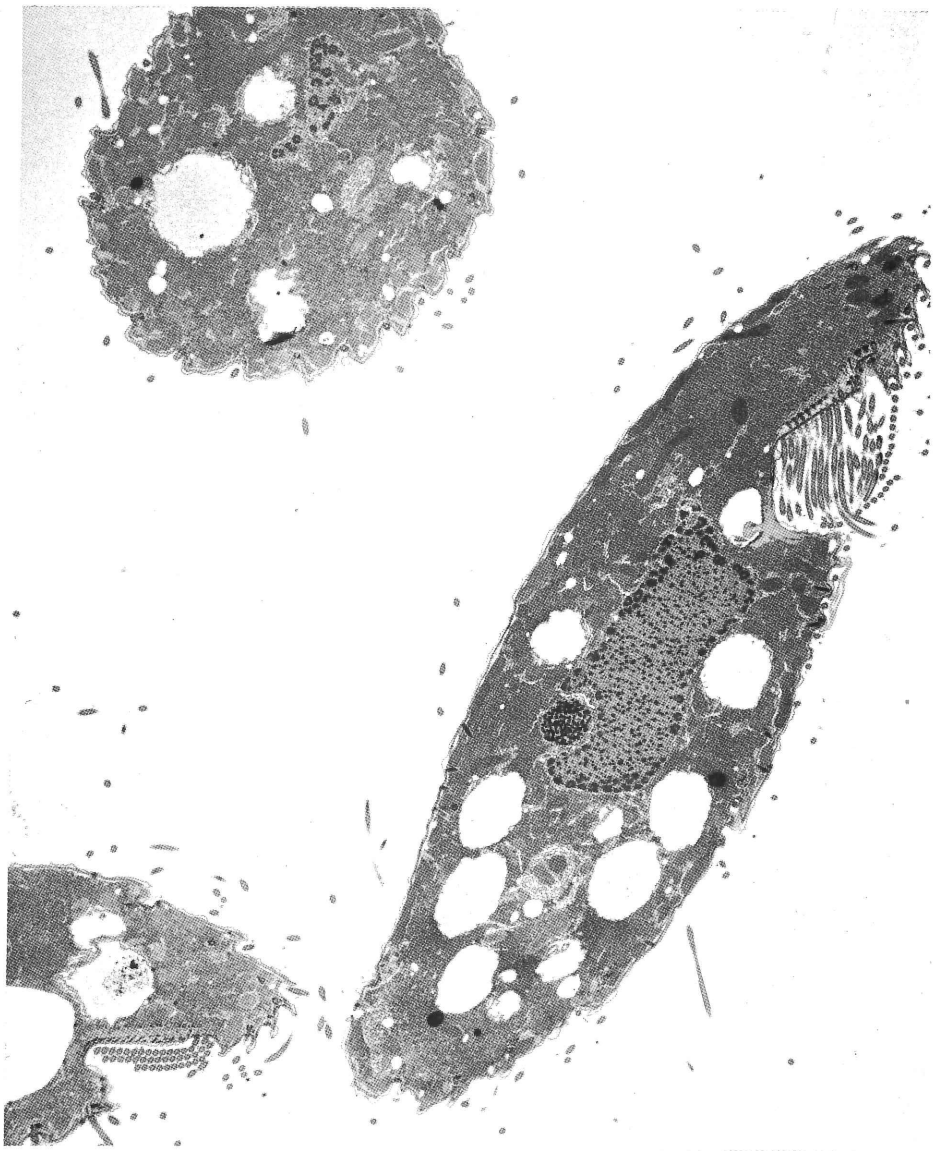
TSEM Texas Society for Electron Microscopy NEWSLETTER



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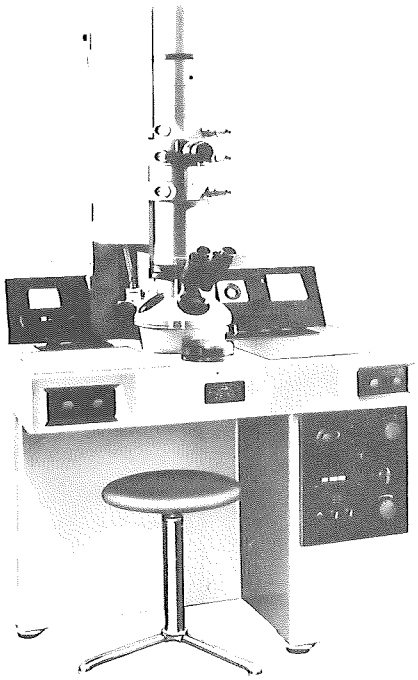

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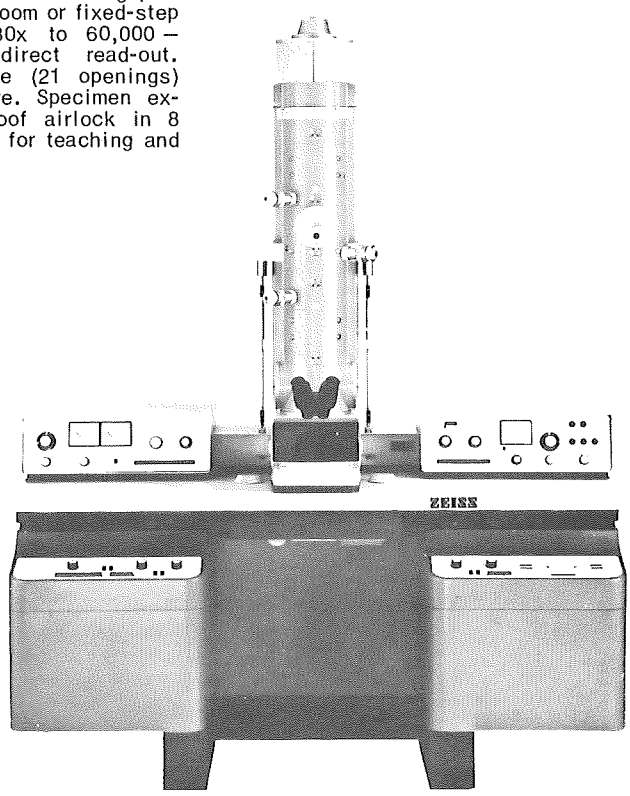

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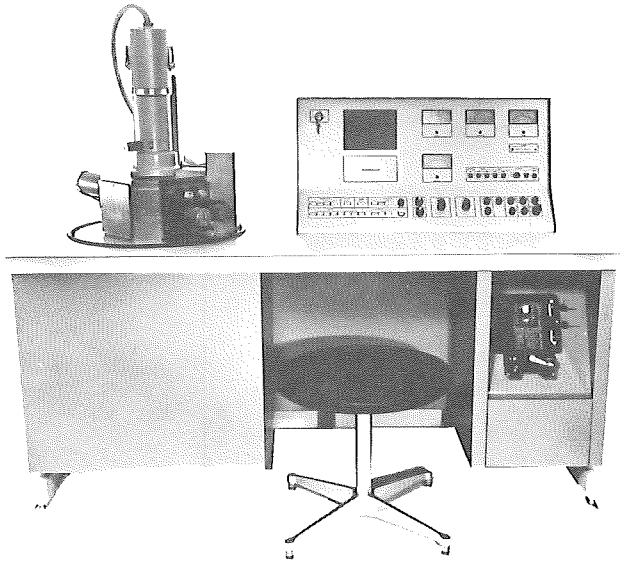

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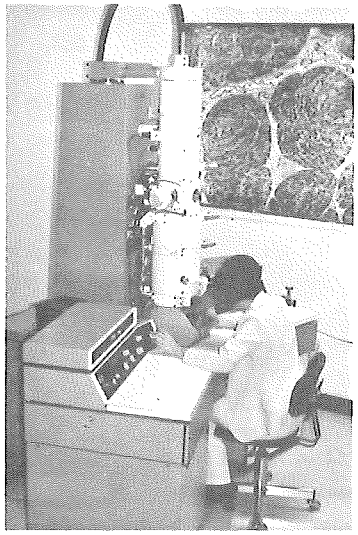

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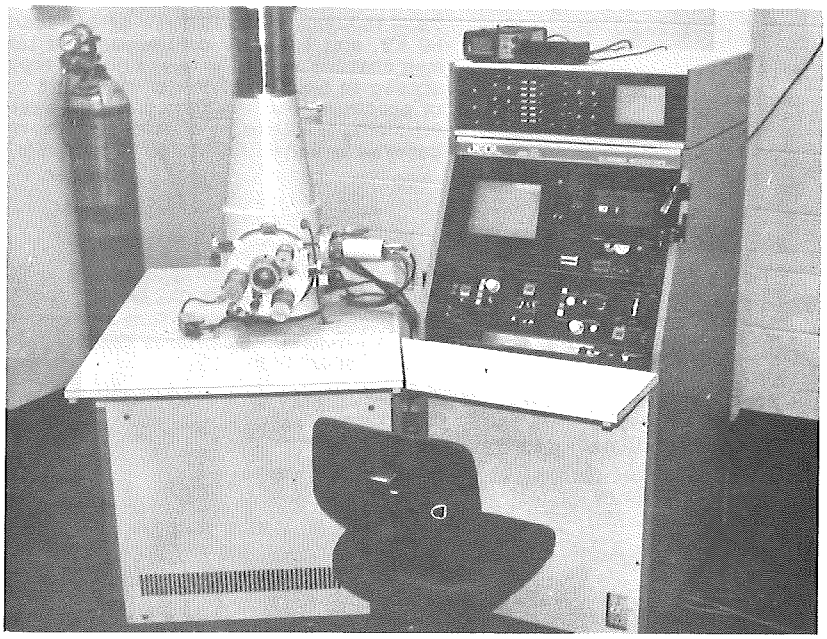

