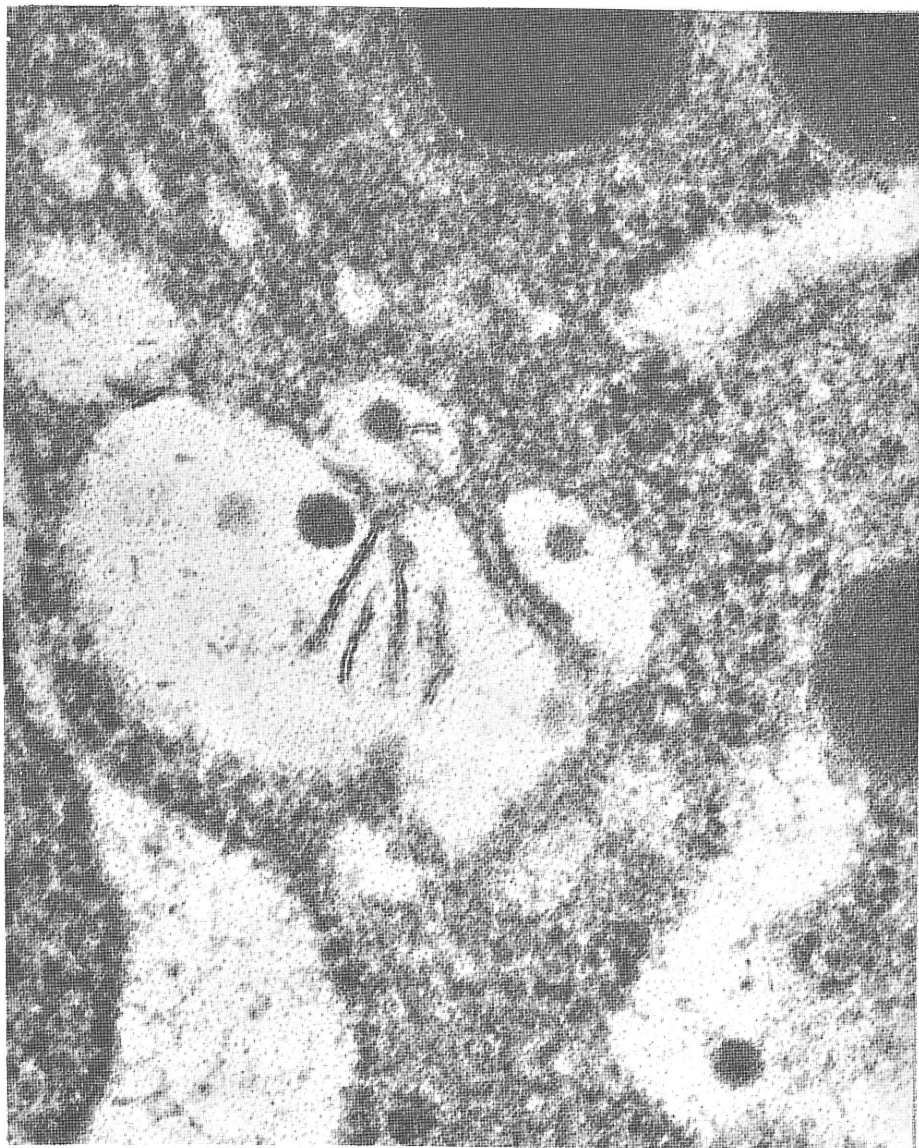


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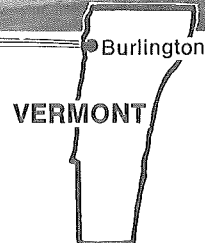
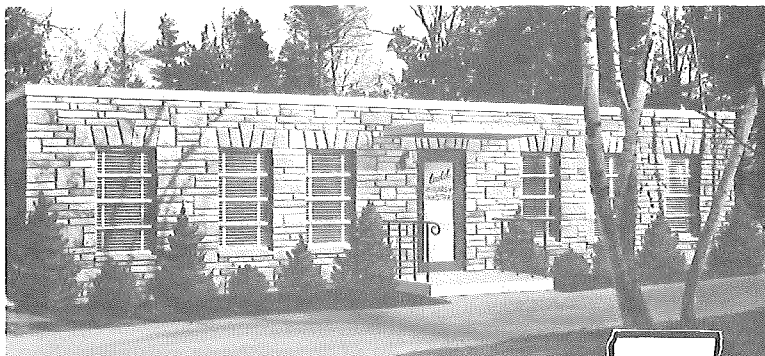
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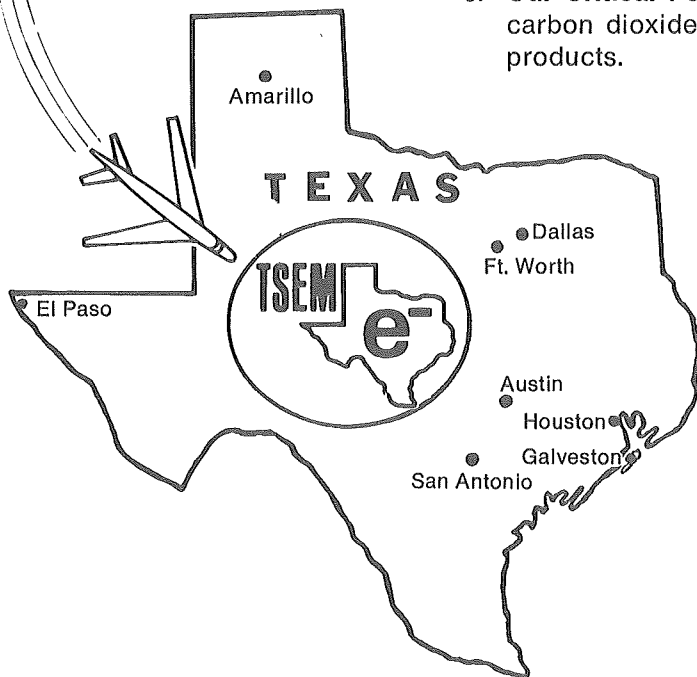
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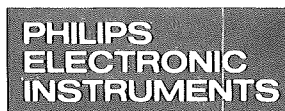
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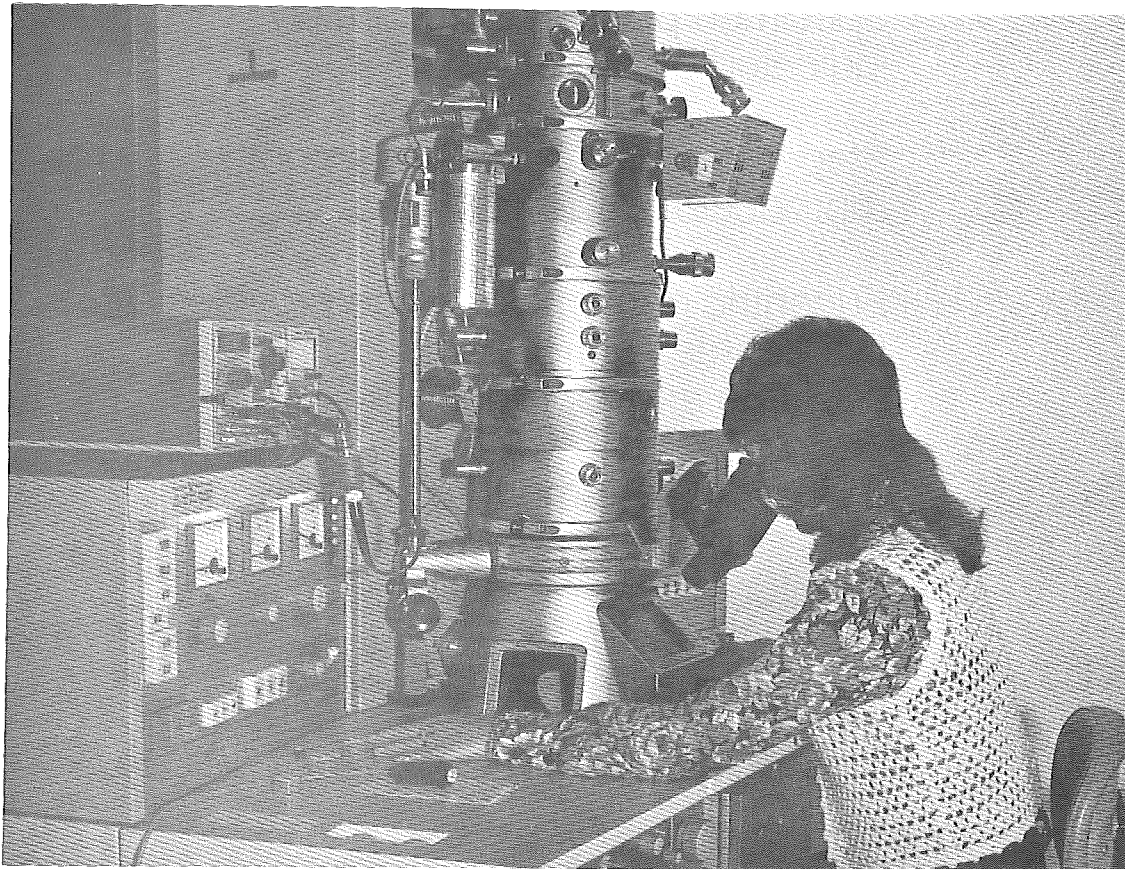


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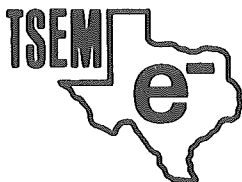
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VOLUME 6

NUMBER 1

WINTER 1975

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COMMENT

In these times of economic stress and rampant inflation there remains one commodity that has remained stable--the \$5 TSEM membership dues. Immediately one might ask if this fee merely reflects the lack of service to the membership, thus not warranting the percentage increase in cost that has been evident in other areas of the economy. Let us look at the benefits of TSEM membership for \$5:

1. An excellent newsletter of top quality mailed to members three times each year.
2. Literature reviews, area news, recent publications, editorials and current short notes are presented.
3. Three meetings each year that satisfy recreational as well as educational and research interests.
4. A social meeting that allows contacts to be renewed and nurtured.
5. Encouragement of graduate student studies and research through awards and travel stipends.
6. A ready contact with major E. M. suppliers with a collective voice to enhance service in this area.
7. A membership certificate.
8. A voice in the National EMSA through our affiliate representative.
9. A medium for job placement.
10. An hour discourse from either Bob Turner or Joe Wood, just by asking (this might be a minus rather than a plus).

So, as you can see, this membership is one of the last REAL bargains. It is suggested that you, the members, each get a colleague or graduate student to join a dynamic, active, professional society that gives you MORE than your money's worth!!

To facilitate this action the next mailout will include membership forms.

PRESIDENT'S MESSAGE

We are nearing the end of the first ten years of existence for The Texas Society for Electron Microscopy. During this time we have grown to a membership of nearly 400 individual and 18 active corporate members. Our triannual meetings attract 100 or more persons each time with 25 to 30 contributed papers being presented along with nationally and internationally known speakers, some of whom come from our own ranks. Along with our growth in membership, our Newsletter has also grown to reflect member interest, participation and pride in a strong professional organization. Our joint symposia with the Louisiana Society combines resources and produces an even better dissemination and exchange of research through the electron microscope. These joint symposia have increased in attendance to more than 200 participants. This bond with our sister society remains strong as we enter our fifth joint meeting. We welcome this association and hope to maintain our mutual exchange for many years.

TSEM is recognized as one of the most successful local societies. We must not, however, rest on our laurels but we must continue to seek improvement in all aspects of our society. Since our society is you, the member, let us celebrate our tenth anniversary at the May meeting by having each current member bring a new member to the Houston spring meeting. Such an effort would generate an even greater TSEM. We must remember that new officers come from the membership and new members provide an active group from whom new ideas and leadership develop. Continued growth and development is also correlated with the active involvement of the corporate members whose sales in Texas are estimated at approximately 20% of their total national sales. Therefore we must increase and support corporate memberships to serve our mutual interests.

I must admit that my leadership has been prodded and helped by the members and executive committee of the TSEM. Thank you for the assistance in maintaining TSEM in a number one position.

TERRY HOAGE

President

T. S. E. M. MINUTES

The T. S. E. M. business meeting was held October 5, 1974 at 1:15 p. m. at Waterwood. President Terry Hoage presided. The minutes of the last meeting were read and approved. The Treasurer's report was read and approved.

Bob Turner reported that the ballot to change the By-Laws was approved 89 for and 3 against. The revised By-Laws were published in the Newsletter (Vol. 5, Number 3). President Hoage commended Brinkley, Turner and Wood for their effort in rewriting the By-Laws.

President Hoage recognized Jeffrey Chang's election to the Academia Sinica, Republic of China (Taiwan).

President Hoage announced that abstracts from both the L. S. E. M. - T. S. E. M. joint meeting and the Graduate Student Meeting will be published in the Texas Reports.

Dick Peterson, Local Arrangement's Chairman, reported that the Hyatt Regency in Houston has been selected for the site of the May 2-4, 1975 meeting.

President Hoage requested that any members interested in a charter flight from Houston to New Orleans for the February, 1975, meeting contact Joe Wood.

President Hoage requested that pictures from previous T. S. E. M. meetings be forwarded to Jerry Berlin for use in compiling a historical document.

President Hoage announced Bob Turner's election to an E. M. S. A. directorship. Specifically, he will represent the local affiliates.

Bill Brinkley complimented Terry Hoage for the excellent Waterwood meeting.

The meeting adjourned at 1:40 p. m.

JERRY BERLIN

Secretary

FINANCIAL REPORT

T. S. E. M. Fall Meeting

Waterwood, Texas

October 4-6, 1974

Receipts:

A. At Waterwood

1. Registration	\$ 290.50
2. Dues received at meeting	69.00
3. Meal tickets	405.00
Sub-total	<u>\$ 764.50</u>

B. Newsletter Advertisements \$ 575.00

C. Dues (other than at Waterwood)

1. Corporate	\$ 450.00
2. Regular and Student	158.00
Sub-total	<u>\$ 608.00</u>
Total	\$1,947.50

Disbursements:

A. At Waterwood

1. Meals	\$ 672.35
2. Miscellaneous expenses	55.76
3. Mailout costs	66.14
Sub-total	<u>\$ 794.25</u>

B. Newsletter

1. Printing costs	\$ 307.25
2. Miscellaneous expenses	55.76
Sub-total	<u>\$ 363.01</u>

C. Other expenses since Waterwood meeting

1. Mailouts and other expenses of secretary	\$ 182.32
Total	\$1,339.58

Summary:

A. Total receipts	\$1,947.50
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B. Total disbursements	<u>1,339.58</u>
Amount of surplus	\$ 607.92

Bank balance (checking) prior to Waterwood meeting	\$1,865.50
--	------------

Bank balance (checking) as of January 17, 1975	2,493.33
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Certificate of deposit	<u>1,077.85</u>
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GRAND TOTAL	\$3,571.18
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WATERWOOD REPORT

For those members who could not find time and/or funds to attend the Fall meeting at Waterwood Resort, I am happy to report that the meeting was quite successful. The panel discussion and NSF speaker provided some insight to grant problems and excellent papers were given. The buffet dinners were well received by all who participated and the Waterwood staff excelled at serving the TSEM members. Jerry Berlin led the assault of the gaming table and came away a million dollar winner (and the personal friend of two lovely feminine gamblers) while Alan Weckerling lost everything. Gary Cole fought the golf course and tennis courts (I don't know who won). Ron Dodson was the runaway winner of the fishing tourney. And you would not believe Paul Enos' form (or lack of it) in the pro tennis display. Joe Wood and Bob Turner opened their palatial residences to an impromptu social that had research continuing for many hours in relation to the flight behavior of the "stork." President-Elect Kischer fell (once again) to the relentless onslaught of Berlin's underhand tennis serves. The Aggie flash even found his way into the wilderness taking four hours for the one hour drive. In general an informative, good time was had by all.

TERRY HOAGE

President

NOTES ON THE AFFILIATE REPRESENTATIVE

As you may recall, the affiliate societies of the Electron Microscopical Society of America elected a representative at the August 1974 meeting. Bob Turner of TSEM was elected and was installed as a member of the EMSA executive council at the February 20, 1975 meeting held in conjunction with the LSEM-TSEM symposium. The question of the role that an affiliate representative would play in EMSA decision making has not been clarified. In a preliminary comment from Bob Turner, he sees his job as primarily a strong representative on the EMSA Council to aid the local societies in any way which would tend to strengthen their organization.

A few more specific ways in which the affiliate representative could assist the local societies, as seen by Bob are:

1. Coordinate speakers from EMSA that would travel to society meetings to serve as main speakers at EMSA expense.
2. Establish a rebate system to local societies for each member that belongs to EMSA.
3. Serve as spokesman for local societies for economic subsidies coming before the EMSA council.
4. Serve as intermediary between local societies and EMSA in terms of needs and complaints that might arise.
5. Generate support from EMSA for worthy students attendance at the annual EMSA meeting.

Since the EMSA council did not specify the duties of the affiliate representative all aspects of the position remain open to discussion and decision by the council. With that in mind, it is asked that all members express to Bob Turner those ideas and needs that they feel would be a reasonable responsibility of the affiliate representative.

Bob Turner has given distinguished service to TSEM and we know that he will be equally effective in his new role as the local societies voice in the EMSA executive council. We give Bob our support and best wishes for continued success. Keep up the outstanding work.

TERRY HOAGE

President

BASIC TESTS FOR SCANNING ELECTRON MICROSCOPE PERFORMANCE

During the last few months we have had the responsibility of evaluating scanning electron microscopes for purchase by our institution. We are pleased to report that all of the companies we have encountered have been most cooperative in allowing us to evaluate and test the performance of their equipment and it is not the purpose of this report to rate different scanning electron microscopes.

During our evaluations we put together some basic performance tests which have proved helpful to us and which we feel may be of value to others. The tests may help in determining problems you are having with the scanning microscope you use or the tests may be used to check microscope performance before and after service work.

To get a fair evaluation of an instrument you are interested in buying we think it is a good idea to have the sales representative make sure that the scope you are evaluating is equipped with the same type of accessory image processing features you will have on the scope you want to buy. In this way you will know just what to expect from the instrument you buy.

