM users. Certainly, the materials scientists have their own societies, but we suffer from not interacting with them. Many advances in instrumentation and techniques often are developed by these scientists, and the rest of us may wait years to learn about new instrumentation or techniques. For example, how many of you are formulating research in your field which might be answered by the scanning tunneling or atomic force microscopes? Whatever we can do to encourage the crossover of materials and physical scientists to our meetings will benefit the Society in the long run. Every member of the Society should concern themselves with actively recruiting new members for TSEM. Whenever a new member joins, each of us gains, and we gain by the active participation of these new members. So be encouraged! Recruit some new members!

The Society is doing well, but I challenge each of you to do your part toward making it even more successful — successful to the point where TSEM becomes not just "the best *little* EM society in the USA," but "the *best* EM society in the USA!"

Howard J. Arnott, President
Texas Society For Electron Microscopy, Inc.
January 27, 1989

Speed, Sophistication and Performance. The New Zeiss EM 900 Transmission Electron Microscope



Speed:
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Sophistication:
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Treasurer's Report

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For Calendar Year 1988

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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:		
Dallas Meeting Registration & Exhibitor Fees	$ 3,305.00	
Galveston Meeting Registration & Exhibitor Fees	2,820.00	
Individual and Corporate Dues	2,528.00	
Journal Ad Revenue 19:1	2,050.00	
Journal Ad Revenue 19:2	1,500.00	
Donations		
Scien-Tech Services	75.00	
Diatome-U.S.	806.88	
Journal Subscriptions	20.00	
Checking Account Interest	220.01	
Interest Earned on Certificates of Deposit	664.02	$13,988.91
EXPENSES:		
Dallas Meeting Expenses	$ 4,488.83	
Galveston Meeting Expenses	5,153.19	
Student Competition/Travel	440.00	
Meeting Announcement/Program		
Printing and Mailing	1,950.00	
Journal Printing & Postage	4,982.17	
Professional Fees	1,000.00	
Journal Subscription Refunds	60.00	
Checking Account Charge	20.00	
Treasurer's Expenses	126.81	$18,221.00
ASSETS ON 31 DECEMBER, 1988:		
Certificate of Deposit No. 10-7199995	$ 2,440.44	
Certificate of Deposit No. 10-8829764	4,119.98	
Certificate of Deposit No. 111-849-6	3,223.28	
Checking Account No. 015210-01	1,597.19	$11,380.89

TREASURER'S REPORT CONTINUED

TREASURER'S REPORT

For Period Ending 9 February, 1989

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Certificate of Deposit No. 11-8829764	4,119.98	
Certificate of Deposit No. 177576	3,223.28	
Checking Account No. 015210-01	1,597.19	$11,380.89
RECEIPTS:		
Austin Meeting Registration & Exhibitor Fees	$ 65.00	
Individual and Corporate Dues	383.00	
Journal Ad Revenue 19:2	375.00	
Journal Ad Revenue 20:1	1,000.00	
Checking Account Interest	7.45	
Interest Earned on Certificates of Deposit	18.20	$ 1,848.65
EXPENSES:		
Meeting Announcement/Program Printing and Mailing	$ 500.00	
Journal Printing & Postage	25.13	
Treasurer's Expenses	25.00	$ 550.13
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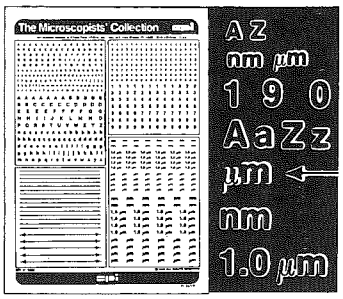

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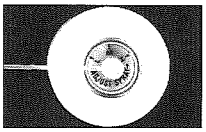

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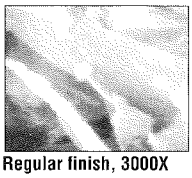
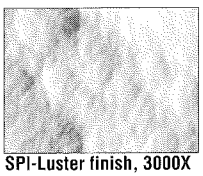

Regular finish, 3000X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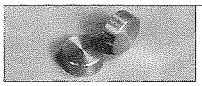

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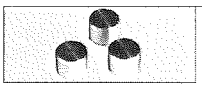

10 mm x 15 mm dia., fits ISI

Regular finish #1504 SPI-Luster #1504L
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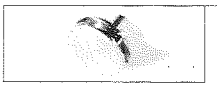

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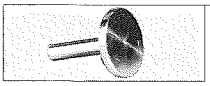

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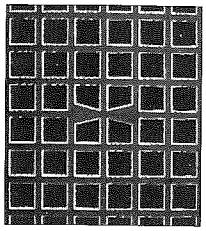
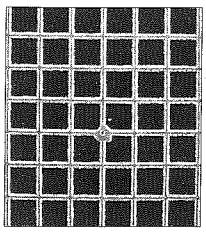


200 mesh regular grid, 50X



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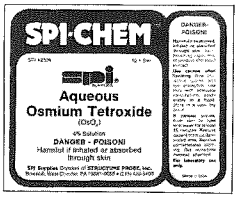

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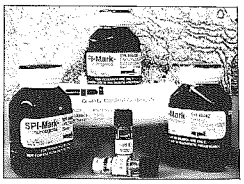

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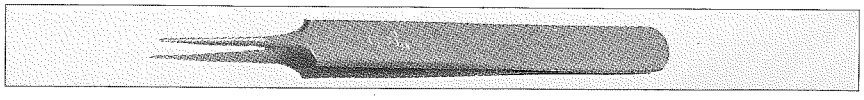

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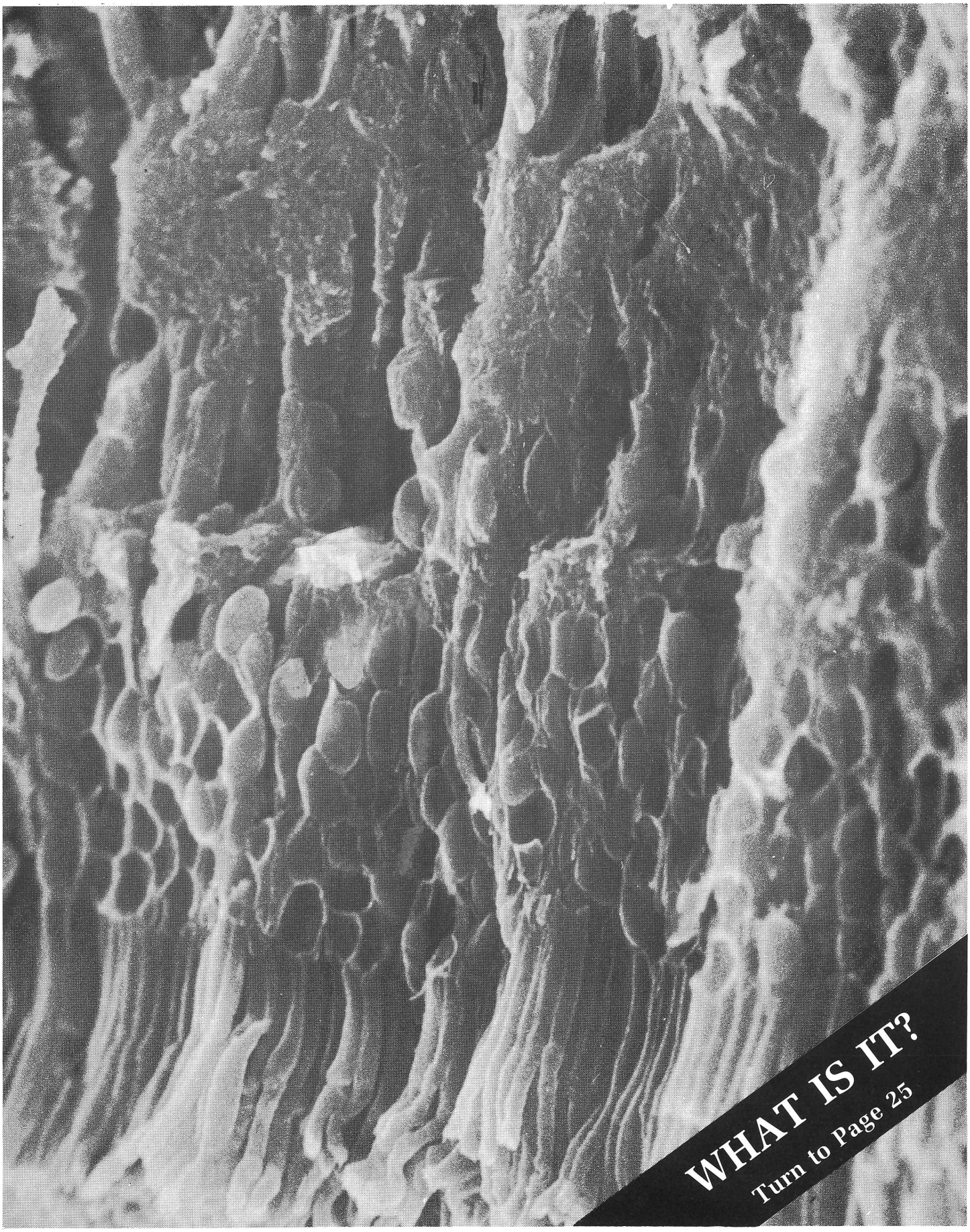
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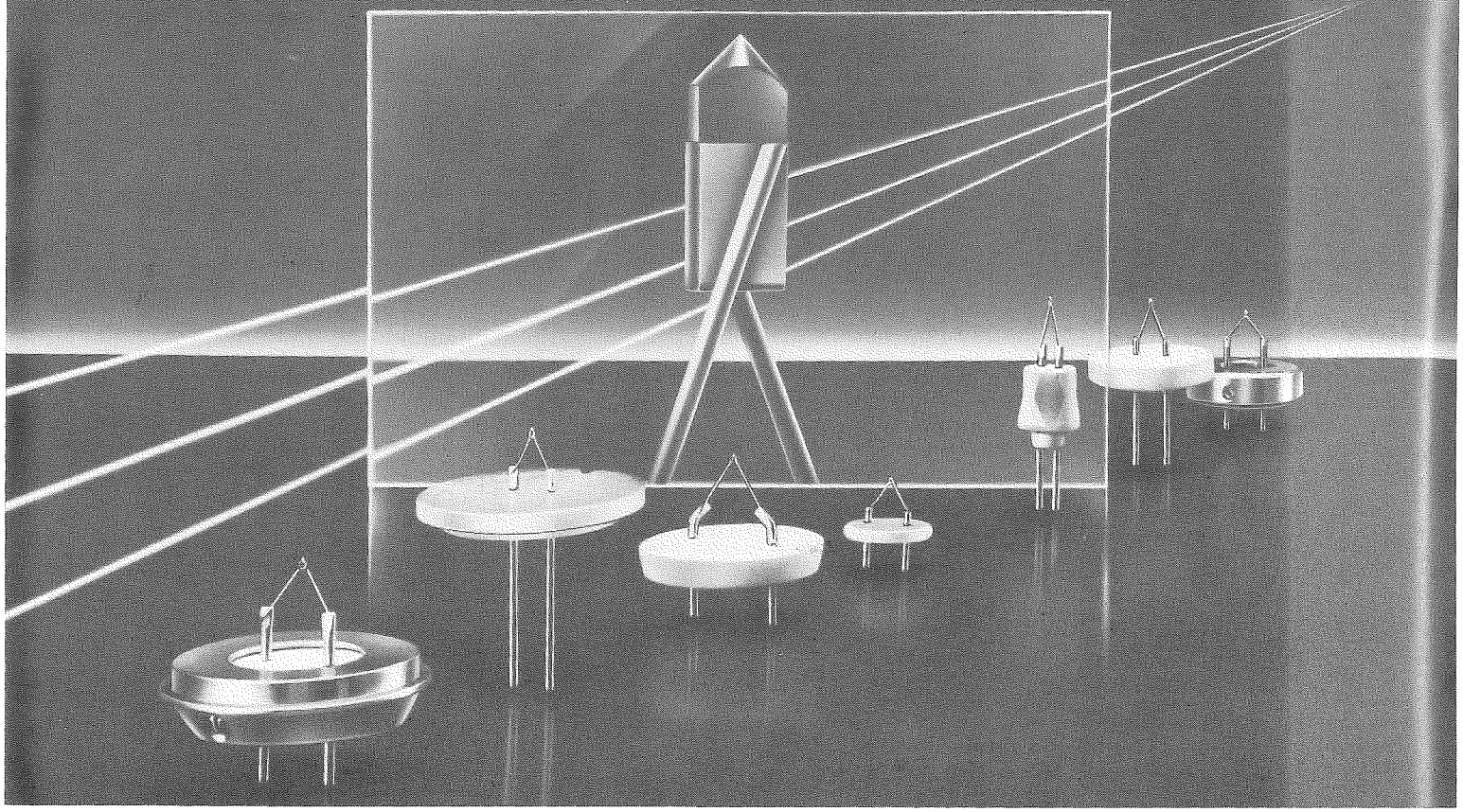

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MORPHOLOGICAL CHANGES IN MAMMALIAN ERYTHROCYTES SUBJECTED TO CONTROLLED HYPOOSMOTIC DIALYSIS AND ULTRAFILTRATION

By

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KEYWORDS: erythrocyte, morphology, scanning electron microscopy.