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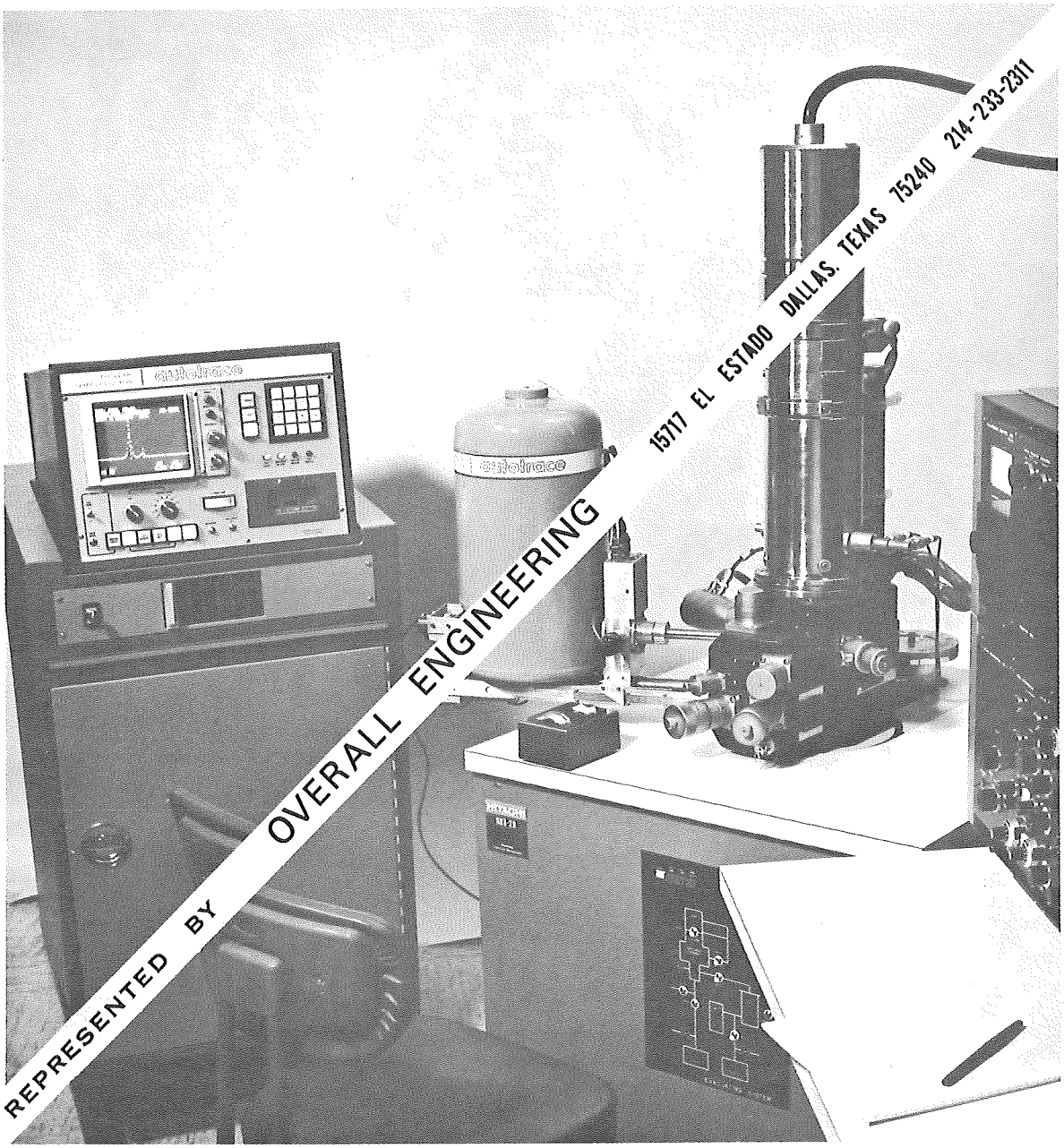

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SPRING 1975

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EDITORIAL

With this issue of the Newsletter I am stepping down as editor. I have been editor for two years. There were two main things I wanted to accomplish as Newsletter editor. First, was a newsletter which was self-supporting. This objective was accomplished by keeping printing costs down by use of our University Print Shop and by sustained advertising from our Corporate Members.

The Society therefore thanks both The University of Texas Health Science Center at San Antonio, as well as the Corporate Members.

My second objective was to establish regional editors around Texas to "beat the drums" for news, new members, articles, contributed micrographs, jobs and to help make editorial policy. This second objective is just starting to work and I wish to recognize and to thank the regional editors listed elsewhere in this issue. The Society thanks you for your service and the new editor, Ron Greuner, is looking forward to your continued support.

Now the entire story behind my editorship can be told. The real editor and worker has been William "Bill" Pavlat. Some of you already realize this and the only thing I can do is to give Bill my thanks. Bill is going to dental school but promises to continue his membership in TSEM (of course at student membership rates). We cannot forget our secretary and friend, Annice Hill, for her two years of previously unrecognized contributions to TSEM.

Well, we in Texas are sometimes known as braggarts, therefore I claim the TSEM has the best Newsletter of any local group around the country.

To all of you let me say that being editor has given me considerable satisfaction and a little heartburn but all in all a happy experience.

Support your TSEM Newsletter for it is, in my opinion, the glue that binds the Society together.

IVAN L. CAMERON

Newsletter Editor

PRESIDENT'S MESSAGE

It has been my pleasure to serve this year as TSEM's tenth president. As you read the special tenth anniversary Newsletter supplement, please note the past presidents and their achievements. They are truly an outstanding group and it is my pleasure to be associated with them.

This past year has been enjoyable but with a few problems as usual. These, I think, have been capably handled by the executive committee. I would like to commend Ward Kischer, Jerry Berlin, Ernie Couch, Ivan Cameron and Bill Pavlat for their work for TSEM this past year. All but Ward are leaving office and I feel that these gentlemen have all done outstanding and extraordinary work for the Society. This hard work has helped to maintain TSEM as a strong regional society structured to serve its members.

As I turn this office over to Ward, I wish him the very best. I know he will continue the quality demanded by the TSEM membership.

Good luck, Ward, and thank you all for your support and help.

TERRY R. HOAGE

President

FINANCIAL REPORT

(Period ending April 7, 1975)

Receipts:

A. Newsletter Advertisements	$ 400.00
B. Dues	
1. Corporate	150.00
2. Regular and Student	69.00
Sub-total	$ 619.00

Disbursements:

A. Newsletter Printing Costs	$ 462.59
B. Newsletter-Miscellaneous Expenses	43.37
Sub-total	$ 505.96

Summary:

A. Total Receipts	$ 619.00
B. Total Disbursements	505.96
Surplus	$ 113.04
Bank balance (checking) as of February 7, 1975	$2,643.33
Bank balance (checking) as of April 7, 1975	2,756.37
Certificate of deposit	1,094.13
GRAND TOTAL	$3,850.50

This is a list of local societies across the nation. Because the officers change, this list is in some cases out of date now or will be in the near future, on the other hand, I'm sure communication with the persons on this list will get anyone in communication with any local society.

We include this list of local societies so as to facilitate intersociety communication.

Name of Society	Corresponding Officer
1) The Electron Microscopy Society of Northwestern Ohio	Dr. J. Chakraborty Department of Physiology Medical College of Ohio P. O. Box 6190 Toledo, Ohio 43614
2) Michigan Electron Microscopy Forum	Dr. Robert Weiss Department of Pathology Wayne State University School of Medicine 540 E. Canfield Ave. Detroit, Michigan 48201
3) The Louisiana Society for Electron Microscopy, Inc.	Joe S. Mascorro Department of Anatomy Tulane University School of Medicine New Orleans, Louisiana 70112
4) Central States Electron Microscopy Society	Ray Faup Department of Plant Pathology 108 Waters Hall University of Missouri Columbia, Missouri 65201
5) Southeastern Electron Microscopy Society	Gene Michaels Department of Microbiology Biological Sciences Building University of Georgia Athens, Georgia 30602
6) New England Society of Electron Microscopy	Elinor M. O'Brien Associate Director Cancer Research Institute of Boston College Chestnut Hill, Massachusetts 02167

Name of Society	Corresponding Officer
7) Washington Society for Electron Microscopy	Marshall L. Rennels Departments of Anatomy and Neurology University of Maryland School of Medicine Baltimore, Maryland 21201
8) Northern California Society for Electron Microscopy	Bob Warner General Electric Company Vallecitos Nuclear Center Pleasonton, California 94566
9) Southern California Society for Electron Microscopy	Barbara G. Bystrom Environmental Neurobiology Lab. Brain Research Institute U. C. L. A., Sclichter Hall Los Angeles, California 90024
10) Midwest Society for Electron Microscopy	Ruth A. Becker Department of Neurological Sciences Rush-Presbyterian St. Luke's Medical Center 1753 W. Congress Pkwy. Chicago, Illinois 60612
11) Minnesota Electron Microscopy Society	Mary P. Ooka Department of Urological Surgery Box 394, Mayo University of Minnesota Minneapolis, Minnesota 55455
12) New Mexico Society of Electron Microscopy	Dr. Scott Jordon Department of Pathology University of New Mexico Albuquerque, New Mexico 87106
13) New York Society of Electron Microscopists	Dr. George Schidlovsky Pfizer, Inc. 199 Maywood Avenue Maywood, New Jersey 07607

Name of Society	Corresponding Officer
14) Ohio State University Microscopy Seminar	Nobushisa Baba Department of Pathology Ohio State University Columbus, Ohio 43210
15) Philadelphia Electron Microscopy Society	Lolly Marchant Fallon Franklin Institute Research Lab. 20th and Parkway Philadelphia, Pennsylvania 15213
16) Electron Microscopy Society of Northeastern Ohio, Inc.	Ralph G. DePalma 2065 Adelbert Road Cleveland, Ohio 44106
17) Mountain States Society	Patricia McMurry Department of Pathology Colorado State University Ft. Collins, Colorado 80521

Cover:

The ciliate Tetrahymena pyriformis in a dog eat dog world.
I. L. Cameron and G. M. Williams, Department of Anatomy,
The University of Texas Health Science Center at San Antonio.

