TSEM

Texas Society for Electron Microscopy, Inc.

JOURNAL
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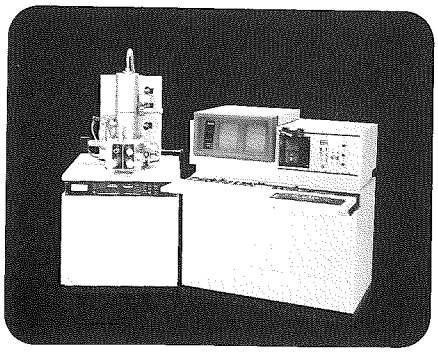
ISSN 0196-5662

LOOK INTO HITACHI'S BROAD LINE OF FIELD EMISSION SEM'S



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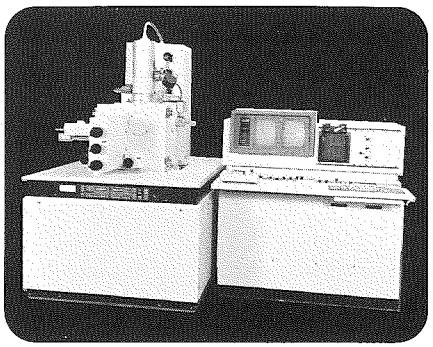

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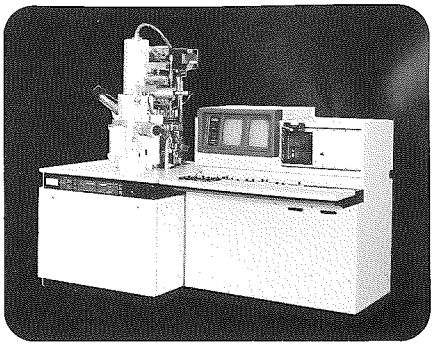

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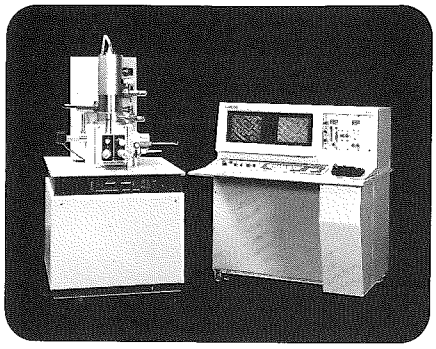

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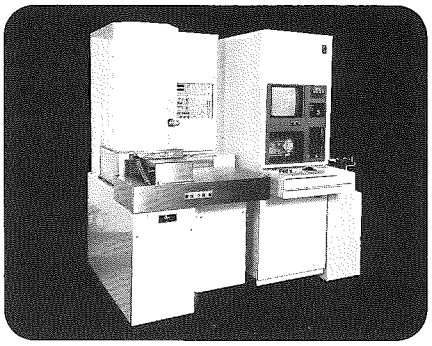

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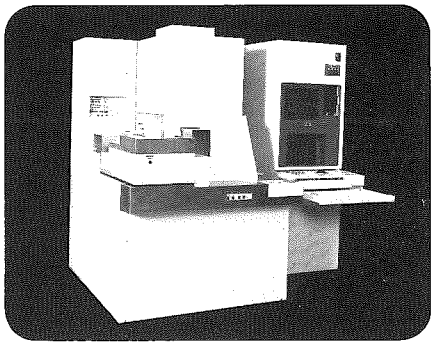

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TEXAS SOCIETY FOR ELECTRON MICROSCOPY
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Ronald W. Davis, Editor
Department of Medical Anatomy, Texas A&M Univ., College, Station, TX 77843

Texas Society for Electron Microscopy

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

ON THE COVER

Scanning electron micrograph showing the attachment of the front and rear wings of a honey bee. The rear wing has a row of hooks called hamuli that hold onto a fold on the rear edge of the front wing. Presumably, this allows the fore and rear wings to beat together and increases flying efficiency. Hamuli are a characteristic of the insect order Hymenoptera, which includes bees, wasps and ants.

Micrograph by Ronald W. Davis, Dept. of Medical Anatomy, Texas A&M University, College Station, Texas 77843. (Magnification approximately 150X.)

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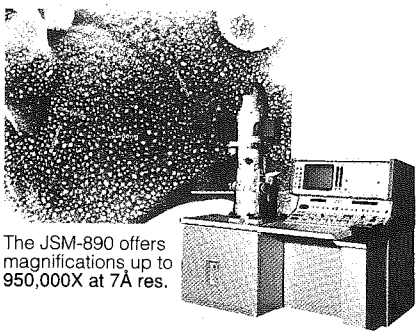

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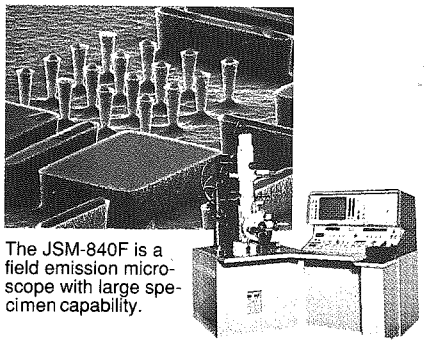

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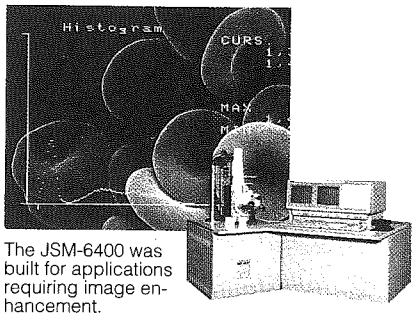

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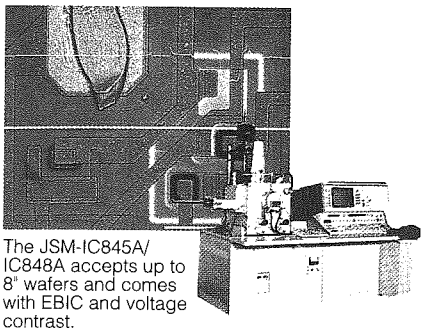

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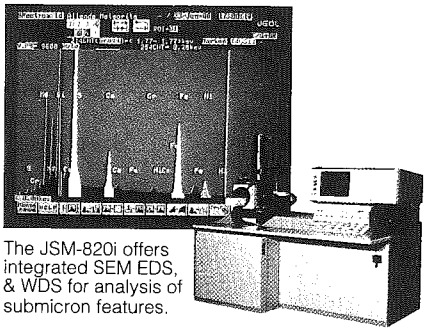

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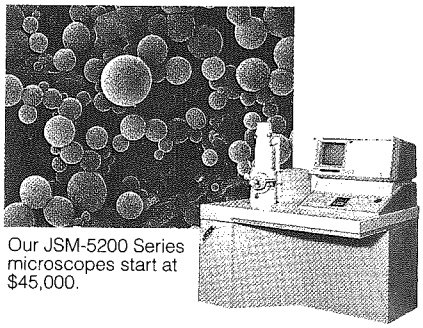

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President's Message

The Spring meeting of TSEM, Inc., in Austin was a huge success. Howard Arnott, Ron Davis and participating TSEM members are to be commended for putting together such a fine program and workshop. I can still hear the clicking of the counters that were being used in the stereology/morphometry workshop. It sounded a lot like a high school typing class, and I kept waiting to hear the bell "ding" at the end of the line. Wayne Fagerberg was a vibrant, energetic teacher who did a fantastic job and who recognized our inabilities without embarrassing us.

I would encourage the continued use of workshops such as the one in Austin, possibly in autoradiograph interpretation or immunogold labeling or electron diffraction interpretation. If you have suggestions for future workshops or for vibrant workshop instructors or you would like to teach one yourself, please tell someone on the executive board. This is your society and can only function properly with your input and participation.

We have had communication with past president Joe Wood, who has recently moved to Oklahoma and he says there is interest in future joint meetings with Oklahoma such as we have had with Louisiana. I believe this could lead to some very interesting and productive meetings. I should have more information concerning this soon. I hope that you and your graduate students and technicians are planning on sharing their work with us in Arlington and Kerrville.

H. Wayne Sampson
President, TSEM, Inc.

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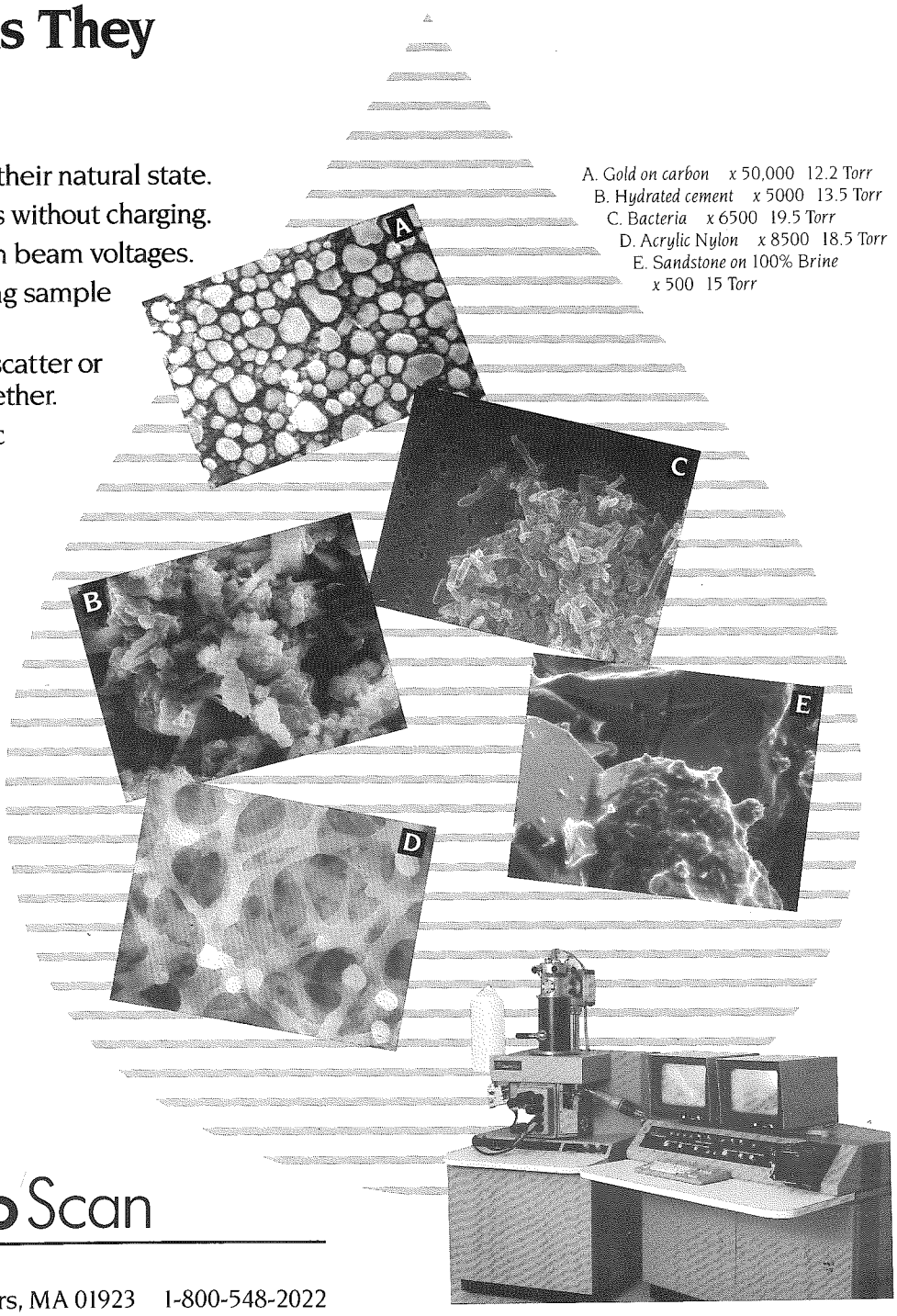

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Treasurer's Report

TREASURER'S REPORT

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Certificate of Deposit No. 11-8829764	4,119.98	
Checking Account No. 015210-01	1,597.19	$11,380.89
RECEIPTS:		
Austin Meeting / Registration & Exhibitor Fees	$ 2,427.00	
Stereology Workshop	600.00	
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Certificate of Deposit Interest	294.66	
Donations	74.68	$ 8,443.42
EXPENSES:		
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Journal Printing & Postage	1,973.82	
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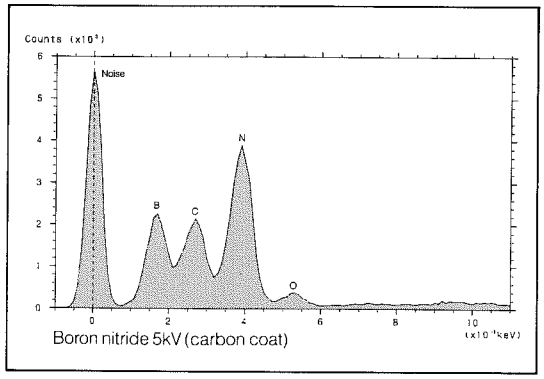
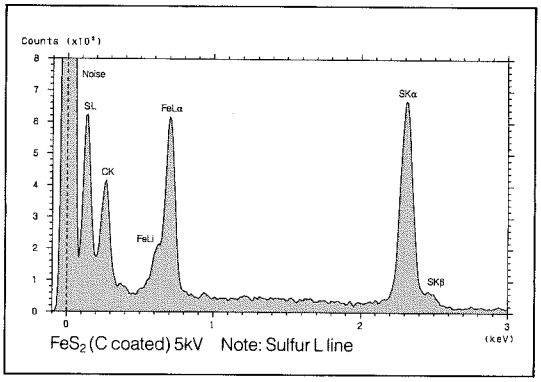
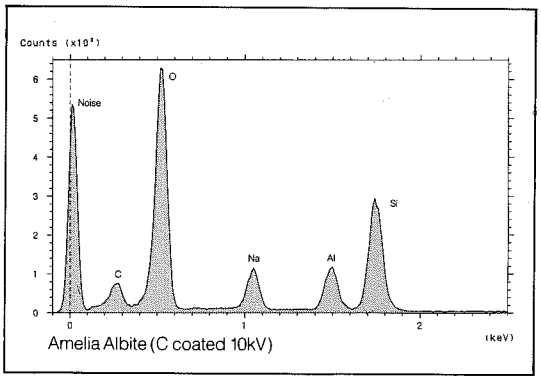


Boron nitride 5kV (carbon coat)



FeS_2 (C coated) 5kV Note: Sulfur L line



Amelia Albite (C coated 10kV)