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ABSTRACT

Scanning electron microscopy was used, for quality control, to examine erythrocytes subjected to controlled hypoosmotic dialysis and ultrafiltration processing for morphological changes. Erythrocytes from three species (cattle, sheep, and human) were examined for morphological changes associated with the hemoglobin isolation process. Each stage of the process produced a characteristic morphological change in the erythrocyte. However, erythrocytes from all species exhibited the same stage-specific morphological changes.

INTRODUCTION

Hemoglobin is the primary, protein component of erythrocytes and one of the most studied mammalian proteins (1-3). One aspect of hemoglobin research which has received vast attention is its potential for use as a blood substitute (3-5). The lack of a simple, efficient, and cost-effective process for isolating large quantities of ultrapure hemoglobin has been a major hinderance in its development as a satisfactory blood substitute for human and veterinary use.

Our research has been concerned with developing a method in which large quantities of ultrapure, lipid-free hemoglobin can be isolated. The method we developed uses controlled hypoosmotic dialysis followed by ultrafiltration. This provides a nondestructive means of opening pores in the erythrocyte membrane, through which hemoglobin can be extracted. The most critical step in this procedure is the hypoosmotic dialysis (6-11). We found that Scanning Electron Microscopy (SEM) allowed us to monitor each step of the process quickly and accurately, thus enabling us to fine tune the hemoglobin isolation process and maximize our success.

METHODS AND MATERIALS

Stage One — Blood Collection and Erythrocyte Isolation

Cattle and sheep whole blood (2-3 liters) was collected by venipuncture into evacuated containers. Heparin (Sigma, St. Louis, MO) at a concentration of 0.1 mg/ml of whole blood was used as an anticoagulant. Heparin was chosen because it is the least morphologically altering anticoagulant available (12). Human blood (2-3 liters) was provided by Dr. Ralph Green of the Cleveland Clinic Foundation; Cleveland, Ohio. It was collected in blood bags which contained 63 ml of a commercially prepared citrate-phosphate-dextrose anticoagulant for every 450 ml of blood.

Red blood cells were isolated by centrifugation at 1000g for 30 minutes. The cell pellet was resuspended in a phosphate buffered saline (ph 7.2 - 7.4, 300 mOsmol/Kg) to the original whole blood volume and recentrifuged four times. The hematocrit of the washed pack cell suspension was then adjusted to 55-60% with wash buffer. After the final wash, aliquots were taken for SEM evaluation.

Stage Two — Hypoosmotic Dialysis

The apparatus used for this process has been previously described (8-11). Briefly it consists of a commercial kidney dialysis unit, three ultrafiltration units (0.1μm pores), a peristaltic pump, a replenishing buffer chamber and a hemoglobin collection vessel. Erythrocytes were dialyzed against 12 liters of hypoosmotic buffer (ph 7.4, 50-100 mOsmol/Kg) at 4° C and recirculated until the optimal osmotic pressure (human - 132 mOsmol/Kg, cattle - 170 mOsmol/Kg, sheep - 204 mOsmol/Kg) was achieved. Aliquots were taken at this point for SEM evaluation.

Stage Three — Ultrafiltration

Dialyzed erythrocytes were circulated through the ultrafiltration portion of the apparatus at 4° C. The volume of the dialyzed Erythrocyte suspension was kept constant by the addition of replenishing buffer with the same osmotic pressure and pH as the cell suspension. The retentate from the ultrafiltration process contained 3 groups of cells, intact red blood cells, pink ghosts, and white ghosts depending on how much hemoglobin remained in the cells. Only the white ghost cells were taken for SEM evaluation.

For comparison cattle erythrocytes were lysed and the resulting stroma or membrane protein lipid aggregate, was collected for SEM evaluation.

SEM — Preparation and Examination

Erythrocytes (0.1 ml) from stage one and two of the process were added to 10 ml of 0.5 - 1.0% glutaraldehyde prepared in wash buffer or replenishing buffer respectively. In order to maintain buffering capacity, in the case of the stage three and stroma samples, the glutaraldehyde concentration was lowered to 0.5% and phosphate buffer was used. The buffer concentration was adjusted to yield a fixative containing 0.5 - 1.0% glutaraldehyde at the same osmotic pressure as the sample. Samples were fixed under constant agitation for 1 hour at room temperature. After fixation, they were refrigerated for short-term storage. Fixed samples were centrifuged and the supernatant was discarded. During all centrifugations the g-force used was kept to the minimum needed to sediment the sample in 8-10 minutes. The sample was resuspended in sodium phosphate buffer of the same osmotic pressure as the original fixative solution. The sample was centrifuged again, the supernatant was discarded, the pellet was resuspended in distilled water, vortexed to assure complete dispersal of the pellet, held at room temperature for 15 minutes and centrifuged. This water wash was repeated a second time, the sample was resuspended in 50% ethanol, vortexed, held at room temperature for 15 minutes, and then centrifuged. The sample pellet was resuspended in 1-2 mls of 75% ethanol and a drop or two of the 75% suspension was further diluted with 75% ethanol to give a monolayer of sample when a drop or two was allowed to air dry on a prepared stub at room temperature (13-16). This is a modification of the technique by Bessis and Weed (17). The dried sample preparation was placed in a sputter coater and gold was applied. Samples were examined using Cambridge-S4 scanning electron microscope operating at 10 KV (13-16).

RESULTS

Erythrocytes from each species studied, responded to the various stages of the hemoglobin isolation process in a similar and characteristic manner.

Stage one erythrocytes from each species generally exhibited normal morphology, although some crenation was observed (Fig. 1a-c). Human and cattle erythrocytes appeared as biconcave discocytes approximately 7.5μm (Fig. 1a) and 5.9μm (Fig. 1b) in diameter respectively; however some crenated erythrocytes were observed in both cases. Sheep erythrocytes also appeared as normal, somewhat irregularly shaped, discocytes of approximately 4.8μm in diameter; some crenation was observed (Fig. 1c).

At the end of stage two, erythrocytes from each species were characterized by a swollen appearance (Fig. 2a-c). The swelling was less uniform among human erythrocytes, with some echinocytes or stomatocytes present (Fig. 2a). Cattle (Fig. 2b) and sheep (Fig. 2c) erythrocytes were more uniformly swollen, but some echinocytes were also evident.

Stage three resulted in the formation of erythrocyte ghosts (pink and white) for all three species. Only white ghost cells were sampled for SEM examination (Fig. 3a-c). Human (Fig. 3a) and cattle (Fig. 3b) white ghost cells appeared as lace textured, flattened disks with ridges and valleys. Sheep, white ghost cells had a crater like appearance, with dense edges, an extremely flattened center, and small pockets of hemoglobin in some cells (Fig. 3c).

Stroma, which is made up of lysed erythrocyte membrane components, appeared as amorphous masses of cross-linked proteinaceous material (Fig. 4).

DISCUSSION

The success of our hemoglobin isolation technique depends on absolute quality control. We must monitor the erythrocytes at each stage of the process to insure that they are being properly manipulated and not damaged. The most reliable method we have found is scanning electron microscopy. The use of scanning electron microscopy for studying morphological changes in erythrocytes is widely accepted (13-21). SEM also allowed us to identify morphological features, such as non-uniform erythrocyte swelling or the presence of stroma, which are key indicators of problems with the process.

By studying the morphology of the erythrocytes at each step of the dialysis/ultrafiltration process, we are able to adjust the procedure and ensure the isolation of an ultrapure hemoglobin preparation, that is lipid and membrane-stroma free. This evaluation can be completed within 24 hours as compared with lipid analysis or High Performance Liquid Chromatography, each of which requires 7-10 days for completion. The speed of analysis and generation of detailed information combine to make SEM a tool well-suited to quality control.

Generally, all erythrocytes from stage one exhibited their normal morphology. However, some crenated erythrocytes were present (Fig. 1a-c). This crenation was probably caused by prolonged exposure to the anticoagulant and/or loss of ATP during storage (3,12). The presence of large numbers of crenated cells indicated an erythrocyte population that was unsuitable for processing because of the unstable

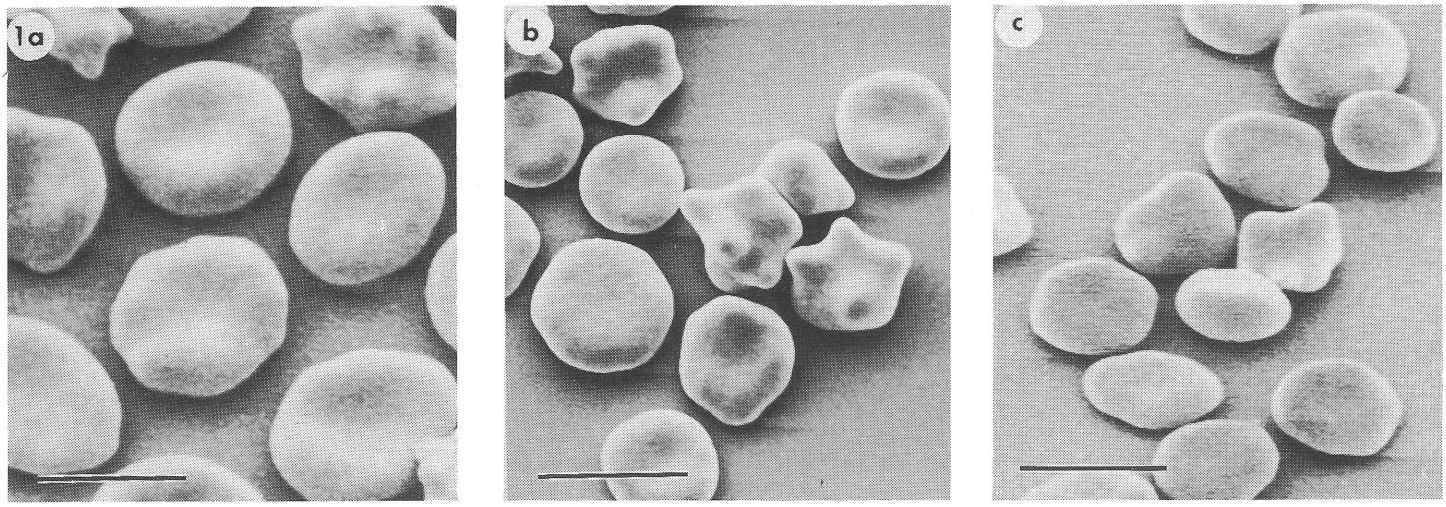

Fig. 1a-c. Human, cattle, and sheep washed erythrocytes. Mag. 3900X.

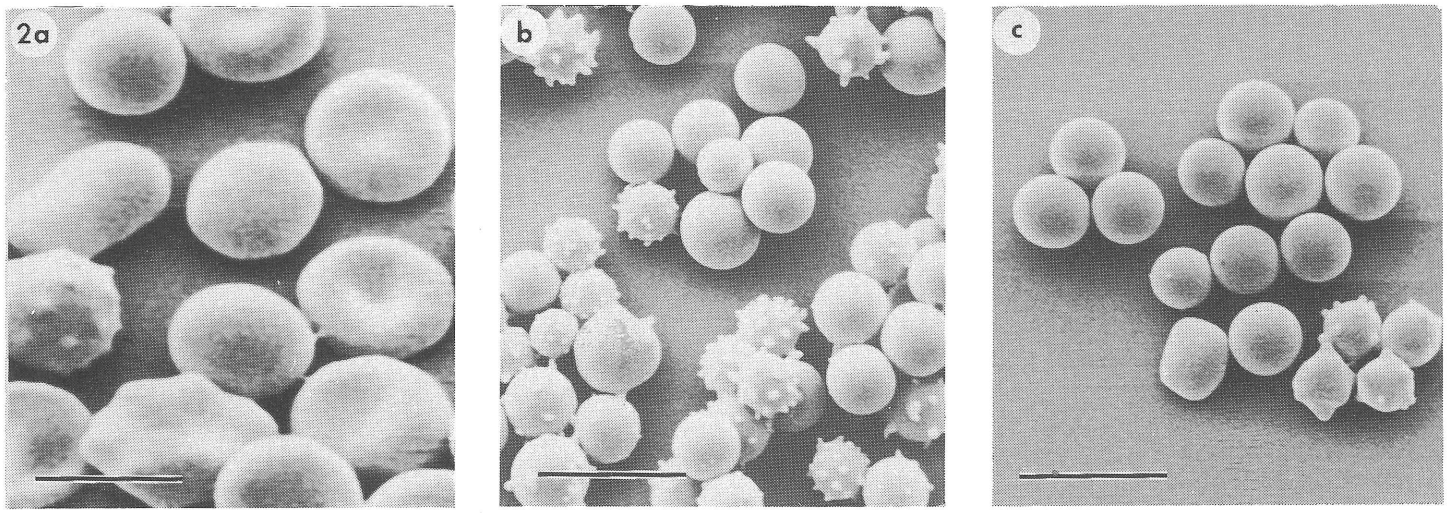

Fig. 2a-c. Human, cattle, and sheep dialyzed erythrocytes. Mag. 3900X.