Protocol for Ultrastructural Immunocytochemistry

Damon C. Herbert
Department of Anatomy, The University of Texas
Health Science Center at San Antonio, San Antonio, Texas

Enzyme labelled antibodies have become an effective means by which one can identify specific cell products at their site of origin as well as at the level of the target tissue. With improved techniques which obviate the use of elaborate and very expensive equipment, the method of immunocytochemistry is now available to anyone interested in employing this highly sensitive method in their research. The following is an outline of the method for use at both the light and the electron microscopic levels.

For Paraffin Sections

1. Fix the tissue in formol-sublimate or Bouin's solution for 8-24 hr.
2. Embed in paraffin, section at 4-6μ and hydrate the tissue.
3. Wash twice, 15 min each time, in 0.01 M or 0.05 M phosphate saline buffer (PSB) pH 7.2-7.4. Use a staining dish with slide carrier and stirring bar for adequate washing.
4. Wipe off the excess PSB and place the slide in a petri dish containing moist filter paper. Place a drop of first antibody over the tissue section and incubate for 1 hr at room temperature.
5. Rinse the slides in a Coplin jar containing PSB and wash as in step 3.
6. Wipe off the excess PSB. Place a drop of second antibody (the second antibody is an antiserum prepared against the gamma globulin fraction of serum of the host animal in which the first antibody was prepared) over the tissue section and incubate for 1 hr at room temperature. The second antibody is labelled with horseradish peroxidase and is available in 5 cc lots for $75 from Cappel Laboratory, Downington, Pa.
7. Rinse and wash as in steps 3 and 5.
8. The histochemical reaction is carried out in a staining dish or Coplin jar. Mix 10 mg of diaminobenzidine (DAB) with 20 ml of 0.05 M Tris buffer pH 7.6. Add 20μl of 3% H_2O_2. This is stable for no more than 1 hr so prepare it just before use. React the tissue for a few minutes or until it takes on a brown hue. Rinse the slide in Tris buffer and examine under the microscope. The histochemical reaction is characterized by a brown reaction product which becomes more intense the longer it remains in the DAB-H_2O_2 mixture. If the reaction product is dark enough, proceed to step 9. If not, return to the DAB-H_2O_2 mixture until the reaction product is of desirable intensity. To enhance the intensity of the reaction product, place a drop of 2% OsO_4 over the tissue section for 30 sec and rinse with water. A new procedure for this step was recently published which

may produce a more specific reaction product - Weir et al., J. Histochem. Cytochem. 22: 1135, 1974.

9. Wash in 0.05 M Tris buffer for 15 min. Clear and mount.

10. It is important that both the first and second antibodies be diluted before use for greater specificity and to decrease background staining.

For Electron Microscopy

Thick and Thin Sections

1. Fix the tissue in a paraformaldehyde-picric acid mixture (Nakane, 1971) or in a mixture of lysine, paraformaldehyde, and periodate (McLean and Nakane, 1974) for 8 hr.

2. Embed in araldite or epon. Cut 1μ thick sections and place on glass slides.

3. Warm the slides for 1 hr at 45° C. Etch the tissue with 3% NaOH in 80% alcohol for 5 min and then neutralize the NaOH with 1% acetic acid for 2 min.

4. Follow the procedure for paraffin sections, steps 3-9. Use the adjacent ultrathin serial section stained with uranyl acetate and lead citrate for examination under the electron microscope. Correlate the ultrastructural findings with the observations made on the thick section.

5. This method can also be employed with a specific differential light microscope stain as shown in fig. 1.

Ultrathin Sections

1. Fix and prepare tissue as in steps 1 and 2 - "Thick and Thin Sections."

2. Cut ultrathin sections and mount on coated grids.

3. Etch the tissue by placing the grids face down on a drop of 10% H_2O_2 for 10 min.

4. Wash for 5 min on a drop of PSB.

5. Place the grid on a drop of normal serum (1:10 dilution-source is the same as the host animal used to generate the second antibody) for 5 min.

6. Wash as in step 4.

7. Place the grid on a drop of the first antibody which has been diluted within a range of 1:10 to 1:100 for 1 hr. The antiserum can be used at a dilution greater than 1:100 and the reaction conducted at 4° C for up to 48 hr. Wash as in step 4 and repeat step 5.

8. Place the grid on a drop of peroxidase-labelled antibody which has been diluted within a range of 1:10 to 1:100 for 1 hr. Wash as in step 4.

9. Repeat with DAB-H_2O_2 mixture as described above for 5 min. Keep the mixture moving over the surface of the section and rinse well with water. Stain with 1% OsO_4 for 2 min.

10. As an alternative method, the enzyme antibody bridge technique of Mason et al. (1969) or the unlabelled peroxidase-antiperoxidase method of Sternberger et al. (1970), fig. 2, can be employed.

References

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Sternberger, L. A., Hardy, P. H., Jr., Cuculis, J. J., and Meyer, H. G. (1970) J. Histochem. Cytochem. 18: 315.

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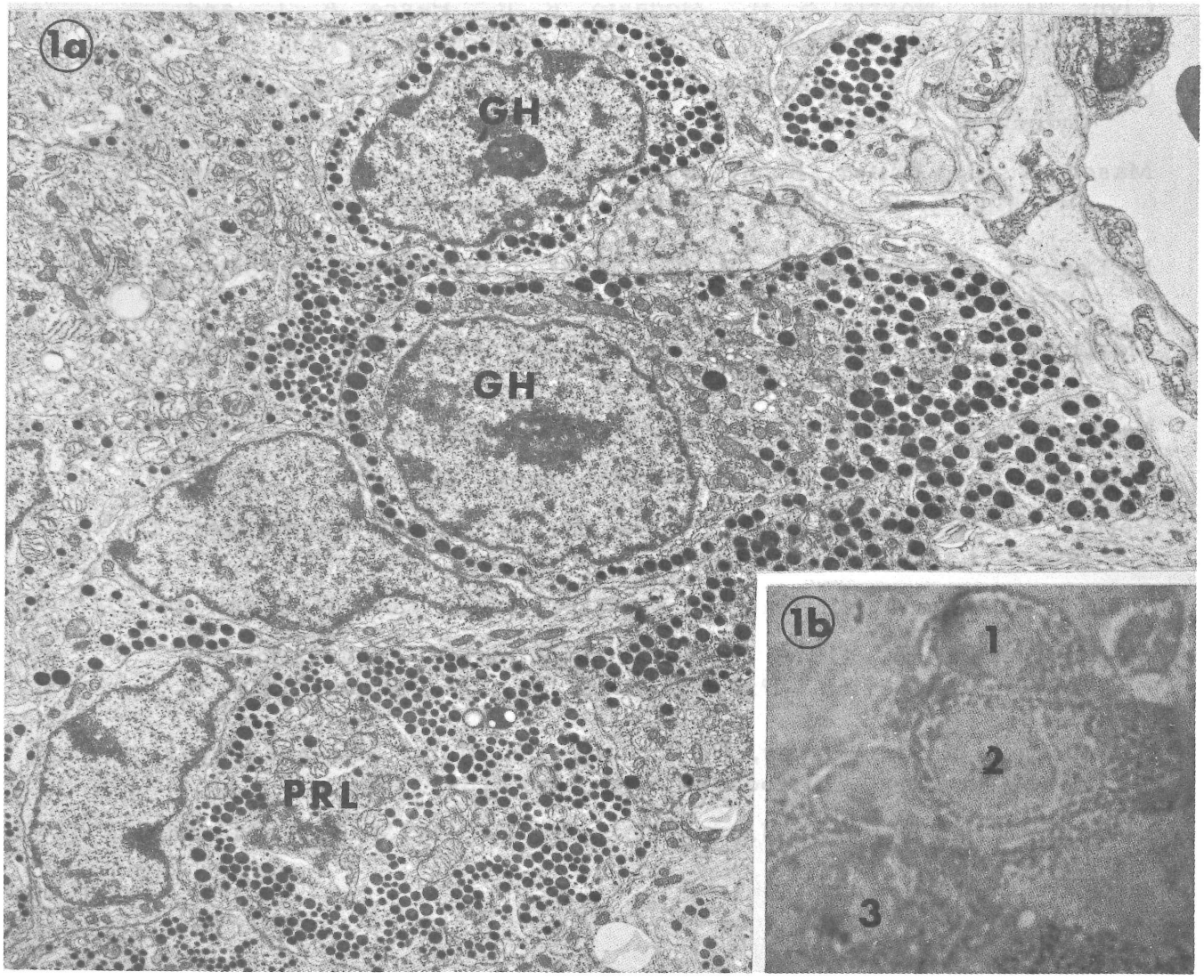
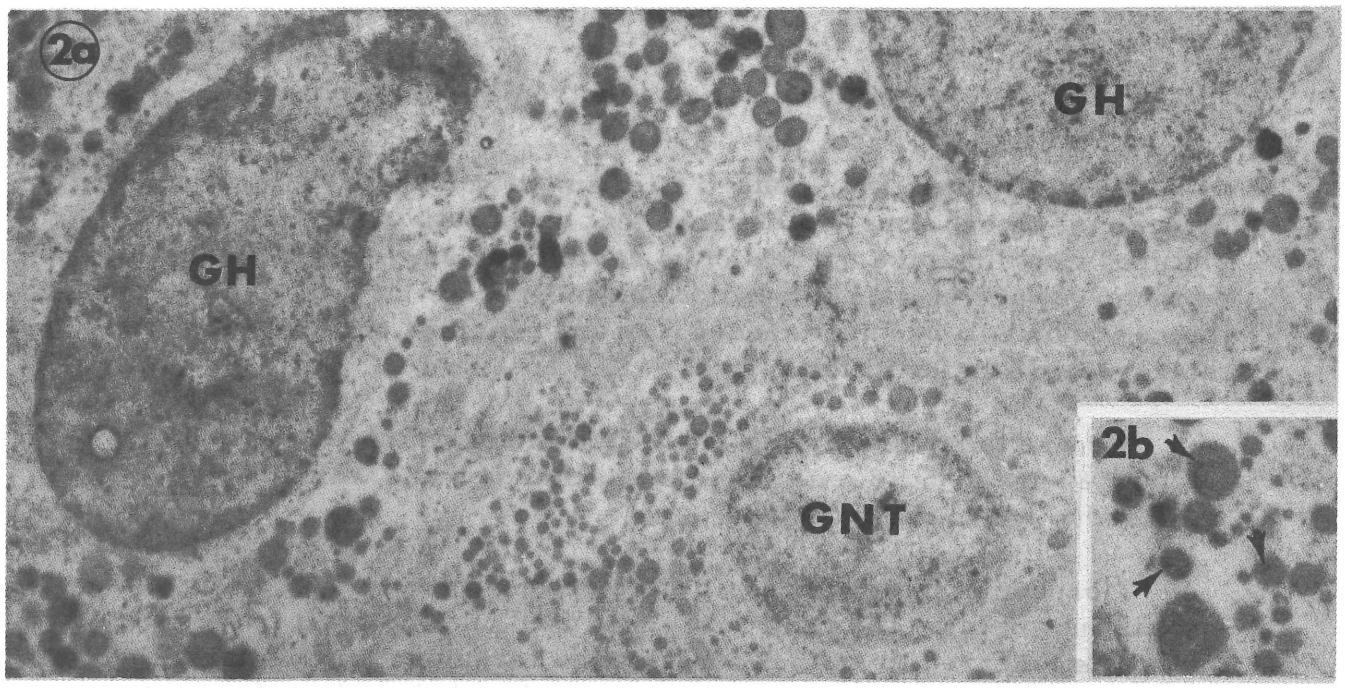
Figure 1. a) Ultrathin section of the rhesus monkey pituitary gland showing two growth hormone (GH) cells and one prolactin (PRL) cell. X7400. b) Adjacent 1μ thick serial section stained with Brookes' trichrome technique. Cells 1 and 2 stained with orange-G and correspond to the two GH cells in fig. 1a while cell 3 stained with carmoisine and corresponds to the PRL. X3000.