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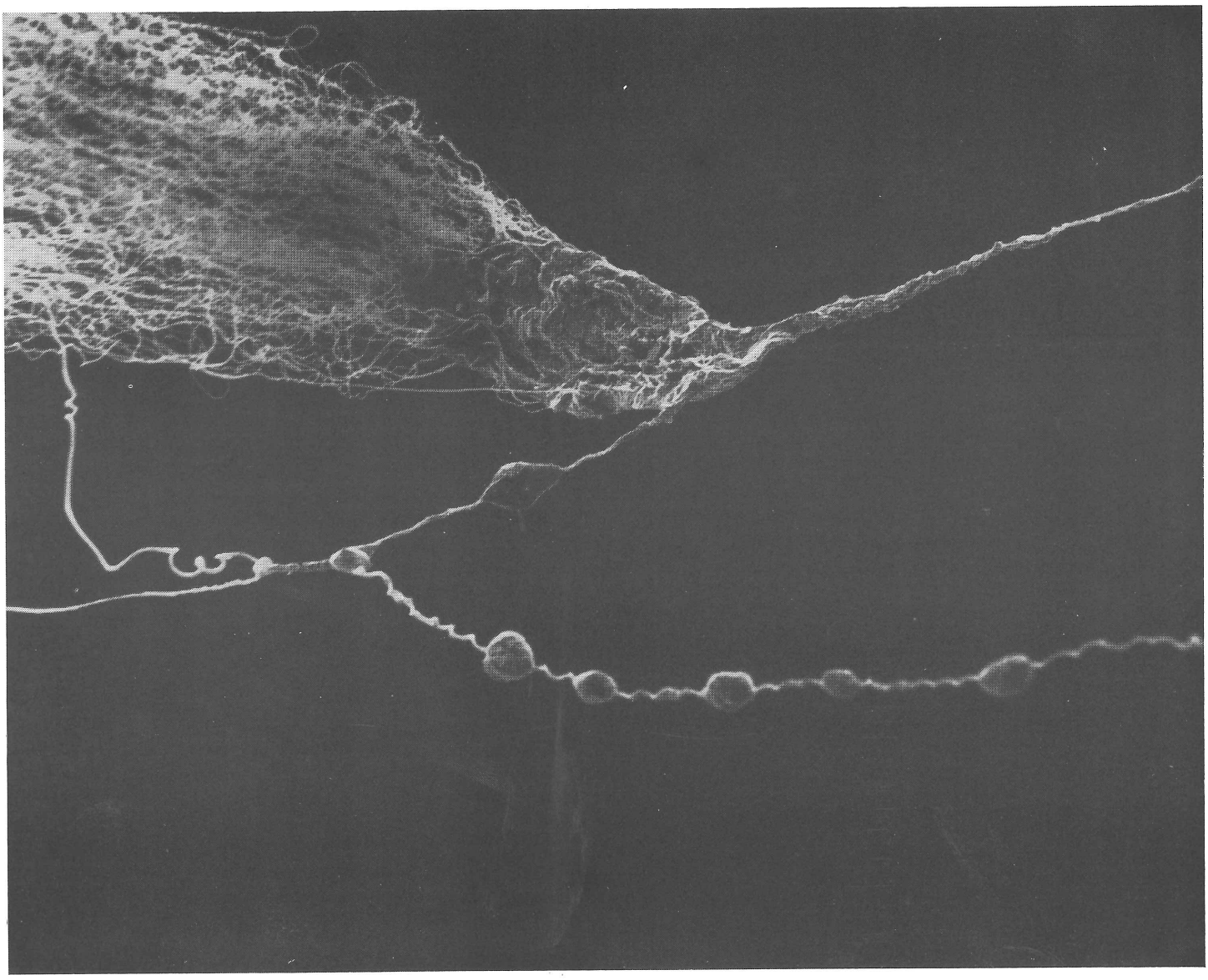

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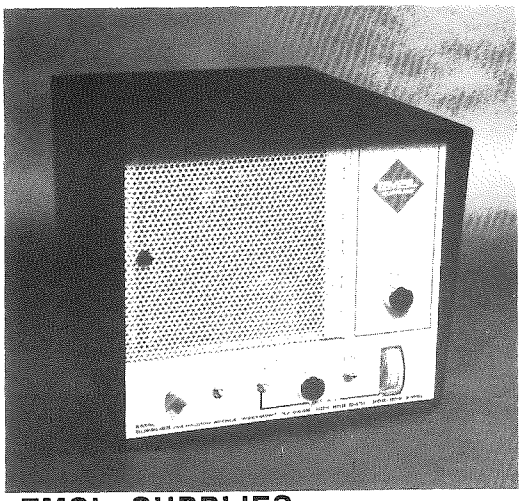

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VARIABLE IMMUNOLABELLING PATTERNS OF T-ag AND p53 IN A VARIETY OF SV40 TRANSFORMED CELL LINES

By

L.S. Stein, K.A. Neck, M.S. Frey and R.C. Burghardt
Department of Veterinary Anatomy
Texas A&M University
College Station, Texas 77843

The small DNA tumor virus, Simian virus 40 (SV40), has been used as a model system for the study of tumorigenesis, transformation and lytic infection of cells in culture. Many approaches have demonstrated that the SV40 large T-antigen (T-ag) has a role in both the immortalization and transformation of cells in tissue culture and the induction of tumors in animals. Most of T-ag (more than 95%) is located in the nucleus where the majority of its functions are exerted (1). One important feature of nuclear T-ag is its ability to form tight complexes with the cellular oncoprotein p53 in SV40-transformed and infected cells. The association of p53 with SV40 T-ag results in a stabilization of p53 and consequently an enhanced level of this cellular protein. It has been suggested that p53 T-ag complexes may be involved in the SV40-mediated transformation process (2).

A clonal line of SV40-transformed granulosa cells (DC3) was generated for study as a potential model system for use in studies of granulosa cell differentiation and ovarian carcinogenesis. In the course of characterizing the properties of the cell line, it became apparent that DC3 cells may also be used as a novel model for the study of the subcellular sites of action of T-ag (2). Indirect immuofluorescence studies using a panel of monoclonal antibodies directed against T-ag and p53 indicated that both antigens were concentrated in discrete areas of the nucleus and appeared as patches of intense fluorescence in DC3 cells (Figure 1A). Parallel labelling studies with an established SV40-transformed cell line (COS) indicated that the antigens in this cell line were distributed in a homogeneous granular pattern, distinctly different from the pattern observed in the DC3 cell line. The pattern of labelling in COS is similar to that described previously for T-ag and p53 in a variety of cell lines (Figure 1B). Optical sectioning of DC3 and COS cells using a confocal microscope supports the variable labelling patterns described above and suggests that the antigens in

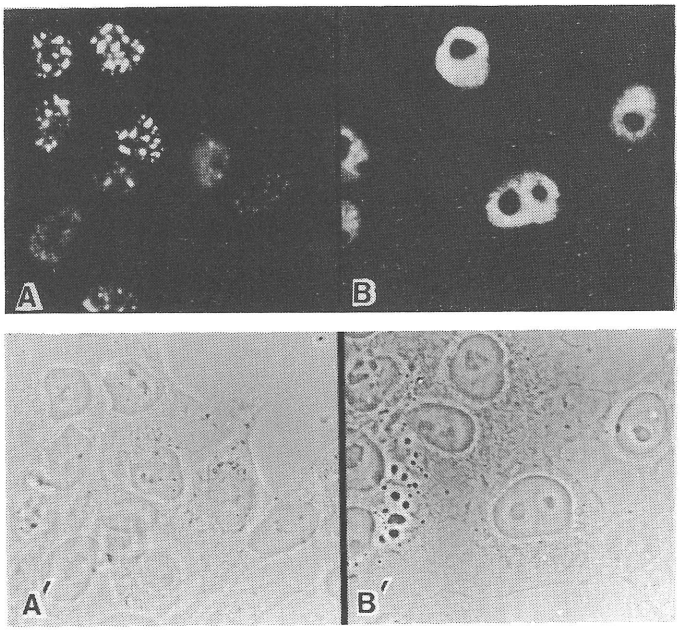

FIGURE 1. Localization of T-ag by immunolabelling of DC3 cells (A) and COS cells, a positive control (B). Phase contract correlates (A' and B') show the label to be confined to the nucleus with an absence of label in the nucleoli.

both cell types are localized in packets present in specific planes of the nucleus (Figure 2). Further characterization of these antigens in the DC3 cell line by Western blot analysis identified T-ag and p53 as single bands of 90K and 53K respectively (Figure 3). These results suggested a unique nuclear pattern of T-ag and p53 in DC3 cells even though protein bands were consistent with those previously described for these antigens. These observations were extended to the ultrastructure level using immunogold electron microscopy. Labelling with 10 nm gold particles in DC3 cells was observed in association with nuclear filaments, and at the boundary between the heterochromatin and the euchromatin (Figure 4).

In order to further understand the significance of the labelling pattern of these antigens, they were characterized in several other SV40-transformed cell lines by indirect immunofluorescence. The results suggested that localization of these antigens may be cell type specific (Data not shown). Published studies of T-ag and p53 characterized by immunofluorescence have reported that labelling of these antigens is restricted to the nucleus with an absence of labelling in the nucleolus. Our studies confirm this general labelling pattern but also suggest that there is a variability of labelling patterns within the nucleus at the light microscopy level. This may indicate a cell type specific function for T-ag which could conceivably be involved in the expression of the transformed phenotype. Extension of these observations to the ultrastructure level in currently in progress and should provide more information as to the subcellular localization of these antigens. In DC3 cells the label is found in association with nuclear filaments and at the boundary between the heterochromatin and euchromatin which is also the site of hnRNP production. Recent data using ultrastructural immunocytochemistry suggest that the subnuclear distribution of T-ag in transformed cells is different from that found during lytic infection of cells (4). This structural difference is thought to correspond to a functional difference between the transforming and lytic functions of T-ag. Based on the variability of labelling patterns by light microscopy studies in several transformed cell lines, studies in progress are directed at extending these observations at the ultrastructure level in order to determine the relationship between the patterns of labelling and transformation-specific alterations in cellular function.
(Supported by a BRSG and Research Enhancement Funds, Texas A&M University, College of Veterinary Medicine).

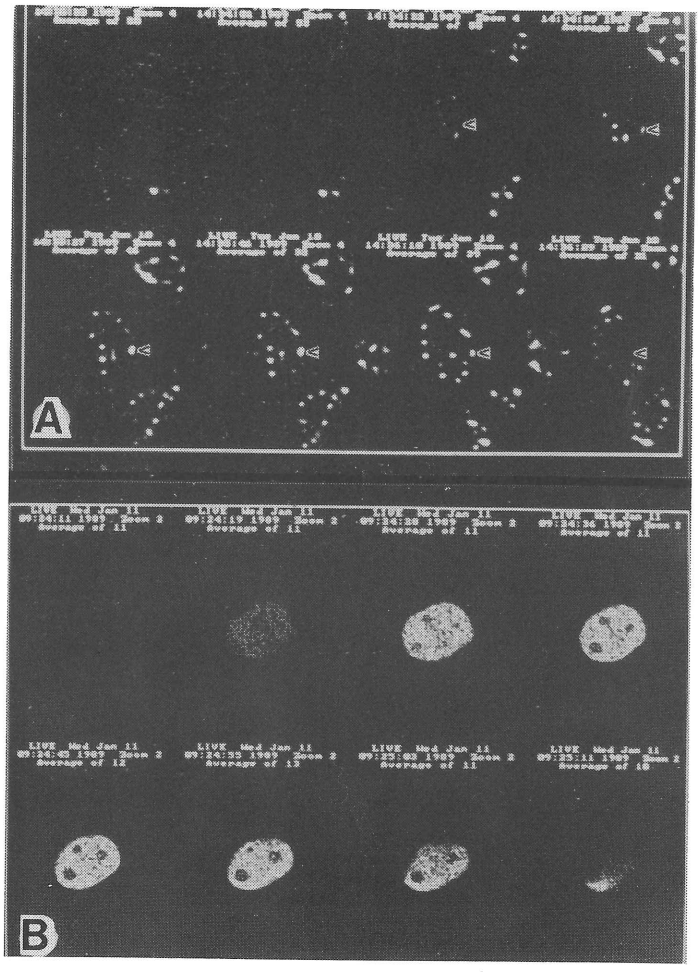

FIGURE 2. DC3 (A) and COS (B) cells immunolabelled with an antibody to T-ag were optically sectioned using the MRC-500 confocal microscope (BioRad). In DC3 cells, concentrated discrete packets of label are observed to appear and then disappear as one sections through the cell (arrowheads). The label in COS cells appears to be distributed within small packets which are localized more homogeneously in any given plane within the nucleus than that seen in DC3 cells.

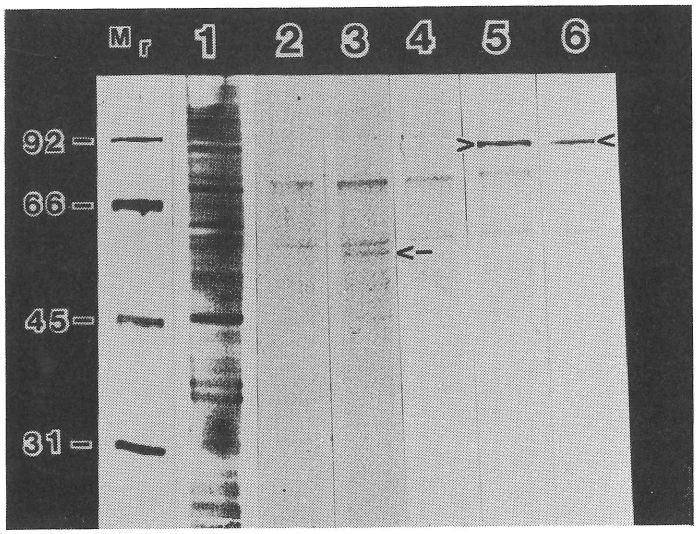

FIGURE 3. Photograph of a DC3 whole cell lysate separated by 1-D Page (10% mini) transferred to nitrocellulose, incubated with indicated primary antibodies, biotinylated secondary antibodies and visualized with a silver enhanced gold-strepavidin conjugate. Control lanes were silver enhanced for the same time period as corresponding experimental lanes. Arrow indicates position of p53 band and arrowheads the positions of T-ag bands. **LANES: M_r**, Aurodye forte stain of protein standards; **1**, Aurodye forte stain or whole cell lysate; **2**, p53 Control - no primary antibody; **3**, Incubation with antibody to p53 (Pab 122); **4**, T-ag Control - no primary antibody; **5**, Incubation with antibody to T-ag (Pab 419); **6**, Incubation with antibody to T-ag (Pab 405).

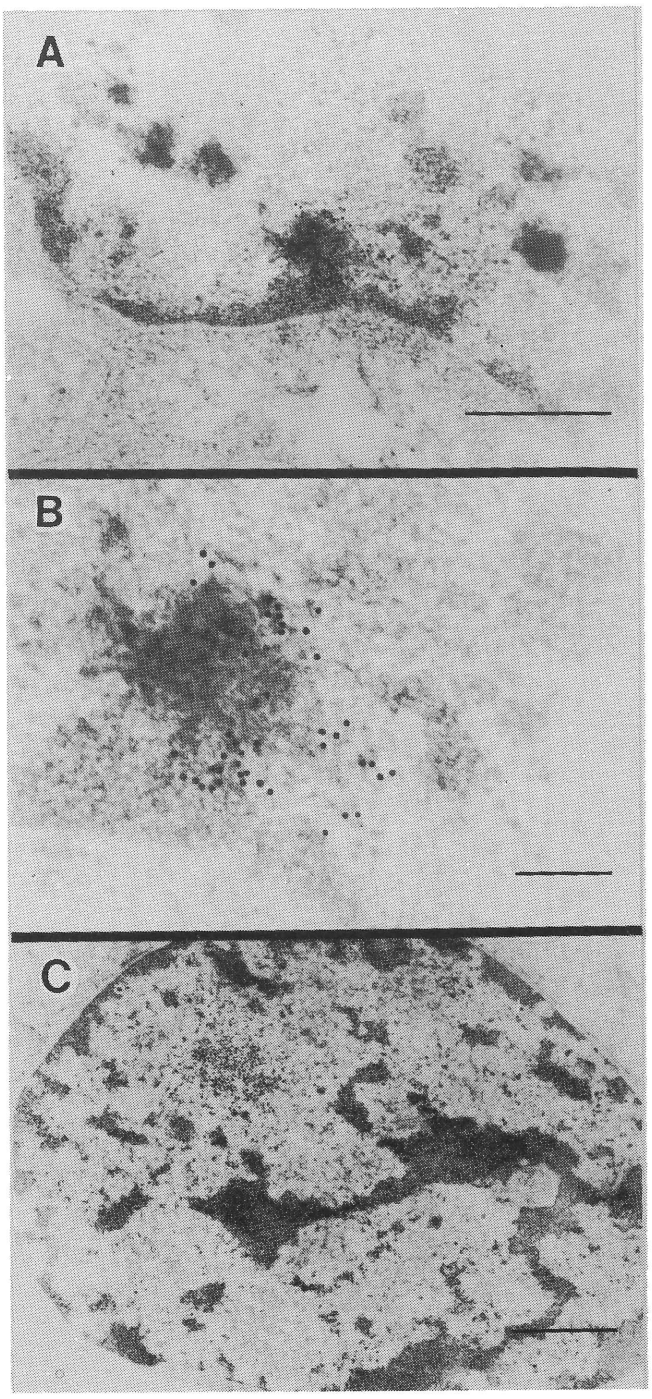

FIGURE 4. Electron micrographs of DC3 cells incubated with antibody to T-ag (Pab 405) and 10 nm goat anti mouse IgG. **A** identifies a concentration of label in the nucleus. (Bar = 0.5 μm). **B** is a higher magnification of **A** in which 10 nm gold particles are seen. (Bar = 0.125 μm). **C** is a control with no primary antibody. Only one gold particle was seen in the field (circle). (Bar = 1.0 μm).