1) Resolution Test for the Secondary Imaging Mode

The specimen should be mechanically stable, have fine detail, absence of any charging, and a long shelf life. We have used TDK magnetic tape (see figure 1, mag. 100,000X) which was recommended to us by JEOL, Inc. This tape will be supplied free from JEOL by writing Mr. R. T. Santorelli, JEOL, Inc., 447 Riverside Avenue, Medford, Mass., 02155. It may be a good idea to call companies you are dealing with to ask them about the specimens they are currently using to determine resolution and to decide on one specimen that will be acceptable to them as well as to you. All of the companies we have dealt with have been willing to take all such calls on a collect basis.

The test is usually performed at a magnification of 100,000X where $100\text{\AA} = 1\text{mm}$. All accessory image processing should be turned off and basic performance tested. Take two pictures of each field to validate fine detail. It is even better if stereo pairs of pictures of a given field are produced for later stereoscopic viewing. When dealing with companies allow each to produce their best pictures and record all microscope conditions so that the results can be reproduced and compared. The smallest distance that can be measured between two closely spaced particles gives the resolving power.

2) Drift Test

The above specimen can be used for a drift test which may be performed by taking a series of 100,000X pictures at zero time, at 5 min and finally at 10 min. The specimen should not be moved during the test, the beam should remain on and the microscope should not be refocussed. The image movement in Å per min is then measured.

3) Distortion and Magnification Test

The specimen we used was a cross lined grating replica ruled with 2160 lines/mm in the X and in the Y perpendicular axis (figure 2, mag. 20,200X). Such a grating replica is sold by Ernest F. Fullam, Inc. (catalog #1002X). The grating replica is mounted on a 300 mesh copper grid. It is recommended that at least two such grating replicas be purchased since they are easily damaged. A microscope stub with ruled lines can be used as a specimen for low magnification tests where larger distortions may be expected. The diffraction grating replica is useful up to magnifications of 100,000X. Deviations in the straightness of the lines indicate distortion. Because the absolute number of lines/mm is known for the grating replica the magnification can be checked for sequential magnification stops in both the X and the Y directions from the same pictures.

4) Contamination Test

Good results have been obtained using the recently reported test by W. A. Knox (1974) A study of contamination caused by a very narrow electron beam, Proceedings of the 32nd Annual EMSA meeting, p. 560.

Besides considering the results of such performance tests, we are taking into account a number of other factors in our decision of which scanning electron microscope to buy. Such factors include: space it will require, who will be using the microscope and for what purposes, who will be in charge of the microscope and take responsibility for it, ease of microscope operation, training programs and applications expertise the company may provide, the cost of the basic microscope including needed accessories, replacement parts and service contracts, past operation reports such as "down time" and service work performance reports from others who are users (most companies will supply a list of owners and users of their instruments and contact with these users has proved of considerable value to us), what specimen preparative instruments and facilities will be needed to make the microscope useful for various individuals.

For general background information on the scanning electron microscope the following two books have proved especially helpful to us: "The Use of the Scanning Electron Microscope" by J. W. S. Hearle, J. T. Sparrow and P. M. Cross, Pergamon Press, Oxford (1972) and "Scanning Electron Microscopy" by P. R. Thronton, Chapman & Hall, London (1968).

Acknowledgements for helpful discussions and comments are extended to C. Lane, S. Moll, L. Thurston, A. Kabaya, J. Geller and G. Bruno.

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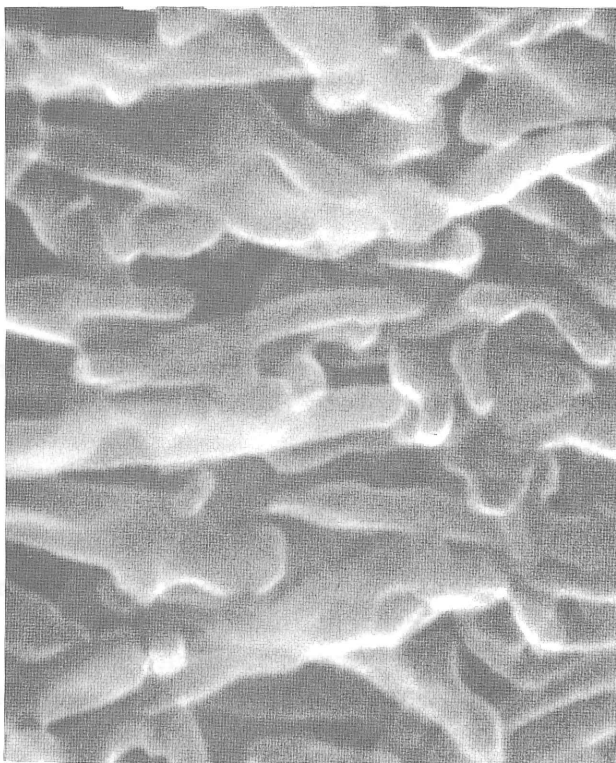


Figure 1

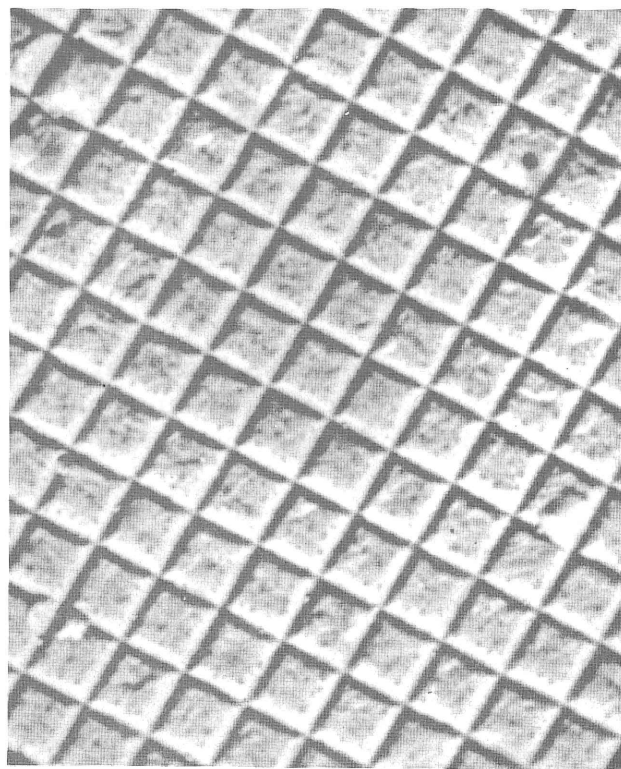


Figure 2

PIGEON POXVIRUS

Poxviruses are the largest of animal viruses and they can be divided into subgroups on the basis of their morphology, specific antigens, and natural hosts. Pigeon pox is included with other Avian viruses in "Group 2" of poxviruses, and is not pathogenic for man. Manifestations of the pigeon poxvirus may be in the form of proliferative epithelioma-like growths on the skin of the head, yellowish membranous lesions in the oral cavity, and/or roup (a watery discharge from the nose and eyes). This virus can easily be seen as an eosinophilic inclusion body in the epidermal cell of pigeon skin.

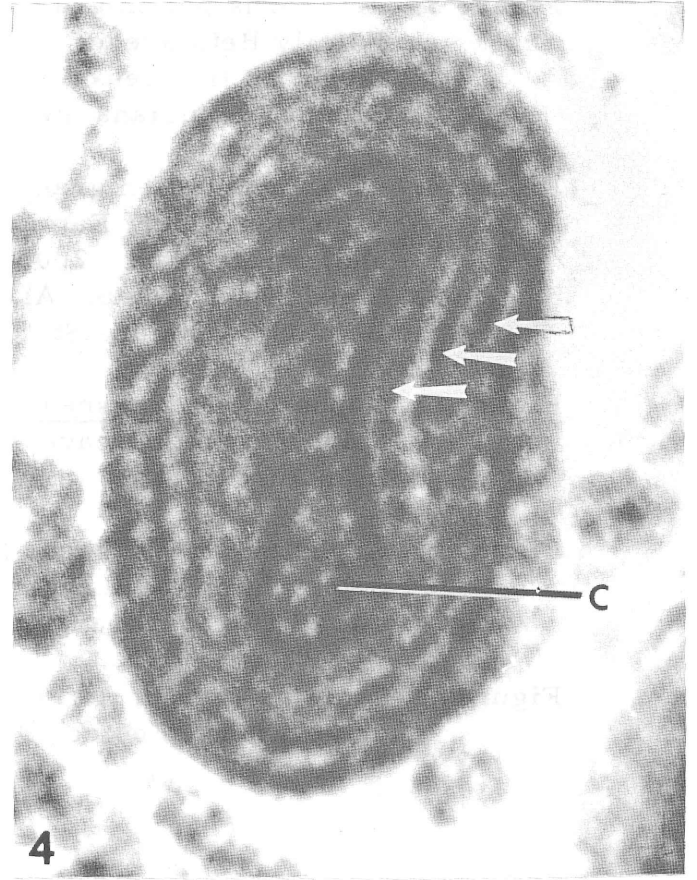
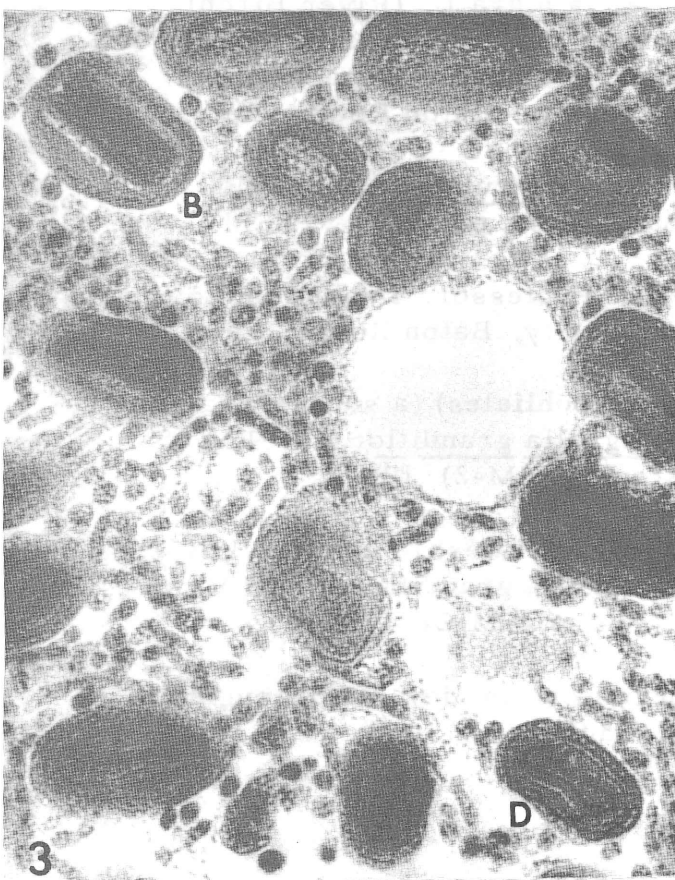
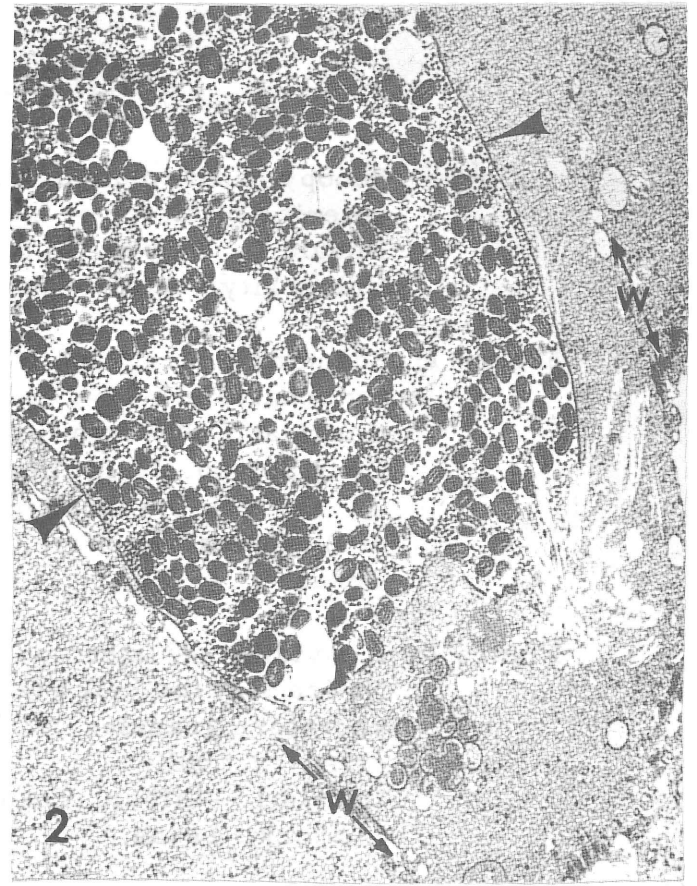
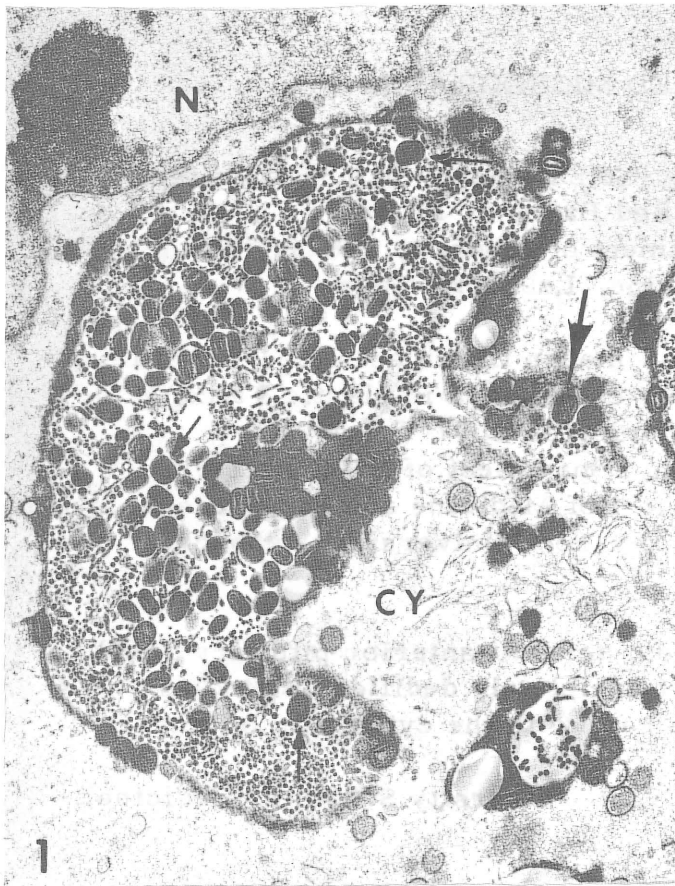
(Fig. 1) The virus is found in the epidermal cell cytoplasm (CY) outside of the nucleus (N). The small arrows point to several brick-shaped viruses enclosed in a large single membrane, while the large arrow points to a typical brick-shaped pigeon poxvirus free in the cytoplasm. 10,200X

(Fig. 2) The large arrows indicate a well defined single membrane surrounding a mass of viral particles. Note that this structure in the cytoplasm is separate from the cell wall (W). 10,200X

(Fig. 3) The morphology of the virus in a longitudinal section is considered brick or ovoid shaped (B), and reveals a dense DNA core. This central core in cross section has a distinctive dumbbell shape (D). 71,000X