Figure 2. a) Rat anterior pituitary gland immunochemically stained with antiserum to GH using the method of Sternberger et al. (1970). Two GH cells are reacting with the antibody while the adjacent gonadotroph (GNT) is unreactive. X12,200. b) Enlargement of the GH granules showing the reaction product, which are small electron dense particles, over the secretory granules (arrows). X16,600.





ANNOUNCEMENT

Spring 1975

A Seminar Series

FIELD EMISSION SCANNING ELECTRON MICROSCOPY

May 13 and 14

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Recommended Hotel: Galveston Holiday Inn, 600 Strand,
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Morphometry of Electron Micrographs

There is much more information in our micrographs than many of us realize. Most of us would agree that quantitative analysis of our pictures would be nice but few of us actually do any numerical analysis on them. It seems safe to predict that morphometric analysis of electron micrographs will become a necessary and required part of electron microscopic studies in the future.

We are therefore listing a few references which we have used to introduce ourselves to morphometric techniques and which can serve as an introduction to the techniques.

Morphometric techniques can be broken into two rather separate and distinct fields. Currently the most used and the least expensive method is referred to as the Stereological technique (particularly the point counting method). The less developed technique is referred to as the Microstereophotogrammetry technique.

Stereological techniques are used to collect data from pictures of electron microscopic sections and then to relate this data to an idealized "average" object, such as the average volume of mitochondria in an average cell, whereas Microstereophotogrammetry concerns accurate determination of X-ray take off angles, surface contours and the surface area or the volume of individual and real objects. Microstereophotogrammetry is desirable because it deals with individual determinations of real objects but requires stereoscopic pairs of pictures which can be taken in electron microscopes with stereoscopic capabilities. The stereological point counting technique does not require stereoscopic pairs of pictures nor does it require any special equipment. Using stereological point counting, which requires only the aid of appropriate grids (which can be easily made) and a ruler, one can characterize structures such as the organelles of a specific type of cell; for example, the volume of mitochondria per volume of cytoplasm, the surface area of endoplasmic reticulum per volume of cytoplasm, the number and average volume of particles such as secretory granules in an average sized cell, the length of linear features such as fibers, the mean caliper diameter and the volume of an object (such as the nucleus) and the average volume and surface area of the cell type being studied.

Annotated references dealing the the Stereological techniques:

Perhaps the best known worker in the field is Ewald R. Weibel at the Universitat Bern in Switzerland and perhaps the best description of the technique is a chapter entitled Stereological Techniques for Electron Microscopic Morphometry, by E. R. Weibel in Volume 3 of Principles and Techniques of Electron Microscope: Biological Applications, edited by

M. A. Hayat, Van Nostrand Reinhold Co., New York (1973) pp. 237-296. A representative example of stereological description of liver cells is given in a paper by A. V. Loud (J. Cell Biol. 37: 27-47, 1968), and a stereological description of parotid gland acinar cells is given in a paper by G. H. Cope and M. A. Williams (J. Cell Biol. 60: 292-297, 1974). Recently H. U. Rätz et al. have developed an on-line computer system for point counting stereology (J. of Microscopy 101: 267-282, 1973), while Boyde et al. have developed a stereological grid method for scanning electron microscopy using a superimposed grid on the cathode ray tube display screen (J. Microscopy 101: 261-266, 1974).

Annotated references dealing with the Microstereophotogrammetry technique.

The most recent papers discussing biostereometrics, and in particular microstereophotogrammetry, are published in the Proceedings of the Symposium of Commission V International Society for Photogrammetry entitled "Biostereometrics '74'", edited by R. E. Herron and H. M. Karara, American Society of Photogrammetry, 105 North Virginia Avenue, Falls Church, Virginia 22046 (1974). Among the active workers in the field of microstereophotogrammetry are A. Boyde at University College London, and S. K. Ghosh at Ohio State University. Both authors have contributed to the above proceedings on pages 483 and 495 respectively.

The following basic papers deal with microstereophotogrammetry in general:

Boyde, A. Practical problems and methods in the three dimensional analysis of scanning electron microscope images. In: Scanning Electron Microscope (ed. by O. Jokari), pp. 105-122, IITRI, Chicago (1970).

Boyde, A. Quantitative Photogrammetric Analysis and Qualitative Stereoscopic Analysis of SEM Images. J. of Microscopy 98: 452 (1973).

Boyde, A. Photogrammetry of Stereo Pair SEM Images Using Separate Measurements from the Two Images. In: Scanning Electron Microscopy (ed. by O. Jokari), pp. 101-108, IITRI, Chicago (1974).

Lane, G. S. The application of stereographic techniques to the scanning electron microscope. J. of Scientific Instr. 2: 265 (1969). The latter report is concerned with the basic theory of the technique.

A report by A. Hepworth and J. Sikorski deals with a theoretical study of a model (Stereoscopy of cylindrical objects in the scanning electron microscope. J. of Microscopy 98: 436, 1973).

Two other reports, one by Unsworth and Hepworth (A new stereo-adaptor for use with the scanning electron microscope. J. of Microscopy 94: 245, 1971) and the other by Hudson and Makin, (The optimum tilt angle for electron stereo-microscopy. J. of Physics Sci. Instr. 3: 311, 1970), give a more practical approach to stereomeasurements.

If one desires a general introduction to the elements of photogrammetry and to stereoscopic principles in particular, we recommend "Elements of Photogrammetry" by W. H. Baker, The Ronald Press Co., New York (1960).

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AREA NEWS

Fort Worth

TEXAS CHRISTIAN UNIVERSITY

Department of Biology

Recent visiting lectures were given by W. Brinkley, University of Texas Medical Branch, Galveston; H. Mollenhauer, Texas A & M; J. G. Wood, University of Texas Health Science Center at Houston.

Houston

BAYLOR COLLEGE OF MEDICINE

Department of Anatomy

New Members:

Mr. Ronald W. Scates has joined the Department of Neurology and the Baylor-Methodist Center for Cerebrovascular Research as a member of Dr. Ronald Dodson's lab. Mr. Scates came to Baylor in October from Dr. R. V. Blystone's lab at Trinity University.

Mrs. Laura L. Burgess has also recently joined Dr. Dodson's lab and will coordinate the ultrastructural research program in neuromuscular disease in conjunction with Dr. Bernard Patten's group.

Lectures:

Mr. Ronald W. Scates presented a paper at the Zoological Session of the Texas Academy of Science entitled "The Morphology of the Ependymal Complex in the Lateral Ventricle of the Squirrel Monkey". The paper was co-authored by Dr. Ronald Dodson and Mrs. Lena Wwi-Fong Chu. Mr. Scates also presented another paper the following day entitled "The Fine Structure of Tarebia granifera Spermatogenesis" at the same meeting.

Recent Publications:

Ott, Erwin O., J. Abraham, J. S. Meyer, C. A. F. Tulleken, N. T. Mathew, A. N. Achari, M. Aoyagi, and R. F. Dodson: Regional Cerebral Blood Flow Measured by the Gamma Camera after Direct Injection of $Xenon^{133}$ into the Distal Stump of the Occluded Middle Cerebral Artery. Stroke, 1975.

Dodson, R. F., Y. Tagashira, and L. W. F. Chu: The Effects of Glycerol on Cerebral Ultrastructure Following Experimentally Induced Cerebral Ischemia. Journal of Neurological Science (in press).

Department of Microbiology

Grant:

Dr. H. D. Mayor from the National Cancer Institute, "Growth and Maturation of Adeno-Associated Satellite Viruses".

Visitors:

Dr. Ilse Fischer, Robert Koch Institute, Berlin, Germany visited Liane Jordan, TSEM member.

THE UNIVERSITY OF TEXAS HEALTH SCIENCE CENTER AT HOUSTON

Department of Neurobiology

Grants:

Dr. Dianna Redburn received a general research support grant from The University of Texas Medical School.