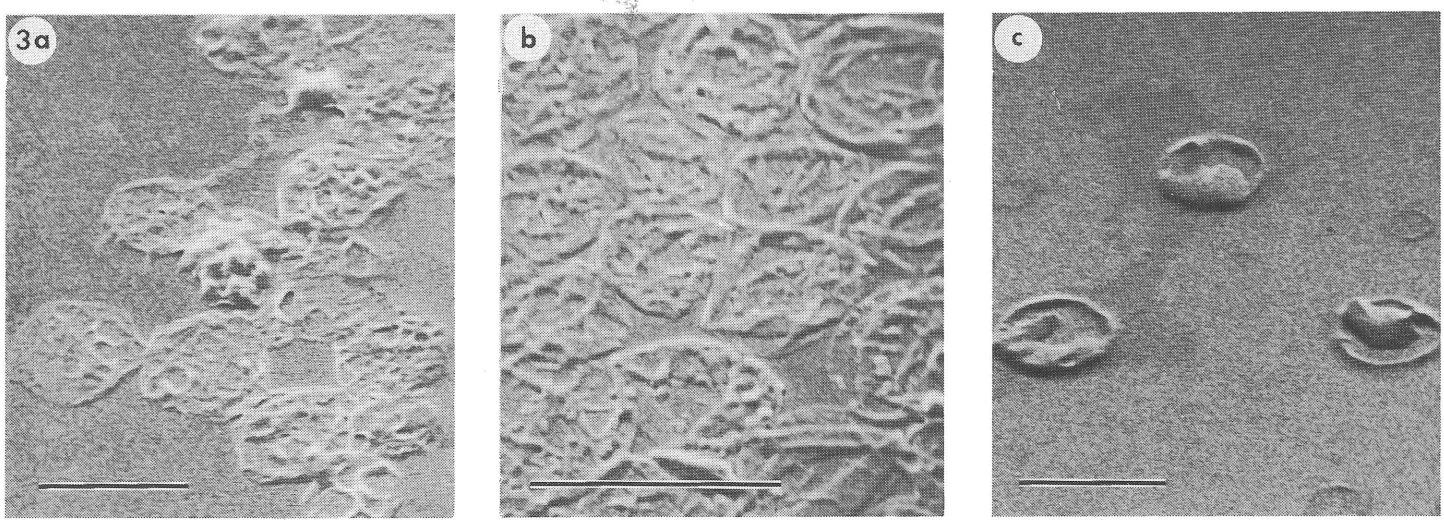

Fig. 3a-c. Human, cattle, and sheep white ghost erythrocytes. 3a,c — Mag. 3900X; 3b — Mag. 6660X.

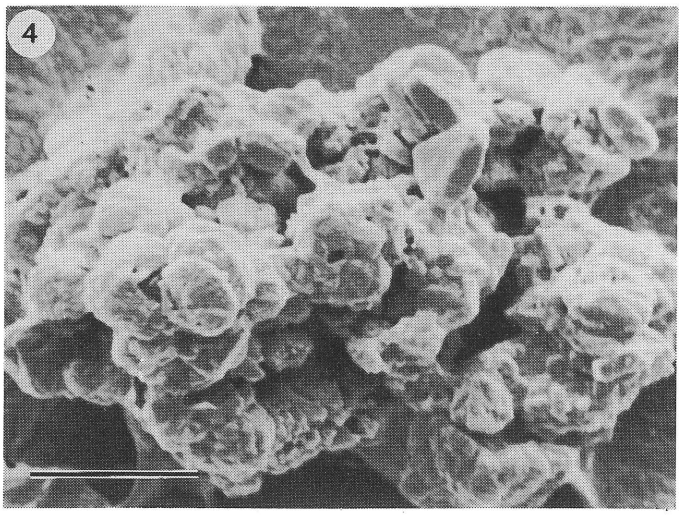

Fig. 4. Stromal material. Mag. 4400X.

nature of their membranes. The erythrocytes from all species showed no signs of membrane stress, other than the crenation mentioned above, at the end of stage one.

Thus, we can be relatively assured that this portion of the process does not alter the normal morphology of the erythrocytes (8-11, 13-18).

During stage two, pores are formed in the membrane of the cells (18). However, these pores cannot be visualized using scanning electron microscopy. Erythrocytes from all three species exhibited a generally swollen appearance however, some other morphologies were also evident (Fig. 2a-c). Some cells did not appear swollen at all, this could result from incomplete dialysis or individual differences in erythrocyte resistance to dialysis. This is somewhat dependent on the age of the erythrocyte, with younger erythrocytes being more resistant (6,7,12). A large population of unswollen erythrocytes could indicate that the cells were dialyzed for an insufficient period of time or that the osmotic pressure of the dialysis solution was not sufficiently low to promote complete dialysis.

The white ghost cells, resulting from the hypoosmotic dialysis and ultrafiltration process, appear to be empty sacks which have collapsed onto themselves. The appearance of various ghost cells differ somewhat according to species (Fig. 3a-c). Human (Fig. 3a) and cattle (Fig. 3b) ghost cells had a diffuse, lacy, central portion surrounded by a thin outer membrane edge. Sheep ghost cells exhibited a quite different morphology than either those of human or cattle. The outer edges of these ghost cells appeared to be thickened, while the central portion was devoid of texture. However, presumptive pockets of hemoglobin may be seen near the edges in some cells. We examined only white ghosts because these cells had been subjected to the most stress and would, therefore, be most sensitive to lysis.

Stromal material (Fig. 4) produced when erythrocytes are lysed can result in contamination of isolated hemoglobin.

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"Student-Technician Award Entries Fall, 1988"

ELECTRON MICROSCOPY OF SINDBIS VIRUS INFECTION IN VERTEBRATE AND INVERTEBRATE CELL CULTURE

By

M.L. Miller and D.T. Brown
Cell Research Institute and Dept. Microbiology
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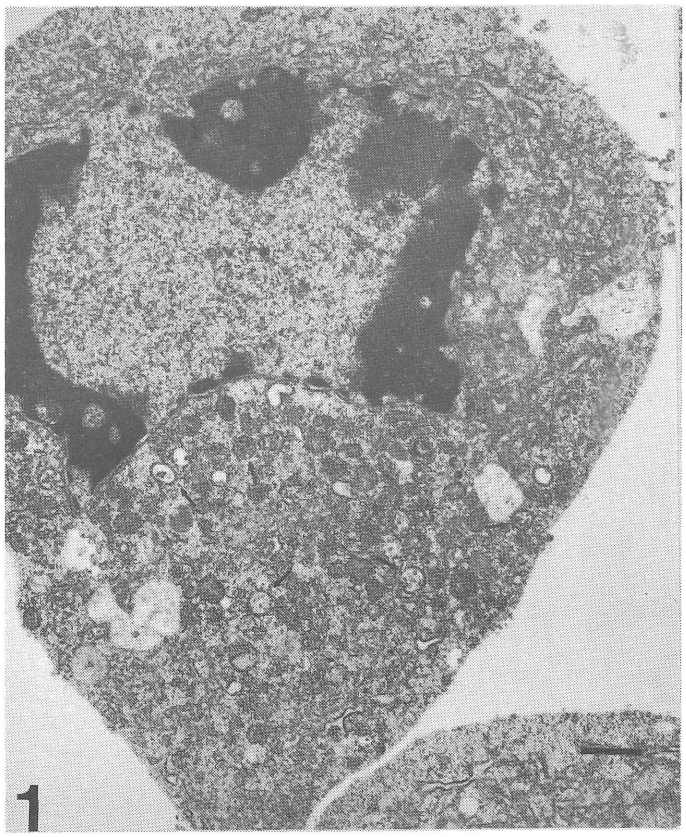
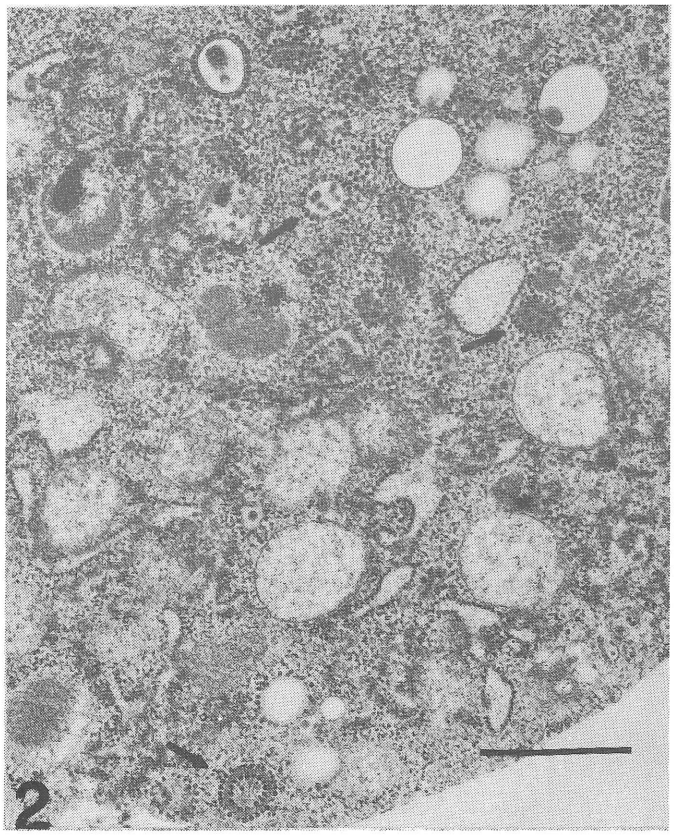
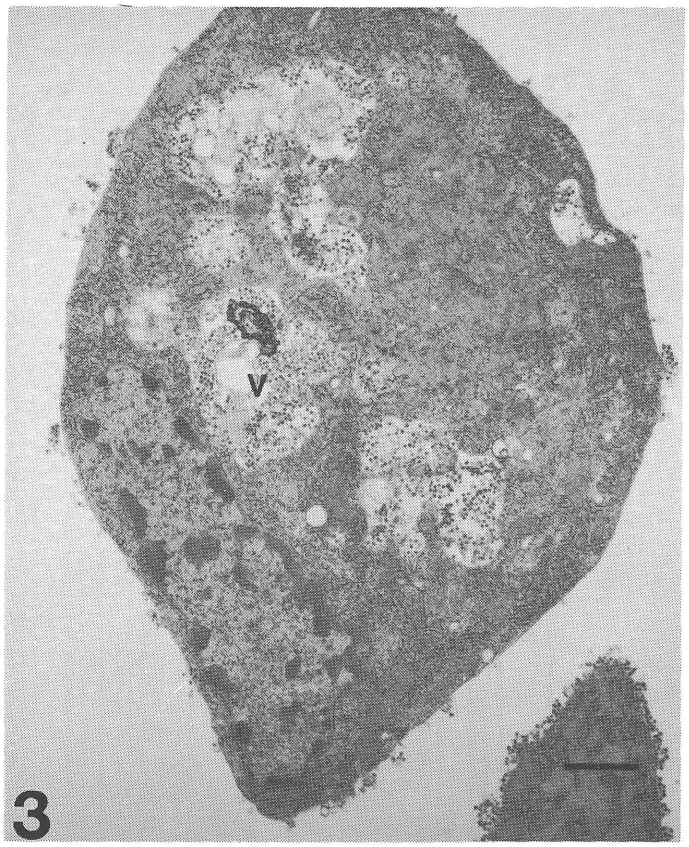
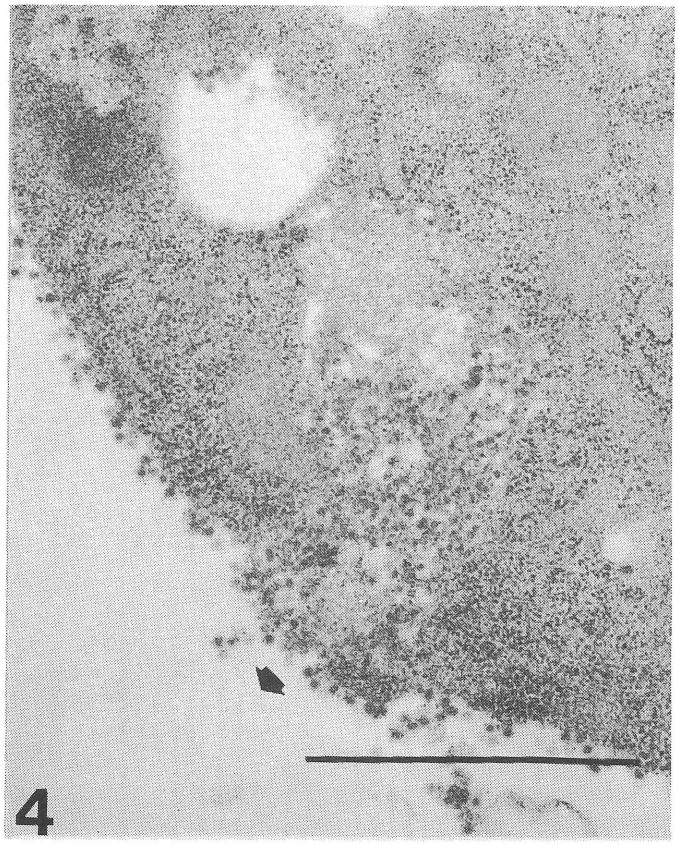
Sindbis virus, the type species of the alphavirus genus, is cycled in nature through insect and animal hosts which include mosquitoes, birds and rodents (1). The virus is also successfully cultivated in the laboratory in vertebrate and invertebrate cultured cells. Infection in either cell type yields equivalent progeny virus and similar production kinetics (2) and virions are identical in infectivity and antigenicity (3,4). However, virus synthesis and processing mechanisms diverge in these two phylogenetically distinct systems. Host protein and RNA synthesis shuts down in vertebrate cells 1.5 to 3.0 hours post-infection with mortality and lysis occurring within 24 hours (5). Infected invertebrate cells do not exhibit shutdown of host functions, instead such populations become persistently infected with no detectable





FIGURES 1 & 2. Sindbis virus infected BHK cells at 12 hours PI. **Fig. 1.** Infection activity appears localized in the cytoplasm beneath the nucleus. Cytopathic vacuoles (arrows) and viral capsids are visible. **Fig. 2.** Enlargement of a region showing capsids dispersed throughout the cytoplasm and different types of cytopathic vacuoles (arrows).