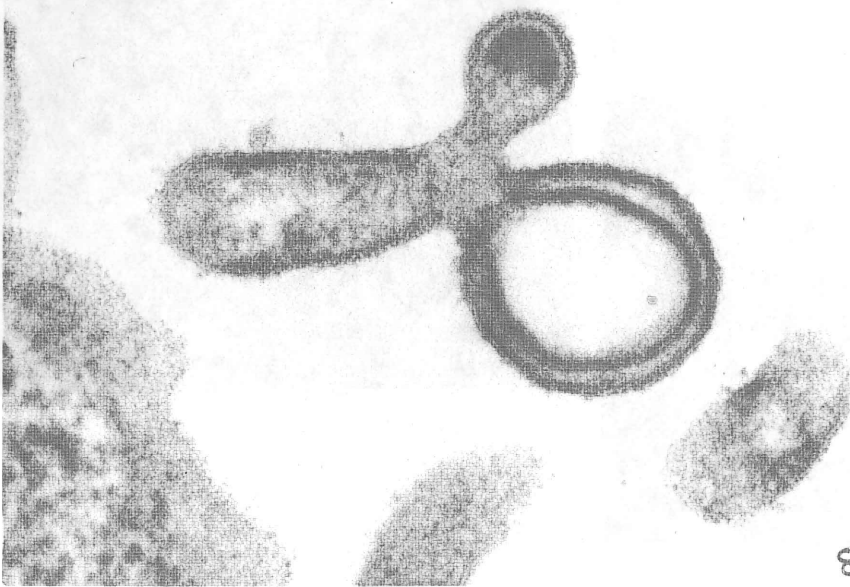
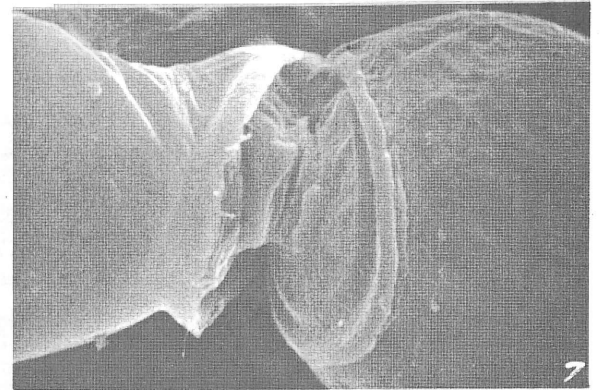
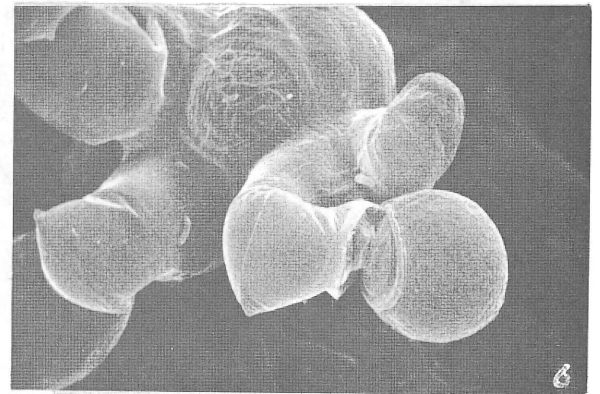
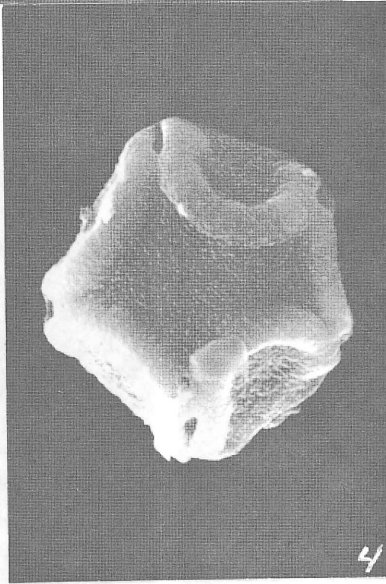
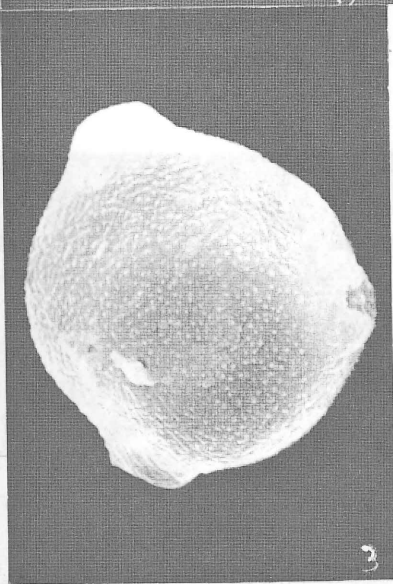
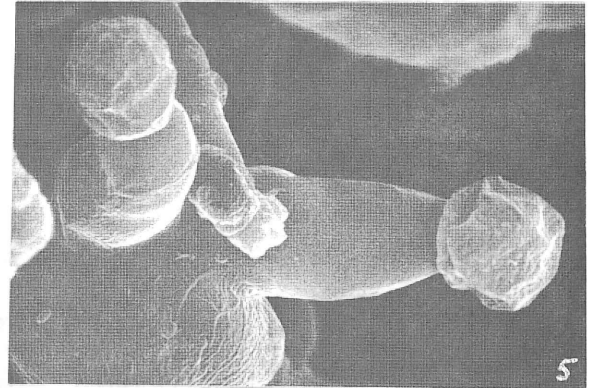
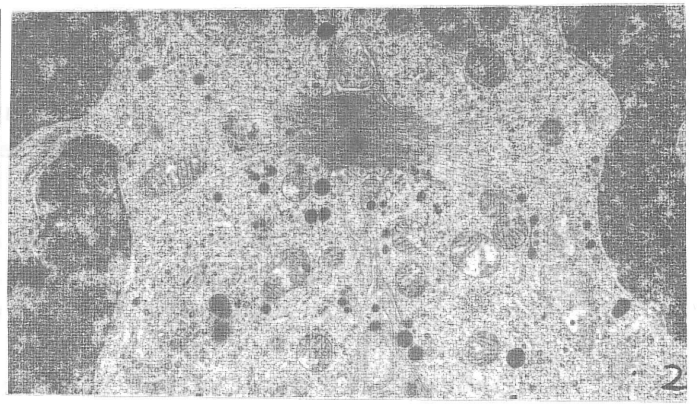
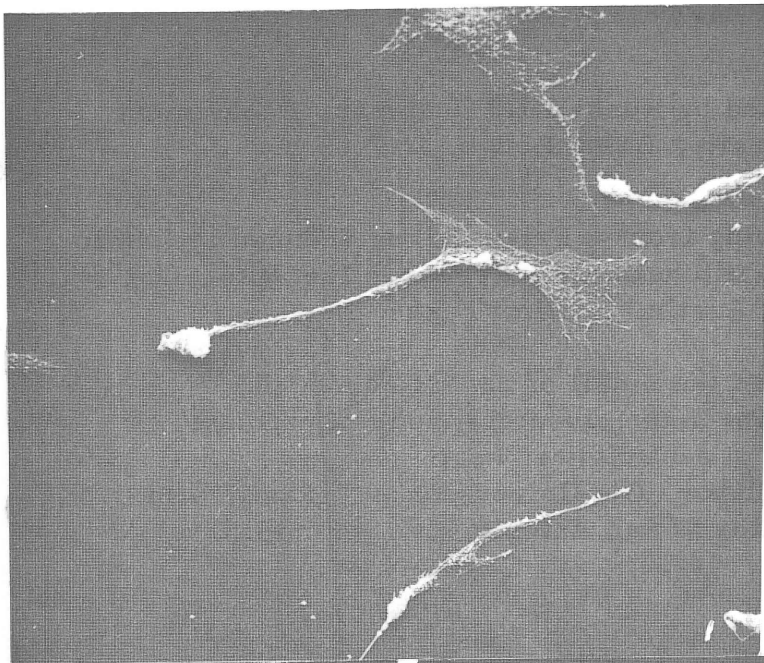
(Fig. 4) A high magnification of a pigeon poxvirus shows the central viral DNA core (C) surrounded by several lipoprotein layers (arrows) which form the outer membrane. 200,000X

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CONTRIBUTED MICROGRAPHS

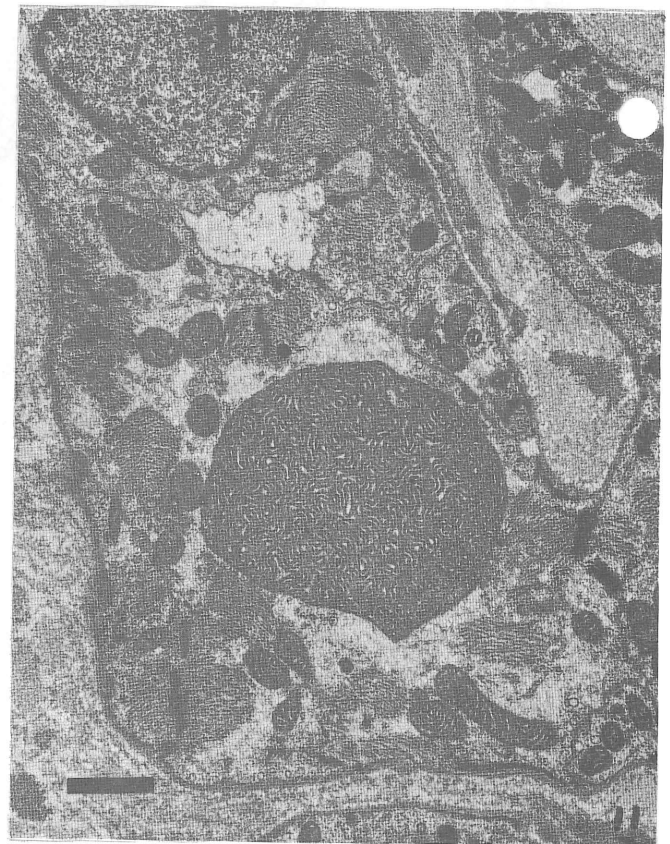
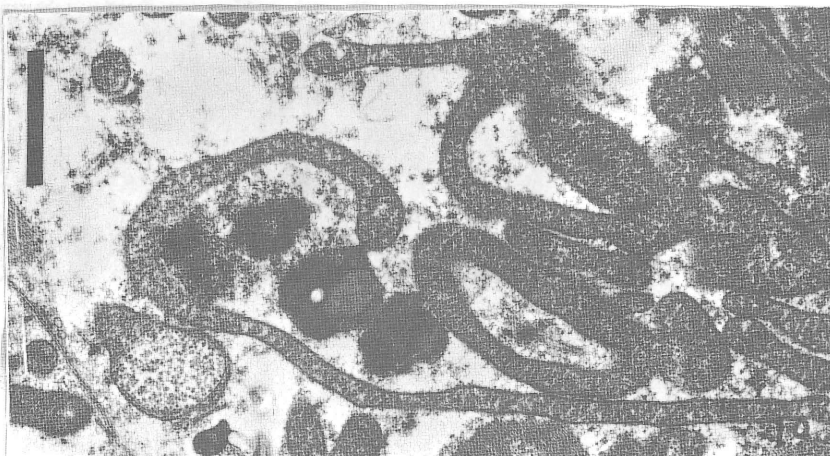
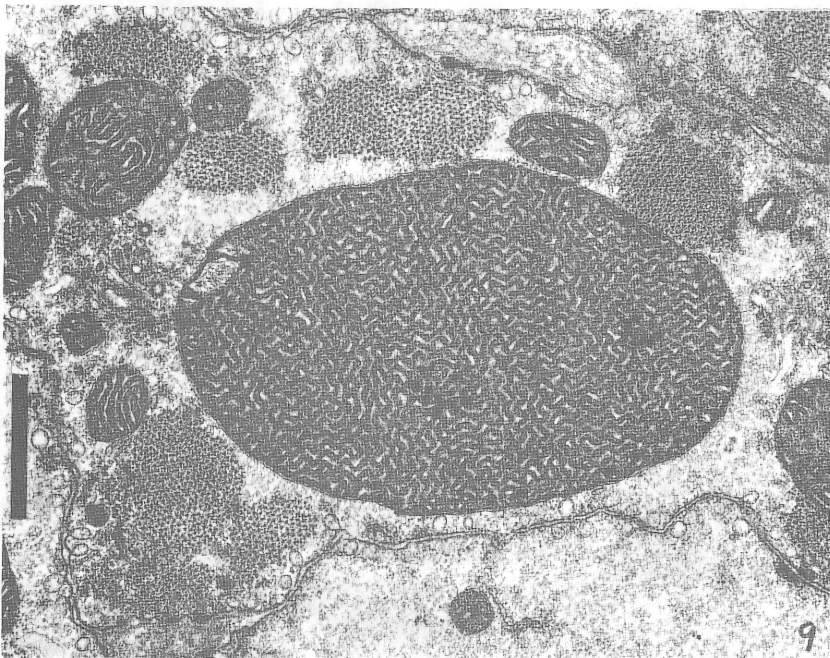
- Cover: Snoopy about to be hit with "Somatotroph" bombs from the anterior pituitary of the "Red Baron." 21,500X
Donald L. Wilbur, Department of Anatomy, Texas Tech University School of Medicine, Lubbock, Texas.
- Figure 1: Scanning micrograph of A-9 mouse fibroblasts treated with Cytochalasin B 10 μ g/ml and centrifuged in plastic Falcon flasks at 7,000 rpm for 30 minutes at 37°C. 500X
Doug Stocco, J. D. Berlin, Department of Biochemistry, Texas Tech University School of Medicine and Department of Biological Sciences, Texas Tech University, Lubbock, Texas.
- Figure 2: A dividing somatotroph from an adult anterior pituitary gland. Notice that the cell has not completely dedifferentiated as secretory granules can be seen with the cytoplasm of both cells. 9,400X
Donald L. Wilbur, Texas Tech University School of Medicine, Lubbock, Texas.
- Figure 3: Air-borne pollen grain of Betula nigra L. (River Birch), Family Betulaceae; polar view; SEM photomicrograph, 3000X.
Meredith H. Lieux, Assistant Professor, Department of Botany, Louisiana State University, Baton Rouge, La.
- Figure 4: Air-borne pollen grain of Alnus serrulata (Ait.) Willd. (Common Alder) Family Betulaceae; polar view; SEM photomicrograph, 2000X.
Meredith H. Lieux, Assistant Professor, Department of Botany, Louisiana State University, Baton Rouge, La.
- Figure 5: Cephaleuros virescens (Trentepohliales) (a subaerial alga, epiphytic on the leaves of Magnolia grandiflora) - SEM of immature zoosporangia, (Jeolco JSM-2) 1200X.
- Figure 6: Cephaleuros virescens (Trentepohliales) (a subaerial alga, epiphytic on the leaves of Magnolia grandiflora) - SEM of a mature zoosporangium, (Jeolco JSM-2) 1000X.
- Figure 7: Same as Fig. 6, higher magnification view of sporangium detachment, (Jeolco JSM-2) 3000X.



Figures 5, 6 and 7 were contributed by Russell L. Chapman, Department of Botany, Louisiana State University, Baton Rouge, La.

Figure 8: A C-type particle budding from a Friend virus-induced leukemic cell. Ruben Ramirez-Mitchell, Cell Research Institute, The University of Texas at Austin, Austin, Texas.

Figures 9, 10 and 11: The large and the small of it: Mitochondrial pleomorphism in the sinoatrial node of primate heart. Bar in each figure: 1 μ m.
Gene L. Colborn, The University of Texas Health Science Center at San Antonio, San Antonio, Tx.



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American Optical Corporation
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Scientific Instrument Division
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Dallas, Texas 75201

Brinkman Instruments, Inc.
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3100 West Alabama St.
Houston, Texas 77006

Carl Zeiss, Inc.
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Bldg. 2, Suite 191
3233 Weslayan
Houston, Texas 77027

Curtin Matheson Scientific
Mr. Jack Classon
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Mr. Charles Walsh
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Prairie View, Illinois 60069

Ernest F. Fullam, Inc.
Mr. Thomas P. Turnbull
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Mr. Steve Pinson
3392 Investment Blvd.
Hayward, California 94545

George Lange, Inc.
Mr. William Lange
P. O. Box 20150
Houston, Texas 77025

Harper & Row Publishers, Inc.
Mr. David P. Miller, Publisher
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New York, New York 10016

International Scientific Instruments
Mr. Don Evins
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970 San Antonio Rd.
Palo Alto, California 94303

Ivan Sorvall, Incorporated
Mr. Martin Wannberg
National Sales Manager
Pearl Street
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214 S. Sheppard Drive
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8020 Austin Avenue
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Mr. Ted Willmorth
1209 Dogwood Dr.
Kingston, Tenn. 37763

LKB Instruments, Inc.
Mr. Al Doty
6600 W. Irving Park Road
Chicago, Illinois 60634

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Mr. Don Gordon
P. O. Box 44008
Dallas, Texas 75234

Perkin-Elmer Corporation
Mr. Mike Mullin
411 Clyde Avenue
Mountain View, California 94040

Philips Electronic Instruments
Mr. George Brock
7302 Harwin Drive
Suite #106
Houston, Texas 77036

Polysciences, Incorporated
Mr. B. David Halpern, President
Warrington, Pennsylvania 18976

Siemens Corporation
Mr. Dietrich Voss
4101 San Jacinto
Houston, Texas 77004

William H. Talley Company
Mr. William H. Talley
P. O. Box 22574
Houston, Texas 77027

ANNOUNCEMENT

SPRING TSEM MEETING

HYATT REGENCY HOTEL
HOUSTON, TEXAS

MAY 2-3, 1975

AREA NEWS

Austin

THE UNIVERSITY OF TEXAS AT AUSTIN

Department of Botany

Dr. Garry T. Cole has been recently promoted to Associate Professor.

New Member: Dr. Stefan Kirchanski (Ph. D., University of California at Berkeley), Assistant Professor, Department of Botany

Seminars Given: Dr. Brian Fineran, University of Canterbury, Christchurch, New Zealand: Ultrastructural studies on Utricularia.

Recent papers: Garry T. Cole, Ultrastructure of Drechslera Sorokiniana (Hyphomycetes) grown on cellophane films. Contribution to the Colloquium on Biodegradation and Humification, University of Nancy, France, 1975.

Cell Research Institute

Visitors: Dr. Brian Fineran, University of Canterbury, Christchurch, New Zealand, worked at the C. R. I. and at the Marine Science Institute at Port Aransas on the ultrastructure of some teleosts' retinas. He was invited by Dr. Colin Nicol, Marine Science Institute, Port Aransas.

Dr. R. N. Kapil, Department of Botany, University of Delhi, India, is working at the Institute under a UNESCO Fellowship.

Seminars Given:

In the summer of 1974 Dr. W. Gordon Whaley, Director of the Cell Research Institute, delivered a series of lectures and participated in seminars on the Golgi apparatus and the cell surface at the Children's Hospital of the University of Hamburg, the Universities of Geneva and Basel and the Federal Institute of Technology at Zurich, the University of Paris-Sud, and the Strangeways Laboratory and the Department of Biochemistry at Cambridge. The purpose was a preliminary evaluation of the participation of the organelle in the formation of substances with immunological properties. Additionally, he spent considerable time in Golgi's Laboratory of Histology and the Institute of General Pathology, which has been renamed for Golgi, at the University of Pavia in Italy, studying both Golgi's materials and the general advance of biomedical science in that institute. Later, he returned to Europe to deliver a symposium address on the Golgi apparatus and the cell surface as part of a program dealing with cell differentiation held by the University of Lisbon in Portugal. In the course of the first trip, he discussed the final stages in the preparation of a book-length monograph on the Golgi apparatus with the editors of Springer-Verlag in Vienna.

El Paso

THE UNIVERSITY OF TEXAS AT EL PASO

Mr. Michael Postek, a master's graduate from Texas A & M, became an EM technician here in August, 1974.

New instruments: Denton Vacuum Evaporator DV-502 and Denton DFE-3 Freeze Etch Apparatus.

Visiting seminar: Dr. David E. Comings, "Mechanisms of Chromosome Banding," December 17, 1974.

Denton

TEXAS WOMEN'S UNIVERSITY

The Department of Biology is establishing an electron microscopy facility with the purchase of a Siemen's Elmiskop 101.

Freeport

DOW CHEMICAL USA, TEXAS DIVISION

New Equipment: JEM 100C analytical electron microscope equipped with STEM accessory and KEVEX 5100 energy dispersive X ray system.

Galveston

THE UNIVERSITY OF TEXAS MEDICAL BRANCH

Department of Anatomy

New Members: Marvin Canon, Ph. D.

Recent Publications: "Predictability of Resolution of Hypertrophic Scars by Scanning Electron Microscopy,"
C. Ward Kischer (J. of Trauma).

Department of Pathology

New Instruments: Philips 201

Grants: Renewals relating to Renal Pathology and cellular immunity.

Dr. William McCutnick and Dr. Sidney Schochet have joined the Department. One of their major research activities is the application of EM to neuropathology and muscular diseases.

Dr. Marshall with Dr. Morales (Univ. of Miami School of Medicine) and Dr. Alexander (Univ. of Florida Medical School) presented an expanded workshop on Applications of Electron Microscopic Techniques in Diagnosis of Disease at the fall meeting of The American Society of Clinical Pathology.