Recent Talks:

Recent lecturers to the Neuroscience Society included Dr. J. A. C. Nicol and Dr. A. C. Coats. Other speakers to the department were Dr. J. A. Besso on "Structure and Function of the Escape System of Myxicola infundibulum", Dr. J. M. Schaeffer on "Solubilization and Characterization of the GABA Receptor", and Dr. Harry Grundfest from Colombia University spoke on the "Natural History of Neurons".

Papers:

Dr. R. G. Peterson recently had a paper published in the Journal of Neurocytology entitled "Electron microscopy of Trypsin digested peripheral nerve myelin."

New Equipment:

Perkins-Elmer Infra Red Spectrophotometer

New Staff:

Miss Helena Stuler recently joined the technical staff in Dr. J. G. Wood's lab.

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

Department of Virology

Seminars:

Dr. Elwin E. Fraley, Professor and Chairman, Department of Virologic Surgery, University of Minnesota Medical School, Minneapolis, Minn., visited the Department of Virology on January 7-8 and presented a seminar entitled, "Studies on the Etiology of Human Urogenital Cancer."

Dr. R. C. Nairn, Professor and Chairman, Department of Pathology and Immunology, Alfred Hospital, Monash University Medical School, Prahan, Victoria, Australia, visited the Department of Virology on January 11-19, 1975, and presented a seminar entitled, "Recent Developments in Immunofluorescence."

Dr. David Baltimore, Professor of Biology at the Massachusetts Institute of Technology, presented a seminar entitled, "The Growth of Leukemia Viruses" on February 12, 1975.

Dr. Jeffrey Schlom, Chairman, Breast Cancer Virus Segment, NCI, presented a seminar entitled, "Biochemical Evidence for a Viral Involvement in Breast Cancer" on February 13, 1975.

Papers Accepted for Publication from EM Laboratory:

A chapter entitled, "Ultrastructural Characteristics of Human Tumor Cells in Vitro," by G. Seman and L. Dmochowski, will appear in Human Tumor Cells in Vitro in April 1975, to be published by Plenum Publishing Corporation.

"Virologic and Immunologic Studies of Human Prostatic Carcinoma," by L. Dmochowski, K. Maruyama, Y. Ohtsuki, G. Seman, J. M. Bowen, W. A. Newton, and D. E. Johnson, will appear in Cancer Chemotherapy Reports, January/February 1975.

Other:

An abstract entitled, "Lectin Binding Studies on RNA Tumor Virus-Infected Cells," by D. C. Hixson, was submitted to The Electron Microscopy Society of American for presentation at the Annual Meeting in

August 1975. This abstract has been selected as a winner in the competition for EMSA Presidential Scholarships and Mr. Hixson will receive an award with a cash value equivalent to the round trip airfare to the meeting.

Lubbock

TEXAS TECH UNIVERSITY

Department of Biological Sciences

Seminars given:

Principles and Techniques of Critical Point Drying, Workshop presented to EM people on campus and Medical School by F. Bailey and J. Berlin.

Publications:

Pizzolato, T., and C. Heimsch 1975 Ontogeny of the Protophloem fibers and Secondary Xylen Fibers within the Stem of Coleus. I. A light Microscopic Study. Canadian Journal of Botany (in press).

Pizzolato, T., and C. Heimsch 1975 Ontogeny of the Protophloem Fibers and Secondary Xylem Fibers within the Stem of Coleus. II. An Electron Microscopic Study. Canadian Journal of Botany (in press).

Burbano, J., T. Pizzolato, P. Morey, and J. Berlin 1975 An application of the Prussian Blue Technique to a light microscope study of transpiration in leaves of cotton (Gossypium hirsutum L.). Submitted Journal of Experimental Botany.

Pizzolato, T., J. Berlin, J. Burbano, P. Morey, R. Pease 1975 An Electron Microscope Study of the Path of Water in Transpiring Leaves of Cotton (Gossypium hirsutum L.). Submitted Journal of Experimental Botany.

San Antonio

THE UNIVERSITY OF TEXAS HEALTH SCIENCE CENTER AT SAN ANTONIO

Department of Anatomy

Faculty, researchers, and graduate students who presented papers at the American Association of Anatomists meeting in Los Angeles included Drs. Erle Adrian, Damon Herbert, Hou-Chi Dung, Ivan L. Cameron, Masataka Shiino, N. Hagino, Alexis Burton and R. J. Reiter. Also Leonard Seelig, Jr., John Carter, Kevin Rudeen, David Blask, Mary K. Vaughan and Linda Johnson.

Recent Publications:

Shiino, M., J. B. Warchol and E. G. Rennels 1974 Microtubules in prolactin cells of the rat anterior pituitary gland. Proc. Soc. Exp. Biol. Med. 147: 361-366.

Shiino, M., A. Arimura, and E. G. Rennels 1974 Effects of blinding, olfactory bulbectomy, and pinealectomy on prolactin and growth hormone cells of the rat, with special reference to ultrastructure. Am. J. of Anat. 139: 191-208.

Shiino, M., and E. G. Rennels 1974 Ultrastructural observations on pituitary gonadotrophs following gonadectomy or administration of LH/FSH-releasing hormone in neonatal rats. Tex. Rep. Biol. Med. 32: 561-567.

Shiino, M., and E. G. Rennels 1975 Effects of TRH on pituitary grafts in the rat. Electron microscopic concepts of secretion ultrastructure of endocrinal and reproductive organs (ed. M. Hess). Published by John Wiley & Sons, Inc.

PLACEMENT SERVICE

Position Wanted:

Biological Electron Microscopist
B.S., Biology, 1961, Oglethorpe University; M.S., Biology, 1966, Tulane University; Ph.D., Biology, 1969, Tulane University

Broad experience with TEM--Research and technical
Pre-doctoral Cell Biology Trainee
Post-doctoral Fellow in Cardiology - 2 years - electron microscopic research in cardiovascular diseases.
Research experience-6 years, 33 publications.
Technical experience-6 years as Technical Research Specialist in Electron Microscopy Laboratory, Biology Department, Tulane University

Present Appointment: Assistant Professor of Medicine, Department of Medicine, Tulane Medical School. Electron Microscopist-Cardiovascular Section.

Desire a position with teaching, teaching/research, or research in EM, Cell Biology, General Biology, Zoology. I would like to expand experience into SEM, STEM and continue TEM. Would be interested in directing present EM facility and assist in the development or establishment of a course in electron microscopy. Industrial inquires invited.

Geographical location: open, preference Houston, Texas.
Available: July 1, 1975, or before.
Salary: negotiable, but should take into account experience.

Contact: Joseph M. Harb, Ph.D.
Feazel Laboratory
Tulane Medical Center
1430 Tulane Avenue
New Orleans, La. 70112
Telephone (504) 588-5185

4621 South Johnson St.
New Orleans, La. 70125
Telephone (504) 821-0404

Positions Available:

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) Postdoctoral training, molecular and physiological aspects of cardiovascular research. Program encompasses purification and characterization of Na^+, K^+-ATPase, contractile proteins and sarcoplasmic reticulum. Other areas of interest include RNA and DNA metabolism of the developing heart, smooth muscle metabolism, mitochondrial transport systems and metabolism, electron microscopy and laser beam diffraction analysis. The position is available immediately, salary is negotiable, depending on experience. Dr. Jeanie M. Wood, Associate Director for Postdoctoral Training, Department of Cell Biophysics, Baylor College of Medicine, Houston, Texas 77025. An equal opportunity affirmative action employer.

4) EM Technician - B. S. or M. S. in Biological Sciences. Expertise in ultramicrotomy, darkroom procedures, tissue preparation, operation of a high resolution transmission microscope and freeze-etch. Skill in teaching and supervision is important. An equal opportunity/ affirmative action employer. Send three letters of recommendation and vita to: Dr. Joanne T. Ellzey, Biological Sciences, University of Texas at El Paso, El Paso, Texas 79968.

Abstracts of Papers

and

Scientific Exhibits Presented

at the

Spring 1975 Meeting of the

TEXAS SOCIETY OF ELECTRON MICROSCOPY

Hyatt Regency Hotel
Houston, Texas

May 2 - 3, 1975

Pathology of Acute Canine Myocardial Infarction and its Relationship to the Scintigraphic Detection of Myocardial Infarcts with Technetium-99m Stannous Pyrophosphate (^{99m}Tc-PYP)

L. Maximilian Buja, M.D., Jane H. Dees, B.A. and Anna Reynolds, H.T. (ASCP). (Sponsor: Rolland C. Reynolds, M.D.). Department of Pathology, Southwestern Medical School, University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

Histologic, histochemical, ultrastructural and energy dispersive x-ray spectrographic (EDS) studies were performed on 22 dogs with myocardial infarcts produced by proximal occlusion of the left anterior descending coronary artery for 1 to 13 days. The infarcts were identified premortem by a recently developed diagnostic technique, the ^{99m}Tc-PYP myocardial scintigram. Positive scintigrams showed doughnut patterns with zones of marked radioactivity surrounding central zones of decreased uptake. At necropsy, the infarcts exhibited subendocardially located central zones and surrounding peripheral zones, both of which showed distinctive morphologic features, including the selective occurrence in the peripheral zones of many muscle cells with histochemical and ultrastructural evidence of mitochondrial calcification. Presence of calcium and phosphorous in the mitochondrial densities was confirmed by EDS in 2 dogs. This study establishes a relationship between calcium accumulation in myocardial infarcts and the scintigraphic detection of the lesions with ^{99m}Tc-PYP.

ULTRASTRUCTURE OF NORMAL, ISCHEMIC AND ANOXIC MYOCARDIUM.

Margaret A. Goldstein, David L. Murphy and Arnold Schwartz. Department of Cell Biophysics, Baylor College of Medicine, Houston, Texas 77025.

A comparison of canine papillary muscles is presented. In the model for ischemia, the left posterior papillary muscle is examined at various times after ligation of the left circumflex artery. In the model for anoxia, the left posterior papillary muscle is examined 30 minutes after perfusion of the left circumflex artery with blood equilibrated with 95% N_2-5%CO_2. In both models the left anterior papillary muscle is the control. The control muscle is indistinguishable from normal muscle.