FIGURES 3 & 4. Sindbis virus infected mosquito cells at 25 hours PI. **Fig. 3.** Virions accumulate within vacuoles (V). **Fig. 4.** Release of virus at the cell surface in direction of arrow. (Bar = 1 micron)

aberrations in growth rate or metabolic function (6).

To correlate these biochemical differences with ultrastructural changes, the time course of Sindbis virus infection was contrasted in baby hamster kidney (BHK) and mosquito *(Aedes albopictus)* cell cultures. Cell monolayers were infected with virus at a multiplicity of infection (MOI) of 100 plaque forming units (pfu) per cell or mock infected and incubated at 37° C (BHK) or 28° C (mosquito). At designated times post-infection (PI) cells were processed for routine examination by transmission electron microscopy.

Virus is first seen budding from the plasma membrane of BHK cells at 4 hours PI and a few viral capsids are visible in the cytoplasm. At 6 hours PI, cytopathic vacuoles (7) appear in the cytoplasm and become more numerous over the next 9 hours as greater amounts of virus can be observed budding from the cell surface. By 15 hours PI, BHK cells show obvious cytopathic effect as cells begin to die. Breakdown of the nucleus and cytoplasm is apparent. Extracellular virus is abundant but evidence of intracellular involvement in the infection is diminished. Most cells have disintegrated by 36 hours PI.

In contrast, limited budding of virus from the mosquito cell plasma membrane is detectable by 6 hours PI. Both infected and mock infected cells contain numerous vacuoles at all time points. Viruses appear to collect within these structures around 10 to 12 hours PI and virus packed vesicles are visible by 20 to 25 hours PI. Release of virions from the cell appears to result primarily by exocytosis of these vesicles. Such observations suggest that compartmentalization of virus multiplication in insect cells protects them against the cytopathic effects observed in animal cells.

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ELECTRON MICROSCOPY OF THE LIPID-RICH CORE REGION OF HUMAN ATHEROSCLEROTIC PLAQUES, UTILIZING OSMIUM-TANNIC ACID-PARAPHENYLENEDIAMINE (OTAP) AND OSMIUM-THIOCARBOHYDRAZIDE-OSMIUM (OTO) TECHNIQUES

By

Keith F. Klemp and John R. Guyton
Departments of Medicine and Cell Biology
Baylor University of Medicine
Houston, Texas 77030

Of the three major extracellular lipid phases found in human atherosclerotic plaques only crystalline cholesterol monohydrate is adequately identified by routine electron microscopy. Neutral lipid droplets and membranous vesicles are poorly distinguished. An osmium-enhancing mordant techinque developed in our laboratory allows examination of all three forms simultaneously.

Tannic acid after osmication greatly enhances membranes (Simionescu 1976) and paraphenylenediamine retains neutral lipid very effectively (Ledingham 1972). Our new technique uses these agents in combination after osmication to demonstrate vesicles (tannic acid) and droplets (paraphenylenediamine). The OTO procedure (Seligman 1966), which reveals surface defects as pits, is a perfect complement to the OTAP method.

Human aortic fibrous plaques from autopsies were used in developing the OTAP method. The procedure is as follows: overnight fixation in 3% glutaraldehyde in .1 M cacodylate buffer, pH 7.4, at 4°C, buffer wash (all subsequent steps at room temperature), 1% OsO_4 in .1 M cacodylate buffer for 120-150 minutes, three

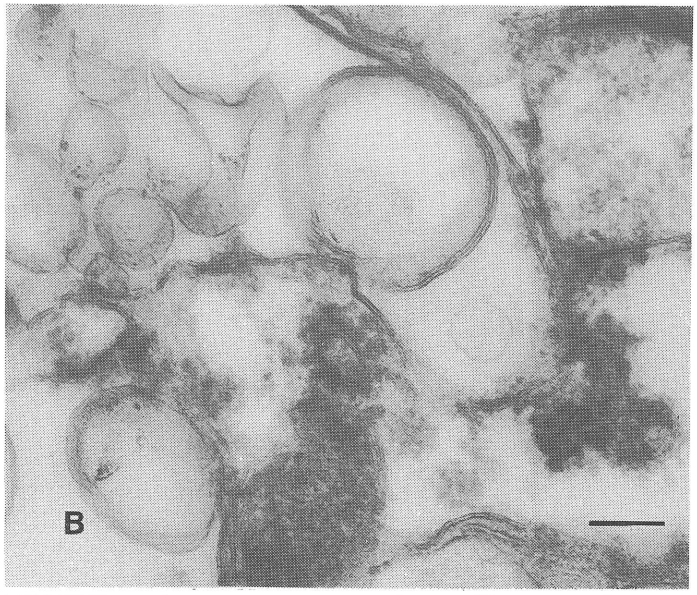

FIGURE B. Osmium-tannic acid, excellent membrane definition but poor retention of nuetral lipid. 99,700X, Bar = 100nm.

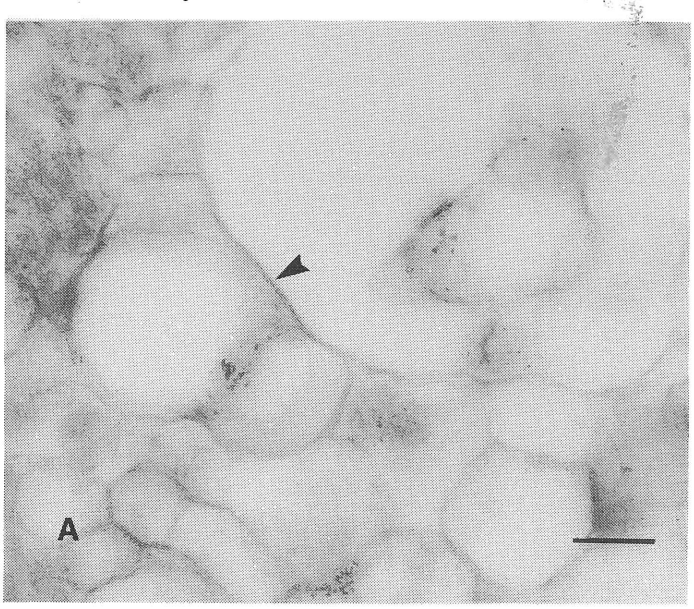

FIGURE A. Routine EM, poor lipid retention and poorly defined membranes (arrow). 72,500X, Bar = 150nm.

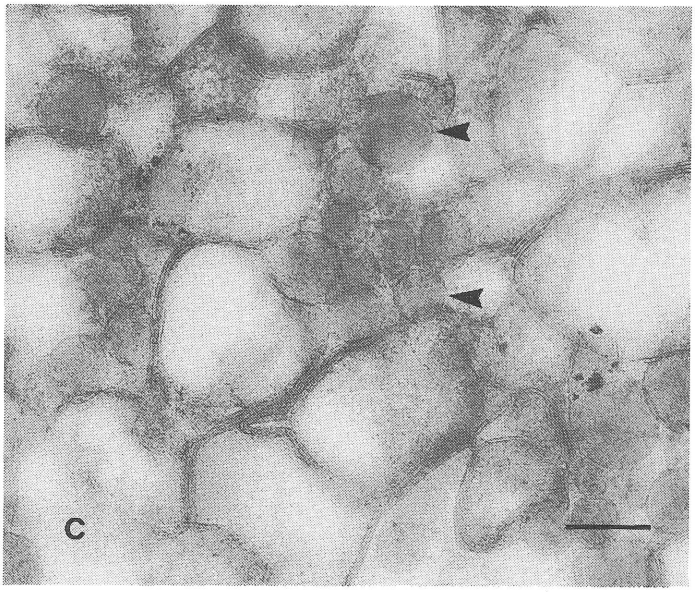

FIGURE C. OTAP, excellent retention of nuetral lipid (arrows) and preservation of membranes. 72,500X, Bar = 150nm.

buffer rinses over 5 minutes, 1% tannic acid in .05 M cacodylate buffer, pH 7.4, for 30 minutes, wash in 1% $NaSO_4$ in .05 M cacodylate buffer for 5 minutes, three rinses in 70% ETOH for 5 minutes each, treatment with 1% para-phenylenediamine in 70% ETOH for 30 minutes, three more rinses in 70% ETOH for 5 minutes each, 95% ETOH for 15 minutes, 100% ETOH for 15 minutes, 1:1 Epon:ETOH for 1 hour, resin overnight, resin 2 hours, resin 2 hours, embedment.

Thin sections were cut at 70-90nm, briefly stained with uranyl acetate and Sato's lead citrate and viewed on a JEOL 200CX electron microscope.

The micrographs reveal the value of the OTAP and OTO techniques when used in complement. The ability to adequately discern lipid phases has promising implications for the study of extracellular lipid deposition and transformation in human atherosclerosis. This technique reveals what we believe is the first comprehensive view of the plaque lipid as it naturally occurs. Among the findings in our current studies are: a predominance of small droplets (.03-.4μm and vesicles, a rarity of large lipid phases (cholesterol crystals and large droplets), the pitting of almost all small droplets, and the intriguing occurrence of a granular precipitate often overlying neutral lipid in droplet-vesicle hybrid structures.

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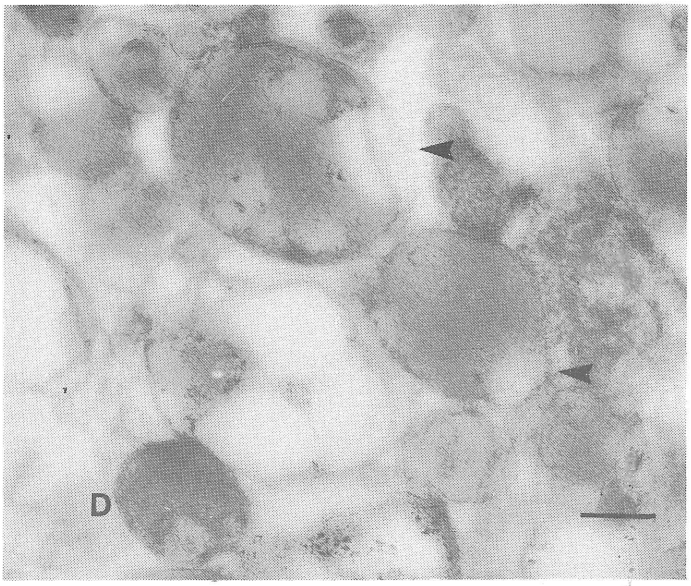

FIGURE D. OTAP, pits and blebs (arrows) associated with nuetral lipid droplets. 98,600X, Bar = 100nm.

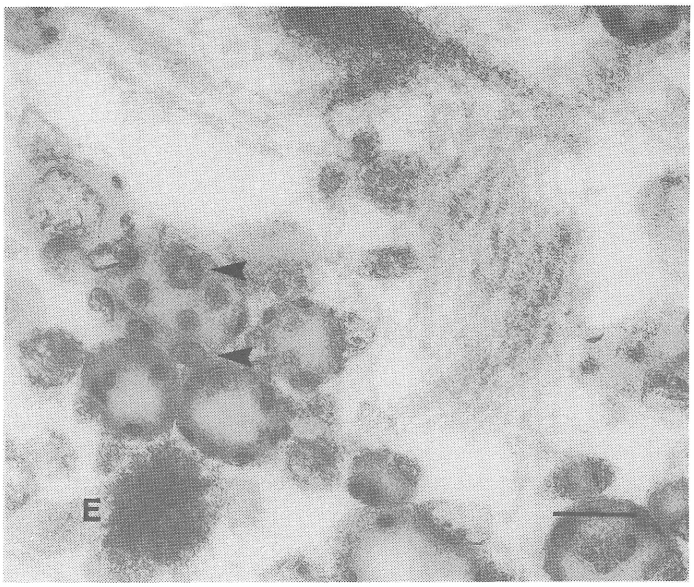

FIGURE E. OTO, enhancement of pits by mordant effect (arrows) but poorly preserved membranes. 72,500X, Bar = 150nm.