Houston

BAYLOR COLLEGE OF MEDICINE

Department of Microbiology and Immunology

Dr. H. D. Mayor attended the International Congress of Electron Microscopy in Canberra Australia August 19, 1974. Dr. Mayor lectured to the Electron Microscope Unit at the University of Sydney on Viruses in Cancer and she also gave a lecture on Electron Microscopy of Tumor Viruses at the Department of Tropical Medicine at the University of Hawaii in September of 1974.

Departments of Neurology and Pathology and the Baylor-Methodist Center for Cerebrovascular Research

Lecture: Dr. Ronald F. Dodson presented a paper entitled "Ultrastructural Responses of Cerebral Tissue Following Periods of Ischemic Insult," at the Fourth Annual Meeting of the Soccity for Neuroscience in St. Louis.

Recent publications:

Dodson, R. S., G. S. Fritz, W. R. Hubler Jr., A. H. Rudolph, J. M. Knox, and L. W-F Chu: Donovanosis: A Morphological Study. The J. of Investigative Dermatology 62: 611-614, 1974.

Dodson, R. F., L. W-F Chu: Ultrastructure of the Ependymal and Subependymal Cells in the Lateral Ventricle of the Squirrel Monkey. Cytobios (in press).

Dodson, R. S., Y. Tagashira, L. W-F Chu: The Ultrastructure of the Middle Cerebral Artery and Its Associated Nerve Fibers in the Squirrel Monkey and Baboon. Tissue and Cell (in press).

Dodson, R. F., Y. Tagashira, and Lena W-F. Chu: Morphological Studies of the Ventricular Wall in Cerebral Infarction. Experimental and Molecular Pathology, April, 1975.

ST. LUKE'S AND BAYLOR

Recent publications: Greenberg, S. Donald, Smith, Marilyn and Spjut, Harlan: Bronchiolo-Alveolar Carcinoma-Cell of Origin, Am. J. Clinical Path. (in press).

THE UNIVERSITY OF TEXAS SYSTEM CANCER CENTER
M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE

Department of Virology

Grants: The Department of Virology has been notified of the renewal of the prostrate grant entitled "Study on oncogenic viruses in human prostrate cancer" for one year.

A research proposal entitled "Relatedness of RNA Tumor Viruses and Human Neoplasia", with Dr. James L. East as principal investigator, was approved and funded by PHS-NCI for a three year period.

Visitors, Lecturers:

Dr. Charles Boone, Chief, Cell Biology Laboratory, Viral Oncology Program, National Cancer Institute, Bethesda, Maryland, visited the Department and presented a seminar entitled "Virus augmentation of tumor transplantation antigens: Possible practical applications."

Dr. Wendell D. Winters, Assistant Professor of Surgery, Microbiology and Immunology, the Center for Health Sciences, University of California, Los Angeles, California, visited the Department and presented a seminar entitled "Immunovirologic studies of human sarcomas."

Mr. Bob Mason, V. A. Hospital, Lexington, Kentucky visited the Department for a week and received tutorial instruction from R. L. Hales in various electron microscopy techniques.

Dr. S. S. Kalter of the SW Foundation for Research and Education at San Antonio visited the Department and brought a seminar entitled, "Primate C-type Viruses."

Dr. George Birkmayer, Associate Professor and Leader of the Tumor-Virology Group, Department of Cell Biology at the University of Munich, Munich, Germany, visited the Department and brought a seminar on "Features specific for oncogenic RNA viruses in human melanoma and glioblastoma."

Dr. Johng S. Rhim of Microbiological Associates, Bethesda, Maryland, visited the Department and presented a seminar entitled, "Nonproducer human cells induced by murine sarcoma cells."

Papers published:

The following chapter was published in November 1974: "Studies on Oncornavirus by Immunoferritin and Immunoperoxidase Electron Microscopy" by L. Dmochowski, M. Hoshino, T. Shigematsu, S. Hiraki, and E. S. Priori. Chapter 12 in Viral Immunodiagnosis (E. Kurstak and R. Morisset, eds.), Academic Press, New York, N. Y., pages 183-213, 1974.

"An Immunoelectron Microscopy Study of Soehner-Dmochowski Murine Sarcoma Virus Following Passage in Rats and Hamsters", by S. Hiraki, J. C. Chan, R. L. Hales, and L. Dmochowski, was published in Cancer Research 34: 2906-2910, November 1974.

Chapter 14, "Comparative Morphology, Immunology, and Biochemistry of Viruses Associated with Neoplasia of Animals and Man," by J. M. Bowen, J. L. East, P. T. Allen, K. Maruyama, E. S. Priori, J. Georgiades, J. C. Chan, M. F. Miller, G. Seman, and L. Dmochowski, was published

in Viruses, Evolution, and Cancer. E. Kurstak and K. Maramorosch, Edrs., Academic Press, New York, N. Y., 1974, pp. 403-426.

THE UNIVERSITY OF TEXAS MEDICAL SCHOOL AT HOUSTON

New faces: Diane Saunders is a new technician in Dr. Diana Redburn's lab.

Kathleen Marburger, graduate student in Neuroscience passed her candidacy exam in January.

Recent publications:

"Use of the Analytical Electron Microscope (AEM) in Cytochemical Studies of the Central Nervous System" and "Analytical Electron Microscopic Identification of Cytochemical Products in Thin Sections", papers written by Dr. J. G. Wood, were accepted by The Journal of Histochemistry and Acta Histochemica respectively.

Recent lecturers:

Recent lecturers to the program in Neurostructure and Function included Dr. W. G. Dail from the Department of Anatomy at the University of New Mexico who spoke on "Morphological Evidence for Neural Integration in the Pelvic Plexus", and Dr. Lynn Churchill from the Department of Pharmacology at the University of Wisconsin Medical Center who presented a seminar entitled "Biochemical Composition of Central Nervous System Synapses."

Dr. John E. Dowling, a Grass Foundation traveling lecturer presented a talk on the "Functional Organization of the Vertebrate Retina."

Other visitors who presented lectures to the Society for Neuroscience included Dr. M. W. Van Hof, Dr. R. Coggeshall, Dr. D. Kellaway, Dr. D. Bok, and Dr. M. Igarashi.

Louisiana

News from the Louisiana neighbors:

Dr. Earl Weidner from LSU-Baton Rouge has been awarded a two year NSF grant to continue studies on the biology of microsporidan parasites. Weidner's attention will be directed primarily to microsporidiosis transmission in marine food animals.

Dr. Robert Yates will serve as an invited participant at an NIH Symposium on SIF Cells and Chromaffin Cells. Dr. Yates will present research concerning the carotid body, aortic bodies and abdominal paraganglia done in collaboration with I-Li Chen, John T. Hansen and Joe A. Mascorro. Among other notable speakers at the conference will be Sanford Palay, Olavi Eranka, Floyd Bloom and Terrance Williams.

Dr. James Jeter (San Antonio) and Gerald Kirby (Lubbock) will assume positions as Assistant Professors at Tulane Anatomy effective July 1, 1975.

Drs. John T. Hansen and Peter M. Klara recently received the Ph. D. degree from Tulane Anatomy and are continuing with post-doctoral training and research in electron microscopy in that department.

Publications:

"A Review of Abdominal Paraganglia: Ultrastructure, Mitotic Cells, Catecholamine Release, Innervation, Light and Dark Cells, Vascularity." Joe A. Mascorro and Robert D. Yates. Chapter 27 in Electron Microscopic Concepts of Secretion - Ultrastructure of Endocrine and Reproductive Organs. Edited by Melvin Hess; published by John Wiley & Sons, Inc., 1975.

"The histology and ultrastructure of cat abdominal paraganglia after fixation and localization with glutaraldehyde/potassium dichromate." Joe A. Mascorro and Robert D. Yates, EMSA Proceedings, 1974, 290-291.

"The fine structure and phosphatase cytochemistry of the Golgi complex and associated structures in the Sertoli cells of Syrian hamsters." I-Li Chen and Robert D. Yates, Cell and Tissue Research, in press, 1975.

"Glutaraldehyde perfusion followed by glutaraldehyde/potassium dichromate immersion: A technique for localizing paraganglia." Joe A. Mascorro and Robert D. Yates, Tex. Rep. Biol. Med., in press, 1975.

"Morphological comparisons between aortic and carotid glomuc cells in the rabbit." John T. Hansen, I-Li Chen, and Robert D. Yates, Tex. Rep. Biol. Med., in press, 1975.

Announcement:

Meredith H. Lieux, Assistant Professor, Department of Botany, LSU, Baton Rouge wishes to announce the forthcoming publication of the Catalog of Gulf of Mexico Region Recent and Fossil Microbios. The first contribution to the catalog will be Holocene Tree and Shrub Pollen of Louisiana by Dr. Lieux. The catalog will include a description of the pollen of each species and will be illustrated with light and SEM photomicrographs of each species. The project is sponsored by the Department of Botany, and publication of the catalog will be by the School of Geoscience. Publication of the initial contributions is supported by a grant from the LSU Foundation.

Lubbock

TEXAS TECH UNIVERSITY

Department of Biology

Grant: Continuation of cotton fiber work; renewal from Cotton, Inc., to J. D. Berlin.

Department of Geosciences

Grants: \$10,000 from the Industrial Mineral Ventures Corporation, Colorado.

Seminars given:

"Why are the electron optics so powerful in studying clays and other particulate matter?" presented at the Department of Geology, University of South Florida.

Publications:

"Formation of Laths in Fine-Grained Micas. Mineralogical Magazine 39: 788-792 (1974).

"Evaluation of Bending Effects on Diffraction Intensities. Clay and Clay Minerals (1975).

The above grant, seminar, and publications are by Dr. Necip Guven.

TEXAS TECH UNIVERSITY SCHOOL OF MEDICINE

Lecturers: Dr. Roger Markwald, Medical University of South Carolina, spoke on normal and abnormal cardiogenesis.

Publications: "Ultrastructural observations of anterior pituitary somatotrophs following pituitary portal vessel infusion of dibutyryl cAMP. Donald L. Wilbur, W. Curtis Worthington and Roger R. Markwald, American Journal Anat. 141: 139-145.

San Antonio

BROOKE ARMY MEDICAL CENTER

Department of Pathology

Edward Rappaport, M.D. has joined the lab as our pathologist. He comes to us from Loyola University Hospital in Maywood, Illinois.

Larry Tillman, Ph.D. has also joined the laboratory staff as an electron microscopist. Larry came from the Histology Section of the Department of Biology at the University of Mississippi at University, Mississippi.

SOUTHWEST RESEARCH INSTITUTE

Publications: Davidson, D. L. Electron Channeling: A metallurgical Tool, Research/Development 25: 34-90, September, 1974.

THE UNIVERSITY OF TEXAS HEALTH SCIENCE CENTER
AT SAN ANTONIO

Department of Anatomy

Dr. T. R. Hoage, President of TSEM, is a Visiting Professor in the Department of Anatomy at the Health Science Center.

Recent Publications:

Winborn, W. B., and L. L. Seelig, Jr. 1974 Pattern of osmium deposition in the parietal cells of the stomach. J. Cell Biol. 63: 99.

Winborn, W. B. 1974 Removal of resins from specimens for scanning electron microscopy. In Principals and Techniques of Scanning Electron Microscopy: Biological Applications. M. A. Hayat, Editor. Van Nostrand Reinhold Co., New York. (In Press.)

Temple

SCOTT AND WHITE CLINIC

New members to TSEM: Dr. A. K. Brown - Otolaryngology
Dr. Don Jutzy - Clinical Pathology

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PLACEMENT SERVICE

- 1) Electron Microscope Technician - prefer someone with histological and EM training. Extensive experience not necessary. Position opens about June 1, 1975.
- 2) Electron Microscope Technician II - EM experience required. Will perform limited microscope maintenance, supply ordering, etc. Position opens about September 1, 1975.

For the above two positions apply to Dr. J. R. Hillman or Randy Brackeen, Department of Anatomy, Texas Tech University School of Medicine, P. O. Box 4569, Lubbock, Texas 79409, (806) 742-1178.

- 3) Research Technician - Experience in histochemical techniques necessary. Experience in basic EM techniques and tissue culture desirable. Contact Dr. Frank Bastian, Department of Pathology, St. Luke's Episcopal Hospital, Texas Medical Center, Houston, Texas 77025.

Abstracts of Papers Presented
at the
Fourth Joint Symposium
LOUISIANA AND TEXAS SOCIETIES FOR ELECTRON MICROSCOPY

Delta Towers Hôtel
New Orleans, Louisiana

February 20 - 22, 1975

FINE STRUCTURE OF THE ARMADILLO SALIVARY BLADDER

Allen, E.R., and Ruby, J.R., Louisiana State University Medical Center, Department of Anatomy, 1100 Florida Avenue, New Orleans, Louisiana 70119.

An ultrastructural study of the salivary gland system in the nine-banded armadillo revealed two storage organs or salivary bladders positioned between the bilateral submandibular glands and the rear of the mouth. They are in contact with and connected by ducts to the submandibular but appear to have no association with the parotid glands. The bladder walls are composed of three well-defined layers of tissue. The outermost layer and majority of the wall thickness is composed of striated muscle, while the innermost layer is composed of epithelium with a collagenous layer intervening between the two. The striated muscle contains cross striated patterns typical to those found in adult vertebrate muscle. According to the fast, slow, intermediate classification, it is highly vascularized, intermediate type striated muscle containing numerous nerves and myoneural junctions. The epithelial layer is cuboidal to low columnar with microvilli on the luminal surface. There are three distinct cell types, easily differentiated according to their histological characteristics. The morphology of the epithelial cells has also been correlated with the functional state of the salivary bladders. The intervening dense connective tissue layer is composed of numerous longitudinal bundles of collagen fibers and contains many scattered blood vessels and nerves, along with some nerve ganglia.

ONTOGENY OF THE STAMINAL SHIELD OF

SCHISANDRA GLABRA (BRICKELL) REHDER

J. Allen Borland, III and Shirley C. Tucker

Botany Department, Louisiana State University, Baton Rouge

A comprehensive study is being made of the habit, vegetative ontogeny, and floral ontogeny of Schisandra glabra, a member of the primitive ranalian family Schisandraceae. Schisandra glabra is a monoecious or apparently often dioecious, woody, high-climbing vine native to the southeastern United States. The ontogeny of the stamens and staminal shield in this species is significant because of the combination of primitive and advanced characteristics. In addition to light microscopic studies, scanning electronmicrographs have aided greatly in determination of developmental sequences. The floral apex has a tunica-corpus configuration; the apex increases in height and width throughout appendage initiation. The apical residuum persists and constitutes the central portion of the staminal shield. This type of shield formation is of interest in that most of the Asiatic species in this genus lack such a structure.

A TECHNIQUE FOR FIXATION OF ARTIFACT-FREE STRETCHED MYOCARDIUM

Wilhelmina Butcher, B.A.
William F. Mac Kenzie, D.V.M., M.A.
Clinical Pathology Branch
Veterinary Sciences Division
USAF School of Aerospace Medicine
Brooks Air Force Base, Texas 78235

We developed this method of fixation for myocardium to provide large blocks of artifact-free stretched myocardium so that extensive areas of tissue could be surveyed for subsequent electron microscopy. Myocardium is stressed when it is minced and real lesions are masked by artifactual contraction bands. Large slices of tissue fixed under tension prevent the occurrence of contraction bands and provide well demonstrated I-bands. Pig hearts are perfused with 2% buffered glutaraldehyde at 80 mm of mercury through the thoracic aorta and at the same time through the left ventricle to keep the heart stretched. Slices of myocardium 2 mm wide and 2 cm long are taken from the left and right papillary muscle and from the left ventricular wall. Each slice is stretched longitudinally and pinned to a small-meshed screen and allowed to fix by immersion an additional hour. Each slice, in turn, is cut into 5 mm blocks and care is taken to embed each block so that sections taken will show the myofibrils in a longitudinal plane.

A SIMPLE ATTACHABLE ILLUMINATOR FOR FACILITATING ULTRAMICROTOME KNIFE ORIENTATION AND BLOCK APPROACH

James K. Butler

Biological Ultrastructure Laboratory
Biology Department
The University of Texas at Arlington
Arlington, Texas 76019

The construction and operation of a simple, inexpensive illuminator that produces high quality dark field-like illumination of the ultramicrotome knife edge and the edge-to-block face-gap is described. Use of the illuminator greatly speeds knife adjustment and reduces the likelihood of specimen or knife edge damage. The illuminator utilizes an eighteen volt grain-o-wheat light bulb and an adjustable bulb holder fashioned from bent paper clips. The holder permits both lateral and axial adjustment of the bulb position which is necessary to achieve satisfactory illumination with different specimens and knives. The illuminator, with slight modifications, can be adapted for use on any ultra-microtome.

CHROMATOID BODIES AND ASSOCIATED STRUCTURES IN EARLY SPERMATIDS
OF SYRIAN HAMSTERS: AN ELECTRON MICROSCOPIC STUDY

I-Li Chen and R.D. Yates, Department of Anatomy, Tulane Medical
School, New Orleans

In spermatids of the Syrian hamster during the Golgi and early cap phases, the chromatoid body is a prominent structure which appears to consist of aggregations of extremely fine granules and/or filaments, forming a compact mass or anastomosing strands. Tubular invaginations of the general cytoplasmic matrix into the former are not uncommon. Aside from displaying a close proximity to the nucleus the chromatoid bodies are in association with small vesicles or tubules of various sizes as well as clusters of small particles, presumably, ribosomes. The vesicles or tubules often show an intimate affinity to the compact masses and clusters of ribosomes to the anastomosing strands of the bodies. Other structures seen frequently in the vicinity of the chromatoid bodies are multivesicular bodies, the membrane of which are often seen to be continuous with small tubules. Acid phosphatase activity was localized in many of the small vesicles and tubules associated with the chromatoid bodies and in the multivesicular bodies. The significance of this close relationship of the chromatoid bodies to those structures mentioned above is not completely understood; however, acid phosphatase activity in this area does not seem to be involved in the autolysis of the chromatoid bodies.