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AN ULTRASTRUCTURAL IMMUNOCYTOCHEMICAL STUDY OF RETINAL GANGLION CELLS USING A CELL-SPECIFIC MONOCLONAL ANTIBODY

By

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The development of hybridoma technology has greatly facilitated our ability to study the structural organization of neuronal tissue. Using this technology, it is possible to produce highly specific monoclonal antibodies against previously unidentified antigens, many of which are cell-specific. Previous studies in this laboratory have described the development and use of the AB5 monoclonal antibody as a cell-specific label for ganglion cells in the mammalian retina (for review see Fry and Lam, 1989). At the light microscopic level, the AB5 antibody has aided in studying the structure, distribution, development, regeneration and neurochemical specificity of these cells. In the current study, an electron microscopic immunocytochemical analysis was performed to determine the efficacy of using the AB5 antibody as an ultrastructural probe for ganglion cell processes and to subsequently study their synaptic relationships in the retina.

Adult New Zealand White rabbits were sacrificed and enucleated. The posterior eyecup was processed for electron microscopic immunocytochemistry according to a procedure, developed by Eldred et al. (1983), involving the use of sodium borohydride enhancement and ethanol permeabilization techniques. A pre-embedding indirect immunocytochemical method employing the ABC-peroxidase technique was used to visualize the AB5 labeling sites in the rabbit retina. Silver-gold sections (approximately 70-80 nm.) were cut, counterstained and examined with an Hitachi H7000 transmission electron microscope.

The localization of AB5-labeling using electron microscopical immunocytochemical techniques was consistent with the results of previous light level studies indicating specificity of the antibody for the ganglion cells of the retina. AB5-immunoreactivity was localized in the ganglion cell perikarya, axons and dendritic processes in the ganglion cell layer, nerve fiber layer (NFL) and inner plexiform layer (IPL), respectively. Labeling appeared as an electron-dense precipitate in assocation with microtubules and the inner aspect of the plasma membrane.

AB5-labeled ganglion cell dendritic processes were observed in a variety of synaptic relationships in two distinct bands in the IPL. These bands represent the connections of the "on" and "off" types of ganglion cells in the retina (Famiglietti and Kolb, 1976). In all cases, the ganglion cell dendritic processes formed the postsynaptic element of the synapse. Labeled processes were observed receiving "conventional" synapses from amacrine cells (Fig. A), "ribbon" synapses from bipolar cells (Fig. B) and "serial" synapses involving both "conventional" and "ribbon" synapses (Fig. C). Although the ganglion cell axons in the NFL were heavily labeled (Fig. D), they were not observed to either receive or make synapses within the retina.

The results of this study demonstrate that the AB5 monoclonal antibody is an effective marker for ganglion cells and their processes at the ultrastructural level. Use of the AB5 monoclonal antibody in multiple-level studies will prove useful in determining the synaptic relationships of retinal ganglion cells with other neurochemically distinct retinal cell types. Studies such as these are critical to our understanding of how the retina processes visual information and in general how information processing in other areas of the brain occur.

Supported by NIH Grant EYO 6469 and the Retina Research Foundation.

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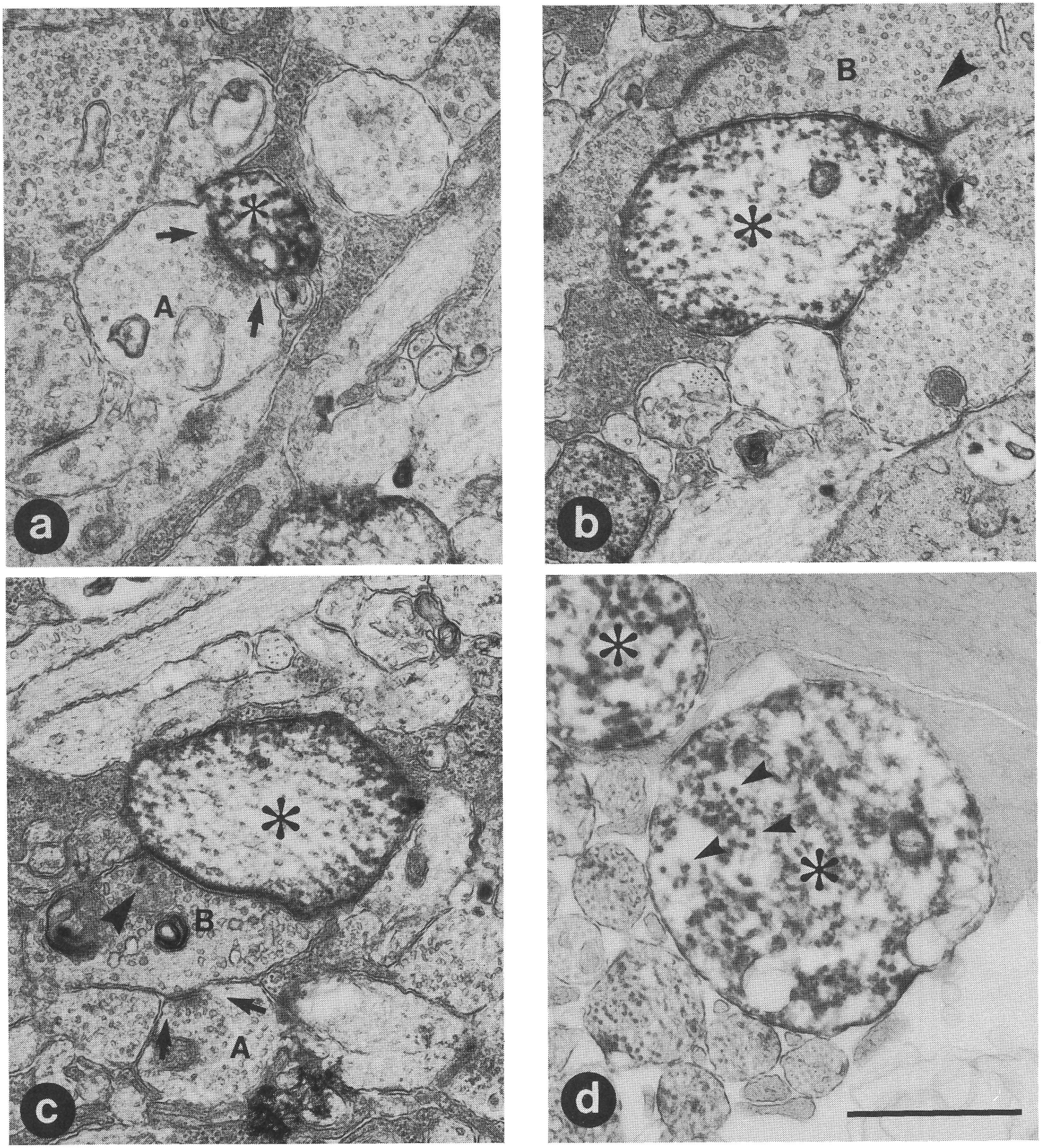

FIGURES. AB5-labeled ganglion cell processes in the rabbit retina. **a)** Labeled dendritic process (asterisk) in the inner plexiform layer (IPL) receiving a conventional synapse (arrows) from an amacrine cell process (A); **b)** Labeled dendritic process (asterisk) in the IPL receiving a ribbon synapse (arrowhead) from a bipolar cell process (B); **c)** Labeled dendritic process (asterisk) in the IPL receiving a serial synapse, (A) amacrine cell process, (B) bipolar cell process, (arrowhead) ribbon synapse, (arrows) conventional synapse; **d)** Labeled axons (asterisk) in the nerve fiber layer. Note AB5 label associated with microtubules (arrowheads). Bar = 1.0 μm.

FINE STRUCTURAL FEATURES OF CORONARY VASCULOGENESIS IN COLLAGEN LATTICES

By

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Many of the developmental events of the heart are better understood than the development of the coronary circulation. The latter process is still without a definitive and commonly accepted theory of formation. To better understand this process, we used an *in vitro* model to discover whether these structures developed from precursor cell differentiation or from the migration of endothelial cells from pre-existing vessels.

Intact embryonic chick hearts between stages 17-22 were grown for a period of four days on collagen gels. At that time the explanted tissue was removed, leaving cells that had migrated onto and into the gel; these remaining cells were grown for three more days.

Examination of the gels revealed layers of plated cells on the surface and mesenchymal-like cells migrating through the gel, which was similar to that observed by Runyan and Markwald (Runyan et al., 1982). In addition, vascular-like structures were found running parallel with the layer of plated cells, within the center of the gel and a few were continuous with the surface while extending deep into the gel.

The lumina of vessels were lined with a continuous layer of highly attenuated cells that were connected by cell junctions (plasma membrane densities). These cells are thought to be endothelial cells because they possess characteristics of endothelium: cell junctions, multivesicular bodies, attenuated cytoplasm, dilated rER and fenestrations (Ausprunk et al., 1974). Within the lumina of smaller vessels, a large amount of cellular debris was observed; however, in the larger structures, the lumina were free from debris.

Other cell types often associated with vessels during angiogenesis, among which are granulocytes (Cliff, 1962) and thrombocytes. The latter have been described as primary phagocytic cells in the chicken circulation (Chang and Hamilton, 1979). The presence of these phagocytic cells would explain the removal of the cellular debris.

To determine the area of origin of the precursor cells, dorsal mesocardia (DMC), and ventricular and atrial tissues from stage 15-16 hearts were grown separately. Extensive plating, seeding and vascular structures were observed in the DMC explants; however, the ventricular and atrial explants showed little seeding and no vessels. Therefore, it appears highly likely that the precursors originate in the region of the DMC.

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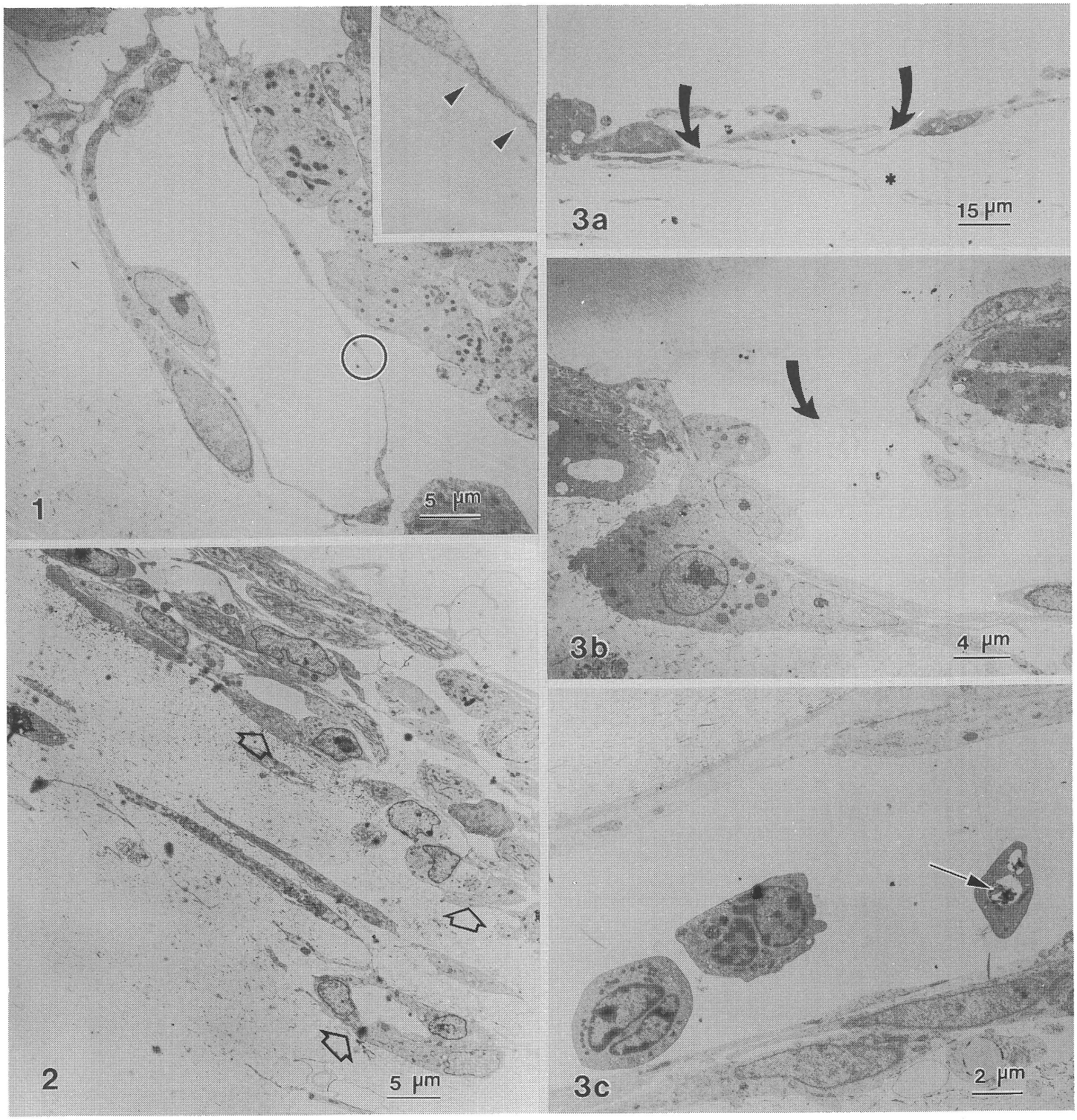

FIGURES Capillaries grown from dorsal mesocardial **(Figs. 1 & 3)** and whole heart **(Fig. 2)** explants. In **Figure 1**, the endothelium is highly attenuated and possesses fenestrae (inset, arrowheads). In **Figure 2**, three small capillaries (arrows) appear to be at various stages of development. Smaller vessels contain intraluminal cellular debris while larger vessels are virtually free of material. **Figure 3a** illustrates two capillaries that are continuous (arrows) with the surface epithelium. Note the extensive penetration of the longer capillary into the collagen, where it leaves and returns to the plane of section (*). **Figure 3b** is an electron micrograph of the longer vessel shown above. Cells resembling thrombocytes **(Figure 3c)** were found in the lumen of the above vessel. In birds such cells are phagocytic and thus may be responsible for the removal of intraluminal debris. (Note phagosomes: arrow).