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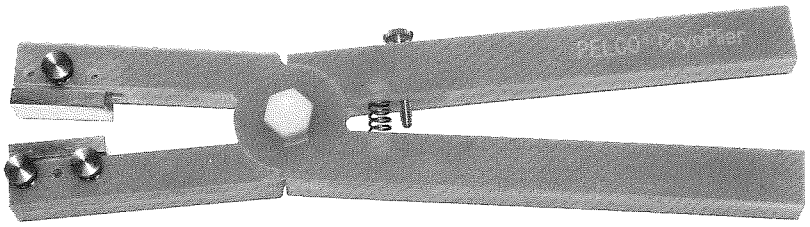

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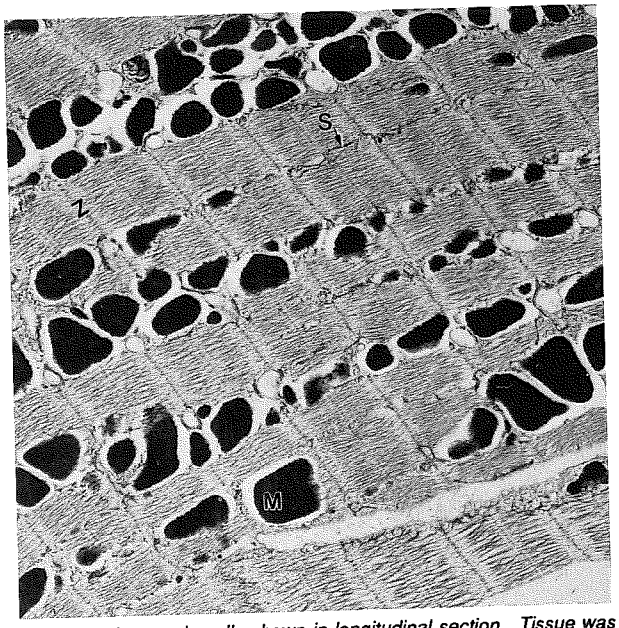

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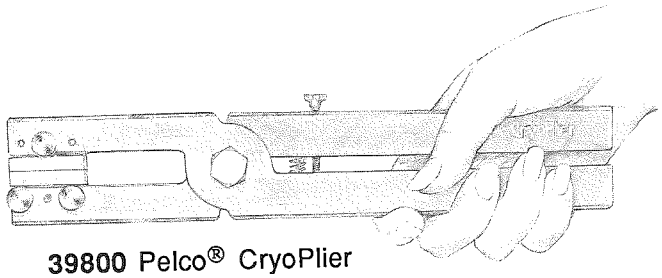

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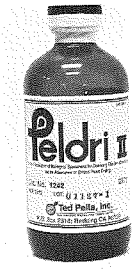

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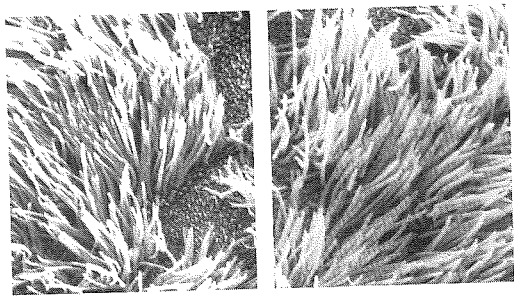

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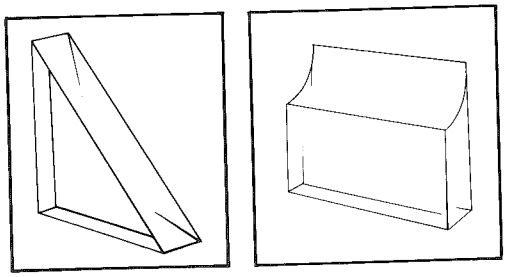

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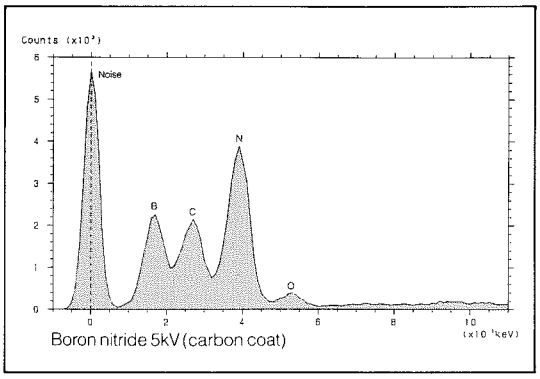

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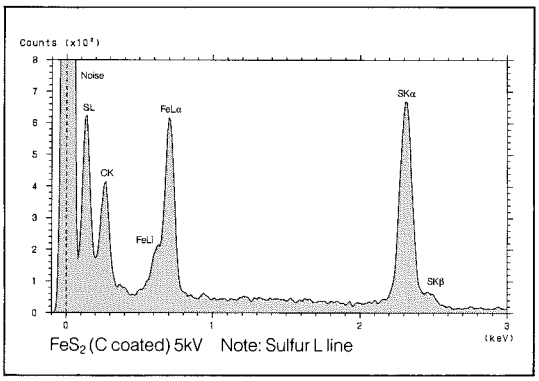

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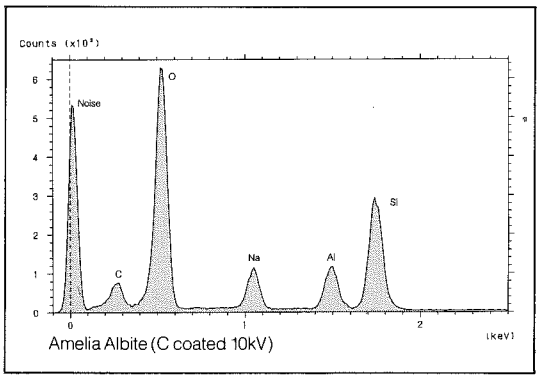

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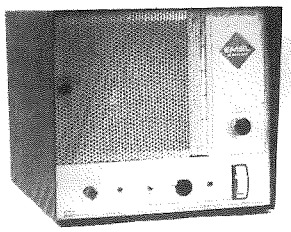

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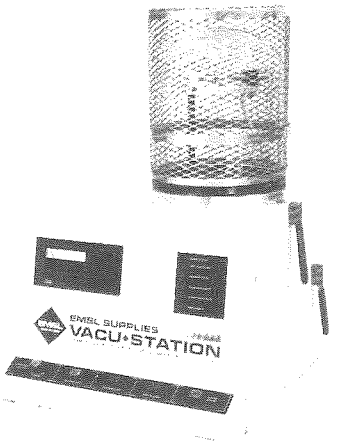

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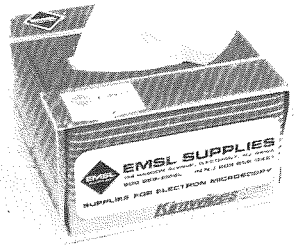

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Abstracts

BIOLOGICAL SCIENCES

PLATFORM PRESENTATION — SPRING 1989

COMPARATIVE STUDIES OF IN VIVO AND IN VITRO HUMAN CHONDROCYTES, J.T. Ellard, M.A. Machado, J.A. Sanford, E.A. Putnam, D.F. Campbell, and W.A. Horton, Department of Pediatrics, Division of Medical Genetics, The University of Texas Medical School, Houston, Texas 77225.

A two-step method of cell culture was developed to study differentiated human chondrocytes. Cells were obtained from human costochondral cartilage and cultured first in monolayer to allow for dedifferentiation and amplification in cell number. Dedifferentiated cells appeared either stellate or spindle-shaped and produced type I procollagen, fibronectin, and small non-cartilagenous proteoglycans. Redifferentiation occured over one to two weeks following culturing in agarose. The cells became spherical-shaped, closely resembling chondrocytes found in vivo. Abundant rough endoplasmic reticulum, golgi, transport vesicles, and lipid were observed both in vitro and in vivo. Cultures stained with ruthenium red displayed numerous proteoglycan granules within the matrix secreted around the cells, as well as along the cell membrane. Cells cultured for extended periods of time (i.e. four and five weeks) demonstrated an increase in matrical proteoglycan content. Type II collagen fibrils were also observed within the matrix. Chondrocytes from two human cartilage disorders, achondrogenesis and pseudoachondroplasia, were also cultured. Ultrastructural and matrical similarities were observed between the in vitro and in vivo conditions of each disorder. The striking similarities between cultured and in situ human chondrocytes provides additional evidence for the validity of this model. As a result, this system will provide a more direct approach for the study and evaluation of the various human chondrodystrophies.

FINE STRUCTURAL FEATURES OF CORONARY VASCULOGENESIS IN COLLAGEN LATTICES. J. KEVIN LANGFORD, DON A. HAY and DAVID L. BOLENDER, Department of Biology, Stephen F. Austin State University, Nacogdoches, Texas; and Department of Anatomy and Cellular Biology, Medical College of Wisconsin, Milwaukee, Wisconsin.

We have developed a culture model for studying the mechanisms of coronary angiogenesis in which mesenchymal precursors derived from dorsal mesocardia (stage 18) penetrate the collagen gel and form vascular-like structures. Using correlative light and electron microscopy, the objective of the present study was to further characterize the cellular components of these putative blood vessels. Intact chick embryo hearts and/or dorsal mesocardia (stages 18-22) were grown on collagen lattices for 4 days, at which time the explant was removed. An epicardial outgrowth remained on the surface of the gel, from which subsequent "capillary" development occurred. Many of the cells within the lattice showed signs of increased secretory activity as revealed by accumulation of rER, Golgi and large secretory vacuoles. The earliest vessels appeared to be filled with cellular debris possibly resulting from autolysis, while the lumina of larger vessels were free of debris. Within the cells lining the lumina of the forming vessels the following endothelial-like characteristics were observed: attenuated cytoplasm, fenestrae, poorly developed cell junctions (plasma membrane densities) and multivesicular bodies. Unidentified cells peripheral to the endothelium exhibited highly attenuated cytoplasm that completely surrounded most of the vessel. The nature of these cells as well as the extent of vascular development within the collagen lattice is presently being investigated.

VARIABLE IMMUNOLABELLING PATTERNS OF T-ag AND p53 IN A VARIETY OF SV40 TRANSFORMED CELL LINES. L.S. STEIN, K.A. NECK, M.S. FREY, AND R.C. BURGHARDT, Dept. Veterinary Anatomy, Texas A&M University, College Station, TX 77843

Labelling patterns of the large tumor antigen (T-ag) and the cellular oncoprotein, p53, in several SV40 transformed cell lines were characterized by immunofluorescence and suggested that localization of the antigens may be cell type specific. In all cases labelling was restricted to the nucleus with an absence of labelling in the nucleolus. Using confocal microscopy, the antigens were observed in discrete packets within specific planes of the nucleus. However, both the size and the nuclear pattern of the label varied between cell types. Studies were extended to include immunogold localization of the antigens in order to identify the specific nuclear structures associated with these antigens. In one cell line derived from rat ovarian granulosa cells (DC3) the label was found to be associated with nuclear filaments and at the boundary between heterochromatin and euchromatin. Studies in progress are directed at determining the significance of variability in labelling patterns at the ultrastructural level. (Supported by a BRSG).

ENAMEL STRUCTURE IN AN EXTINCT AND AN EXTANT FISH. J.E. McINTOSH, Department of Anatomy, Baylor College of Dentistry, Dallas, TX 75246.

The ultrastructural examination of enamel is a useful taxonomic tool in the study of extinct and extant species of fauna which contain teeth. The lack of prisms, or when they are present, the prism packing pattern, and the presence or absence of enamel tubules can be used to determine the proper classification of teeth of unknown origin. Teeth utilized in this study (provided by Dr. C.E. Baugh & D.R. Patton) were prepared (1), by making a small groove with a plain tungsten carbide fissure bur on the mesiodistal side of the crown or incisal/occlusal edge (fossil teeth), and (2), by grinding and polishing midsagittal or cross sectional areas (non-fossil teeth). All teeth were etched with 5% HCl for 10 seconds, sputter-coated with gold and examined at 20 kv in a JEOL 35CF scanning electron microscope. A single fossil tooth found during an archeological dig along the Paluxy River was grossly identified by numerous dental experts as being humanoid; most probably a human deciduous maxillary incisor. SEM analysis of the enamel revealed a prism pattern which did not fit any of three established prism packing patterns, but was similar to that observed in incisor and pavement teeth of an extinct pyncnodont fish (Pycnodus sp.). Additionally, enamel tubules were present in the "Paluxy" tooth and the pyncnodont teeth. The sheepshead fish (Archosargus probatocephalus), has incisor and pavement teeth identical to, but smaller than, the pyncnodont. SEM analysis of sheepshead enamel also revealed enamel tubules and a prism pattern very similar to the pyncnodont. The "Paluxy" tooth can be removed from consideration as a human tooth since its enamel structure is totally different than human enamel. The data suggest that it is a pyncnodont incisor tooth.

SEQUENTIAL EVENTS IN THE EARLY DEVELOPMENT OF THE PROSOPIS GLANDULOSA SEED COAT. Rebecca S. Westover and Louis H. Bragg. Department of Biology, Univ. of Texas at Arlington, Arlington, Tx. 76019.

Prosopis glandulosa (mesquite) is a representative member of the woody legumes within the subfamily Mimosoideae. A pleurogram and a cracked "tile" surface with distinct microsurface patterns are characteristic of the Prosopis seed coat. Little is known about the sequence in which the pleurogram, surface cracks, and patterns are formed. We have observed seed coats during the early stages of development to determine the sequence in which these characters become evident.

MORPHOLOGICAL AND IMMUNOLOGICAL ASSESSMENT OF TISSUE PREPARED BY SELECTED CONVENTIONAL AND CRYOFIXATION TECHNIQUES.
E.S. Griffey, R. Chiovetti, A.A. del Campo, S.A. Livesey, LifeCell Corporation, The Woodlands, TX 77381

Electron microscopists have a wide variety of tissue processing techniques at their disposal. Conventional chemical processing techniques, though routinely used for ultrastructural studies, are not always suitable for immunoelectron microscopic localizations. These techniques allow chemical crosslinking and solvent extraction which result in alteration or loss of cellular antigens. Cryofixation methods in which the tissue is rapidly frozen, have the potential to minimize extraction by solvents or buffer solutions and cause less alteration in cellular antigens. Yet, these methods are generally less standardized and often yield tissue with unfamiliar structural features.