PRELIMINARY ELECTRON MICROSCOPIC OBSERVATIONS ON LICHENIZED CEPHALEUROS (STRIGULA)

R. L. Chapman, Department of Botany, Louisiana State University, Baton Rouge

Cephaeleuros virescens (Trentepohliales), a foliicolous epiphyte of Magnolia grandiflora and other angiosperms, occurs as the phycobiont of Strigula complanata and S. elegans which are obligately foliicolous (generally epiphyllous) pyrenocarpous crustose lichens. Transmission electron microscopic examination of ultrathin sectioned material confirms the subcuticular position and homiomeric morphology of the ecorticate lichen S. complanata foliicolous on M. grandiflora. The phycobiont is characterized by the presence of plasmodesmata (similar in form and arrangement to those observed in the free-living alga), an abundance of lipid-like material in the cytoplasm and (to a lesser extent) in the chloroplasts, and a relative paucity of interthylakoidal starch grains. Observations on the ascomycetous mycobiont include the apparent absence of crystalline inclusions following fixation in glutaraldehyde-osmium, the presence of "Moronin-like bodies" (Pevling) at the septal pores of haustorial hyphae, and the occurrence of prothallus and apparent absence of these cytoplasmic inclusions in vegetative portions of the thallus. As seen in thin-section, the alga-fungus relation is either close apposition without wall thinning or dissolution, or fungal penetration of the phycobiont by one or more walled haustoria. Haustorial invasion is not restricted to senescing or decaying algal cells in S. complanata, although it is reported to be thus restricted in other pyrenocarpous lichens. The protrusion of naked (membrane-bound) fungal protoplast into phycobiont cells (following localized disintegration of both algal and fungal walls) which has been observed in Dermatocarpon minutum, D. hepaticum, and Verrucaria spp. (pyrenocarpous lichens) by Galun and coworkers has thus far not been observed.

POLYCATIONIC FERRITIN-INDUCED REDISTRIBUTION OF INTRAMEMBRANE PARTICLES IN
RAT ERYTHROCYTE GHOST MEMBRANES.

Jeffrey Day and Charles R. Hackenbrock, Department of Cell Biology, University
of Texas Southwestern Medical School, Dallas, Texas, 75235.

Freeze-cleave electron microscopy of erythrocyte ghost membranes has revealed intramembrane particles that are currently thought to represent integral glycoproteins partially or entirely embedded in the membrane lipid bilayer. Utilizing freeze-cleaving followed by deep etching, we have determined that polycationic ferritin binds to the surface of erythrocyte ghost membranes resulting in a redistribution of intramembrane particles. Most likely polycationic ferritin binds to the negatively charged carbohydrate moiety which projects from the glycoproteins onto the surface of the membrane. Rat erythrocyte ghost membranes, untreated with polycationic ferritin, revealed intramembrane particles in a chain-like arrangement in the "A" fracture face of the membrane. The "B" fracture face revealed fewer intramembrane particles but corresponded closely in their pattern of distribution to intramembrane particles on the "A" fracture face. After binding of polycationic ferritin to the outer surface of the membrane at pH 7.4, the intramembrane particles of both the "A" and "B" fracture faces redistributed into large clusters which appeared in register with large clusters of polycationic ferritin bound to the membrane surface. These results suggest that integral proteins associated with both halves of the membrane lipid bilayer undergo coordinated aggregation through crosslinking of their exposed negative surface charges by polycationic ferritin. Prior exposure of the membrane to trypsin, neuraminidase, or low pH is not required for the polycationic ferritin-induced aggregation.

(Supported by NSF Grant # BMS 72-02372 A02).

ABSTRACT

THE FINE STRUCTURE AND PHYLOGENETIC POSITION OF THE COTYLOCIDIUM
LARVA OF COTYLOGASTER OCCIDENTALIS (TREMATODA: ASPIDOGASTRIDAE)

David W. Frederickson, Tulane University, New Orleans, Louisiana.

The previously little known cotylocidium larva of C. occidentalis Hickerson 1902, was studied with the aid of light, scanning(SEM) and transmission(TEM) electron microscopes. The tegument of this larva expresses a surface of short cytoplasmic extensions and a presumed glycocalyx. Both light and SEM microscope studies show that two types of small structures appear in the larval tegument, uniloculate and dome-shaped. TEM reveals the dome-shaped forms to be openings of gland cells and the uniloculate structures to be sensory. Identical uniloculate sensory structures are numerous in juveniles and adults of C. occidentalis, especially in the ventral adhesive disc. The adhesive disc develops from a small sucker located posteriorly on the cotylocidium larva. Cilia are distributed among 14 circular patches on the larva of this species, a number greater than is present on other known larval species in the Subclass Aspidobothria Burmeister 1856. When compared morphologically with other known members of its subclass, the cotylocidium of C. occidentalis appears to be among the most primitive.

Supported in part by NSF Research Grant GB-23057, under the direction of Dr. Martin J. Ulmer; and NIH Research Grant AI-08673, under the direction of Dr. Richard D. Lumsden.

ULTRASTRUCTURAL CYTOCHEMISTRY OF THE TEGUMENTAL SURFACE
MEMBRANE OF PARAGONIMUS KELLICOTTI

Francis Cress, Tulane University, New Orleans, Louisiana

Digenetic trematodes of the genus Paragonimus parasitize the bronchiolar tissues of a variety of mammals, including man. The presence of the worms elicits an allergic inflammation which leads to the formation of fibrotic pulmonary lesions, but the worms are not themselves rejected by the host's immunological/inflammatory response. We report here observations on the body surface fine structure and topochemistry of P. kellyi which may relate to this and other features of host-parasite interactions in paragonimiasis. As has been noted for other trematodes, the body surface of P. kellyi is not a cuticle, in the strict sense, but a cytoplasmic syncytium. The free surface plasma membrane is invested with a hirsute coat. Cytochemical staining with ruthenium red, concanavalin A, ferritin and colloidal iron provides evidence for the presence of neutral saccharide and acidic moieties within the substance of this coat. As for the glycocalyx of certain other cell types, these components of the Paragonimus surface membrane may contribute significantly to its adsorptive properties and immunogenicity.

Supported by grants (AI 08673, AI 0002) from the National Institutes of Health.

FLUORESCENCE HISTOCHEMICAL AND ELECTRON MICROSCOPIC
LOCALIZATION OF BIOGENIC AMINES IN THE SUBCLAVIAN GLOMUS.
John T. Hansen and Robert D. Yates, Department of Anatomy,
Tulane University School of Medicine, New Orleans, Louisiana
70112.

In the aortic arch region there occur several aggregations of specialized epithelioid cells which are referred to collectively as "aortic bodies." They function as chemoreceptors and morphologically are similar to the carotid body. In the present study the subclavian glomera (aortic bodies) from newborn New Zealand white rabbits were studied utilizing both the light and electron microscopes. Glomus tissue was prepared for electron microscopy according to standard glutaraldehyde perfusion and Epon embedment procedures. At the ultrastructural level, the subclavian glomus cells possessed the usual complement of cytoplasmic organelles, including rough endoplasmic reticulum, mitochondria, Golgi complexes and multivesicular bodies. In addition, the cytoplasm contained numerous electron-opaque granules. These granules were surrounded by smooth surfaced membranes and appeared similar to those observed in the cells of the adrenal medulla, carotid body and abdominal paraganglia. Evidence suggests that these granules are storage sites for catecholamines. Following perfusion with phosphate buffered paraformaldehyde, the cells of the subclavian glomera fluoresced yellow-green. Individual cells

displayed yellow, yellow-green, or green fluorescence. The specificity of the fluorescence was validated chemically using sodium borohydride. The electron-opaque granules observed ultrastructurally are probably the storage sites of biogenic amines responsible for the intense fluorescence observed in the subclavian glomera.

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SCANNING ELECTRON MICROSCOPY AS A TOOL IN THE TAXONOMY OF OLIGOCHAETES

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Two subspecies of Pristina longiseta (Oligochaeta: Naididae) are present in the Western Hemisphere: Pristina longiseta leidy in North America and Pristina longiseta bidentata in South and Central America. One characteristic separates the two subspecies: P. l. leidy has a simple-pointed needle in its dorsal setal bundles, whereas P. l. bidentata possesses a bifid needle. Examination of the South American form by phase contrast light microscopy has revealed that the teeth of the bifurcation of the needle often are less than 0.50 μ in length and consequently not easily detected or studied except in the case of perfectly prepared specimens; therefore, scanning electron microscopy has been utilized to study the structure of the needle. Electron photographs confirm the presence of a bifurcate needle in North American specimens as well as those from South America. A re-examination of the subspecies concept for Pristina longiseta in the Americas therefore is necessary. The study also revealed that the hair setae of the dorsal bundles possess a surface modification which may require revision of species descriptions.

VARIATIONS IN ULTRASTRUCTURE OF PERIPHERAL NERVE SCHWANN CELL TUMORS OF MAN

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Surgical specimens of tumors of peripheral nerves were processed for transmission electron microscopy. Included were schwannomas, plexiform neurofibromas, ganglioneuromas and tumors we have tentatively classified as plexiform schwannomas. The tumor Schwann cells resemble hyperplastic Schwann cells found in zones of nerve regeneration in having convoluted plasma membranes with complex pseudopodia and a prominent basement membrane paralleling the plasma membrane and also extending irregularly into the extracellular region as a redundant structure. Long-spacing collagen as well as regular collagen is found adjacent to both the neoplastic and the non-neoplastic Schwann cells. In well differentiated schwannomas cytoplasmic loops may make as many as three spiral wraps around a bit of Schwann cell cytoplasm thereby creating a pattern superficially resembling the loose myelin seen in earthworm and other species. In the plexiform schwannoma the complexity of Schwann cell cytoplasmic attenuation and infolding reached a much more extreme pattern than that found in any other tumor; the striking layering of the cells in some regions suggests the pattern of layers of cells seen in the normal perineurium. Possibly schwannomas arise from the perineurium.

OBSERVATIONS ON THE CAUDA EPIDIDYMIIS IN THE NORMAL AND VASECTOMIZED DOG

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Since the cauda epididymis is recognized as a site of fluid resorption, it became of interest to assess alterations in fine structure following vasectomy and the consequent accumulation of fluid and sperm within the male excurrent duct system. As part of an overall research program to determine the effect of vasectomy upon the dog genital system, sixteen sexually mature, male mongrel dogs were vasectomized and were subsequently sacrificed in groups at 1, 4, 8 and 16 weeks post-operatively. Tissues were fixed for light and electron microscopy, as well as for histochemistry. The normal canine cauda epididymis is lined by a tall, pseudostratified columnar epithelium, underlain by a circularly disposed layer of smooth muscle. As in other mammalian species, the epithelium bears tall stereocilia on the free surface, and exhibits numerous vesicles and lysosomes within the apical cytoplasm and a well developed rough endoplasmic reticulum and Golgi apparatus apical to the nucleus. Crystalline inclusions associated with the Golgi complex and the endoplasmic reticulum are exceptionally numerous and well developed in this species. Crystalloids up to 3.7 microns in length and 0.2 microns in width were commonly observed; these exhibited a 125 Angstrom periodicity of linear subunits. The epithelial response to vasectomy is similar to that of the vas deferens, and

includes by 4 weeks postoperatively a decrease in the height of the epithelium, apical blebbing of cells and loss of stereocilia, and the accumulation of electron opaque material within large apical dense bodies. Such changes were accompanied by an increase in acid phosphatase activity. No evidence of phagocytosis of intact sperm by epithelial cells has been seen, necessitating alternative hypotheses for the fate of accumulated spermatozoa.

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ULTRASTRUCTURAL ALTERATIONS IN ALCOHOLIC HEPATITIS AND CIRRHOSIS OF THE LIVER

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There are scientific papers and books dealing with morphological changes of the liver under different circumstances and diseases, but this information is not organized for diagnostic purposes. The frequency and specificity of these changes are not correlated to large numbers of routine specimens and the ultrastructural variations have not been related to different clinical situations. In addition, the majority of the information available has been obtained from experimental animals and little from human material.

In the present study, changes found in parenchymal cells, Kupffer's cells and closely related structures like bile canaliculi, sinusoids, and space of Disse are reported from 100 liver biopsies from patients with alcoholic hepatitis and Laennec cirrhosis of the liver. Emphasis is made in the frequency and extension of these changes and the ultrastructural variations according to different clinical situations.

Comments are made on the specificity and diagnostic value of these observations.

MOBILITY OF INTRAMEMBRANE PARTICLES IN THE OUTER MEMBRANE OF ISOLATED MITOCHONDRIA. Matthias Hoechli and Charles R. Hackenbrock. Department of Cell Biology, University of Texas, Southwestern Medical School, Dallas, Texas 75235.

We have investigated the distribution of intramembrane particles in the fracture face of outer membranes of fixed and unfixed, freshly isolated rat liver mitochondria. Mitochondria fixed with 0.2% glutaraldehyde for 5 min and subsequently treated with 30% glycerol for 5 min show a random distribution of intramembrane particles in the concave fracture face of the outer membrane. The same fracture face of unfixed, glycerol treated mitochondria shows intramembrane particles arranged in a network. However, fixation after glycerol treatment results again in a random distribution of intramembrane particles. These rearrangements of intramembrane particles take place at 0° C as well as at 30° C which demonstrates a high degree of fluidity in the outer mitochondrial membrane. Preliminary data indicates that unfixed and non-glycerinated mitochondria show the outer membrane to contain a randomly diffuse distribution of intramembrane particles. It would appear that glycerol causes an aggregation of the intramembrane particles into a discrete chain-like pattern. Glutaraldehyde prevents the aggregation when used prior to glycerol treatment and reverses the aggregation when used after glycerol treatment. No definitive rearrangements of intramembrane particles have been observed in the inner mitochondrial membrane after glycerol treatment. The high degree of mobility of intramembrane particles in the outer membrane compared to the inner membrane is consistent with the high lipid to protein ratio of the outer membrane compared to the low lipid to protein ratio of the inner membrane. Supported by a Swiss Nat'l. Found. fellowship (M. Hoechli) and NSF Grant #BMS 72-02372 A02.

ULTRASTRUCTURAL CHANGES PRODUCED BY A PTE LEAD AND CADMIUM ADMINISTRATION IN THE RAT

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Among natural substances that man concentrates in his immediate environment, lead and cadmium are known to have deleterious biological effects. Both metals, regardless of the route of administration, are known to accumulate initially in the liver with obvious physiological alterations. Ultrastructural studies of this organ in the acute phase of these intoxications are scanty and incomplete. In the present experiment, the liver of the rat is examined in the electron microscope following intravenous administration of sublethal doses of either lead or cadmium. Lead produces vacuolization and abundant deposition of a finely granular, electron dense material (iron, lead) in the cytoplasm of Kupffer's cells. There was also formation of autophagic vacuoles and degenerative changes of the mitochondria of these cells. Parenchymal cells were less altered and presented depletion of glycogen granules, mild dilatation of the endoplasmic reticulum and occasionally few vesicles containing the same finely granular, electron dense material and fragmented organelles.

The most prominent changes after acute cadmium administration appeared in parenchymal cells and consisted of single cell necrosis, focal areas of cytoplasmic degradation, deterioration of ER, malformation of SER, and mitochondrial degenerative changes and prominent

REPLICATION OF CORONAVIRUS 229-E IN HUMAN FORESKIN FIBROBLAST CELLS

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Infection of human foreskin fibroblast (HFF) cells with Coronavirus 229-E results in production of high titers of progeny virus, if the cells are incubated at 33°C [permissive infection (PI)]. However, incubation of the infected cells at 37°C allows only limited viral gene function and infectious progeny cannot be detected [non-permissive infection (NPI)].

This study represents a preliminary investigation of the morphological aspects of the NPI of HFF cells by Coronavirus 229-E.

The PI of HFF cells was shown to be complete 24 hours after inoculation. Numerous virus particles could be detected in the cytoplasmic compartment, free and enclosed in vesicles, and in the extracellular spaces. Particles were of at least three morphological types: uniformly dense, ring form, and bulls' eye; all approximately 100nm in diameter. Although virus particles were observed to be within the outer nuclear membrane, they were never observed in the nucleus itself.

The morphological changes during NPI were obvious. Most dramatic was the relative lack of recognizable virus particles. Representatives of each type could be demonstrated, however infected cells produced only a few particles. Many particles appeared to be irregular in shape. Other changes typical of the NPI were 2) an increase in lysosomes, b) production of osmiophilic grey bodies, and c) disorganization of mitochondria.

autophagocytosis. Kupffer's cells appeared to be less altered although they were frequently found detached from the parenchymal cells. Occasional cytoplasmic degenerative changes were also found in these cells. Sinusoids presented scattered areas of platelet deposition, cellular debris and few inflammatory cells. This study shows that acute intoxication by lead in the rat produces more prominent changes in Kupffer's cells than in parenchymal cells and that cadmium appears to have more prominent toxic effect on parenchymal cells.

INSTANT, CHEMICAL DEHYDRATION FOR ELECTRON MICROSCOPY. T.J. Jacks and L.L. Muller, Southern Regional Research Center, New Orleans, La.

Dehydration of samples for electron microscopic examination is generally accomplished by physically exchanging water for organic solvents. We developed a simpler and quicker procedure in which acidified 2,2-dimethoxypropane reacts chemically with water in situ to form acetone and methanol. Ultrastructural integrities of plant and animal tissues are maintained after chemical dehydration. Electron micrographs of these materials will be presented.

Physical Properties of Human Salivary Alpha-Amylases

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Amylases are those enzymes that selectively catalyze the hydrolysis of the α -1-4-glucosidic linkages of polysaccharides such as occur in starch and glycogen. Human salivary α -amylases have long been known to exist in multiple forms, or isoamylases. Studies with polyacrylamide gel electrophoresis have established the existence of one major and four or more minor isoamylases. The major isoenzyme of saliva has 65-70% of the total activity.

To isolate the isoamylases we obtained 3-6 liters of raw saliva, processed this through a two-week crystallization procedure, and placed the α -amylase crystals thus obtained on a Bio-Gel P-100 column equilibrated with TRIS-HCL buffer, pH 7.6, containing 5 mM CaCl_2 . This gel filtration separated the five isoamylases into two families, A and B, which have been differentiated by their carbohydrate content. Family A, glycoproteins of 62,000 m.w., have 13 carbohydrate molecules attached to them, while isoamylases of Family B with a m.w. of 56,000 have no carbohydrate. To separate the isoamylases within each family, we employed an isoelectric focusing column system to simultaneously isolate and concentrate each isoamylase according to its isoelectric point.