THE REPAIR RESPONSE OF DAMSELFLY LARVAE TO GILL DAMAGE

By

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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 distint regions (MacNeill 1960, 1967). Gill autotomy occurs at a breaking joint at the base of each gill and is followed by a repair process which seals the larva's tracheae and hemocoele from the external environment.

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. Pennak and McColl (1944) observed a 50% death rate among gill-injured larvae of *Enallagma*. In our study, however, larvae of *I. posita* exhibited a death rate of less than 10%. 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.

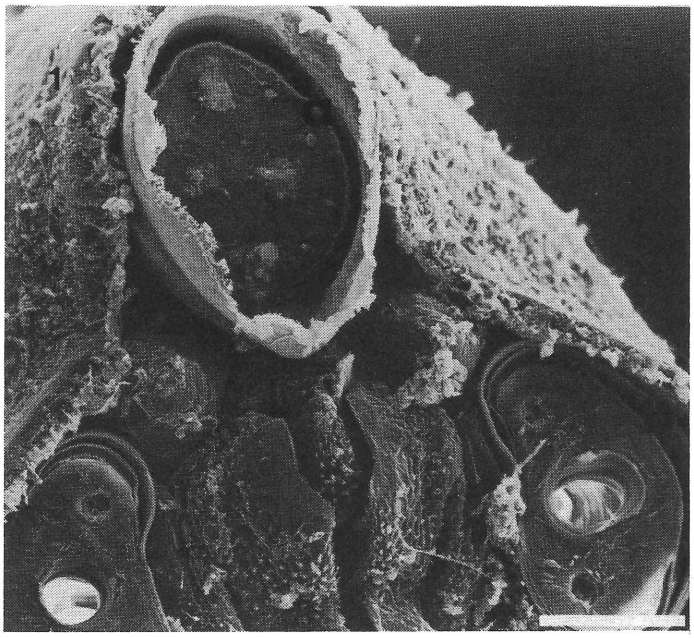

FIGURE 1. Gill stubs of *I. posita*. **(a)** repair following gill autotomy, **(b)** no repair following gill injury. (180X, bar = 100 m)

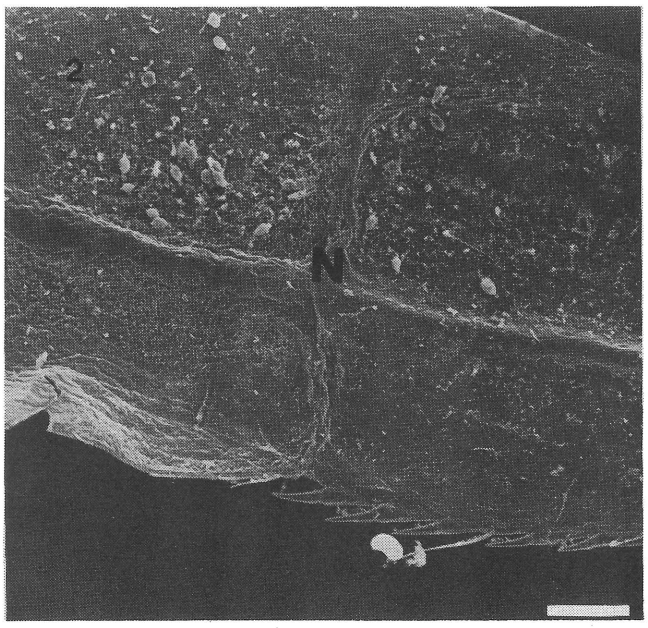

FIGURE 2. Nodal area **(N)** of gill. (100X, bar = 100 m)

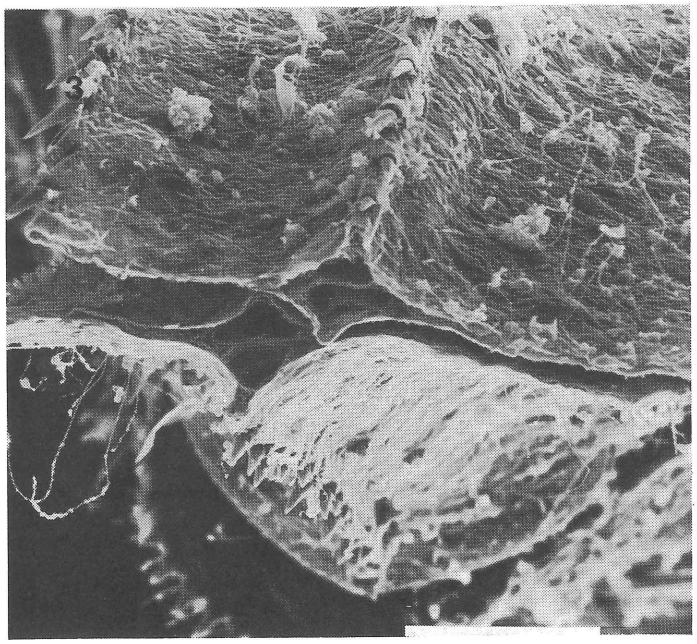

FIGURE 3. Prenodal injury. (240X, bar = 100 m)

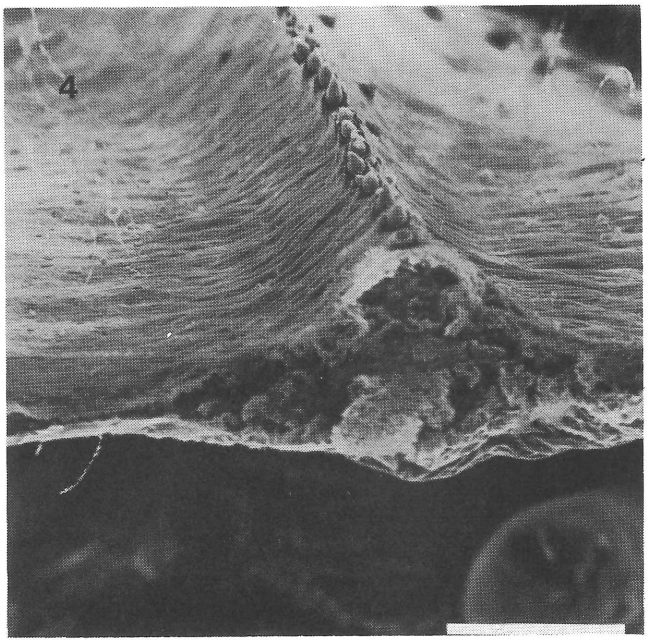

FIGURE 4. Repair following prenodal injury. (220X, bar = 100 m)

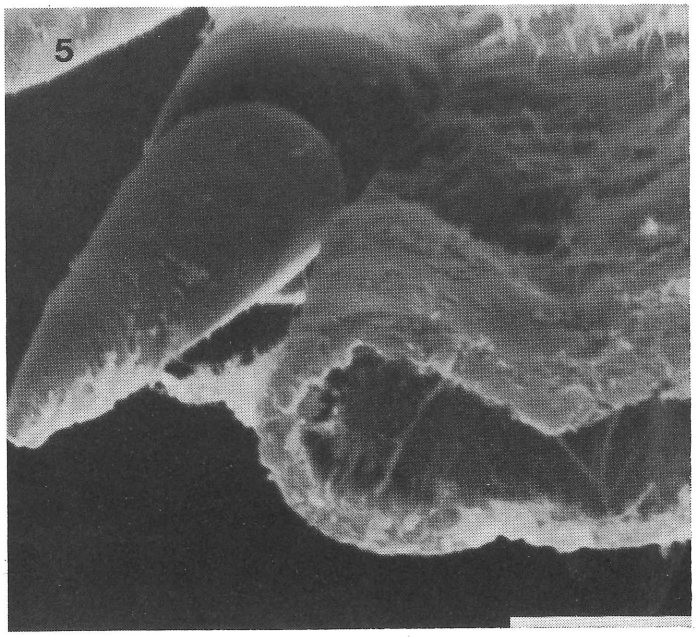

FIGURE 5. Protective spine on gill. (2400X, bar = 10 m)

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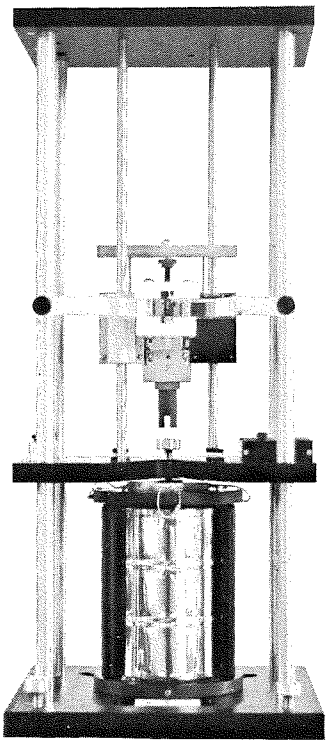

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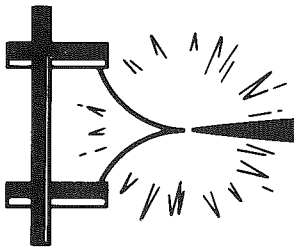

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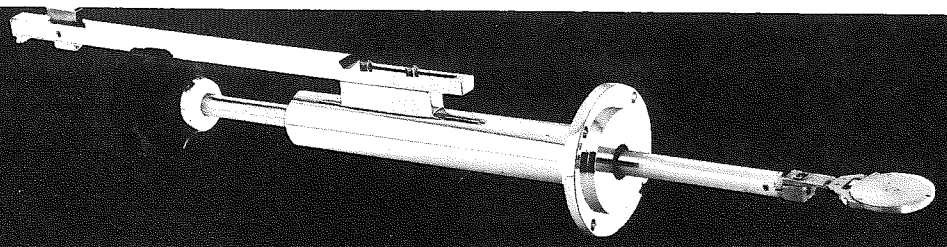

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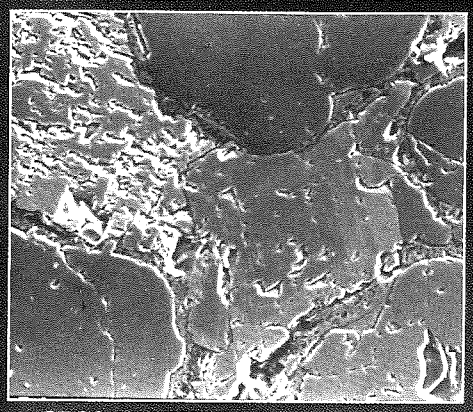

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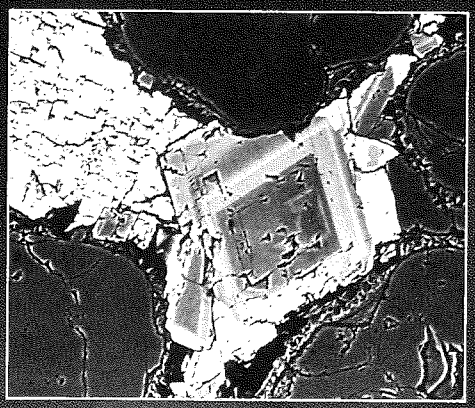

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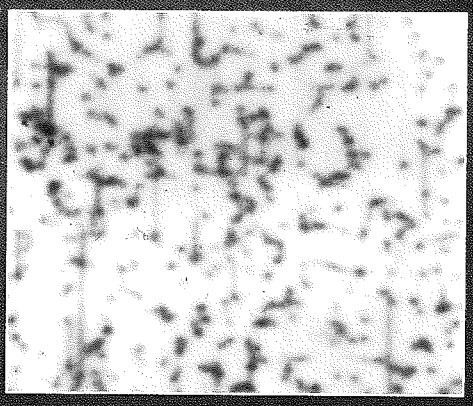

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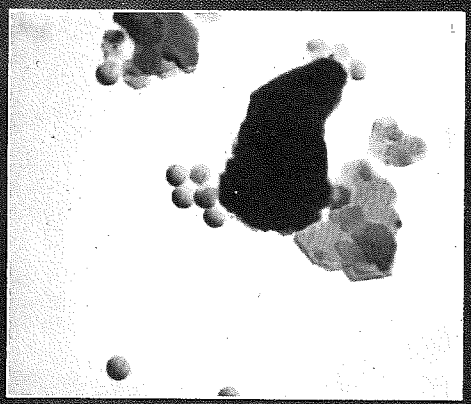

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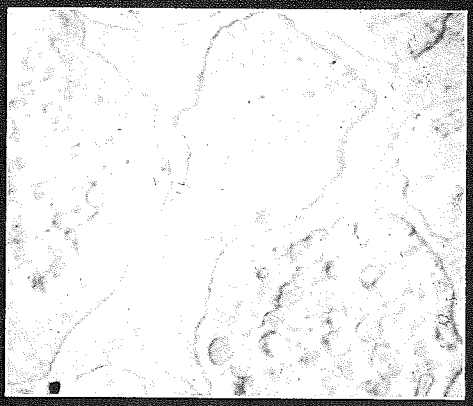

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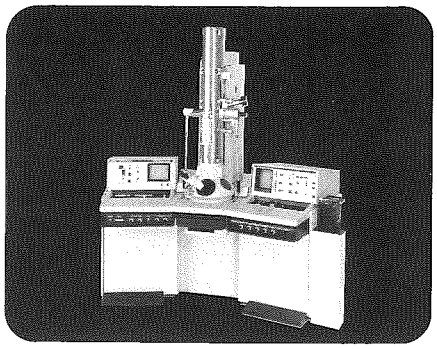

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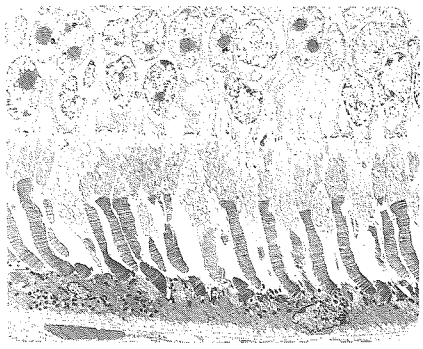

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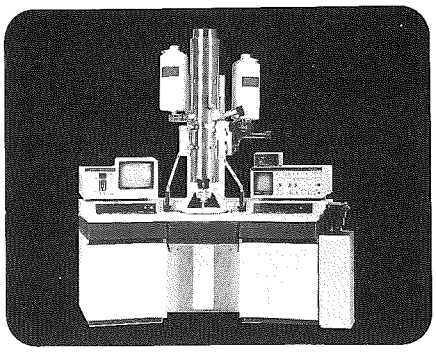

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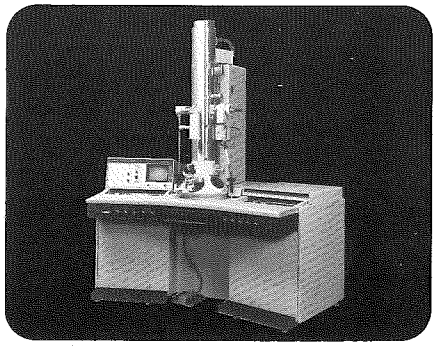

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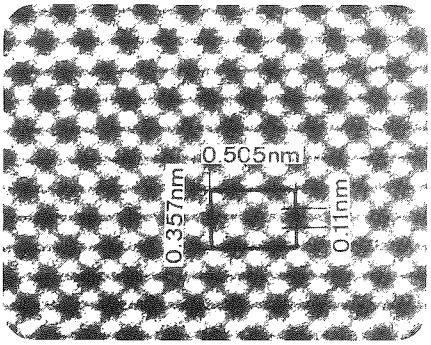