In this study, we report on tissue prepared by conventional chemical fixation, freeze substitution, and the LifeCell® Process, which involves cryofixation followed by the physical removal of water from the samples by incremental heating in an ultrahigh vacuum. These processing techniques are compared with regard to the effect of each step of the sample preparation on the structural and immunological integrity of the final sample. Significant morphological differences were found in rat kidney tissue processed by each technique. Using erythrocytes as a standard, differences in the immunolabling intensity of carbonic anhydrase, a water soluble enzyme, was also observed. Based on the results, a qualitative ranking of the potential detrimental effect of each step of sample preparation on the integrity of the final sample can be made i.e., aqueous contact > solvent extraction > resin embedding > chemical crosslinking > cryofixation.

AN ULTRASTRUCTURAL IMMUNOCYTOCHEMICAL STUDY OF RETINAL GANGLION CELLS USING A CELL-SPECIFIC MONOCLONAL ANTIBODY. Dena M. Edwards and Keith R. Fry, Center for Biotechnology, Baylor College of Medicine, The Woodlands, TX 77381.

Previous light microscopic immunocytochemical studies in this and other laboratories have described the use of the AB5 monoclonal antibody as a cell-specific label for ganglion cells in the vertebrate retina. At the light microscopic level, AB5 has aided in studying the distribution, development, regeneration, and neurochemical specificity of ganglion cells. In the current study, electron microscopic immunocytochemical studies using the AB5 antibody were performed to determine whether ultrastructural localization of the AB5 antigen in retinal ganglion cells could be demonstrated, and to subsequently study ganglion cell synaptic relationships. A pre-embedding indirect immunocytochemical method employing the ABC-peroxidase technique was used to visualize AB5 labeling in the rabbit retina. AB5 labeling was observed as an electron dense intracellular precipitate associated with the inner aspect of the plasma membrane as well as with microtubules of ganglion cell perikaryons, axons, and dendritic processes. Electron micrographs showed AB5-labeled processes in the retinal inner plexiform layer receiving synapses from both amacrine and bipolar cell processes. No ganglion cell presynaptic relationships were evident in the rabbit retina. These results indicate that the AB5 monoclonal antibody is an effective marker for ganglion cells and their processes at the electron microscopic level. Use of the AB5 antibody in multiple-label studies will prove useful in determining the synaptic relationships of retinal ganglion cells with other neurochemically specific retinal cell types. Supported by NIH Grant EY0 6469 and The Retina Research Foundation.

CALBINDIN-D28K EXPRESSION IN THE CHICK REPRODUCTIVE SYSTEM. N. Inpanbutr and A.N. Taylor, Department of Anatomy, Baylor College of Dentistry, Dallas, Texas

Calbindin-D28K, a vitamin D-dependent calcium binding protein (CaBP), is present in target organs of the vitamin-D endocrine system. The protein was first detected in chick enterocytes and later reported in other chick and higher vertebrate tissues. In this study, CaBP was localized immunohistochemically in the reproductive system of developing and growing chicks at the light (LM) and electron microscopic (EM) levels. Tissues for LM analysis were processed by freeze substitution and reacted immunochemically with Sternberger's PAP technique. In the female, CaBP was first observed at 8 days of incubation in the germinal epithelium of the ovary and subsequently in some of the follicular cells surrounding small oocytes. After hatching and until 5 weeks of age, CaBP was only present in the germinal epithelium of the ovary. In the male, CaBP was first localized in the germinal epithelium of the testes on day 8 of development and diminished in concentration at the time of hatching. CaBP was detected again in 6 week old chick testes in the spermatogonia of the seminiferous tubules. At the EM level, low temperature processing (Lowicryl K4M) and protein-G gold techniques were utilized. Gold beads, the immunomarker for CaBP, were observed in organelle-free areas of cytoplasm and the nucleus of reproductive cells. A similar localization was previously reported for CaBP in enterocytes. Interestingly, the first appearance of CaBP in these cells coincides with the onset of sexually active stages, i.e., division of oogonia in the female and spermatogenesis in the male. The physiological function of this protein in reproductive organs is unknown, but it suggests a potential role in sexual differentiation in the chick. (Supported by NIH grant DE07916).

ULTRASTRUCTURAL MORPHOMETRY OF BRONCHIAL CARCINOID TUMORS. Bruce Mackay, James L. Bennington, and Brian Mayall. Dept. Pathology, University of Texas M.D. Anderson Cancer Center, Houston, TX 77030; Dept. Pathology, Children's Hospital and Adult Medical Center, San Francisco; and Cell Analysis Laboratory, University of California at San Francisco.

The enhanced preservation and minimal distortion of tissue processed for electron microscopy renders it superior to formalin-fixed, paraffin embedded material for morphometric analysis of cells and nuclei. However, structures in electron micrographs must be digitized manually unless they can be highlighted sufficiently to be selectively discriminated by an automated image analyzer. In a study of 50 bronchial carcinoid tumors, the cell and nuclear outlines of 50 cells were traced on transparent white paper with black ink to give high contrast cell and nuclear boundaries. The areas within the boundaries of each cell tracing were scanned using a high-resolution video camera, and each image was digitized using a Leitz TAS Plus Image Analyzer. A total of 16 morphometric tumor cell features was obtained and subjected to statistical analysis. Manual digitizing is preferable for morphometry of cytoplasmic components: the greatest diameters of 200 secretory granules from each case were measured using a stylus with a Jandel digitizing tablet and Sigmascan software. The spectrum of ultrastructural morphology in bronchial carcinoid tumors has been precisely delineated with this approach, and the findings are being correlated with clinical data and with the results of similar studies on the various types of carcinoma of the lung.

INTRACISTERNAL MICROTUBULES IN EXTRASKELETAL MYXOID CHONDROSARCOMA. Irving Dardick, Claire M. Payne, Bruce Mackay: Depts. Pathology, University of Toronto, Canada; University of Arizona, Tucson; University of Texas M.D. Anderson Cancer Center, Houston.

Parallel bundles of straight microtubules within the endoplasmic reticulum occur in approximately one third of extraskeletal myxoid chondrosarcomas. The microtubules vary in quantity but are typically abundant in every cell, filling and distending the cisternae. We have studied a series of 11 cases with transmission electron microscopy and have examined the microtubules morphometrically. Their diameter and linear periodicity are uniform but the spacing can vary. Most are hollow but a few are plugged with electron-dense material. They are confined to the cisternae and are not seen elsewhere in the cytoplasm. Microtubules are common in human tumors, but geometric arrays of straight microtubules within the endoplasmic reticulum are an infrequent finding, encountered in a small percentage of metastatic melanomas but only rarely in other neoplasms. Since intracisternal microtubules have not been reported in normal chondrocytes, their occurrence in such profusion in extraskeletal myxoid chondrosarcomas is surprising.

PREVENTION OF VILLUS EPITHELIAL CELL SLOUGHING IN CHICK INTESTINAL SEGMENTS INCUBATED WITH SALMONELLA TYPHIMURIUM IN VITRO. R.E. DROLESKEY, B.A. OYOFO, and J.R. DeLOACH, USDA-ARS, Veterinary Toxicology and Entomology Research Laboratory, Rt. 5, Box 810, College Station, TX 77840

The presence of the bacteria Salmonella typhimurium on fresh poultry products has become an issue of major public health concern. Research that will produce methods reducing the number of Salmonella organisms colonizing the intestinal tract of chickens are of greatest interest at present. Studies have shown that D-mannose significantly reduces the number of Salmonella organisms colonizing segments of small intestine from one-day-old chicks in vitro (1) and the cecum of broilers in vivo (2). The effects on the ultrastructure of intestinal segments exposed to the following: 2.5% D-mannose, 2.5% D-mannose plus 10^8 Salmonella, PBS, or PBS plus 10^8 Salmonella, as well as the bacterial invasion of intestinal villi, was studied using transmission and scanning electron microscopy. Examination of these samples revealed epithelial cell sloughing in segments exposed to bacteria without D-mannose, while control segments and those incubated with bacteria and D-mannose had similar ultrastructure. Bacteria were observed within the villi of intestinal segments irrespective of D-mannose.

1. B. A. Oyofo, R. E. Droleskey, J. O. Norman, H. H. Mollenhauer and J. R. DeLoach. Inhibition by mannose of in vitro colonization of chicken small intestine by Salmonella typhimurium. Poultry Science (In press).
2. B. A. Oyofo, J. R. DeLoach, D. E. Corrier, J. O. Norman, R. L. Ziprin and H. H. Mollenhauer. Prevention of Salmonella typhimurium colonization of broilers with D-mannose. Poultry Science (In press).

TECHNICAL PROCEDURES FOR DIAGNOSTIC ELECTRON MICROSCOPY OF TUMORS. Mannie C. Steglich, Nelson G. Ordonez, Bruce Mackay: Department of Pathology, University of Texas M.D. Anderson Cancer Center, Houston TX 77030.

The yield from a diagnostic ultrastructural study is largely determined by the quality of the tissue that is available for examination, and the most common reason for failure to reach a diagnosis is inadequate material. It is often difficult and sometimes impossible to derive meaningful information from specimens that are minimally representative, insufficient in quality, poorly fixed or artefactually distorted. Open biopsies or resection specimens of solid tumors provide the most consistently satisfactory specimens, though selection of the tissue for electron microscopy requires the identification of viable areas and avoidance of foci of hemorrhage and necrosis. Pathologists and clinicians inexperienced in electron microscopy are generally not aware of the importance of rapid procurement of the specimen, and of the need to fix small pieces of tissue, and it is important to indoctrinate them. Some types of specimens require special handling in order to harvest tumor cells and retain them in an acceptable state of preservation through processing. Modified techniques are necessary for effusions, cores of bone, aspirates, and endomyocardial biopsies. The methods used at M.D. Anderson Cancer Center will be reviewed.

THE USE OF SCANNING ELECTRON MICROSCOPY TO DETERMINE TAXONOMIC RELATIONSHIP IN SELECTED MEXICAN HAWKWEEDS. J. A. Soule, Deptartment of Botany, University of Texas, Austin TX 78713.

The taxonomic relationships of the highly variable Mexican Hawkweeds, Hieracium abscissum Less., H. dysonymum Blake, H. mexicanum Less., H. pringlei A. Gray, H. schultzii Fries, and two potentially new taxa, H. 'potosi' and H. 'chipinque' were addressed in this study. A survey based on SEM of the external appearence (vestiture) of the plants and statistical analysis of the results by Spearman's Rank Correlation and Average Linkage Cluster Analysis resulted in clear-cut sorting of the taxa. SEM studies of pollen morphology and evaluation of the results aided in the taxonomic determination of this difficult genus. This study indicates that SEM can be a useful tool for examining taxonomic relationships among highly variable, closely related speies.

THE REPAIR RESPONSE OF DAMSELFLY LARVAE TO GILL DAMAGE. MELISA L. MOORMAN, JAMES V. ROBINSON AND DOUGLAS D. HAGEMEIER. The University of Texas at Arlington, Arlington, Texas 76019.

The caudal gills of larval damselflies are important for respiration, swimming, predator avoidance and agonistic behavior. Approximately 50% of field collected Ischnura posita were missing or regenerating at least one of their three caudal gills. I. posita have duplex gills which are laterally subdivided at a node into two morphologically distinct regions. Gill autotomy occurs at a breaking joint at the base of each gill and is followed by a repair process.

The consequences of gill injury versus gill autotomy were studied during this investigation. A comparative study of hemocoele and tracheal closure following gill injury and gill autotomy was performed using scanning electron microscopy. Larvae were experimentally wounded either pre- or post-nodally to simulate natural injury. One gill from each larva was removed while the remaining two were differentially manipulated.

Following autotomy the tracheae and the dorsal and ventral hemocoeles are sealed at the breaking joint. Pre-nodal injury results in the active removal of the gill by the organism. When post-nodal injury is inflicted, approximately 50% of the injured gills are not removed. Behavioral differences were observed between autotomized and injured larvae. The functional value of these behaviors will be discussed in the context of the physical repair process revealed in the micrographs.

BIOLOGICAL SCIENCES

POSTER PRESENTATION — SPRING 1989

THE STABILITY OF SEED COAT CHARACTERS IN PROSOPIS PALLIDA (KIAWE) FROM FIVE HAWAIIAN ISLANDS, L.H. BRAGG, Dept. Biology, The University of Texas, Arlington, TX 76019.

Seeds from populations of P. pallida were examined by SEM to determine the range of variation existing within and between those populations. Whole seeds were examined to determine surface topography and transected seeds were examined to determine differences in layering and kinds of cells composing those layers. The surfaces of the seeds were rugulate with some variation in the size of the rugae. The transected seeds had a single layer of osteosclereids (hour-glass cells) below the palisade cells but a double row of smaller osteosclereids near the lateral edges of the seeds. The layering of the cells were consistent for the individual seeds from different populations. This information was then compared with data from other related species.

IMMUNOGOLD LOCALIZATION OF A PLANT α-GLUCOSIDASE: OCCURRENCE IN THE ENDOCYTIC AND ORGANELLES OF SOYBEAN AND MUNGBEAN CELLS. L.R. Griffing*, R.P. Kaushal# and A.D. Elbein#. *Department of Biology, Texas A&M University, College Station, TX 77843 and Department of Biochemistry, University of Texas Health Science Center, San Antonio, TX 78284.