Anoxic muscle cells were swollen; mitochondria were swollen and the nucleus showed clearing of the nucleoplasm; the sarcoplasmic reticulum and T system were of normal size. Similar changes were seen in acute ischemia (30 minutes after ligation). In anoxic muscle, the myofibrils appeared normal except for some increased density in the I band. In "chronic ischemia" at 8 days after ligation, some cells in the marginal zone were severely damaged. Most of the cells, however, showed less severe changes such as widening of the Z band and appearance of an N line. In general, the cellular changes observed in acute ischemia are much more variable than those seen in the same region in acute anoxia and the damage appears to be greater in acute ischemia.

ULTRASTRUCTURAL ASPECTS OF CULTURED CHINESE HAMSTER CELLS TREATED WITH RESPIRATORY INHIBITORS. S. S. Barham and B. R. Brinkley, University of Texas Medical Branch, Galveston, Texas 77550.

Inhibitors of mitochondrial respiration, phosphorylation inhibitors, and uncoupling agents have been reported to delay or inhibit mitosis in cultured mammalian cells. Although the molecular mechanism by which mitosis is delayed in the presence of most respiratory inhibitors presumably involves lowered ATP production for mitotic requirements, one respiratory inhibitor, rotenone, was found to arrest mitosis by an unrelated mechanism. Scanning electron microscopy revealed that cells were blocked in an early stage of mitosis. No anaphase, telophase, or early G_1 phase cells were observed in cell populations treated for 3 hrs with 1.2×10^{-5}M rotenone. Transmission electron microscopy revealed that rotenone prevented the formation of complete mitotic spindles and blocked cells in a mitotic configuration similar to that of colcemid arrested cells. Sodium amobarbital, which inhibits electron transport at the same site as does rotenone, failed to block cell progression through mitosis. Rotenone was found to inhibit ^{3}H-colchicine binding to the mitotic spindle protein and prevented in vitro assembly of a purified tubulin extract. These data suggest that rotenone arrests cells in mitosis in a manner like colcemid and related mitotic spindle assembly inhibitors.

THE EFFECT OF INTRAPERITONEAL COLCHICINE INJECTIONS ON SCIATIC NERVE IN MICE. Margaret E. Bell; Dept. of Neurostructure and Function, The Univ. of Texas Health Science Center at Houston, Houston, Texas 77025

Previous studies have shown that topical application of colchicine to nerves causes changes in neurotubular ultrastructure. It is currently believed that colchicine inhibits axonal transport by binding to the neurotubule subunit, tubulin. The present experiment was designed to investigate the possibility of showing that ultrastructural changes in neurotubules can be obtained with intraperitoneal injections. Three groups of mice (Sprague-Dawley) were given intraperitoneal injections of 0.05mg to 5mg of colchicine per animal. Animals were sacrificed 6-8 hours or 24 hours after injection. Tissue for electron microscopy was fixed with buffered aldehydes, post-fixed in OsO_4 and embedded with epon. Upon examination one sees a dose-related decrease in the number of neurotubules in myelinated fibers. Also, the mice treated with the 0.25mg dosage retained normal breathing patterns, normal gait and posture and good reflexes, while most of the animals receiving 5mg died within the day.

THE USE OF PIPES BUFFER IN THE FIXATION OF MAMMALIAN AND MARINE TISSUES FOR ELECTRON MICROSCOPY. Paul S. Baur, Jr., Ph.D., University of Texas Medical Branch, Galveston, Texas 77550.

Various buffers have been suggested for the preparation of tissues for E.M. study. Most of these are inorganic buffers and contain elements of physiological importance. Therefore, cellular details may be altered by these buffer constituents if they enter the cell prior to the actual chemical fixation. Likewise, fixative-tissue interactions may be affected by the inorganic cations contained in these buffers. The use of the organic based Pipes buffer (Piperazine-N-N'-bis (2-ethanol-sulfonic acid) has been successfully employed in our laboratory with a variety of different tissues. These include: neurological tissues from the marine mollusc Aplysia, human skin, and various species of mammalian tissue culture cells. Ultrastructural details, as revealed by transmission electron microscopy, and topological features, as observed by means of scanning electron microscopy, were deemed to be of excellent quality. Additionally, microchemical determinations, by means of energy dispersive X-ray analysis, of fixed and embedded or fixed and dried samples was readily achievable due to the fact that no extraneous inorganic cation or anion, save for Sodium, is employed during the preparative procedure.

BASIC STUDIES OF THE MECHANISM OF INTERACTION OF MICROORGANISMS WITH MINERALS.

V.K. BERRY, Department of Metallurgical and Materials Engineering, New Mexico Institute of Mining and Technology, Socorro, New Mexico 87801.

Considerable effort has been directed towards an understanding of the unique properties of microorganisms and their reaction on minerals with hydrometallurgical processes. Though some workers have reported the mode and character of attachment on different surfaces, the attachment location of these microorganisms as it relates to minerals has not been reported.

In the current investigation an attempt has been made to describe the interaction of a chemoautrophic, thermophillic and acidophillic microorganism caldariella with mineral molybdenite. The attachment appears to be adsorptive. There appears to be necessarily no correlation of bacterial attachment to dislocation density in molybdenite in which dislocations run parallel to the surface and do not end on the surface. However, microbes do seem to sit on dislocation lines and are also selective in sitting on the voids on the molybdenite surface.

STEREO ELECTRON MICROSCOPY OF ISOLATED MITOTIC CHROMOSOMES. Arthur Cole and Sandra Robinson, Physics Department, The University of Texas System Cancer Center, Houston, Texas 77025.

Chromosome structure is being studied using a number of techniques including stereo electron microscopy, sucrose gradient ultracentrifugation, and short penetration particle beam cell irradiation. The electron microscopic studies utilize a high resolution specimen tilt holder, a special centrifuge cell providing for selective sedimentation directly onto microscope grids, and a number of treatment techniques designed to extend and chemically dissect mitotic chromosomes. Such extended structures exhibit a quasi parallel array of some 64 duplex DNA molecules. Intermediate levels of chromosomal condensation, similar to the "ν bodies" of interphase chromatin, are being investigated.

Supported in part by ERDA Contract AT-(40-1)-2832.

THE ACCUMULATION OF LIPOFUSCIN IN THE MIDGUT EPITHELIUM OF THE AGING HOUSEFLY, *Musca domestica*. Duncan, S. L., R. S. Sohal and V. F. Allison. Department of Biology, Southern Methodist University, Dallas, Texas 75275.

The somatic tissues of adult dipteran insects are composed entirely of post-mitotic cells, making them especially suited for the study of age-related structural alterations. The present study is concerned with the electron microscopic study of the midgut epithelium of aging houseflies, giving special attention to the formation of lipofuscin and the localization of acid phosphatase.

Epithelial cells of the midgut of young animals show few dense bodies associated with the Golgi regions. The epithelium of the 8 day old flies contain increased accumulation of lipofuscin in the perinuclear region. In tissues from animals 13 days of age, opaque lead precipitates widely scattered among the components of the lipofuscin complexes, indicate acid phosphatase activity. Certain dense bodies observed in the epithelial cells from 18 day old flies appear to have undergone further degradation and may represent residual bodies.

The present study has shown an age-related accumulation of lipofuscin in the midgut of the housefly similar to the accumulation of autophagic vacuoles reported in *Calliphora* (Priester, Z. Zellforsch. 129, 430, 1972). Presently, studies are underway to further define the genesis and fate of these lipofuscin bodies.

OPTICAL DIFFRACTION ANALYSIS OF THE Z LATTICE IN STRIATED MUSCLE. Margaret A. Goldstein, John P. Schroeter* and Ronald L. Sass. Dept. of Cell Biophysics and Medicine, Baylor College of Medicine and Dept. of Chemistry*, Rice University, Houston, Tx.

As a first step in understanding three dimensional aspects of the Z band we have constructed a model lattice based on diffraction data from cross sections. We are testing the assumption that regardless of the thickness of the Z band at rest length as observed in longitudinal section, the lattice unit cells as viewed in cross section will be simply related. The data reveal two types of cross sectional lattices.

Type 1 is an uncentered lattice found at the edge of the Z band which exhibits the same ordering as adjacent thin filaments. Type 2 is a centered lattice found in the interior of the Z band. Both centered and uncentered lattices have been indicated by optical diffraction patterns of very wide, "anomalous" cardiac Z bands, normal cardiac Z bands (900-1200Å) and normal Z bands in skeletal muscle (500-900Åthick). The data so far strongly suggest that the Z band is not rigid even at rest length. Optical diffraction analysis of Z bands provides more precise measurement of lattice dimensions and gives a method for studying short range variations within a single Z band.

SURFACE ULTRASTRUCTURE AND CYTOCHEMISTRY OF ISOLATED NUCLEOLI FROM NOVIKOFF HEPATOMA ASCITES CELLS. Y. Daskal, A. W. Prestayko, J. B. Hughes and H. Busch, Dept. Pharm., Baylor Coll. Med., Houston, Texas 77025.

The surface ultrastructure and cytochemistry of isolated Novikoff hepatoma cell nucleoli was examined using scanning electron microscopy (SEM). Nuclear preparations were examined at 15 second intervals during the sonication procedure for isolation of nucleoli. In the initial stages of nuclear disruption the nucleoli were attached to large chromatin masses. A compact nucleoprotein nucleolar stalk relatively resistant to shear was observed in association with many nucleoli. Further sonication disrupted these structures and left tightly coiled, helical filaments still attached to the purified nucleoli. These filaments were removed by DNase digestion but were resistant to RNase digestion. The present study provides a new perspective of nucleolar ultrastructure, its surface organization, and its relationships to other nuclear components.

OBSERVATIONS ON THE EFFECT OF ANDROGEN ON SEMINAL VESICLE EPITHELIUM IN AGING RATS. Hein, C.E., G.C. Fink and V.F. Allison. Department of Biology, Southern Methodist University, Dallas, Texas 75275.