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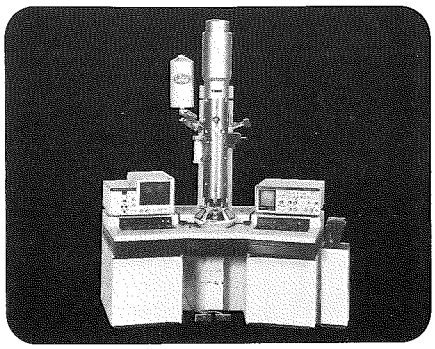

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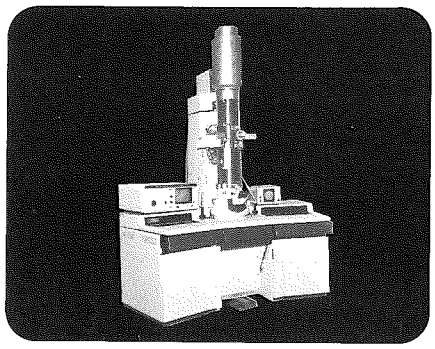

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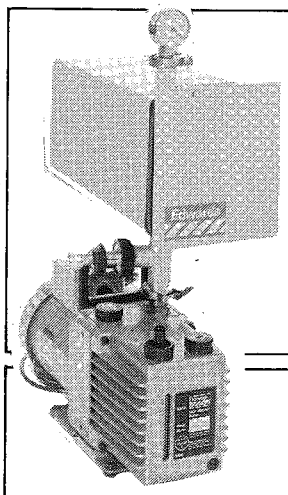

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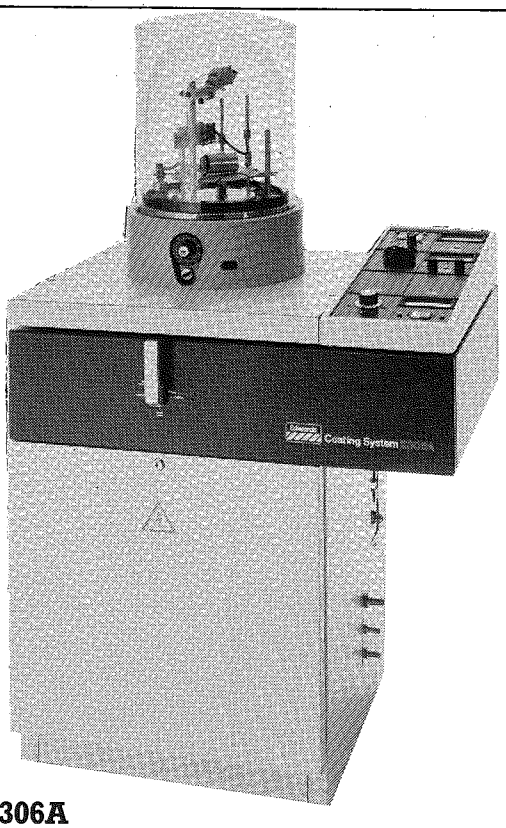

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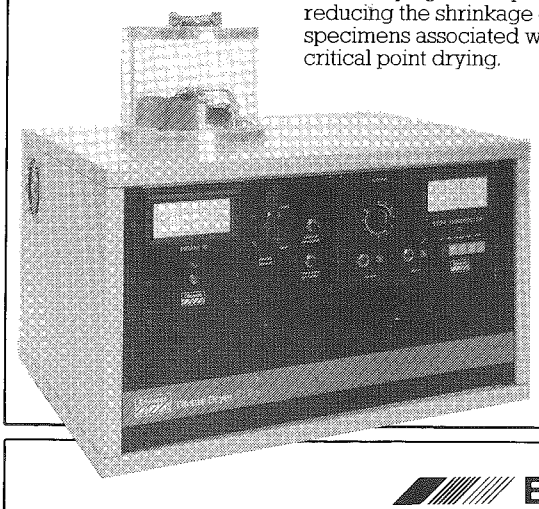

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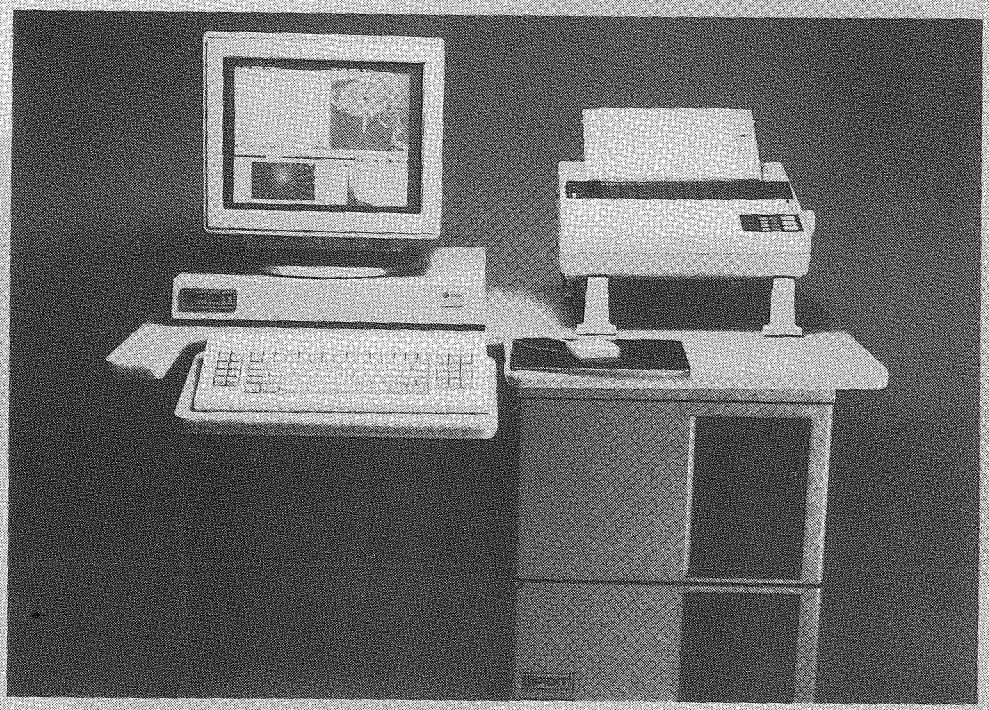

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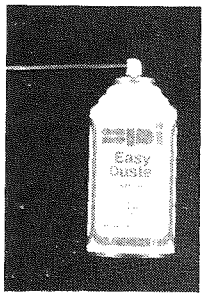

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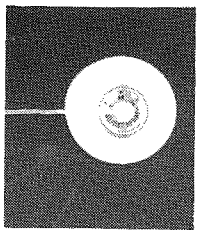

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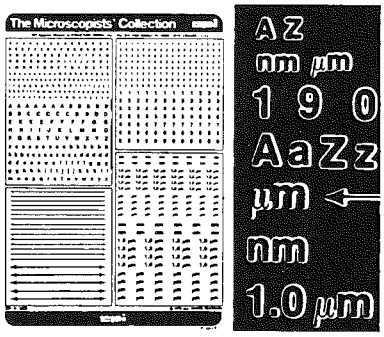

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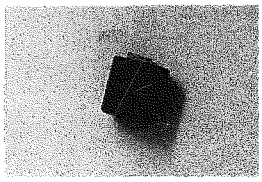

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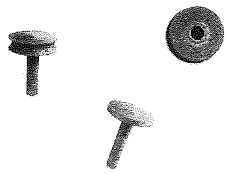

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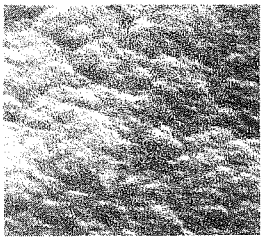

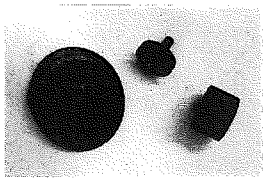

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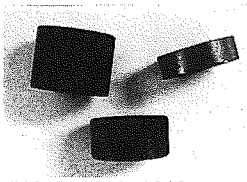

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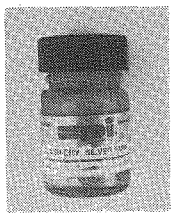

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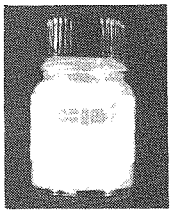

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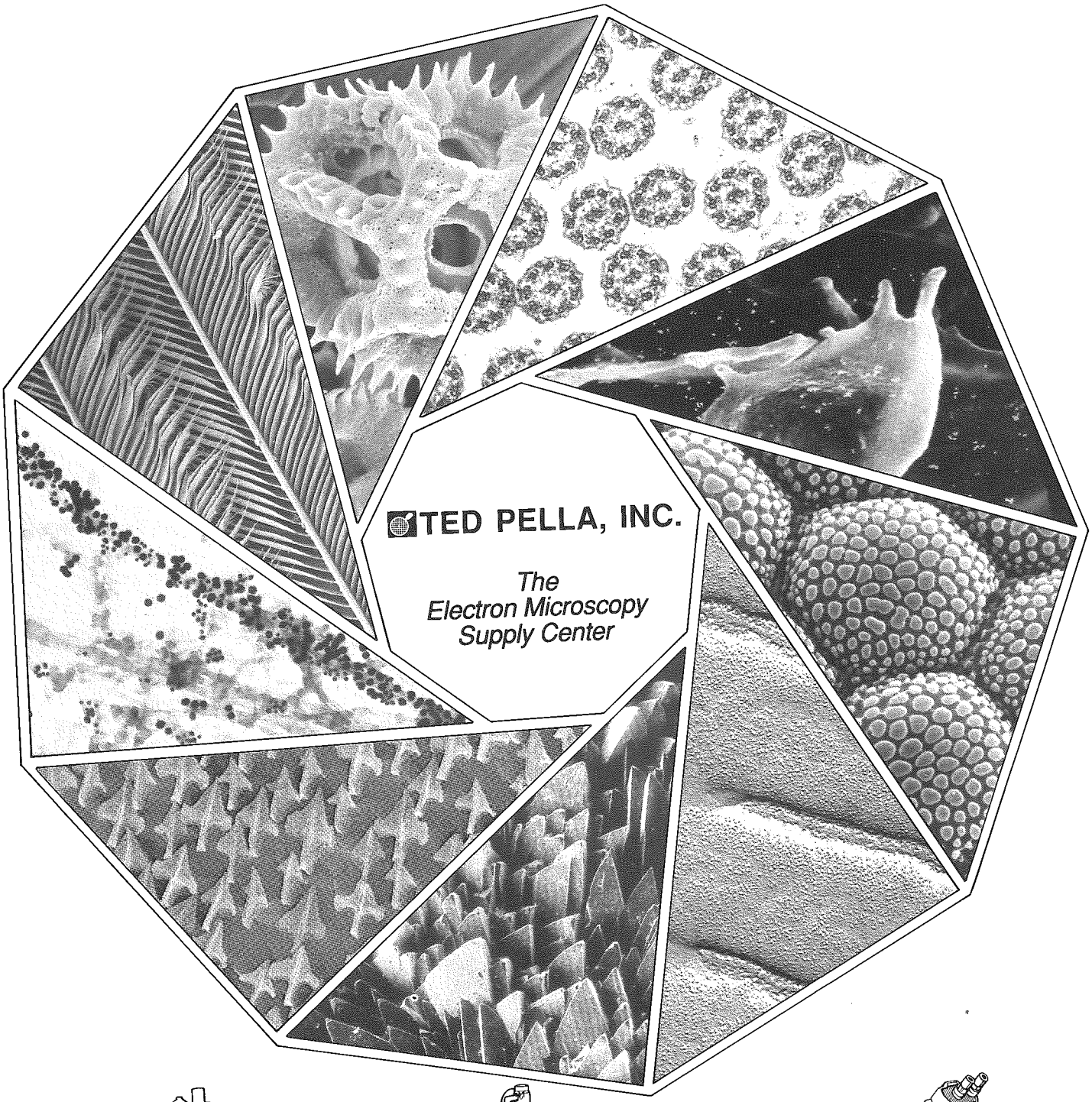

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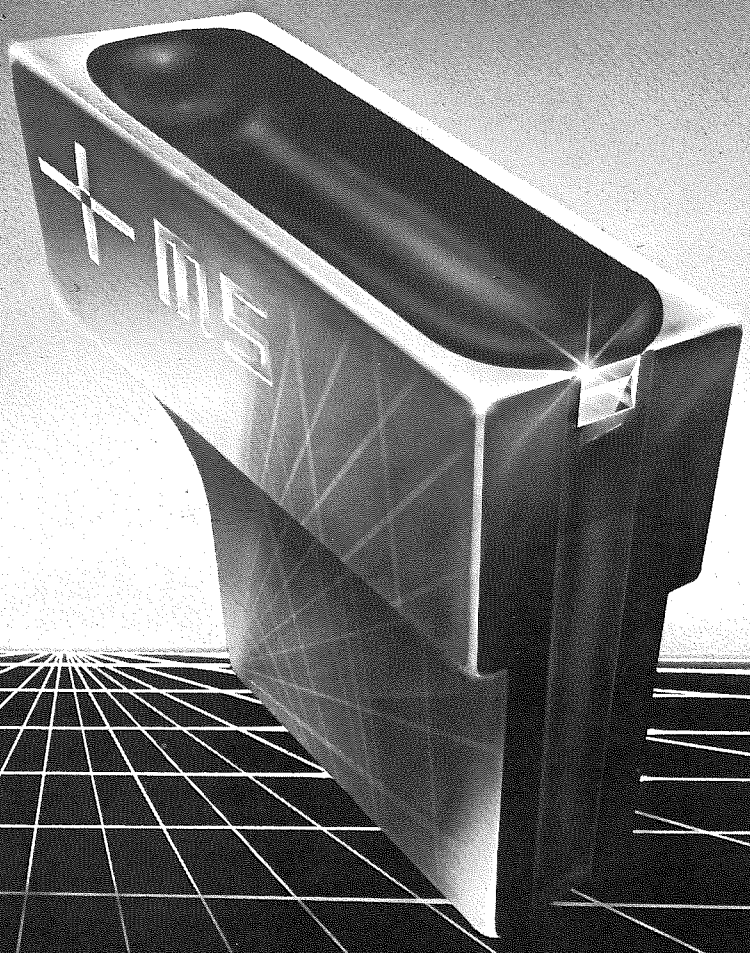

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What Is It?

Answer from Page 11

This issue's scanning electron micrograph shows part of an orb web, probably spun by a spider from the family Araneidae (orb weavers). This group is very large, widely distributed, and responsible for the round-type of web you can find in your backyard. Actually, this web was found in my backyard.

The reason I collected the web was to see if I could sputter coat it, bombard it with the electron beam, and still be able to see the sticky droplets. A lot of the web was destroyed, but a small portion of it did survive.

Some of the visable structures are: 1) A projection of the multi-stranded central portion of the web and its attachment to the single strands of the radial part, 2) the sticky droplets on the single strands, and 3) the coil-spring structure of some portions of the silk. This last feature probably increases the elasticity of the web to help protect it from breaking.

The webs are spun by female spiders, from fingerlike projections called spinnerets, on the back of the abdomen. Each spinneret has many, sometimes hundreds, of spinning tubes from which the web silk emerges. The spider spends most of its time head down in the center of the web where there is no sticky material. I asked a friend why a spider does not get caught in its own web and his answer was, because it knows where to step.

Micrograph by R.W. Davis, Dept. of Medical Anatomy, College of Medicine, Texas A&M University, College Station, TX 77843. (Magnification unknown.)