An α-glucosidase purified from mungbean hypocotyls has been immunolocalized at the subcellular level using a monospecific antibody and protein A-colloidal gold. The α-Glucosidase can be found in abundance in the Golgi complex, at the plasma membrane, and in the cell wall of mungbean cells. The antibody crossreacts with a protein from soybean suspension culture (line SB1) cells. The subcellular distribution of the protein in SB1 cells is similar to that found in mungbean cells. Praotoplasts made from SB1 cells also contain the protein at the plasma membrane and Golgi complex. In addition, the α-glucosidase was found in multivesicular bodies and endosomes in soybean protoplasts. The endocytic compartment of the plant protoplasts was mrked with the electron-dense probe, cationized ferritin. This observation is consistent with the hypothesis that plant cells can support entry of extracellular or plasma membrane-bound hydrolytic enzymes via an endocytic pathway similar to that found in animal cells. Supported by NSF (L.R.G.) and NIH (A.D.E.).

THE PARTIALLY COATED RETICULUM OF MAIZE ROOT SECRETORY CELLS. Hilton H. Mollenhauer[1], D. James Morré[2] and Lawrence R. Griffing[3]. [1]Veterinary Toxicology and Entomology Research Laboratory, USDA-ARS, Route 5, Box 810, College Station TX 77840; [2]Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, IN 47907; [3]Department of Biology, Texas A&M University, College Station, TX 77843

Outer cells of the maize root cap were examined for the presence of a partially coated reticulum (PCR) which has been suggested as the plant equivalent of the Trans Golgi Network of animal cells. Such a network was found near the trans poles of the Golgi apparatus (i.e., dictyosomes). This network appears in two distinct forms which we will arbitrarily label PCR_1 and PCR_2. PCR_1 and PCR_2 are separable from one another by shape, types of associated vesicles, position with respect to the dictyosome, and reaction to the plasma membrane marker, PTA/chromic acid. PCR_1 is formed when trans cisternae are sloughed from the trans poles of the dictyosomes and vesiculate into a network containing tubules, smooth vesicles, and coated vesicles. Thus, the primary function of PCR_1 appears to be dissipation of sloughed cisternae which is estimated to be the equivalent of 100 cisternae/cell/minute. PCR_2 is associated with the secretory vesicles during vesicle condensation. The secretory vesicles of this cell type are large and very elongate structures attached to the trans cisternae by tubules. When these secretory vesicles separate from the dictyosomes (and from their attached cisternae), they loose more than half of their membrane, and their secretory product undergoes distinct alterations in density. Therefore, the function of PCR_2 appears to be the condensation and final maturation of secretory vesicles before their discharge from the cell.

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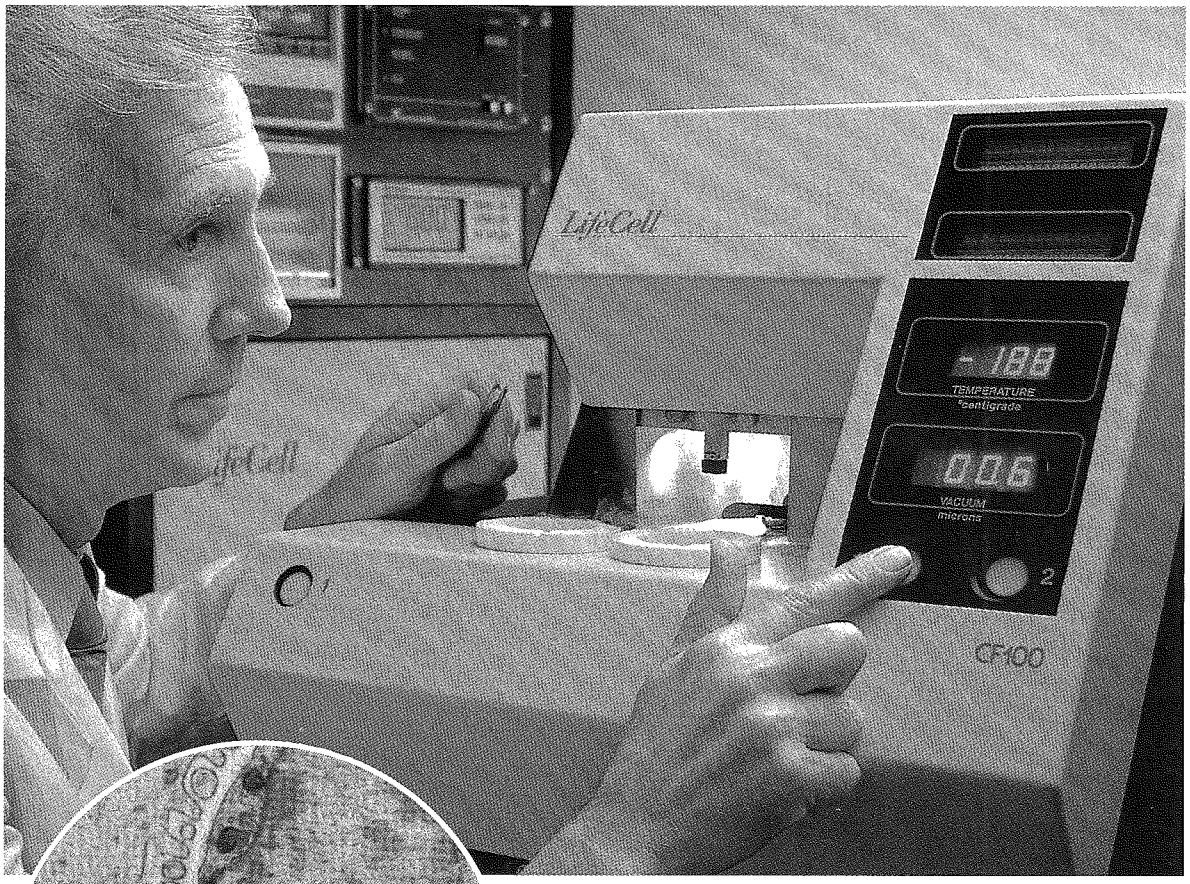

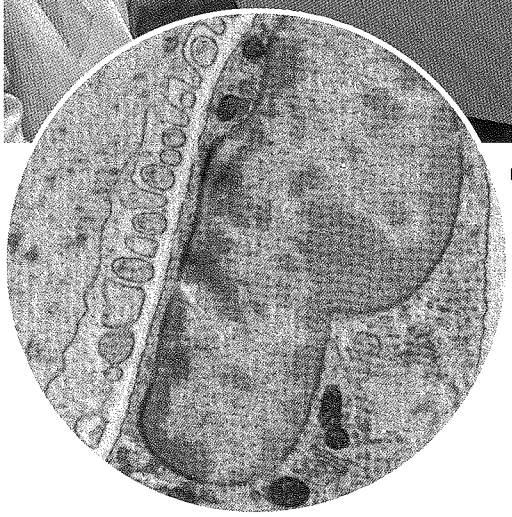

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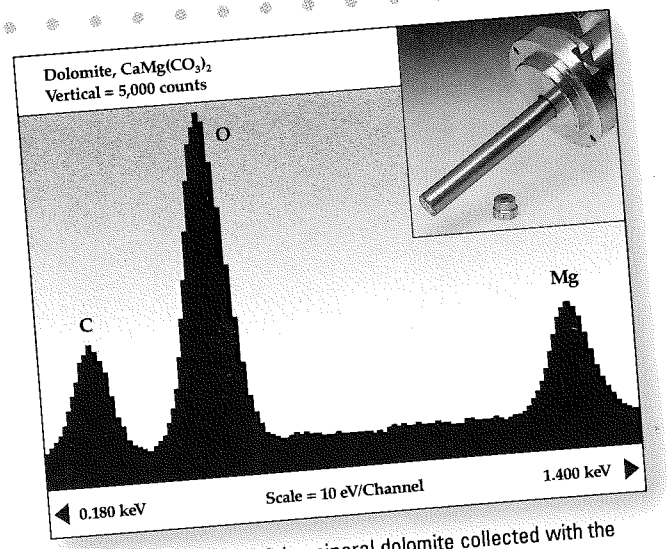

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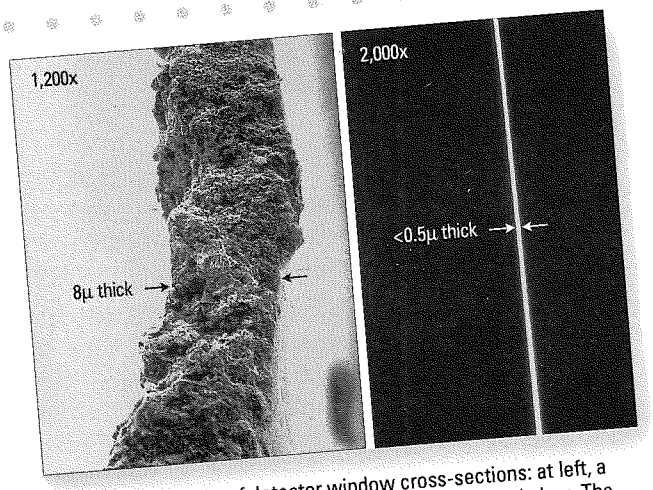

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(1) A. Glauert, Practical Methods in Electron Microscopy. Vol. 2 (North-Holland. Amsterdam, 1974) 82-88.
(2) P.S. Baur, Jr., G.F. Barratt, G.M. Brown and D.H. Parks. Ultrastructural Evidence for the Presence of "Fibroclasts" and "myofibroclasts" in Wound Healing Tissues. J. of Trauma. 19 (1979) 774-756.
(3) D. Gabor. Information Theory in Electron Microscopy, in: Quantitative Electron Microscopy. Eds. G.F. Bahr and E. Zeitler (Williams and Wilkins, Baltimore, 1956) 63-68.

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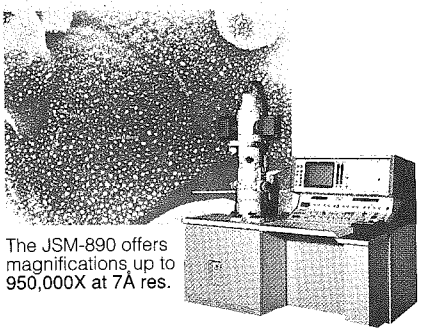

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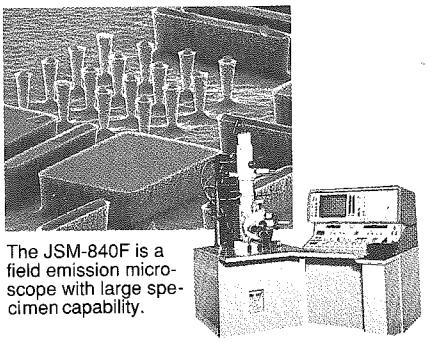

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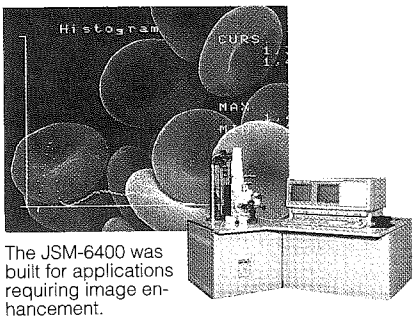

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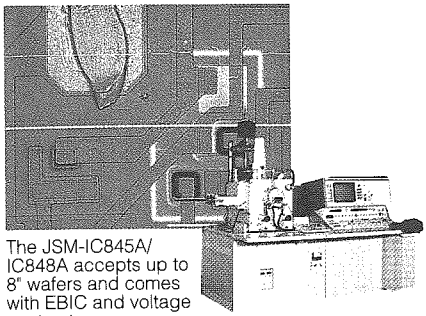

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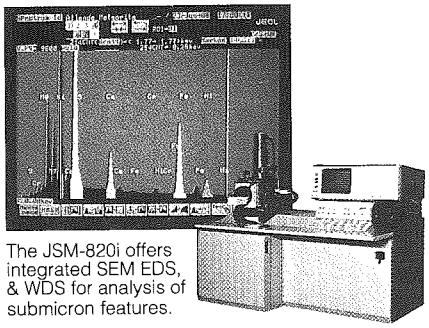

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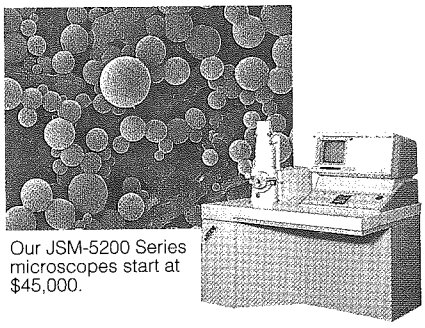