Having thus isolated the isoamylases we will attempt to differentiate each isoenzyme on the basis of its crystals by both light and electron microscopy.

The results of these experiments will be presented.

SELECTIVE ULTRASTRUCTURAL LOCALIZATION OF SEROTONIN
STORAGE SITES IN RAT PINEALOCYTES. Lance Kirkegaard* and

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77025.

In glutaraldehyde fixed tissue, the primary amine reaction with potassium dichromate has been used for a number of years in the subcellular localization of biogenic amines. This technique which utilizes chromium as an electron dense reaction product does not, however, distinguish between catechol- and indoleamines. Such differentiation can be accomplished with incubation of the tissue in paraformaldehyde prior to the glutaraldehyde treatment since in vitro and in vivo studies have demonstrated paraformaldehyde-selective blockage of the glutaraldehyde-catecholamine reaction. Thus, this study reports the localization of cytoplasmic 5-hydroxytryptamine (serotonin) storage sites in pineal parenchymal cells. Selected electron dense reaction sites were bombarded with an electron beam in an analytical electron microscope and subsequent qualitative analyses of the emission spectra reveal chromium peaks indicative of a positive glutaraldehyde-indoleamine-dichromate (GID) reaction. This energy dispersive instrumentation is capable of high sensitivity and precision, and coupled with the GID reaction provides the system for in situ morphological correlation by X-ray analysis.

Supported by HEW Grant NS 10326.

KLARA, P.M. and K.R. BRIZZEE, Department of Anatomy, Tulane University School of Medicine and Delta Regional Primate Research Center, New Orleans, Louisiana. THE FINE STRUCTURE OF THE SQUIRREL MONKEY AREA POSTREMA.

The area postrema (AP) of the squirrel monkey Saimiri sciureus was examined by transmission electron microscopy. This circumventricular organ consisted of 2 cell types; neuronal and glial. The neurons were not arranged in any discernable pattern but were frequently closely related to perivascular spaces. These relatively small cells contained enfolded nuclei with a single characteristic nucleolus. The neuronal cytoplasm displayed the normal complement of organelles including rough and smooth endoplasmic reticulum, lysosomes and multivesicular bodies. Synaptic endings were numerous and were of both axo-somatic and axo-dendritic varieties. Frequently, both clear and dense cored vesicles were observed in the same ending. Unmyelinated processes were the rule although myelinated processes were also observed within the AP. Non-neuronal cells in the AP resembled astrocytes; while the ventricular surface was covered with a modified ependyma which lacked kinocilia but demonstrated numerous microvilli. Occasionally bulb-like protrusions containing mitochondria were observed on the ependymal surface. The AP vasculature was characterized by perivascular spaces bounded by distinct basal laminae. These spaces contained collagen, fibroblasts and free nerve endings. The vascular endothelium was fenestrated and pinocytotic vessels were numerous and routinely present. Since the squirrel monkey demonstrated an emetic response to 5-HTP and the AP reportedly functions as a chemoreceptor trigger zone for emesis, a morphological comparison of squirrel monkey AP to that of other species (eg. rat, rabbit) which fail to demonstrate an emetic response will be considered. Also, the possibility of a neurosecretory role for the AP will be discussed.

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FIBRIN AND HUMAN GRANULATION TISSUES FROM THERMAL BURNS

-C. Ward Kischer, Department of Anatomy, University of Texas Medical Branch, Galveston, Texas 77550.

Biopsies of human granulation tissues resulting from thermal burns were examined by light and electron microscopy for cellular and extracellular characterization, and for comparison to the known parameters of hypertrophic scarring. A few of the tissues were classified as "normal" granulations and a few others demonstrated hypertrophic patterns. Most progressed to hypertrophic scarring. Variations occur in the fine structure of the fibroblasts. Some appear friable while others have well developed rough endoplasmic reticulum. Ceroid-like material is frequently found in the interstitium. There are variations in the magnitude of inflammatory cells present. Some granulation tissues show well developed collagen tracts but usually in a hypertrophic pattern. Virtually all of the tissues examined demonstrate fibrin. Often it is found in vessel lumina and vessel walls. It is found in macrophages and sometimes appears "sticking" to fibroblasts and platelets. In some tissues the granular fibrin phase is demonstrated and small patches of similar looking material have been observed in hypertrophic scars. The possible integral involvement of fibrin in development of the hypertrophic scar will be discussed.

MESOTHELIOOMA - WHAT KIND OF TUMOR?

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Interest in pleural tumors increased during the last two decades and mesotheliomas became more frequently diagnosed; however, clinicians, radiologists, and pathologists are aware of the difficulties to render such a diagnosis on light microscopy alone. Electronmicroscopy contributed significantly to the diagnosis of this tumor as well as to a better understanding of its histogenesis.

Seven cases of pseudoglandular and five cases of sarcomatous malignant mesotheliomas were examined. The most characteristic ultrastructural features were: long microvilli on the surface forming occasionally brush boarder, focal presence of basal membrane, occasional desmosomes, and intimate surface relation to collagen. The cytoplasmic organelles were not conspicuous. Mitochondria were the most frequent organelle, and pinocytotic vesicles were abundant in some cases. Also, different amounts of intracytoplasmic fibrils, tonofilaments, and glycogen were present. The comparison with normal mesothelial cells as well as cells from pleural plaques and with re-active mesothelial cells showed definite similarities. In contrast to that, cells in a case of benign (fibrous) mesothelioma and pleural fibrosarcoma were indistinguishable from benign and/or malignant fibrocytes.

The observations indicate that an electronmicroscopist can distinguish between malignant mesothelioma and other types of tumors involving the pleura. The study confirms that malignant mesotheliomas arise from the mesothelial cell. It brought about a question if the benign (fibrous) mesothelioma also develops from the mesothelial cell because of the pluripotentiality of this cell, or, if it represents a fibroma, the benign counterpart of fibrosarcoma.

THE DUBIN JOHNSON SYNDROME: ULTRASTRUCTURAL STUDIES.

Jaime Lamberty and Joan Coover, Veterans Administration Hospital, New Orleans, Louisiana 70112.

Needle biopsies of the liver in two brothers with increased two-hour BSP and intermittent episodes of jaundice were examined by light and electron microscopy. The predominant alterations seen by light microscopy consisted of mild hepatocellular alterations, increased lipofuscin-melanin type pigment in centrilobular areas, mild hemosiderosis, and minimal portal chronic inflammatory infiltration with round cells.

Histochemical studies confirmed the presence of a non ferric, sudanophilic pigment in the centrilobular areas of the hepatic lobule.

A characteristic granular electron dense material surrounded by a single membrane was identified in the sinusoidal pole of the hepatocytes. Ultrastructural changes of cytoplasmic organelles consisted mainly of degenerative changes of the mitochondria characterized by ballooning and fibrillary degeneration. A moderate increase in glycogen as well as endoplasmic reticulum was seen.

The differential diagnosis of congenital hyperbilirubinemias will be discussed.

THYMOMA: CORRELATION OF ULTRASTRUCTURAL PATTERN WITH THREE

DIFFERENT CLINICAL SYNDROMES. Jaime Lamberty and Joan Coover, Veterans Administration Hospital, New Orleans, Louisiana 70112.

The association of thymomas with a wide variety of clinical syndromes is a well established fact. Its relationship to myasthenia gravis, pure red cell aplasia, dysgamma or hypogammaglobulinemia and disturbances of the immune mechanism have been reviewed by several authors.

In the literature, the correlation of a specific structural pattern to different syndromes is conflicting. Some authors indicate a predominance of lymphocytic component in thymos associated with myasthenia gravis while others report an increase in the epithelial or spindle like cell component. The same controversy applies to reported cases of thymomas in association with pure red cell aplasia and dysgammaglobulinemias. Both benign and malignant neoplasms have been reported in the literature in association with the above described syndromes.

In an attempt to clarify conflicting reports, the author studied three cases of thymomas and correlated the light and electron microscopic findings with the clinical syndrome involved.

Two of the cases presented, clinically, as anterior mediastinal masses, while the third and most interesting presented initially as an anterior mediastinal mass attached to the myocardium and was diagnosed by x-ray as an aortic aneurysm. The patient later developed a syndrome characterized by pure red cell aplasia and hypogammaglobulinemia,

predominantly IGA deficiency.

Two of the cases behaved biologically and morphologically as malignant reoplasms. Predominance of spindling pattern was seen in one of the cases although all three cases were of the mixed type.

This project was funded totally by the Veterans Administration Hospital.

CATION BINDING AND PHOSPHOHYDROLASE ACTIVITY IN THE
BODY SURFACE BRUSH BORDER OF THE TAPEWORM HYMENOLEPIS
DIMINUTA

Richard D. Lumsden, Tulane University, New Orleans, Louisiana

Like many other absorptive surfaces, the syncytial body coveri
of tapeworms includes a brush border with phosphohydrolase
activity against a wide variety of substrates. The orientatic
of the enzyme(s) is such that the hydrolysis of p-esters
occurs on the external face of the plasma membrane. This
phosphatase activity is markedly inhibited by chelating
agents, the inhibition being largely reversible by calcium,
magnesium, and zinc. The initial binding of these cations
to the brush border plasma membrane appears to involve
the electronegative charges of the glycocalyx, since
neutralizing these charges (by lanthanum adsorption)
blocks Ca/Mg/Zn reactivation of the chelator-inhibited
phosphatase activity. Moreover, magnesium, zinc, and
lanthanum inhibit ⁴⁵Ca binding to the tegument surface,
the degree of inhibition increasing non-linearly with the
magnitude of the concentration difference between calcium
and the aforementioned metal ions. These observations are
taken as further evidence for common binding sites for
these cations in their initial adsorption from the ambient
medium.

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STUDIES ON ENTEROCHROMAFFIN CELL SEROTONIN BY ANALYTICAL ELECTRON MICRO-
SCOPY. Kathleen Marburger* and Joe G. Wood, Ph.D. Program in Neuro-
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In the past, numerous histochemical techniques have added to the accumu-
lating evidence that 5-hydroxytryptamine (5HT, serotonin) is localized in
the enterochromaffin (EC) cells of the gastrointestinal epithelium. The
5HT is believed to be concentrated within the cytoplasmic granules of these
cells. In order to confirm that it is 5HT which has been detected in these
cells and not other biogenic amines such as norepinephrine (NE) or dopamine
(DA), which also give positive results with some of the histochemical tech-
niques used to demonstrate EC cells, rat intestine was fixed first with para-
formaldehyde and then with a combination of glutaraldehyde + dichromate
(G+DC). It has been shown by others that this fixation method results (in
vivo and in vitro) in a positive reaction (electron density due to the
presence of chromium, Cr) for 5HT but is negative for NE and DA, in that the
pretreatment with paraformaldehyde blocks the reaction of NE and/or DA with
G+DC but not of 5HT. Control tissue was fixed with G+DC only. Examination
of thin sections in the analytical electron microscope has shown that, al-
though the Cr peaks are substantially above background, the pretreatment
with paraformaldehyde reduces the amount of Cr in EC cell granules compared
to that in G+DC fixed tissue. Experiments are being designed to determine
if this difference is the result of partial blockage of the 5HT reaction by
the paraformaldehyde or if it represents a heterogeneity in 5HT content
within EC cells and/or their granules. Supported by HEW grant NS10326.

"CATECHOLAMINE RELEASE FROM CAT ABDOMINAL PARAGANGLIA: MORPHOLOGICAL OBSERVATIONS." Joe A. Mascorro and Robert D. Yates, Department of Anatomy, Tulane University School of Medicine, New Orleans, Louisiana 70112.

Catecholamine release in the adrenal medulla can occur via exocytosis, a process whereby granules are extruded following fusion with the plasma membrane. Other reports indicate that catecholamines are released into the cytoplasm and diffuse thru the plasma membrane. Exocytosis involves loss of the entire granule components, including catecholamines, ATP, proteins and lipid. Cytoplasmic diffusion implies that only the catecholamines are lost with binding material remaining within the granule. This study attempts to elucidate the mechanism of catecholamine release from the adrenal medullary homologues, the voluminous abdominal paraganglia.

Young cats (110-400 grams) received reserpine (5 mg/kg, IP, 2 animals) or acetylcholine chloride (1 mg/kg, via saline perfusion, 2 animals); two were placed in an ether atmosphere and two others served as controls. Nonetherized cats received Nembutal (35 mg/kg, IP) and all were perfused with 3% glutaraldehyde in 0.1M sodium phosphate, pH 7.3. The retroperitoneal tissue block was further fixed in the perfusion fluid and the paraganglia were localized by immersing the block in phosphate buffered 3% glutaraldehyde/2.5% potassium dichromate, pH 6.8. Potassium dichromate produced a gross chromaffin reaction and the paraganglia were easily traced. The bodies were post-fixed in osmium, dehydrated and embedded in Epon. Thin sections were stained and viewed in a Siemens 101 Elmiskop.

Paraganglion cells of untreated animals contained catecholamine granules with very dense cores, but cells from reserpinized cats displayed granules which showed various degrees of reduced electron opacity indicative of catecholamine loss. Ether anesthesia caused the granules to become less dense, fragmented and obviously swollen. Acetylcholine chloride caused the appearance of very few exocytotic figures, even when calcium chloride (1 mg/ml) was added to the perfusate to further stimulate catecholamine secretion.

Many thin sections representing different planes and containing innumerable granules were studied, with emphasis on the plasma membrane areas as well as the intercellular and perivascular spaces where exocytosis could occur. The appearance of limited exocytotic figures and many less dense granules led us to believe that, under the present conditions, exocytotic profiles did not occur in sufficient numbers to account for a significant catecholamine loss. Therefore, most catecholamine possibly was released by a cytoplasmic diffusion process not involving whole granule extrusion.

ELECTRON MICROSCOPIC STUDY OF ACID PHOSPHATASE ACTIVITY IN CULTIVATED FIBROBLASTS FROM CYSTIC FIBROSIS PATIENTS

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Fibroblasts from cystic fibrosis (CF) patients, heterozygous CF carriers, and normal persons were cultured on Teflon-coated coverglasses (Chang, 1971) in Falcon dishes. The monolayer cells at both exponential and plateau phases were fixed in situ for 30 to 60 min by immersion of the coverglasses in 2% glutaraldehyde in 0.1 M cacodylate buffer containing 8% sucrose at pH 7.4 at 4°C. After fixation, cells were washed for 30 to 60 min in 0.01 M cacodylate buffer with 0.25 M sucrose and 10% (V/V) DMSO. For demonstration of acid phosphatase activity, the cells were incubated for 30 to 90 min at 37°C in the following mixture: 0.05 M acetate buffer (pH 5.0), 10 mM Sodium β -glycerophosphate, 3 mM lead nitrate, 10% (V/V) DMSO, and 0.25 M sucrose. Then, the cells were rinsed with buffer, post-fixed with 1% OsO₄ for 60 min, rinsed again, immersed in buffered 1% yellow ammonium sulfide, and processed for light and electron microscopic observations.

Acid phosphatase activity was demonstrated on lysosomes, Golgi-endoplasmic reticulum-lysosome (GERL) systems, Golgi apparatus, and the so-called metachromatic granules in all cells under various growth conditions. However, the strikingly well-developed GERL systems appeared more often in heterozygous and in normal cells than those in CF cells. On the other hand, strong acid phosphatase activity in the metachromatic granules is found mainly in CF cells at plateau phase.

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ULTRASTRUCTURAL LOCALIZATION OF CYTOCHROME OXIDASE ON THE MITOCHONDRIAL INNER MEMBRANE AND ON CYTOCHROME OXIDASE LIPOSOMES.

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Charles R. Hackenbrock. Department of Cell Biology, University of Texas
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Affinity purified immunoglobulin (IgG), highly specific for cytochrome oxidase, was conjugated to ferritin (Fer) with glutaraldehyde to yield an electron opaque, immunospecific probe (IgG-Fer). The use of this probe has allowed us to determine the molecular distribution of cytochrome oxidase on the surface of outer membrane-free rat liver mitochondrial inner membranes. We have also determined the distribution of cytochrome oxidase on the surface of detergent-prepared cytochrome oxidase liposomes. Membrane preparations were examined in thin sections, whole mounts, and freeze-etched replicas with the electron microscope. The IgG-Fer probe shows cytochrome oxidase to be randomly distributed in the horizontal plane of the inner mitochondrial membrane, both on the inner crystal membrane and on the inner boundary membrane. In addition, the use of inverted inner mitochondrial membranes has revealed binding of the IgG-Fer probe to the membrane's inner surface. We found that addition of IgG-Fer inhibits the function of cytochrome oxidase in electron transport. These observations reveal that cytochrome oxidase is located in a transmembrane position. The distance between the molecules of cytochrome oxidase can be as little as 400 Å. Freeze-cleave electron microscopy of cytochrome oxidase liposomes reveals intramembrane particles may represent individual molecules of cytochrome oxidase. (We thank Dr. Tsao King for cytochrome oxidase; supported by NSF Grant # BMS72-02372-A02).

RECENT CONTRIBUTIONS OF TRANSMISSION ELECTRON MICROSCOPY

TO THE DESIGN OF TOUGH Fe-Ni STEELS*

by

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While structural materials are normally used in thick sections, their essential mechanical properties are often controlled by sub-microscopic microstructural features. For this reason transmission electron microscopy plays a central role in research toward new alloys with superior mechanical properties. In our own research, which addresses the problem of designing new steels which retain toughness with high strength at cryogenic temperature, thin foil TEM has recently led to two significant observations. The first is the observation and identification of hitherto unknown ordering reactions in Fe-Ni alloys of moderate Ni content. This ordering may prove a major source of the beneficial microstructure established on quenching these alloys. The second is the identification and morphological characterization of induced second-phase islands in Fe-Ni alloys. These observations allowed us to establish a close experimental control over the distribution of second phase (austenite) material, which led to a major improvement in the cryogenic toughness of Fe-8Ni-2Mn-0.25Ti alloys.

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MORPHOMETRIC ANALYSIS OF PANCREATIC ACINAR CELLS FROM

ORALLY FED AND INTRAVENOUSLY FED RATS. Pavlat, W. A., Rogers, W., and Cameron, I. L. Departments of Anatomy and Surgery, The University of Texas Health Science Center at San Antonio, and the Audie Murphy Memorial Veterans Hospital, San Antonio, Texas.