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Abstracts

BIOLOGICAL SCIENCES

PLATFORM PRESENTATION — FALL 1989

THE DEVELOPMENT OF ANAPLASMA MARGINALE IN MALE DERMACENTOR ANDERSONI THAT WERE TRANSFERRED FROM INFECTED TO SUSCEPTIBLE CALVES. K.M. KOCAN, D. STILLER, W.L. GOFF, W. EDWARDS, S.A. EWING, T.C. MCGUIRE, J.A. HAIR, AND S.J. BARRON, College of Veterinary Medicine, Oklahoma State University; USDA-ARS Animal Disease Research Unit, University of Idaho; and College of Veterinary Medicine, Washington State University.
Laboratory-reared male Dermacentor andersoni were allowed to feed for 7 days on a calf with ascending Anaplasma marginale parasitemia. The ticks were removed and held at room temperature in a humidity chamber for 5 days after which they were allowed to feed on a susceptible calf for 9 days. Gut and salivary glands were collected from ticks on each of the 21 days of the experiment and examined with LM and EM. Development of A. marginale was observed in midgut epithelial cells on the 7th day of feeding on the infected calf. While ticks were held between feeding periods many A. marginale colonies developed in gut cells; the first colonies contained one large reticulated organism that subsequently divided into many others. These smaller organisms became more electron-dense over time. When ticks were feeding on the second (non-infected) calf, A. marginale colonies were found from days 5-9 on the hemocoel side of the gut basement membrane. Final development occurred in salivary glands. Colonies were first seen in acinar cells on the third day that ticks fed on the second calf, and the highest percentage of infected host cells occurred on days 7-9 of that feeding. Salivary gland acinar cells often contains several large colonies and organisms within the colonies were initially electron-lucent but became electron dense by the end of the feeding period.

THE ROLE OF ELECTRON MICROSCOPY IN DIAGNOSIS OF INTRACRANIAL TUMORS IN CHILDREN. S.C. Bauserman, M.D.,Scott and White Clinic, Texas A&M University College of Medicine and R.T. King, Scott and White Clinic, Section of Electron Microscopy.

Brain tumors in infancy and childhood present the second most common neoplasm encountered in this age group and frequently present difficult diagnostic problems for the clinician, radiologist and pathologist/neuropathologist. Adjunctive studiers including immunohistochemistry (IHC) and electron microscopy (EM) are more commonly brought into use for such cases than in the adult.

We present representative case material from the files of the sections of Neuropathology and Electron Microscopy which illustrate the invaluable adjunctive role which is often played by EM in establishing the precise diagnosis for some unusual brain tumors of infancy and childhood. Examples include: PRIMITIVE NEUROECTODERMAL TUMOR (PNET), INTRA-VENTRICULAR FIBROMA AND TANYCYTIC EPENDYMOMA.

In each instance, the ultrastructural study of the biopsy material was used as an adjunctive investigation in order to properly identify the process and its possible histogenesis. Some of these unusual cases can be managed with simple surgical excision with excellent long-term survival without subjecting these young patients to radiation therapy.

FLORAL DEVELOPMENT - AN SEM EXAMINATION. PAULA S. WILLIAMSON, Department of Biology, Southwest Texas State University, San Marcos, TX., 78666.

Scanning electron microscopy was used to examine organogenesis of the flower of Ondinea purpurea subsp. purpurea. The flowers are solitary. There are 4 hypogynous sepals, 14-23 stamens which at anthesis are attached near the top of the ovary, and 3-7 carpels which form a compound ovary. Petal primordia are not initiated in this subspecies. Floral organs arise acropetally, in centripetal sequence, from the floral apical meristem in a condensed helical anthotaxy. Sepal primordia and most of the stamen primordia arise from the floral apical meristem in a manner typical of hypogynous flowers. At a later stage of development meristematic activity shifts to a subapical cup-shaped ring meristem, surrounding the floral apex. The floral apex elongates, by diffuse growth, forming a conspicuous central projection. The ring meristem is responsible for initiation of the more centripetal stamens and the gynoecium. The ring meristem forms radiating ridges which initiate locules and form stigmatic carpellary lobes. The proximal portions of the meristem are active in further development of the locular region and production of outer ovary wall tissue.

PARACLOACAL GLANDS OF ALLIGATOR MISSISSIPIENSIS. H.W. SAMPSON and P.J. WELDON, Dept. Anatomy, College of Medicine and Dept. Biology, Texas A&M University, College Station, TX 77843.

The histology of the paracloacal 'musk' glands of adult American alligators (Alligator mississipiensis) is described. The gland is a single secretory sac with a single duct and a central lumen partially occluded by a central, cylindrical conglomerate of cells and secretion product. The capsule of the gland consists of an outer layer of smooth muscle and an inner layer of connective tissue containing collagen and elastin fibers. Septae carrying blood vessels radiate from the connective tissue layer of the capsule to the border of the central conglomerate. Parenchymal cells containing lipid droplets enlarge from the periphery to the center of the gland. Secretions formed by degeneration of cells in the central cylinder are concentrated near the secretory duct. Histochemical tests indicate lipids but not mucopolysaccharides in the glandular exudate.

MORPHOLOGICAL CHANGES THAT OCCUR IN ERYTHROCYTES DURING CARRIER CELL PREPARATION. R.E. Droleskey, E.G. Moore, K. Andrews, and J.R. DeLoach, USDA-ARS, Veterinary Toxicology & Entomology Research Laboratory, Rt. 5, Box 810, College Station, TX 77840

When erythrocytes are subjected to controlled hypotonic dialysis for the production of carrier erythrocytes they undergo stage specific morphological changes. During carrier cell preparation for erythrocytes from several species, samples were taken for examination by scanning and transmission electron microscopy. Samples included nondialyzed control, dialyzed, resealed, and carrier erythrocytes. The majority of erythrocytes were fixed for morphological evaluation with a stage specific isoosmotic glutaraldehyde fixative. Isoosmotic fixatives using osmium tetroxide alone or in combination with glutaraldehyde were also tested. Nondialyzed control erythrocytes fixed by glutaraldehyde appeared mainly as biconcave diskocytes, and erythrocytes in the dialyzed state appeared mainly as spherocytes with some echinocytic projections. Osmium tetroxide fixed dialyzed erythrocytes usually had a flattened appearance. Regardless of the fixative used, the majority of carrier cells in a preparation usually exhibited morphology similar to that of nondialyzed control erythrocytes. In addition to these SEM observations, the results of the TEM examination of these preparations will also be presented.

SEED COAT MORPHOLOGY AND TAXONOMY OF NAMA SECTION CONANTHUS, John D. Bacon, Department of Biology, The University of Texas at Arlington, Arlington, TX 76019.

Nama sect. Conanthus is presently treated as housing three species, N. aretioides, with two varieties, N. densum and N. parviflorum. Chromosome numbers for N. aretioides and N. densum portray them as consistently diploid while N. parviflorum has both diploid and tetraploid populations. Examination of seed coat morphology revealed four seed forms in N. aretioides, the forms correlated with the four entities recognized by Brand in 1913. Seed coat morphology as N.densum is uniform in all populations examined, while the diploid and tetraploid populations of N. parviflorum have distinctive seed coats. However one of the seed forms in N. aretioides is identical with the seeds of diploid N. parviflorum and another is identical with the seed pattern in N. densum. Seed coat morphology suggests that additional entities should be recognized within sect. Conanthus and that relationships among these taxa may be quite different than now held.

CALCIUM HYDROXYAPATITE CRYSTAL ACCUMULATION IN THE INTERVERTEBRAL DISK OF PROGRESSIVE ANKYLOSIS MOUSE. H.W. SAMPSON, Dept. Anatomy, Texas A&M University, College Station, TX 77843.

Serum, intervertebral disks and vertebral bodies were removed from mice with the progressive ankylosis trait and normal sibling controls that were from 4 to 13 weeks of age. Whole vertebral columns were studied by techniques using KOH digestion and alizarin red staining. Serum calcium (8.44±1 mg/dl vs. 7.48±2) and phosphorus (6.66±1 mg/dl vs. 5.53±2) levels were within normal limits, but the levels in ankylosing mice were slightly lower than in control animals. Ankylosing mice had an increase in calcium content and a decrease in phosphorus content of the intervertebral disks. The magnitude of these changes increased with age. There was no significant difference in mineral or water content of the vertebral bodies. Gross morphological KOH-alizarin red studies reveal a progression from syndesmophyte formation through joint bridging to total fusion. Light microscopic techniques demonstrate the presence of small, irregular, eosinophilic, acellular foci of necrosis in the fibrocartilage disks that stain positive for calcium with alizarin red stain and Von Kossa. This is followed at 6 weeks by a proliferation of hyaline cartilage at the periphery at the end plate; the cartilage spans the disk and becomes necrotic. The adjacent vertebrae lay down new bone on their ventral surfaces which occasionally advances across, but does not completely span the intervertebral disk. Electron microscopic techniques reveal the necrotic foci seen in light microscope studies to be massive accumulations of mineral deposits within the extracellular matrix. Chondrocytes of older animals demonstrated post-mortem changes and contained numerous large vacuoles.

TWO ANOMALIES ENCOUNTERED DURING X-RAY MICROANALYSIS OF MINERALIZED BIOLOGICAL TISSUES. R. W. Davis, Editor, Department of Medical Anatomy, College of Medicine, Texas A&M University, College Station, Texas 77843-1114.

1) Osmicated, but unpoststained tissue sections of corn seeds contained small accumulations of Ca and P along the plasma membrane. After routine poststaining the optical density of these areas was increased, but Ca and P appeared to have been leached from the sections. By carefully comparing energy count rates in the Ca and background regions of stained and unstained sections, two conclusions were made: 1) Ca and P were not being leached from the sections, 2) the low levels of Ca and P were being obscured by an increased background after Pb and U poststaining.

2) Sections from mineralized areas of corn seed and bone showed the presence of Pb on an irregular and infrequent basis. The occurrence of Pb in these areas was considered to be an artifact, but the source was not apparent. Two observations were made: 1) accumulation of the Pb took place over about 5 minutes as the sections floated on water in the diamond knife boat, 2) accumulation was detectable only in mineralized (calcified) areas. It was concluded that the plastic filter holder used to filter the water for the diamond knife boat had been previously used for filtering Pb-citrate, that the Pb had been absorbed into the porous plastic and was subsequently being leached out.

FINE STRUCTURE OF THE PARANANUCLEUS IN DICYEMID MESOZOA. P. HORVATH, Dept. Biology, Texas A & M University, College Station TX 77843

Specimens of the genus Dicyemennea (Mesozoa: Dicyemida) from host Octopus bimaculatus were used to study the ultrastructure of the dicyemid paranucleus. The paranucleus is a structure closely associated with a multicellular hermaphroditic reproductive complex, the infusorigen. This entire complex resides confined within the cytoplasm of the huge cylindrical central axial cell of the rhombogen adult dicyemid. The rhombogen is a vermiform adult in the sexual reproductive stage. In the rhombogen, the infusorigen complex produces the oocytes and sperm that form zygotes. These develop into infusoriform dispersal larvae, which have a body structure very different from that of the vermiform larvae produced by the asexual adult stage, the nematogen. The paranucleus has all the conventional histological properties characteristic of nuclei at the light microscopic level. It does not appear to have a cell membrane or cytoplasm of its own. To date, transmission electron microscope studies on the infusorigen complex have not included the paranucleus. In this study, the fine structure of the dicyemid paranucleus is described. A double membrane nuclear envelope with nucleopores is observed. Vast amounts of electron-dense material is seen both within the paranucleus and passing through its pores. A peculiar relationship exists between the membranes of the paranucleus and the cell membrane of the oocytes it touches in that the membranes appear to fuse.

MORPHOLOGY OF ISCHNURA POSITA MALE GENITALIA AND ITS ROLE IN REPRODUCTIVE BEHAVIOR. MELISA L. MOORMAN AND JAMES V. ROBINSON, Department of Biology, University of Texas at Arlington, Arlington, Texas 76019.

A primary role of adult damselflies is to reproduce. Eggs are fertilized with sperm stored by the female in the bursa copulatrix and spermatheca. Females will often mate multiple times in a single day. In zygopterans, copulation is accomplished by the male grasping the female's first thoracic segment with his anal appendages, and then both the male and female bend their abdomens into the mating position. Three post copulatory behaviors are exhibited in zygopterans, following copulation the pair 1) will fly in tandem to the oviposition site and remain in tandem while the female oviposits, 2) will fly in tandem to the oviposition site, the female will then oviposit alone, but the male will remain nearby to "guard" her, 3) will release and the female oviposits alone, usually at a later time. In many species males are able to displace sperm from a female's previous mating using spines located on the distal segment of the penis. Non-contact guarding and ovipositing in tandem would prevent a competing male from removing the current male's sperm. Copulation in I. posita has not been documented, however, females oviposit without the male being present. Female I. posita also have an effective avoidance behavior to mating. In response to an approaching male the female curves her abdomen downward. The male then ceases his pursuit. She may also use a "wing warning" behavior to discourage contact. The fact that males recognize this behavior and do not pursue these females suggests that they may not be able to displace sperm from previous matings. In this study the distal segment of the penis in I. posita has been examined using SEM to determine if the penis has the appropriate morphology for sperm displacement.

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

Earlier observations of Prosopis glandulosa (mesquite) seeds revealed the presence of a pleurogram approximately 8 weeks after floral initiation. The beginning of surface cracks were also observed some 6 weeks after the pleurogram became evident. Further developmental events such as surface patterning and more extensive cracking in the fully developed seeds are being determined.

ABNORMAL HUMAN CILIA: SOME EXAMPLES FROM UTHCT. L.D. GRAY and D. SUEZ*, Dept. of Cell Biology and Environmental Sciences, *Dept. of Allergy and Immunology, The University of Texas Health Center at Tyler, P.O. Box 2003, Tyler, TX 75710.

Primary ciliary dyskinesia (The Immotile Cilia Syndrome) is an inherited condition in which defects of the ciliary and sperm flagellar axonemes cause these structures to be non or dysfunctional. Patients with this genetic defect present with chronic respiratory infections and are usually infertile regardless of sex. Ultrastructural assessment of axoneme structure in respiratory cilia and/or sperm flagella can, in combination with the clinical picture, be helpful in diagnosis. Although axonemal defects are a hallmark of this syndrome, not all types of malformations are considered to be genetic. Certain defects are likely symptoms of chronic respiratory irritation and for others, their origin is not known. Also, the percent of defective cilia and flagella in the general population is not known at this time, making diagnosis on the basis of the ultrastructural appearance of a few cilia and a questionable patient history a difficult if not impossible task. A defect that affects dynein arms and is recurrent in virtually all axonemes is likely genetic in origin, however, variable defects or a consistent malformation present only in a moderate number of cilia present difficulty in diagnosis. Examples from pediatric patients at the Health Center will be presented.

SYNOVIAL SARCOMA. Bruce Mackay and Nelson G. Ordonez: Department of Pathology, University of Texas M.D. Anderson Cancer Center, Houston.

The soft tissue sarcoma known as synovial sarcoma is so named because of a resemblance in light microscopic sections to normal synovial membrane, but its histogenesis continues to be controversial. Many of the tumors arise some distance from the nearest synovial joint, and an epithelial component is not always present. We have examined a series of synovial sarcomas including biphasic and monophasic tumors with electron microscopy and a battery of immunocytochemical stains, and have compared the tumor cells to those of normal synovium. The epithelial component of a biphasic synovial sarcoma closely resembles an adenocarcinoma ultrastructurally and appears quite different from the superficial layer of cells in normal human synovium. The latter have slender cytoplasmic extensions that ramify on the exposed surface, and a similar appearance is seen in the lining cells of the sheath of tendons. The validity of monophasic synovial sarcoma as a specific entity has been questioned, but it is given support by the findings from our study. The spindle cells are similar to those in the stroma of biphasic tumors, and epithelial differentiation can sometimes be detected by immunostaining and electron microscopy. Synovial sarcoma is thus a specific type of soft tissue sarcoma which displays to varying degrees of epithelial and mesenchymal components. Neither immunostaining nor electron microscopy has demonstrated a histogenetic relationship with synovium or tendon sheath, but both techniques are useful in resolving the often difficult diagnosis of monophasic synovial sarcoma.