Although the influence of androgen on prenatal, postnatal and adult seminal vesicle epithelial cytology is well known (Dean and Wurzelmann, Am. J. Anat. 117:91, 1965) the effect of androgen depletion and replacement on these target cells in aging animals is relatively uninvestigated.

The epithelial layer remains intact in aging rats castrated and studied four months later; however, epithelial cell atrophy, enhancement of intercellular space, accumulation of pigment and bizarre mitochondria were among cellular changes observed. After androgen replacement, the pseudostratified epithelium of the aging castrated rat is essentially restored except that membranous whorls are numerous, large pigment accumulations are occasionally observed and cell height and quantity of secretion are reduced. Also, macrophages are seen, apparently removing cellular debris.

The influence of androgen depletion and replacement on the seminal vesicle epithelium of aging rats is compared to results from similar studies on young adult rats.

ULTRASTRUCTURAL CHANGES IN THE CHOROID PLEXUS OF DOGS INFUSED WITH BACTERIAL ENDOTOXINS

J. Richard Hillman, R. B. Brackeen and L. S. Holloway, Departments of Anatomy and Physiology, Texas Tech University School of Medicine, Lubbock, Tx.

Artificial cerebrospinal fluid containing 0.1 mg of bacterial endotoxin per ml of artificial cerebrospinal fluid was infused into the cerebral ventricles of adult dogs for 8 hrs after an initial two hour infusion of artificial cerebrospinal fluid. Fixative was then infused into the ventricular spaces and samples of choroid plexus and ventricular wall were taken for scanning and transmission electron microscopy. Ultrastructural examination indicated large numbers of polymorphonuclear leukocytes (PMNs) migrating from vascular channels through the epithelial layer of the choroid plexus into the cerebrospinal fluid space. The polymorphonuclear leukocytes passed between endothelial cells and in many areas the diaphragramned fenestrations characteristic of endothelial cells in the capillaries of the choroid plexus were obliterated. The pericapillary space lying between the vascular channels and the epithelial cells was enlarged and fluid filled. In some areas masses of fibrin-like material were found. The basal lamina of the choroid plexus epithelium was intact except in areas where large numbers of PMNs were migrating through the epithelium. In these areas the epithelium was not present and in its former location was a mass of leukocytes. On scanning E.M. areas of smooth oval cells were seen interspersed between the apices of cells with microvillous borders. These studies indicate that massive migration of PMNs from the vascular channels occurs when bacterial endotoxin is infused into the cerebrospinal fluid space of the dog. Identification of the receptor mechanism by which this occurs awaits further study.

SELECTIVE ULTRASTRUCTURAL LOCALIZATION OF SEROTONIN STORAGE ORGANELLES IN RAT PINEALOCYTES. Lance Kirkegaard; Dept. of Neurostructure and Function, The Univ. of Texas Health Science Center at Houston, Houston, Texas 77025.

The visual localization of biogenic amines provides a major avenue for elucidating nervous and endocrine functioning. The histochemical approach described here combines both pharmacological and fixative manipulations prior to electron microscopic examination of rat pineal gland preparations. 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 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-induced selective blockage of the glutaraldehyde-catecholamine reaction. When combined with drug administration, the selectivity of the reaction is even more apparent. Injections of p-chlorophenylalanine, a potent serotonin synthesis inhibitor, causes disappearance of electron density in the adrenergic terminal vesicles *only* in paraformaldehyde pre-treated tissue. Serotonin is found in two pools in the rat pineal gland, neural and parenchymal. This study reports the first subcellular localization of 5-hydroxytryptamine stores in rat pinealocytes. Indoleamine-specific paraformaldehyde prefixation reveals oval, granular storage vesicles 1500-3000 Å in length. These organelles tend to cluster in "serotonin cells", and appear to possess the ability to release their electron-dense contents. Supported by HEW grant NS-10326

STRUCTURE OF MOLLUSCAN BIVALVE HINGE LIGAMENT, A UNIQUE CALCIFIED ELASTIC TISSUE, Mary Marsh, Department of Chemistry, Rice University, Houston, Tx.

The microstructure of calcified molluscan bivalve hinge ligament has been studied. Similar to bivalve shell and vertibrate dental enamel the ligament is composed of oriented crystal bundles or prisms. Electron micrographs of Spisula solidissima ligament show two types of prisms. The external surface of the ligament is composed of long cylindrical prisms oriented perpendicular to the growing margin of the ligament. The prism crystals are oriented parallel to the prism long axis. In shape and orientation these prisms are similar to those of dental enamel. The prisms observed in the interior region of the ligament are cylindrical but constricted at regular intervals, giving the appearance of 3-dimensional standing waves. The crystal spacings within the prisms vary regularly along the prism axis. Thus, this structure probably responds to compression like an elliptical spring and accounts for the elastic properties of the ligament.

ULTRASTRUCTURAL AND BIOCHEMICAL CHANGES IN PHYSARUM POLYCEPHALUM IN RESPONSE TO HIGH PLASMODIAL DENSITY. McAlister, L.E., V.F. Allison, C. Nations, and J.R. Jeter*
Dept. of Biol., Southern Methodist Univ., Dallas, Tx. 75275
*Dept. of Anat., Tulane Medical School, New Orleans, La.

The developmental transition in response to starvation from metabolically active microplasmodia of the slime mold Physarum polycephalum to a quiescent spherule stage results in major morphological and biochemical changes. Ultrastructural studies (Stewart, P.A., and B.T. Stewart. 1961. Exptl. Cell Res. 23: 471-478.) show spherules are characterized by organelles markedly different both in size and morphology from those observed in plasmodia. Among biochemical changes, the patterns of acidic nuclear proteins differ dramatically and consistently between the two developmental forms.

Nations et. al. (1974. Exptl. Cell Res. 88: 207-215) were able to obtain changes in the nuclear acidic protein patterns similar to those induced by prolonged starvation by gently pelleting and allowing microplasmodia to stand at high densities for 45 minutes. Proteins transferred from the cytoplasm into the nucleus at this time may initiate a quiescent state.

Studies of exponentially growing and pelleted microplasmodia have been undertaken to determine whether the observed changes in nuclear proteins can be correlated with ultrastructural changes in the microplasmodia. Morphological differences between high density microplasmodia and starvation-induced spherules will be discussed.

HUMAN CHROMOSOMES AND CENTRIOLES AS NUCLEATING SITES FOR THE IN VITRO ASSEMBLY OF MICROTUBULES FROM BOVINE BRAIN TUBULIN Manley McGill and B. R. Brinkley, Division of Cell Biology, Department of Human Biological Chemistry and Genetics, The University of Texas Medical Branch, Galveston, TX 77550.

Treatment of HeLa cells with Colcemid at concentrations of 0.06 to 0.10 μg/ml leads to irreversible arrest in mitosis. Colcemid-arrested cells contained few microtubules and many kinetochores and centrioles were free of microtubule association. When these cells were exposed to microtubule reassembly buffer containing Triton X-100 and bovine brain tubulin at 37°C, numerous microtubules were reassembled at all kinetochores of metaphase chromosomes and in association with centriole pairs. When bovine brain tubulin was eliminated from the reassembly system, microtubules failed to assemble at these sites. Similarly, when EGTA was eliminated from the reassembly system, microtubules failed to polymerize. These results are consistent with other investigations of in vitro microtubule assembly and indicate that HeLa chromosomes and centrioles can serve as nucleating sites for the assembly of microtubules from brain tubulin. Both chromosomes and centrioles became displaced from their C-metaphase configurations during tubulin reassembly, indicating that their movements were a direct result of microtubule formation. Although both kinetochore- and centriole-associated microtubules were assembled and movement occurred, we did not observe direct extension of microtubules from kinetochores to centrioles. This system should prove useful for experimental studies of spindle formation and chromosome movement in mammalian cells.

(This work was supported in part by a United States Public Health Service Grant NCI-CA 14675.)

SCANNING ELECTRON MICROSCOPY OF PERIMYSIAL CONNECTIVE TISSUE IN TWO MUSCLES. Mary Lou Percy, T. R. Dutson, and E. L. Thurston. Department of Animal Science, Texas Agricultural Experiment Station; Department of Biology; Texas A&M University.

Little is known about the structural relationship of muscle connective tissue and meat tenderness. Our purpose is to characterize the morphology of perimysial connective tissue in order to better understand the relationship of tenderness to differences in its structure. Samples from psoas major (tender) and biceps femoris (tough) muscles were removed from a bullock carcass at seventy-two hours post-mortem, fixed in glutaraldehyde, and post-fixed in osmium tetroxide. Specimens were critically point dried, coated with gold palladium, and viewed with a scanning electron microscope. Attachment of perimysial connective tissue between muscle bundles was observed, as well as sheets of connective tissue spreading over several muscle fibers. Variation in amount and thickness of connective tissue was observed in these muscles with the psoas exhibiting less connective tissue. Thickness of connective tissue strands ranged from .5μ to 10μ in these muscles. The differences in connective tissue morphology between muscles could contribute to differences in tenderness.

AN UNUSUAL DISTRIBUTION OF GOLGI APPARATUS INTERCISTERNAL FIBERS. Hilton H. Mollenhauer and D. James Morré, Veterinary Toxicology and Entomology Research Laboratory, ARS, USDA, P.O. Drawer GE, College Station, Texas 77840 and Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana 47907.

Oriented fibers, 30-50 Å in diameter, are present within the intercisternal regions of many dictyosomes of plant cells where they are associated with the flattened portions of the cisternae. The fibers normally increase in number from the forming to the maturing face of each dictyosome, and all fibers within a dictyosome are parallel to one another. The function of the intercisternal fibers is unknown, although they probably add an aspect of structural anisotropy or rigidity to the dictyosomes.

An exception to the usual distribution of intercisternal fibers is found in dictysomes of the maize root cap where the fibers are conspicuously associated with the secretory vesicles. These vesicles are unique in the sense that they are elongate (kidney-shaped) during their formative stages. Fibers are present at the earliest stages of vesicle formation and are always oriented parallel with the long axes of the vesicles. Because of their early presence and orientation, we suggest that the fibers may aid in the organization and/or shaping of the forming secretory vesicles.