Pancreatic acinar cells from rats which have undergone two weeks of parenteral (total intravenous) feeding as compared to rats which have been orally fed on solid lab chow were studied by light and by electron microscopy. The intravenous diet of crystalline amino acids, glucose, electrolytes and vitamins provided a higher caloric intake in the i.v. fed animals than in the ad libitum orally fed control animals.

Morphometric (stereological) analysis (Weibel, E. R. in Principles and Techniques of Electron Microscopy, volume 3 ed. by M. A. Hayat, 1973) of sections from the pancreas revealed several significant changes in the acinar cells. For example, there was a 50% decrease in total cell and nuclear volumes of acinar cells in the i.v. fed group of rats. The volume of the ergastoplasmic region (rough endoplasmic reticulum) in the i.v. fed animals was one third that of the control rats. The volumes of the zymogenic granular region in the acinar cells of both groups of animals were equal. Larger nucleoli, indicative of a greater amount of ribosomal RNA synthesis, were also seen in the control animals. The response of the pancreatic acinar cells to parenteral feeding appears similar to that seen by others during starvation. However, with parenteral feeding the normal function of acinar cells is minimized and at the same time the animal is maintained at an optimal nutritional level.

The findings suggest that total i.v. feeding may be of value in the treatment of pancreatic disease or trauma by relieving the acinar cells of their normal function, which involves synthesis of large amounts of zymogen or digestive enzymes, thus allowing the healing process to take place in the pancreas of an animal with adequate nutrition.

Supported by the Morrison Trust and the Veterans Administration Hospital of San Antonio.

ULTRASTRUCTURAL VARIABILITY OF ENCAPSULATED NERVE ENDINGS IN RAT GINGIVA

Pekarthy, James M. and I. Ricardo Martinez, Jr., Departments of Anatomy and Dermatology, Louisiana State University Medical Center, New Orleans, LA.

Encapsulated nerve endings in albino rat gingiva have been described previously (Martinez and Pekarthy, *Am. J. Anat.*, 140:135, 1974). These specialized nerve endings are found almost exclusively in the papillary dermis in proximity to blood vessels. The most frequently observed nerve endings consist of a proximal myelinated axon, a distal unmyelinated axon surrounded by laminar cells, and capsular cells. The more proximal portion of the unmyelinated axon contains mitochondria, many microtubules, and few microvesicles. More distally, the unmyelinated axon contains mitochondria, fewer microtubules, and many microvesicles. The axon in the corpuscular ending is surrounded by tightly-wound cytoplasmic lamellae of specialized cells termed laminar cells. The laminar cells possess a distinct basement membrane and demonstrate extensive pinocytotic activity. Collagen is not found between successive laminar cell wrappings. The capsular cell, located external to the laminar cells, is morphologically identical to a fibroblast. However, ultrastructural variations of this basic type of encapsulated nerve endings have been observed. The variations may include one or more of the following features: 1) absence of capsular cells, 2) loosely wound cytoplasmic lamellae, 3) collagen fibrils between laminar-cell lamellae, 4) branching of encapsulated axon, 5) terminal axonal projections, 6) multiple laminar-cell wrappings.

LOCALIZATION OF PROTEINS IN MYELIN USING WALLERIAN

DEGENERATION. R. G. Peterson, C. P. Sea and R. W. Gruener;

Program in Neurostructure and Function. The University of Texas Health Science Center at Houston, Houston, Texas 77025.

Three predominant proteins are found in PNS myelin. These are the main myelin protein (P_0 , glycoprotein) and two basic proteins (P_1 and P_2). The recent report Wood and Dawson (*J. Neurochem.* 22:631, 1974) has shown that there is a very specific breakdown of the P_0 protein between 5 and 8 days of Wallerian degeneration. The purpose of the present experiments was to follow this protein change at the ultrastructural level, using both biochemical and morphological techniques. Wallerian degeneration in rats was initiated by surgically performing unilateral sciatic neurotomy 4 - 8 days before sacrifice. Tissue for electron microscopy was fixed by both perfusion and immersion. Proteins were analyzed by delipidation of whole nerve in chloroform:methanol 2:1 and acetone and running the remaining proteins by disc gel electrophoresis after the method of Fairbanks, et al (*Biochem.* 10:2606, 1971). Biochemical results on the amount of proteins were similar to those found by Wood and Dawson (*J. Neurochem.* 22:631, 1974). At the electron microscopic level, the initial changes in the myelin bands were in the intraperiod band. These alterations consisted of breaking up, and collapse of the usual double-banded appearance of this structure. During the same period of time, there was no recognizable change in the main period band. The biochemical and ultrastructural results correlated very well. This data supports the hypothesis that the main myelin protein, which is a glycoprotein, is located in the intraperiod band.

TECHNIQUES FOR THE PREPARATION OF PHYTOPLANKTONIC

ORGANISMS FOR SCANNING ELECTRON MICROSCOPY

Michael T. Postek, Department of Biological Sciences, University of Texas at El Paso, El Paso, Texas 79968

Phytoplanktonic organisms suitable for study utilizing the scanning electron microscope can be divided into two broad categories: those capable of withstanding the rigors of air drying and those who are not. Organisms such as the diatoms (Chrysophyta) and certain Gonyaulax, Peridinium and Ceratium species (Pyrrophyta) have a theca heavy enough to withstand the extreme pressures of air drying. The more fragile planktonic species such as Gonyaulax catenella or fresh water green algae such as Cosmarium or Onychonema necessitate more careful preparatory procedures due to the fragility of their outer wall. The first group of organisms may be prepared for air drying by carrying the fixed cells through a number of distilled water washes. This process will remove the fixative and other dissolved contaminants. The cells are allowed to concentrate by sedimentation rather than centrifugation in order to reduce mechanical damage. After washing, a few drops of the suspension are allowed to dry upon a coverslip which is then attached to a specimen stub. The more fragile organisms should be filtered into a specimen processing container, fixed, washed, dehydrated and critical point dried. The dry specimens are then carefully dusted onto a stub covered with double stick tape.

Advantages and applications of each technique will be presented in order to provide a basis for future investigation.

ULTRASTRUCTURE OF THE THYMUS

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Low magnification transmission electron microscopic micrographs, observations with scanning electron microscopy and morphometric assessment permit the study of thymic ultrastructure and to follow the modifications occurring in its post-natal development. The thymus consists of 3 parts: an outer cortex, an inner cortex, and a medulla. The outer cortex contains mainly lymphoblasts, the inner cortex mainly lymphocytes, and the medulla, mainly epithelial cells. Stereologic analysis assesses the respective volumes of these parts and their cell population at different times of thymic involution. The supporting framework of the thymus consists of a maze of anastomosing epithelial cells accompanied by a well-developed basement membrane. This meshwork is attached to the blood vessels by fusion of the respective basement membrane. Scanning electron microscopy indicates that the "perivascular channels" are a non-entity. This false impression is created by the longitudinal sections of trabeculae. The presence of lymphocytes in the wall of post-capillary venules indicates their traffic and the presence of plasma cells close to these vessels implies a possible influx of B-cells. The cells involved in lymphopagocytosis are probably of epithelial origin. A few lymphatic vessels are located in the medulla but do not extend beyond the cortico-medullary junction. During aging, the thymus is invaded by B-lymphocytes from the periphery as well as from the cortico-medullary junction. A similar phenomenon is observed during pregnancy.

ANNULATE LAMELLAE IN HUMAN SPERMIOGENIC CELLS

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Annulate lamellae have been most commonly observed in the cytoplasm of invertebrate and vertebrate germ cells and neoplastic cells. In human testicular tissue, Horstman (Zeitschrift für Zellforsch. 54: 68, 1961, Z. Zellforsch. Mikroskop. Anat. 58: 660, 1963) first described the presence of annulated membranes in Sertoli cells and spermiogenic cells, but no reference was made as to their mode of formation or role in the cellular activity.

Current observations of human spermiogenic cells demonstrate cytoplasmic annulate lamellae associated with highly electron dense cytoplasmic material and membranous continuities with the nuclear envelope and the smooth endoplasmic reticulum. Extranuclear annulate lamellar stacks appear most abundantly in Sb_1 and Sd_1 , maturing human spermatids, positioned either parallel to the nuclear envelope or dispersed in the cytoplasm in close proximity to Golgi bodies. The number of lamellae arrayed into a single stack is variable, but most commonly they are observed in groups of 4 to 6. Annulate lamellar pores in certain stacks appear to be aligned vertically, and occasional annuli are in continuity with those of the nuclear membrane. Enzyme digestion studies are in progress to determine the cytochemistry of the associated electron dense material to the annulate lamellae and to ascertain the role of this structure in human spermiogenesis.

(Supported in part by a grant from the Atomic Energy Commission (AT - (40 - 1) - 4002) and by a summer research grant from the Graduate School of Texas Tech University.)

ULTRASTRUCTURAL ASPECTS OF COTTON FIBER DIFFERENTIATION AS INFLUENCED BY NIGHT TEMPERATURE

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Gossypium hirsutum L. var. dunn 56 C was field grown under four different night temperature regimes (10°, 15°, 20°, and 25° C) by use of field growth chambers. The chambers were rolled onto the plots at sunset and removed at sunrise allowing for ambient daylight temperatures.

Cotton fibers are single cells that arise by differentiation and subsequent elongation of certain cells in the outer epidermal layer of the cotton ovule. This initiation occurs on the day of anthesis and gives rise to the actual elongation stage lasting from anthesis to day 16-19 postanthesis. This stage is followed by cell thickening during which the secondary cell wall is synthesized. This final stage lasts approximately from day 20 to bowl dehiscence 50 to 60 days after anthesis.

Tissue from anthesis to four days postanthesis was examined with both the light and electron microscope. It was found that 15°C was the optimum temperature for fiber elongation. Secondly, the number of cells initiating elongation were not significantly affected by temperature.

At the ultrastructural level, 10° and 25° C caused shorter elongating cells. Electron dense polyphenolic compounds were less apparent at these temperatures; vacuole formation in the developing fiber was retarded; and the amount of rough endoplasmic reticulum was decreased.

(Supported by the U. S. Department of Agriculture with funds made available through Cotton Incorporated.)

Abstract

STRIATED GRANULES OF RAT EPIDERMIS

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Striated granules, also known as membrane-coating-granules, keratinosomes or Odland bodies, are found in the epidermis of many vertebrates. They are increasingly thought to be a form of epidermal lysosome but their origin and functions are not clear. Some involvement in either keratinization or desquamation is suspected. To demonstrate that they are lysosomes, which would favor their role in desquamation, rats were administered various lysosomal releasing and stabilizing agents viz. vitamin A, hydrocortisone and endotoxin, in vivo. The lip was examined by electron microscopy for localization of acid phosphatase. Although variations in the release of lysosomal enzymes resulting from the agents could not be demonstrated, the striated granule was clearly shown to be a form of secondary lysosome. Lysosomal enzymes first collect in granules adjacent to Golgi saccules in basal and prickle cells. Enzymes of these "pure" lysosomes are transferred to the striated granules which form from excess membranes of granular cells. Striated granules disintegrate in the transition cell to bits of membranes with attached enzymes which react with their substrates, keratohyalin and tonofibrils. Hydrolysis of those keratin precursors provides the residues for the synthesis of keratin.

Enzyme-membrane fragments which often aggregate into supergranules continue to promote the hydrolysis of residual keratohyalin in horny cells and intercellular transport of the products probably contributes to cell cohesion. Breakdown of internal supports from continuing intracellular enzymatic activity results in the collapse of the keratinized cell. The loss of cohesion effected by extracellular hydrolytic enzymes brings on desquamation.

RELEASE OF CONTENTS FROM NORADRENERGIC VESICLES BY EXOCYTOSIS.

Åsa Thureson-Klein, Department of Pharmacology, University of Mississippi Medical Center, Jackson, Ms. 39216.

When noradrenergic nerves are stimulated, chromogranin A and dopamine- β -hydroxylase (D β H) are released in addition to the transmitter. It has been concluded that the release mechanism involves partial or complete exocytosis from either the large or small dense core vesicles. However, morphological evidence for exocytosis is meager and consists of descriptions from nerve terminals in rat vas deferens. In these it is a large dense core vesicle which has fused with the neurolemma, in spite of the fact that 95-98% of the vesicles are of the small type.

In splenic nerve axons all, or nearly all, the chromogranin A and D β H is contained in the 'heavy' noradrenergic vesicles. These are assumed to correspond to large dense core vesicles seen in the electron microscope. This type accounts for 50% of the total number of vesicles and about 90% of the entire vesicle volume in the bovine splenic nerve varicosities.

In axons and smooth muscle from bovine spleen capsule, it is possible to find terminal-like areas in which large dense core vesicles are in immediate contact with the neurolemma and in the process of releasing their contents by exocytosis. In a few cases the expelled core can still be seen extra-axonally. The intense sympathetic stimulation in these animals before and during slaughter may account for an increase in transmitter release by exocytosis. In this case the process mainly involved large dense core vesicles, as contacts between small vesicles and neurolemma was rarely observed.

The light and ultrastructural changes in otosclerosis as shown with a special stain (A preliminary report)

By: James C. Stinson, M.D., A. K. Probst, M.D.,
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Stapes removed from patients with otosclerosis were impregnated with silver prior to decalcification. By light microscopy changes were noted in the various locations including the crura, head and cartilagenous footplate. The various changes appear to have some relationship to the amount and location of a silver stained material present in the lacunae and canaliculi of the bone. In some of the stapes, there appeared to be in addition an unusual extrusion of this silver stained material into the cartilagenous portion of the footplate. Normal and abnormal areas might be present in same stapes.

In the electron microscopic examination of the silver stained but uncounter stained bone, minimal amounts of this material were apparent in the abnormal locations. The lacunae or canaliculi were practically empty although some deposits were apparent in the collagen of the matrix. What may have been cell remnants were present in some lacunae. In the more normal appearing locations, black granular material was present in the lacunae and canaliculi and what probably represented cross sections of the canals were prominent. Cells and definite protoplasmic extensions were not definitely observed in the lacunae where the silver granules were prominent.

PERSPECTIVES IN HIGH VOLTAGE, HIGH RESOLUTION ELECTRON MICROSCOPY

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ABSTRACT

The initial impetus for using higher and higher voltages came from the need to examine specimens thicker than could be satisfactorily imaged at 100 kV. Now we have two instruments in the world working at 3 MeV and several 500 kV - 1 MeV installations have been functioning in the USA in recent years. In materials science new advances have been made in studies such as radiation damage, ceramics, minerals and the examination of materials in gaseous environments. In biology advances in using thicker sections for stereo reconstruction seem to be in full swing. Radiation damage limits resolution, especially in crystalline organic objects but there appears to be a 10-20% gain in lifetime at 1 MeV compared to 100 kV. Radiation damage nevertheless seriously impairs studies of living organisms.

Attempts to optimise the high resolution potential of electron microscopy have received considerable impetus recently. Theoretically, atoms are resolvable but again radiation damage may limit the useful resolution of atomic structures of macromolecules. Lattice imaging at 2Å or better is now a powerful high resolution technique for studies of inorganic crystals and examples will be given of recent work at Berkeley on ordered systems.

DISTRIBUTION AND MOBILITY OF ANIONIC SITES ON THE SURFACE OF BABY HAMSTER KIDNEY CELLS ATTACHED TO A SUBSTRATUM. Mary Q. Tobleman, Charles R. Hackenbrock, and Frederick Grinnell. Department of Cell Biology, University of Texas Southwestern Medical School, Dallas, Texas 75235.

The multivalent ligand, polycationic ferritin, was used as a visual marker to detect the distribution of anionic sites on the surface of baby hamster kidney (BHK) cells following cell attachment to a substratum. BHK cells were permitted to attach to the surface of polymerized Epon 812 for time intervals of 15 min., 30 min., and 1 hour. The attached cells were then either prefixed with glutaraldehyde or left unfixed and subsequently incubated with polycationic ferritin (PCF). Prefixed cells exhibited a diffuse and random distribution of PCF on the exposed (upper) cell surface; however, no labeling occurred on the attached (lower) cell surface apposed to the Epon. Unfixed cells exhibited a non-random distribution of PCF on their exposed surfaces. There was a decrease in the bound ligand on cell microextensions and concomitant clustering of the ligand on the cell body. The interval of time that the cells were permitted to attach to the Epon had little effect on the subsequent surface distribution of the bound PCF on either the prefixed or unfixed cells. The random distribution of PCF on the prefixed cells indicates the ubiquitous presence of anionic sites on the exposed cell surface. Clustering of PCF on the cell body of unfixed cells and the simultaneous decrease of the ligand on cell microextensions suggests that a lateral movement of anionic sites occurs on the exposed surface of the attached cells. Similar results have been observed in this laboratory utilizing BHK cells in suspension. (Supported by NIH grant CA14609 and NSF grant EMS72-02372 A02).

CARBOHYDRATE NATURE OF THE INTESTINAL CELL OF ASCARIS LUMBRICOIDES
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The intestine of the parasitic nematode Ascaris lumbricoides constitutes a natural monolayer of tall columnar cells whose apical surface is modified in the form of long microvilli.

Cytochemical tests for carbohydrates indicate a rich storage of PAS positive-diacetase sensitive material in these cells. The microvilli and basement membrane are rich in both neutral and acidic glycans. The general ultrastructural distribution of carbohydrates was shown by the PATCO and Bismuth Subnitrate procedures. Acidic carbohydrates were demonstrated by the colloidal iron and Ruthenium Red procedures. Specific saccharides such as α -D-glycopyranosyl and α -D-manopyranosyl residues were detected by the use of Concanavalin A-Horseradish Peroxidase procedure.

MORPHOLOGICAL AND ECOLOGICAL COMPARISONS OF THE MARINE
 DINOFLAGELLATES CERATIUM FURCA (EHRENBERG) CLAPAREDE
 AND LACHMANN AND CERATIUM HIRCUS SCHRÖDER

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and

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 El Paso, El Paso, Texas 79968

The dinoflagellate Ceratium furca (Ehrenberg) Claparede and Lachmann has been reported to be both abundant and frequently recorded in Gulf of Mexico estuaries and neritic waters. It is likely, however, that Ceratium hircus Schröder may have been incorrectly referred to as C. furca by some investigators.

Scanning electron microscope examinations of both C. furca and C. hircus support the observations of other investigators that C. hircus is characterized by antapical horns of equal length with the right antapical horn curved slightly outward, whereas C. furca has antapical horns of decidedly unequal length with both horns directed toward the posterior. In addition, C. hircus has a broader cingulum width and shorter cell length than C. furca.