COMPARATIVE STUDY OF OXALIS DILLENII SEEDS FROM DIFFERENT GEOGRAPHIC POPULATIONS. Louis H. Bragg and Nikki Matthis. Department of Biology, The University of Texas at Arlington, Arlington, Tx. 76019

Seeds of Oxalis dillenii were obtained from plants collected from different geographical locations in the United States. These plants had exhibited ecotypic differentiation in an earlier study. The seeds were compared for their morphology as well as the occurrence and distribution of calcium oxalate crystals in their seed coats. Seeds were variable in size and shape within and between populations. Calcium oxalate crystals were common to all the seeds observed. The occurrence of these crystals warrant further studies between species within Oxalis to determine their usefulness as taxonomic markers.

ULTRASTRUCTURAL AND FUNCTIONAL PROPERTIES OF A pSV3Neo-Transfected GRANULOSA CELL LINE. L.S. Stein, R.C. Burghardt, K.A. Neck, and M.S. Frey, Department of Veterinary Anatomy, Texas A&M University, College Station, TX 77843.

A cell line derived from primary rat ovarian granulosa cells was generated by transfection with pSV3Neo, a plasmid containing the early region genes of SV40 virus. The cell line, SV-GC, is being characterized as a potential model system for studies of granulosa cell function. The process of follicular development in vivo is under hormonal control and involves the transformation of immature granulosa cells into more differentiated, hormonally-responsive cells culminating in terminally differentiated luteinized granulosa cells which play a key role in the maintenance of early pregnancy. However, these changes occur spontaneously in isolated granulosa cells maintained in vitro, making it difficult to study sequential stages of granulosa cell development. Therefore, there is a need to develop cell lines which are representative of different stages of granulosa cell development. Initial studies using indirect immunofluorescence and Western blot analysis identified T-ag and p53 in SV-GCs which indicate the presence and expression of the early region genes of the SV40 viral genome. SV-GCs exhibit a differentiated morphology and apparent indefinite growth without luteinization in culture. Ultrastructural studies elucidated the differentiated morphology which includes mitochondria characteristic of mature granulosa cells, abundant microvilli and the presence of gap junctional contacts. Functional studies of cell-cell communication using fluorescence recovery after photobleaching revealed extensive intercellular dye transfer which is comparable to that of primary granulosa cells in culture. Because intercellular communication is a hallmark of normal granulosa cell function and ultrastructural studies support a differentiated cell type, SV-GCs may be a good model for in vitro studies of granulosa cell function. Aided by Basic Research Grant No. 1-1052 from the March of Dimes Birth Defects Foundation, a Biomedical Research Support Grant, and Research Enhancement Funds, College of Veterinary Medicine.

BIOLOGICAL SCIENCES

POSTER PRESENTATION — FALL 1989

ULTRASTRUCTURE AND IMMUNOCYTOCHEMISTRY OF LUNG CARCINOMAS. Mannie Steglich, Nelson G. Ordonez and Bruce Mackay: Department of Pathology, University of Texas M.D. Anderson Cancer Center, Houston.

Subdivision of primary lung carcinomas into small cell and non-small cell categories can usually be achieved by routine light microscopy on histologic or cytologic preparations, provided they are representative and acceptably preserved. However, the morphologic features of these two broad groups overlap, as do those of the sub-types of the non-small cell tumors. The information that is obtained by immunostaining and electron microscopy clarifies the nature of the various types of lung carcinoma, and can be helpful in differential diagnosis when conventional light microscopy is inadequate. In this presentation, the following features are illustrated. Tumors classified by light microscopy at the ultrastructural level to possess glandular or squamous differentiation, or both may be evident. A small proportion of non-small cell lung carcinomas show neuroendocrine activity. Morphometric analysis confirms that the cells and nuclei of undifferentiated large cell carcinoma are predominantly larger than those of carcinoids and small cell carcinomas, and that considerable overlap occurs between the latter two tumors. Carcinoid tumors display a range of appearances at the ultrastructural level but the presence of dense-core granules is a consistent finding. Granules are in contrast often sparse or absent in small cell carcinomas, and when present their caliber (approximately 120 nm) is similar to that of some carcinoids: among 50 carcinoids, the mean granule diameter ranged from 95 to 291 nm.

MATERIALS SCIENCES

PLATFORM PRESENTATION — FALL 1989

MEASUREMENT OF MICROSCOPIC DISPLACEMENTS IN GRAPHITE/EPOXY COMPOSITE MATERIALS USING A SEM-GENERATED DIGITAL MAP. R.C. Burghardt[1], J.M. Ehrman[2], T.C. Stephens[2], and M.F. Hibbs[3]. Departments of Veterinary Anatomy[1] and Mechanical Engineering[3] and Electron Microscopy Center[2], Texas A&M University, College Station, TX 77843.

Graphite fiber-reinforced resin composite materials are widely used in aerospace, automotive and sporting goods applications requiring high strength to weight ratios. In order to predict the fracture toughness of a composite material from the constitutive properties of the resin and fibers, experimental methods for the analysis of microscopic displacements and strain fields that develop at the fracture crack tip within the composite material are required. Information derived from measurement of displacements, and calculation of strain fields can then be used to test micromechanical models of matrix dominated fracture. A method was developed in which it is possible to conduct real-time fracture analysis of epoxy-based composite materials, and to subsequently obtain micrometer-scale measurements of displacements in the region of the crack tip. A map matrix was generated on the surface of test specimens in an SEM equipped with a tensile stage, along with an X-ray spectroscopy and image analysis system. A 40 by 40 point digital map was introduced onto the surface of the specimen using the digital X-ray mapping function of the X-ray analysis system which produced a surface matrix with point spacing of 10 μm. The quality of maps varies with test specimens and therefore it is necessary to optimize microscope operation parameters for each resin tested. Reproducible results were obtained with both neat resins and graphite-epoxy composites. In situ analysis of a region of a propagating crack-tip grown using the tensile stage reveals a deformation zone ahead of the crack-tip and images of the stages of microcracking were captured by the image analyzer for subsequent measurement of displacement. Direct measurement of crack-tip displacements from SEM electron beam-induced reference matrices provide an important tool in characterizing the fracture behavior of both neat resin and composite materials.

Procedures for Investigating Corrosion in Retrieved Dental Amalgam Restorations. R.D. Spears, Dept. of Anatomy, Baylor College of Dentistry, Dallas, Texas.

Some investigators subscribe to the theory that corrosion contributes to the failure of dental amalgam restorations. In vitro studies of Ag-Cu-Sn amalgam support this concept (Sarkar et.al, J. Oral Rehab. 1981, 8:401). The in vivo evidence has been less convincing due to difficulties in obtaining in vivo specimens, locating areas of corrosion, analyzing the composition of the corrosion products and assessing the amount of corrosion that occurred. The purpose of this study was to develop a systematic method of examining corrosion in retrieved amalgam restorations.

Amalgam restorations were placed in molars of acrylic teeth in partial dentures and retrieved intact after 96 weeks in situ. The occlusal surface of the retrieved restorations were examined by SEM. Areas at the amalgam-tooth interface were selected for detailed study. The restorations were sectioned at selected sites and mechanically polished to reveal the microstructure of the amalgam. SEM observation was used to screen out potential locations of corrosion, then XEDA analysis was used to distinguish corrosion products from other deposits such as plaque. Amalgam specimens that were corroded in vitro were analyzed by XEDA for use as standards.

The problems encountered in the analysis of these retrieved specimens included: excessive plaque accumulations which coated the restoration obscuring the corrosion products; potential loss of corrosion products during polishing; difficulties detecting small quantities of corrosion products with the XEDA limitations. Overall, the systematic examination of these retrieved in vivo specimens revealed that corrosion products were less evident than predicted by in vitro studies. (Support by NIH DE06539.)

A COMPARATIVE MORPHOLOGICAL STUDY OF THE STINGING EMERGENCES AND TRICHOMES OF FOUR GUATAMALAN FAMILIES OF STINGING PLANTS. Tammy L. Hancock and Dr. John Bacon, Dept. of Biology, University of Texas at Arlington.

Four species of stinging plants representing four plant families, Urticaceae, Euphorbiaceae, Loasaceae and Hydrophyllaceae were examined. Comparisons were made as to stinging emergence and leaf trichome morphology. Stinging emergences of all families represented were found to be similar in overall appearance, yet morphological variations were found to exist in each family. Stinging emergence density was found to vary in each family. Leaf trichome morphology was found to be quite distinct in each family. Leaf trichomes were found to differ markedly from stinging emergences in each of the four families studied.

Information for Authors

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(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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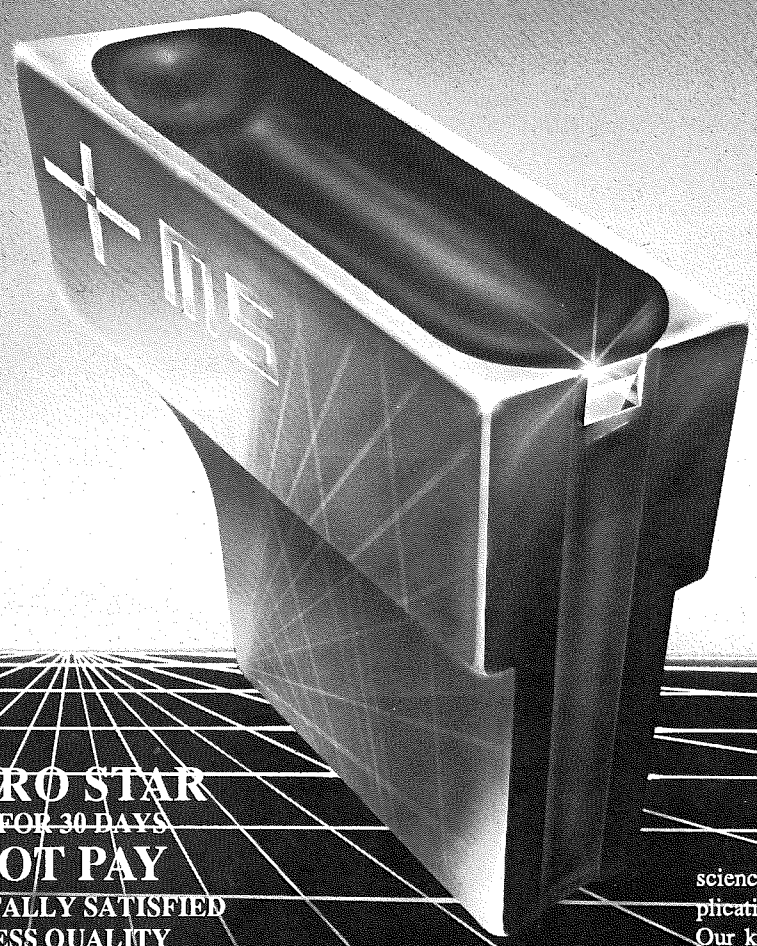

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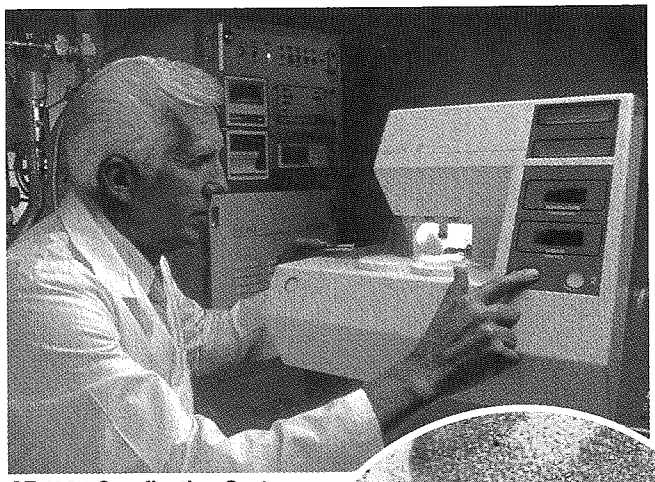

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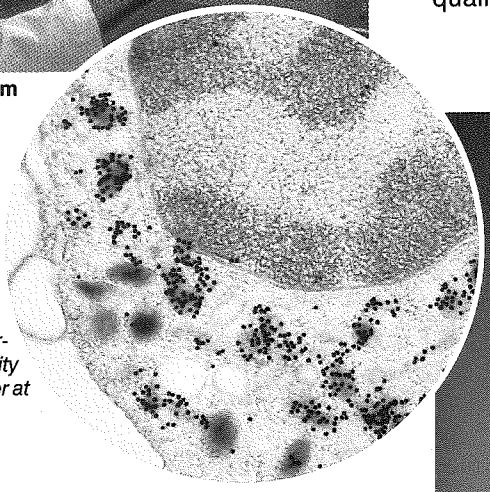

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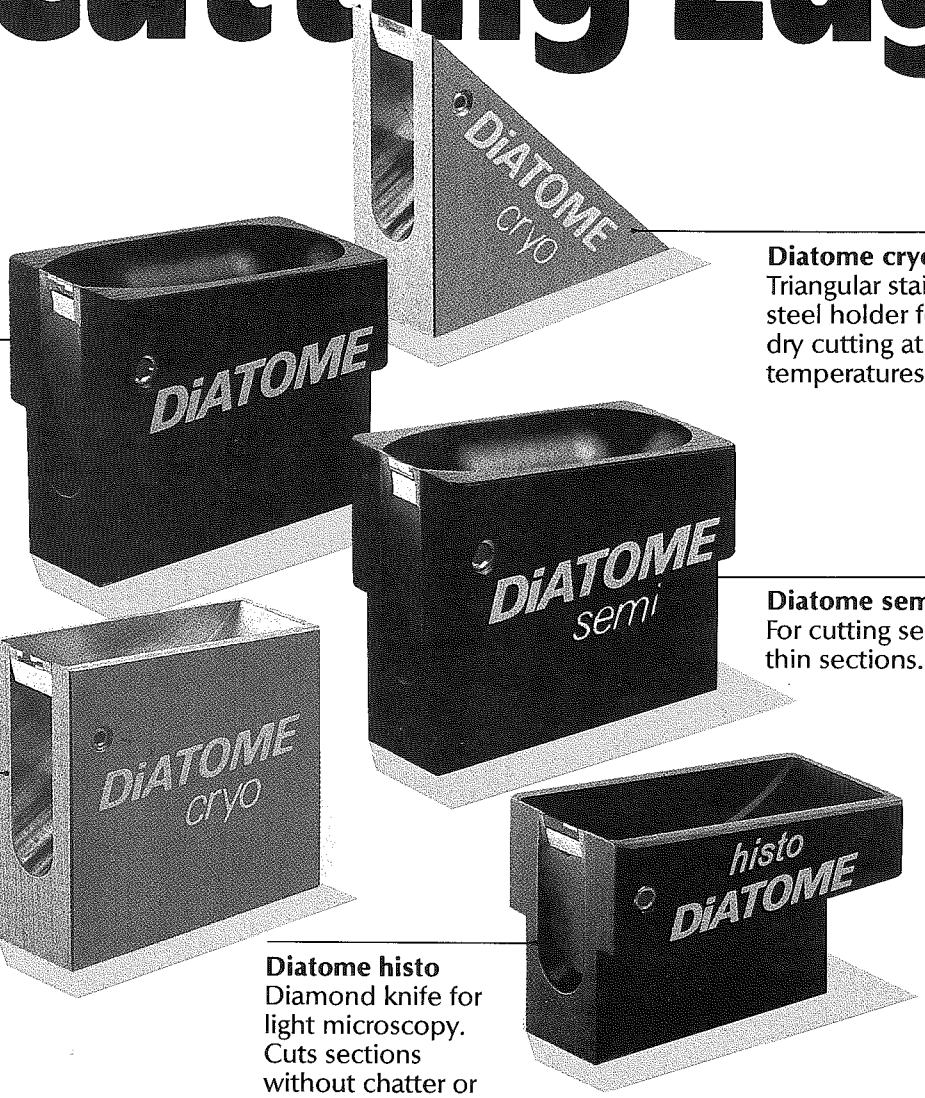