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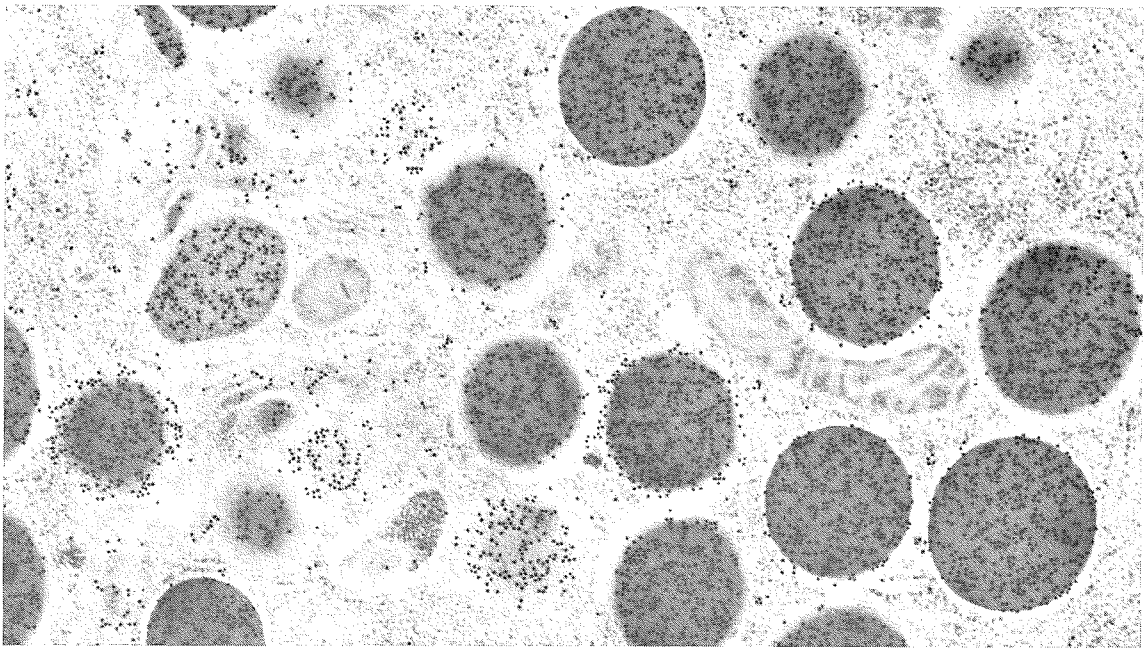

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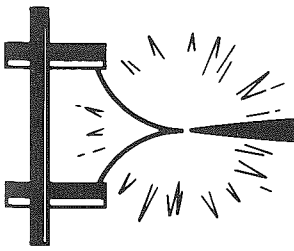

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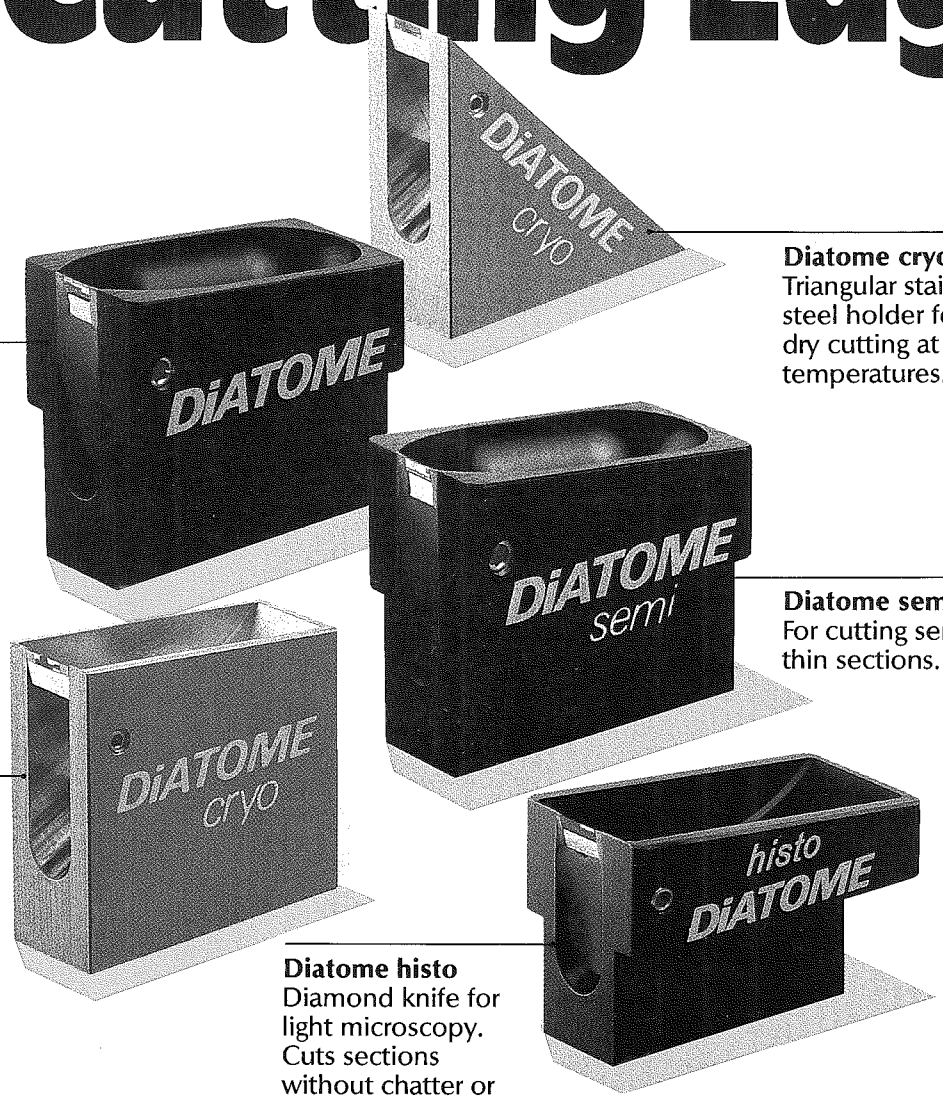

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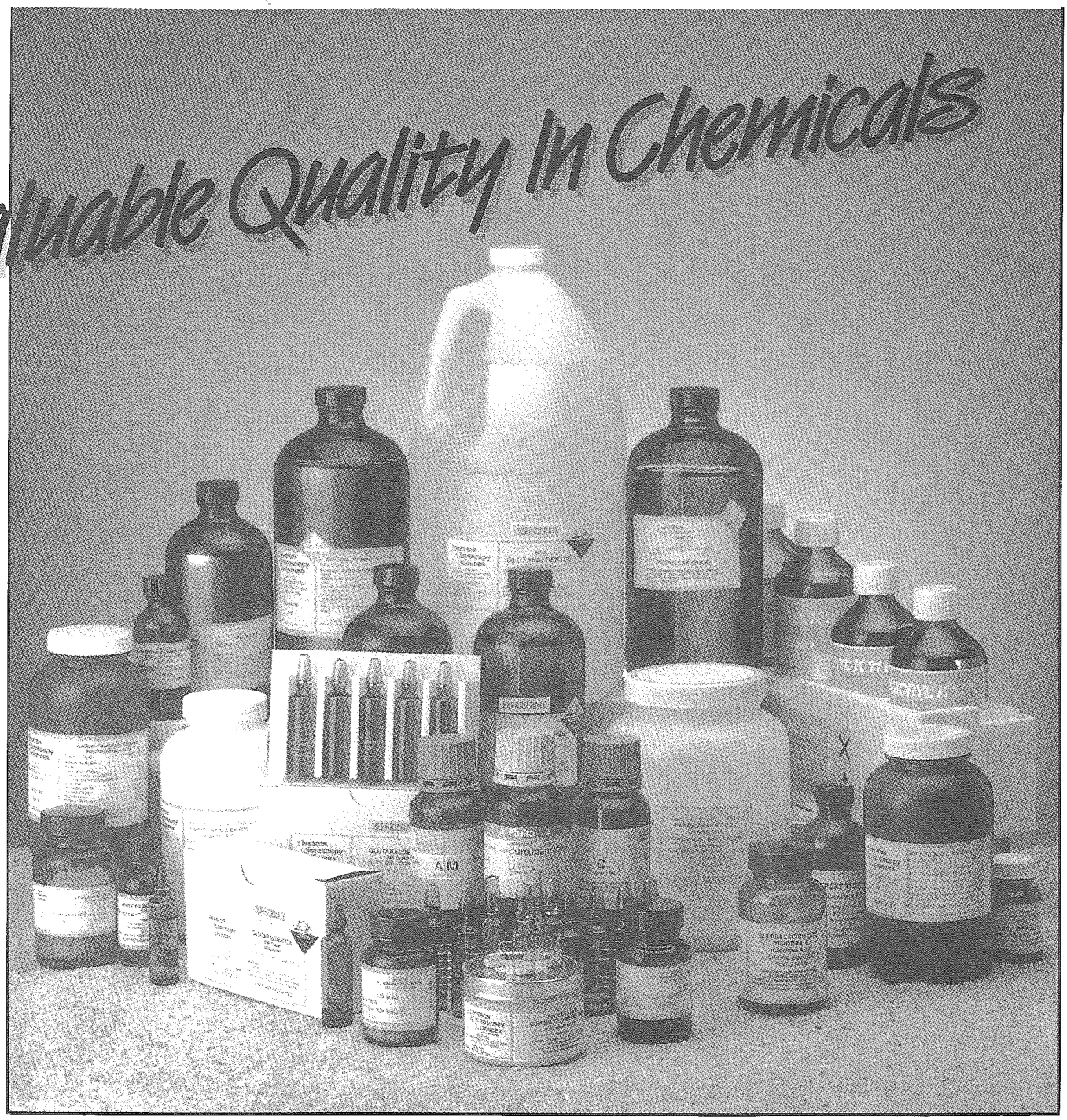

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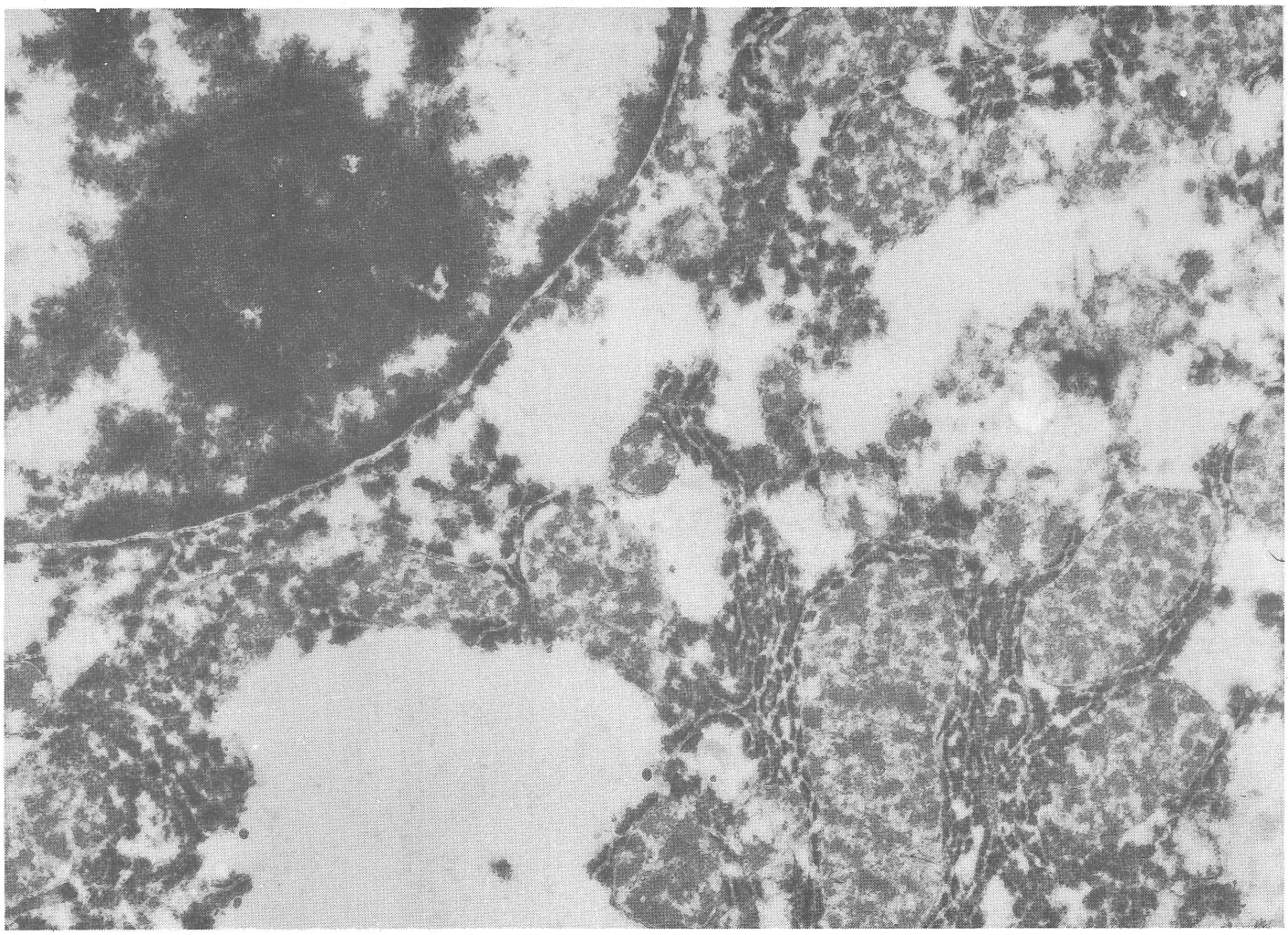

Large vacuolated areas are present, and probably result from the cytoplasmic proteins condensing into a flocculent mass. Even though, by EM standards, the quality of the preservation is not good, there is a surprising amount of detail visible.

Micrograph by R.W. Davis, Dept. of Medical Anatomy, Texas A&M University, College Station, Texas. Magnification approximately 35,000x.

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