An extensive seasonal survey of phytoplankton distribution in the Tampa Bay System, Florida revealed that C. hircus was

the most abundant armored dinoflagellate recorded in all seasons throughout the estuary whereas C. furca was recorded only infrequently near the mouth of the estuary. This suggests that in Tampa Bay, C. hircus is more characteristically an estuarine form than C. furca.

The morphological consistency of C. hircus throughout the seasons and from year-to-year in Tampa Bay, together with records in the literature of its presence in other estuaries and neritic waters of the Gulf of Mexico and Caribbean suggests that C. hircus is not an aberrant form or variety of C. furca but a widely distributed dinoflagellate species.

INTRUSIVE GROWTH OF OIL CELLS IN SAURURUS CERNUUS

By Shirley C. Tucker

Louisiana State University, Baton Rouge, La. 70803

Curious idioblastic oil cells characterize many phylogenetically primitive angiosperms. In Saururus cernuus (Saururaceae: Piperiales) the oil cells are found in leaves, stems, inflorescence axes, bracts, and stamens. Scanning electron microscopy clarifies some of the puzzling aspects of oil cell development revealed by light microscopy. Although oil cells develop in various layers in each organ, those which arise in the layer just below the epidermis are of particular interest. The initials are first recognizable by size and staining qualities; they enlarge and gradually protrude between epidermal cells. Ultimately the oil cells occupy a position completely within the epidermis; certain portions of the cell appear to retract from the subsurface layer. Adjacent cells are distorted in shape and in contents. Intrusive or gliding growth of cells from one layer to another is rare in plants; its implications will be discussed.

ABSTRACT FOR LOUISIANA SOCIETY FOR
ELECTRON MICROSCOPY SYMPOSIUM

SCANNING ELECTRON MICROSCOPY OF THE INTERIOR AND THE
EXTERIOR TOPOGRAPHY OF SINGLE CELLS. (Colcemid-blocked,
Synchronized HeLa Cell in Culture).

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The topographical characteristics of the surface of whole HeLa cells which have been induced to traverse the proliferative cell cycle in a synchronous manner by use of a colcemid block will be described and compared for cells in mitosis at zero time, in G1 phase at 4 hours, and in peak S phase at 16 hours.

The main thrust of the paper will be the description from scanning electron microscopy of the interior topography of cell organelles, which have been made visible, by use of a new and relatively simple technique of specimen preparation to which we have applied the notation "stripping." The stripping is accomplished by pressing a length of adhesive metal tape down firmly upon the surface of the critical point dried culture. The tape is stripped off the surface. In so doing it takes a part of each of the cells with it, leaving the remainder of each individual cell behind, still stuck firmly to the original coverslip. Both the tape and the stripped culture are then coated for examination by SEM.

Stripping provides an important new view of cellular interior topography by allowing the observer to look down into the cell by SEM. Interpretation of

the findings is still in a preliminary stage but certain aspects of the micro-anatomy of single, critical point dried cells have been made directly visible within the stripped cells. Concurrent comparative TEM has indicated from considerations of size, morphology and cellular locations that the Golgi complex, mitochondria, vesiculated bodies, groups of ribosomes, endoplasmic reticulum and bundles of microfilaments within junctional complexes between cells have been visualized and identified in the stripped cells.

The stripping process has also revealed information concerning the tubular nature of filopodia embedded in the substrate, the manner in which the fibrous content of junctional complexes joins and penetrated the cytoplasm of supporting cells, and how the nuclear volume is pulled out of the cytoplasm with cytoplasmic organelles still attached to it. In addition, stripping has given some new information about the undersurface specializations, mostly the reduced number of microvilli and blebs, which populate the undersurfaces of the cells.

HeLa cells were cultured on 10.5 x 32 mm coverslips in Leighton tubes, and inoculated at a density of 50,000 cells/tube in 1.5 ml of Minimal Essential Medium Eagle with 10% calf serum. They were incubated at 37°C in an atmosphere of 5% CO₂/95% air for 48 hours. The medium was removed and replaced with one containing 0.025 ug of colcemid/ml. After

12 hours the colcemid was removed, the cells were washed and fresh growth medium was introduced. The cultures were not shaken. They were fixed at hourly intervals in cacodylate-buffered glutaraldehyde, critical point dried using CO₂, and coated with Au-Pd for SEM. All of the observations reported were made on HeLa cells prepared by these methods. For concurrent comparative TEM the same preparations were post-fixed in veronal acetate-buffered osmium tetroxide, were stained with uranyl acetate (Ph 5), embedded in Araldite, sectioned, and contrasted with uranyl acetate and lead citrate.

ULTRASTRUCTURAL ORGANIZATION OF THE INTERSTITIAL TISSUE OF THE TESTES OF THE NINE-BANDED ARMADILLO

Weaker, Frank J., Department of Anatomy, Louisiana State University Medical Center, New Orleans, LA.

The interstitium of the testes of the nine-banded armadillo contained numerous large lymphatic sinusoids, as well as other connective tissue elements. The space directly between seminiferous tubules contained either blood vessels, lymphatic sinusoids, or connective tissue elements. In contrast, the angular space between adjacent tubules contained lymphatic sinusoids which were intimately related to islands of Leydig cells. Within the islands, several Leydig cells clustered around one or more blood vessels. The cells were generally polygonal in shape with irregular cell surfaces. Frequently, adjacent Leydig cells appeared to be connected by a cell membrane specialization. The cells contained mitochondria with tubular cristae, an abundance of agranular endoplasmic reticulum, and a paucity of granular endoplasmic reticulum which was located adjacent to the nucleus. The agranular reticulum was a network of interconnected tubules which formed concentric whorls of membranes in the peripheral cytoplasm. In addition, the agranular reticulum encircled mitochondria as well as lipid droplets. The cytoplasm also contained polysomes and a Golgi apparatus. There appeared to be no morphological changes in the interstitium between the breeding and non-breeding seasons. Because of the morphological relationship between the Leydig cell islands and the sinusoids, it has been postulated that Leydig cells secrete androgens in the lymphatic system as well as the vascular system. Therefore, androgens needed to maintain spermatogenesis in the seminiferous epithelium could reach the tubules by the lymphatic sinusoids and blood vessels.

ULTRASTRUCTURE OF A HUMAN MESOTHELIOMA

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Department of Pathology, Brooke Army Medical Center, Ft. Sam Houston,
Texas 78234.

A 73 year old male was found to have multiple small nodules on his parietal and visceral peritoneum. These nodules were examined by both light and electron microscopy. The tumor cells had well developed endoplasmic reticulum and bundles of tonofilament-like structures. The nuclei were greatly indented and often gave the appearance of segmentation. There were large nucleoli and occasional intracytoplasmic lipid droplets. The tumor cells were found to be interdigitating and desmosomes were identified. The cell surfaces possessed microvilli which were up to 3 microns in length. These microvilli were also present in small non-lumens formed by the tumor cells. Pinocytotic vesicles were prevalent in many of the tumor cells as were generous deposits of glycogen. The appearance of this tumor is consistent with published morphology of human and non-human, natural and experimentally induced mesotheliomas of the epithelial type.

PROTEIN ANALYSIS OF THE MICROSPORIDIAN SPORE EXTRUSION APPARATUS: ULTRASTRUCTURE, ISOLATION AND ELECTROPHORETIC CHARACTERISTICS

Earl Weidner, Department of Zoology and Physiology
Louisiana State University, Baton Rouge, Louisiana

The infective spore stage of Microsporidia possesses an extrusion apparatus used to inoculate the sporoplasm into cells. The extrusion apparatus contains a protein core. During spore firing, this core protein is rapidly ejected, exteriorized, and assembled as the sheath component of the discharge tube.

Discharge tubes of *Nosema michaelis* were studied by thin sections, carbon replicas and negatively-stained preparations. The sheath protein was easily isolated from hatched spores which was since this was the only component from hatched spores which was SDS insoluble. The protein was reduced with 2-mercaptoethanol, alkylated, and examined by polyacrylamide disc electrophoresis. Molecular weight determinations and amino acid profile analysis indicates the sheath protein is different and smaller than actin.

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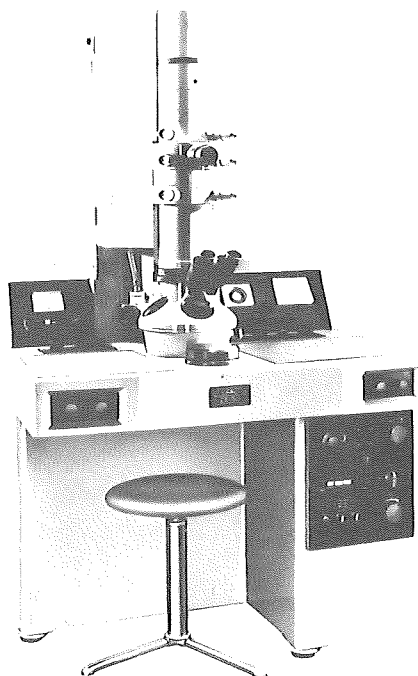
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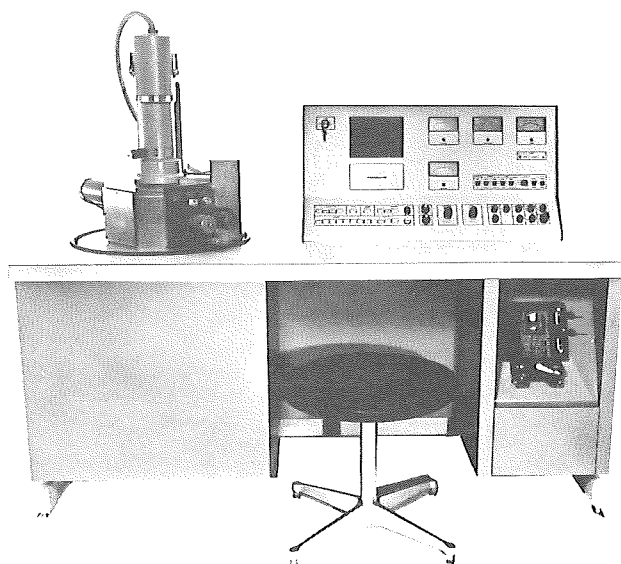
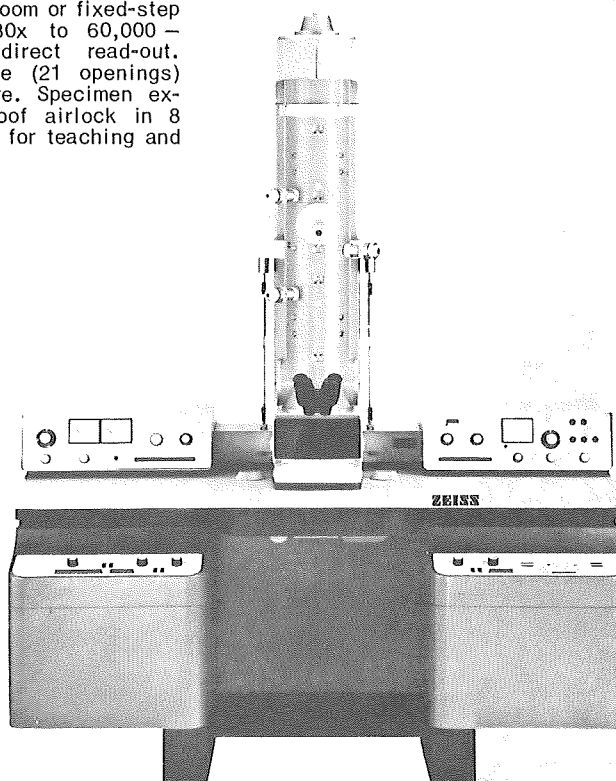
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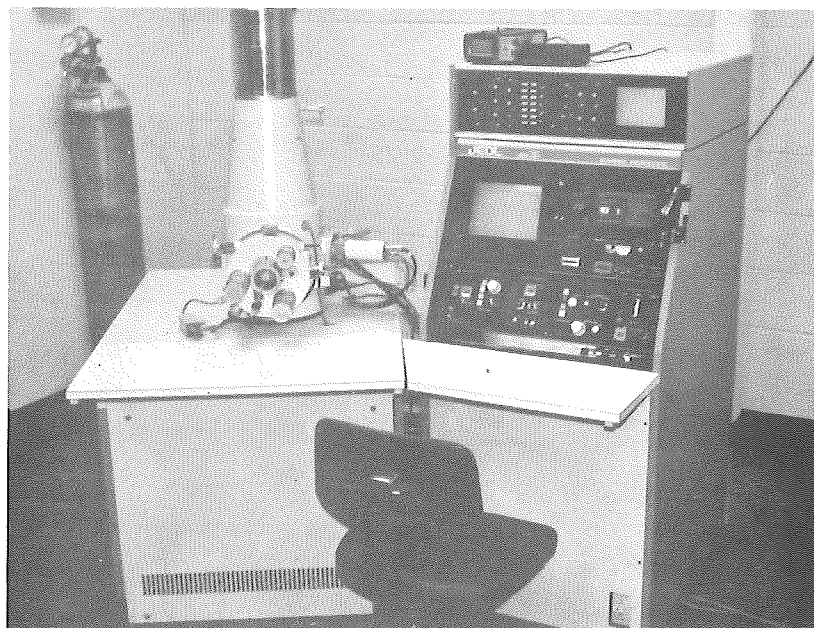


JEM-100C

JEOL's continuing commitment to the design and manufacturing of high voltage, scanning & transmission electron microscopes assures users more value & innovation in each individual instrument.

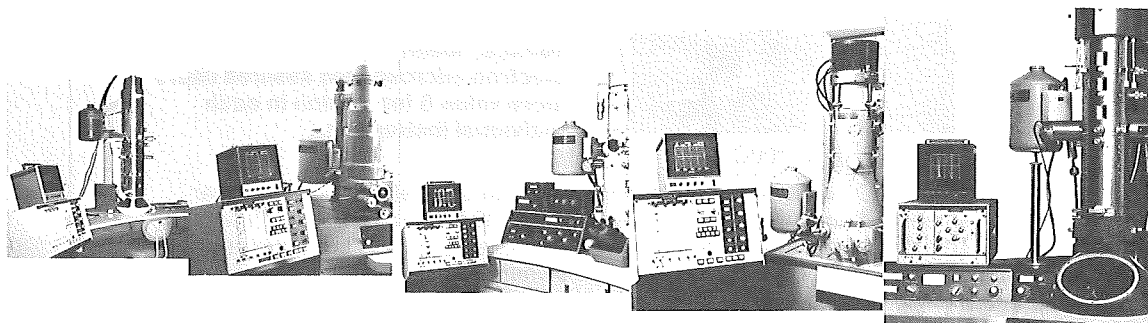
JEM-100C owners appreciate the totally solid-state electronics, the guaranteed 1.4 angstroms in transmission & the 30 angstroms in the STEM mode. The 100C as an analytical instrument, equipped with field emission gun/energy analyser & xray, has no peer. Owners of installed instruments are excited about the JSM-35 self maintaining column, assuring daily the 100A° guarantee, as well as the simultaneous dual image for comparison & astigmatism monitor. 1 – 39 KV provides high resolution 100A° at low 5 KV, as well as sufficient KV for transmission & xray operation. The JEM-100C and the JSM-35 are now being demonstrated in the JEOL Application Laboratory.

Complete information or a demonstration will be arranged by calling 617/391-7240 or writing JEOL Application Laboratory, 477 Riverside Avenue, Medford, Mass. 02155.



JSM-35

WE'RE #1 AND GROWING ONE IMPORTANT CUSTOMER AT A TIME



We're giving xray analysts something they can't get anywhere else — superior energy dispersive systems with backup — and it's paying off — we're growing because we have the widest range and most sophisticated software for qualitative *and* quantitative analysis WITHOUT STANDARDS. Yes, that's what we said — *No standards required for quantitative analysis.*

Write for our Software Catalog and descriptions of our MICRO-EDIT systems which are setting new standards for the industry.

WE'RE #1 BECAUSE WE THINK OUR CUSTOMERS WANT THE BEST.

EDAX
INTERNATIONAL Inc.
P.O. Box 135, Prairie View, Illinois 60069
Phone (312) 634-0600 Telex 72-6407

from: EDAX

the innovators . . . "to get you all the information from your SEM/TEM x-ray analysis"

EDAX/EDIT® systems provide. . .

***Optimized qualitative analysis**

- removal of background and peak overlaps without requiring a library of standards.
- no need to prejudge what elements are present.

***Automatic semiquantitative analysis**

- single pushbutton listing of all statistically significant peaks. True net intensities allow direct comparisons.

***Quantitative analysis with or without standards**

- major breakthroughs in theory handle irregular surfaces normally encountered in SEMs.
- traditional probe standards, or others, or none at all can be used.
- special models developed for thin sections, organics, etc.

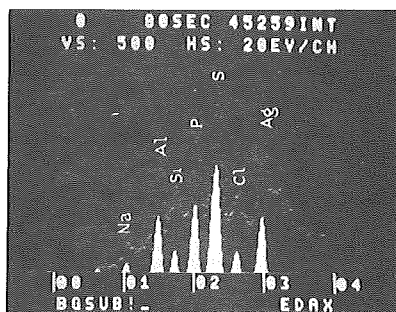
***Computing power for your lab**

- utilize standard high-level languages (Basic, Fortran, Algol, etc.).
- perform general purpose functions while you take x-ray data.
- extensive program libraries.

***Again, a new industry standard from EDAX**

- 4th generation EDAX software shows the continued innovation that has made us the leader since the beginning.
- utilizes microprocessor chips, the latest development in computer technology.

Send for complete information on how EDAX software can help you get the *answers*. . .



Elemental peaks after background subtraction, shown superimposed on original spectrum (kidney tissue)

from EDAX
the innovators

EDAX International, Inc.

P.O. Box 135,
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(312) 634-0600

* ZAF MEM+E NBS SRM #1171		
V=23.5 S=35 T=41		
ELEM	LINE	
FE	K	68.501 %
CR	K	17.312 %
NI	K	11.088 %
MN	K	1.916 %
SI	K	0.443 %
MO	L	0.311 %
CU	K	0.126 %
TI	K	0.092 %

Quantitative analysis of a stainless steel performed without standards and corrected for specimen geometry.