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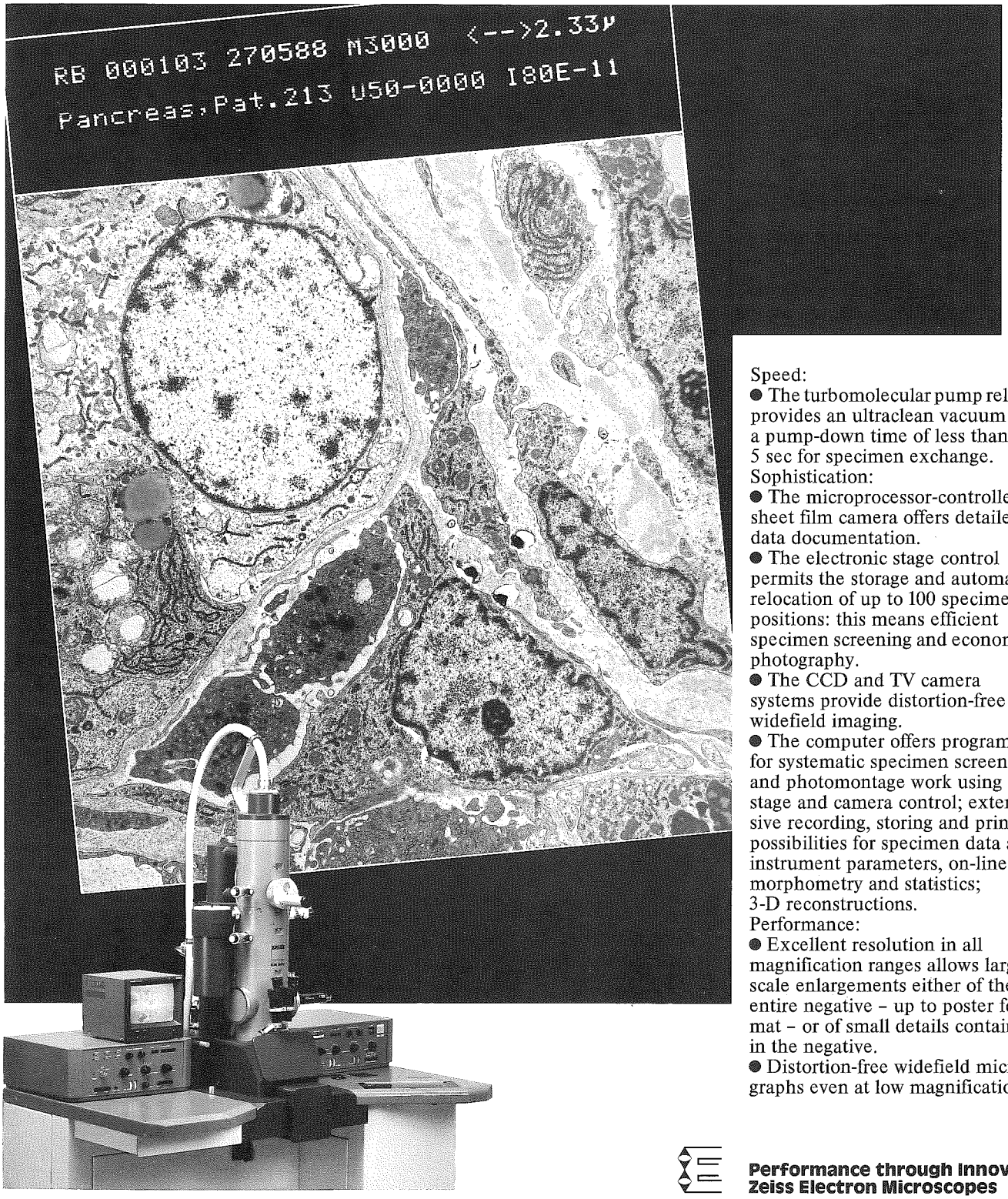

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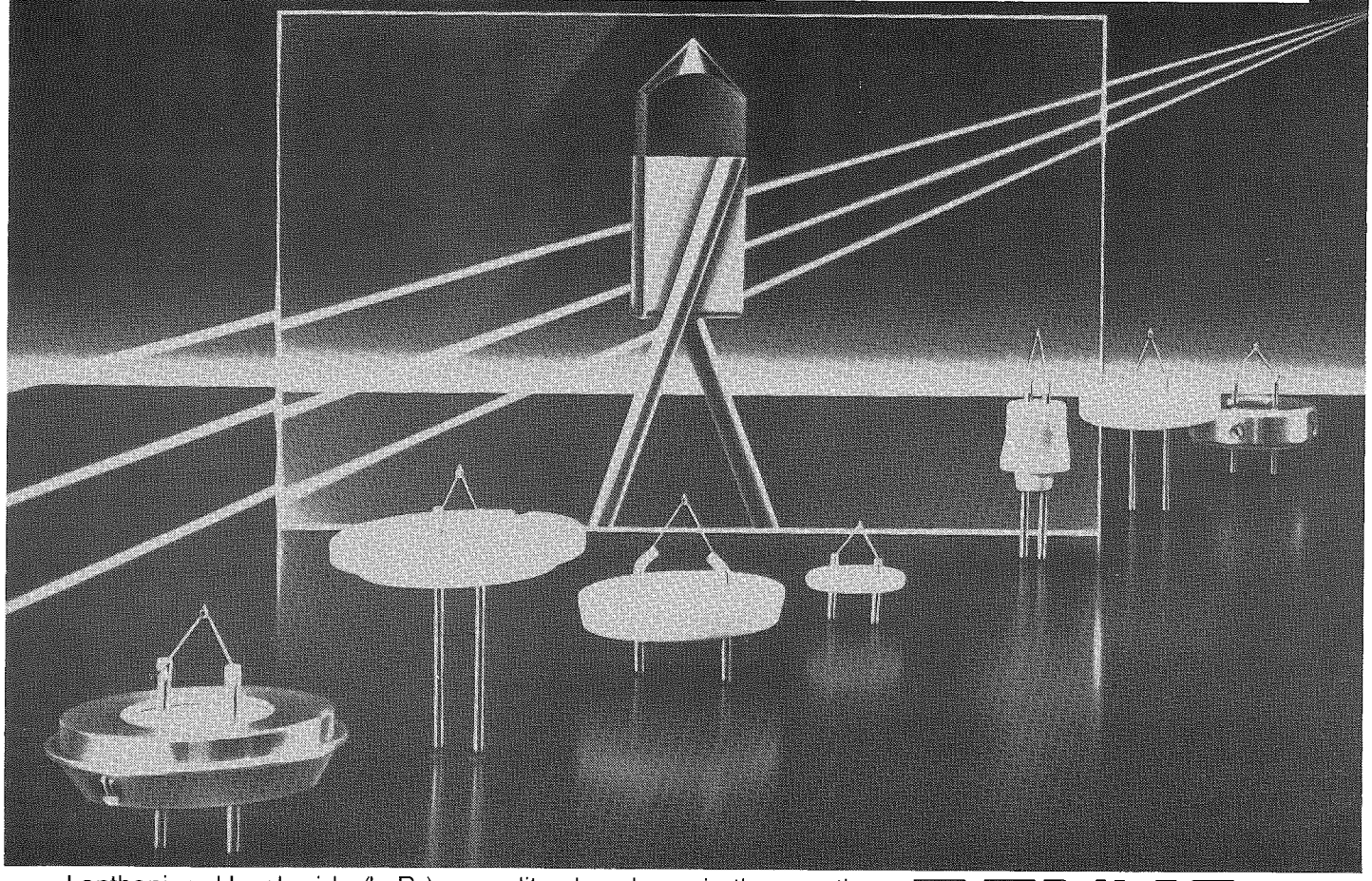

Lanthanium Hexaboride (LaB_6) single crystal cathodes have revolutionized electron beam technology. The intense, coherent beam produced by an LaB_6 cathode is superior in every way to tungsten. But all LaB_6 cathodes are not created equal. To find out who is recognized as the industry leader, just open the wehnelt of a new JEOL, Philips, Cambridge or ISI electron microscope. You'll find only one brand of LaB_6 cathode installed: the DENKA Model 3.

DENKA manufactures the industry's purest, highest quality LaB_6 crystals for use in our cathode tips. We start with crystals larger than any other manufacturer can grow. Our high-quality cathodes represent the pinnacle of precision processing technology and quality control for the precision electronics industry. But quality also shows in the way the DENKA LaB_6 Cathode, Model 3, performs on the job.

Scan our possibilities:

HIGH BRIGHTNESS. An LaB_6 beam is ten times brighter than tungsten, has superior resolution, and features a wide range of acceleration voltages. It provides sharp, clear pictures down to the smallest detail.

LONG LIFE. Heat and vacuum are the final determiners of any cathode tip's life, but at 1550°C and a vacuum of 10^{-7} Torr, a service life of about 500 hours can be expected, far longer than the expected life of other brands.

STABILITY. Thanks to its simple and durable construction, every DENKA LaB_6 Cathode, Model 3, offers a stability of better than 3 percent per hour at 1550°C, encouraging long-term test and research uses.

INTERCHANGEABILITY. There's no need to buy a new electron microscope just to enjoy DENKA quality. Among the many brands for which the DENKA LaB_6 Cathode, Model 3, is available are JEOL, ISI, Hitachi, Philips, Cambridge, Amray, Zeiss, Siemens, and many more.

We're building the best LaB_6 cathodes and we're making them only one way — Precisely, DENKA.

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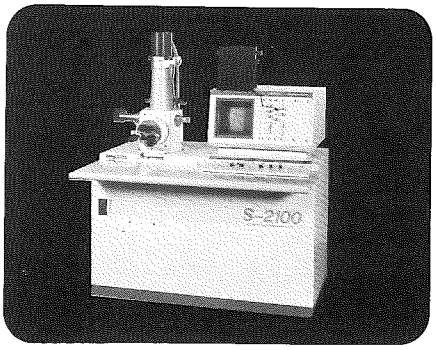
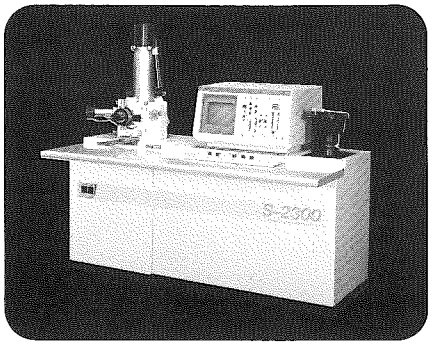
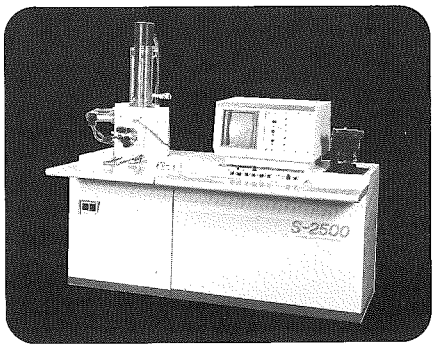
FOCUS ON HITACHI'S WIDE RANGE OF INNOVATIVE SEM'S



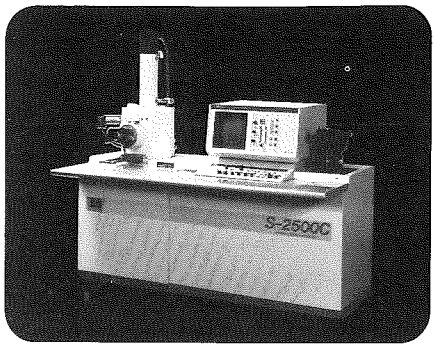
Model S-2100 SEM: ☐ 60 Å Guaranteed ☐ Super Auto Focus ☐ Fully Automatic Computerized Astigmatism Correction ☐ 4" Specimen Capability ☐ Accelerating Voltage Range: 1kV–25kV



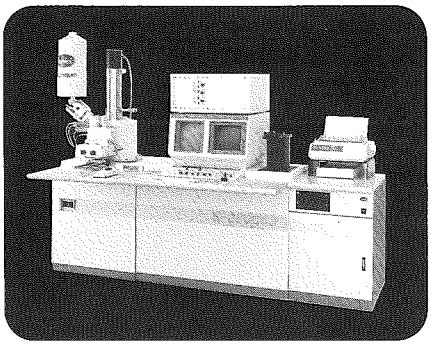
Model S-2300 SEM: ☐ Unique new objective lens design guarantees 45 Å with tungsten source ☐ Accelerating voltage range from 500V to 25kV in 35 steps ☐ Large specimen chamber accommodates 6" specimens ☐ Fully automatic computerized astigmatism correction



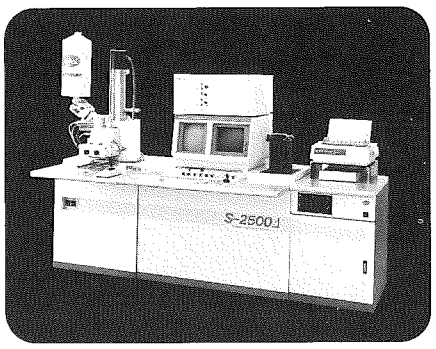
Model S-2500 SEM: ☐ Large specimen chamber, accommodates 200 mm diameter specimens ☐ High take-off angle for both WDX and EDX analysis, expandable to full EPMA ☐ Fully automatic computerized astigmatism correction ☐ In-lens detection system ☐ 35Å guaranteed with W, 25Å with LaB_6 (Option)



Model S-2500C SEM: ☐ 60° conical lens allows high resolution on large samples at high tilts. ☐ High take-off angle for EDX and WDX at 12 mm working distance ☐ Mouse-driven computer stage option ☐ 40Å guaranteed resolution with W, 30Å with LaB_6 (Option)



Model S-2500C ⊿ Delta: ☐ Built-in EDS keyboard and monitor ☐ Reads and sets microscope parameters via EDS ☐ Automated programmable stage ☐ Built-in image processing ☐ 60° conical lens allows EDX and WDX analysis at 12 mm working distance ☐ Light element detector standard



Model S-2500 ⊿ Delta: ☐ Built-in EDS keyboard and monitor ☐ Reads and sets microscope parameters via EDS ☐ Automated programmable stage ☐ Built-in image processing ☐ In-lens detection system ☐ High take-off angle for EDX and WDX analysis ☐ Light element detector standard

Since 1910 Hitachi has pioneered in electrical devices and, later, with electronic instruments and other high technology equipment. It has grown to become one of the 50 largest industrial corporations in the world, and has secured over 20,000 patents through consistent R&D efforts. Through these efforts, Hitachi has developed a comprehensive line of SEMs to fit every application and budget.

Standard Hitachi SEM features:

- ☐ Fully Computerized Operation
- ☐ Super Auto Focus
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