Work supported in part by a grant from NIH CA13145.

SCANNING ELECTRON MICROSCOPY STUDY OF CALCIUM DEPOSITION ON THE SHELL MEMBRANE OF THE DOMESTIC HEN. Hal B. Phillips, Department of Animal Nutrition, Texas A&M University, College Station, Texas.

Egg shell formation begins in the isthmus of the hen, where membrane formation is completed. Small calcium rich clusters are deposited on the membrane in the isthmus and this appears to be the initiation sites for calcium deposition. As the deposition continues, a period which the individual sites become crystalline in appearance is achieved. Further deposition finds the clumps loosing their individual integrity and becoming covered by an amorphous calcium rich layer. When viewed cross-sectionally, the arrangement of the individual sites on the membrane can be viewed as well as their transformation into the more solid outer portion of the shell.

INHIBITION OF STEROID-INDUCED THYMIC PYKNOSIS BY CYCLOHEXIMIDE

Ramah W. La Pushin, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77025 *

The cellular basis for the lytic action of steroids on thymocytes and lymphocytes is poorly understood. Four hours post injection of dexamethasone, 7 mg/100 g body weight, the thymic cortex of the adrenalectomized rat exhibits large numbers of pyknotic cells. These pyknotic thymocytes are distributed as clusters, a phenomenon unique to the thymus (La Pushin, R. & E. de Harven, 1971). Prior administration of cycloheximide, 2.5 mg/100 g body weight reduced the pyknotic index from 30% to 8% and no clusters could be observed. Dexamethasone had no effect on the cortical lymphocytes present in the cuffs surrounding germinal centers present in the submandibular lymph node. The steroid did, however, cause a slight increase in pyknotic debris within tingible body macrophages which was not affected by cycloheximide. The results suggest that an inhibition of protein synthesis can markedly depress the incidence of thymic pyknosis and abrogate cluster formation.

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NUCLEAR INCLUSIONS: DEMONSTRATED RELATIONSHIP WITH CELLULAR HYPERACTIVITY

by L. D. Swain, R. R. Moldenhauer, T. R. Hoage, Sam Houston State University

Cell surface specialization of ion-transporting epithelia in the nasal salt glands of two Rallidae species was studied in relation to differing osmotic stress conditions, with three major cell types evident. Peripheral cells, similar in all experimental and field groups, were found to have relatively few mitochondria and smooth cell surfaces. Transitional secretory cells with lateral plications interdigitating with contiguous cell membranes were intermediate between peripheral and primary secretory cell types. Primary secretory cells had extensive lateral and basal plications with numerous mitochondria trapped in the membrane foldings, the relative abundance of which increased dramatically with osmotic stress. Intranuclear inclusions appeared only after hypertrophy of the primary secretory cells accompanying osmotic stress, thus demonstrating the dependence in some way on an altered or special metabolic state or on cellular hyperactivity.

AN ULTRASTRUCTURAL STUDY OF SPERMATOGENESIS IN THE SNAIL TAREBIA GRANIFERA OF THE FAMILY MELANOIDES.

Ronald W. Scates, Trinity University, San Antonio, Texas.

In his article, Jacob (1957), citing chromosomal studies, emphasized the extreme rarity or possible non-existence of viable males in populations of Melanid snails. The snail populations, nearly totally female in chromosomal count, were presumed to reproduce parthenogenetically; no sexual reproduction in evidence. Similar findings in populations of Tarebia granifera, of the Melanid family, were reported by Abbott (1948, 1952), Pace (1973), and Patterson (1973).

Examination of thin sections of the reproductive tract of Tarebian snails in the San Antonio area has revealed that active spermatogenesis is occurring, documenting the fact that males do exist in the population. All stages characteristic of typical animal spermatogenesis are observed. Mature spermatids are produced in great number and, ultrastructurally, appear viable, bringing into question the theory of parthenogenesis as being the chief or sole means of reproduction among this snail species.

RESPONSE OF LINEAR AND CIRCULAR DNA TO AQUEOUS ETHANOL.

T. Taylor, D. Gray and D. Lang. Institute for Molecular Biology, The University of Texas at Dallas.

Purified DNA of any source undergoes drastic conformational changes when subjected to aqueous ethanol. Electron microscopy of linear, viral DNA of homogeneous size shows compact, highly asymmetric particles. Depending on the ethanol concentration, the particle lengths occur in discrete classes. Their length ratios are given by c^{-n} where $c = 4.3$ and $n = 1, 2, 3$. Although not directly visible, the conformation is concluded to be a family of supercoils of order n. This conclusion has been supported by using covalently-closed and nicked, circular DNA from bacteriophage PM2 which forms circular rings, in aqueous ethanol, of order $n = 1$ and 2 while decreasing in size. The appearance of these rings eliminates the possibility of a folded conformation in favor of supercoiling. The fact that $n = 3$ can not be induced, is interpreted as a topological constraint intrinsic to circular DNA. These results involving dehydration of DNA may have implications for structure, function and electron microscpy of eukaryotic chromosomes.

MELANIN FORMATION IN MICROSCLEROTIA OF *VERTICILLIUM DAHLIAE:* (+)-SCYTALONE, A NATURAL PRECURSOR.

M. H. Wheeler, S. M. Meola, and W. J. Tolmsoff, Agricultural Research Service, U.S. Department of Agriculture, P. O. Drawer JF, College Station, Texas 77840.

Black pigment (melanin) formation in microsclerotia of *Verticillium dahliae* was examined by scanning and transmission electron microscopy. Observations were made on a wild type isolate, a mutant with albino microsclerotia (*alm-1*), and a mutant with brown microsclerotia (*brm-1*). Melanin was present as granules in microsclerotia of the wild type. The granules were embedded in a fibrillar network encapsulating the cell wall and in the outer portion of the wall. Granules were not formed in microsclerotia of the *alm-1* or *brm-1* mutants. When microsclerotia of *alm-1* were treated with (+)-scytalone [3,4-dihydro-3,6,8-trihydroxy-1(2*H*)-naphthalenone), which is a product excreted by the *brm-1* mutant, melanin granules developed in a pattern like that in the wild type. Catechol and L-DOPA, compounds implicated as precursors of melanin in *V. dahliae* and other organisms, did not form melanin granules in microsclerotia of *alm-1*. These observations indicate that (+)-scytalone is the precursor to melanin synthesis in *V. dahliae*.

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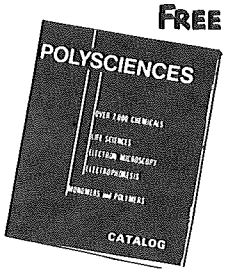

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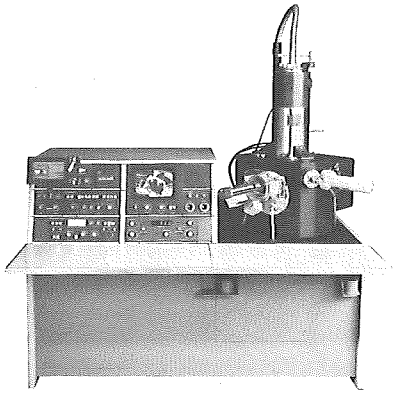

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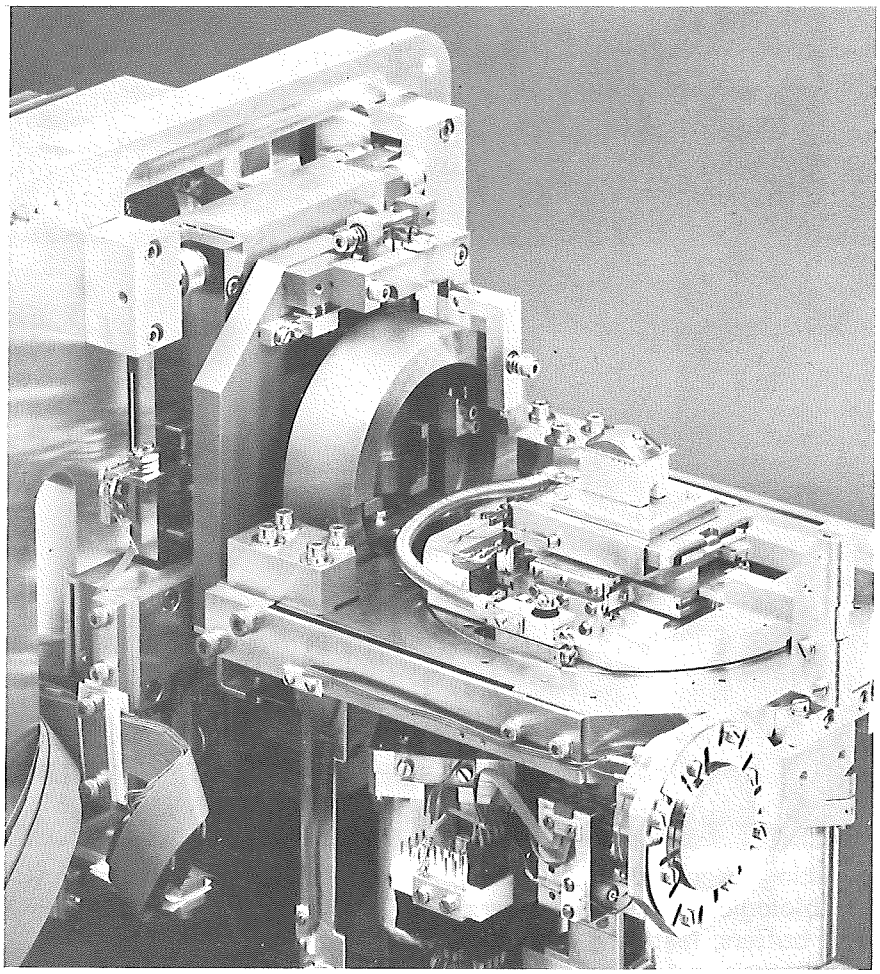

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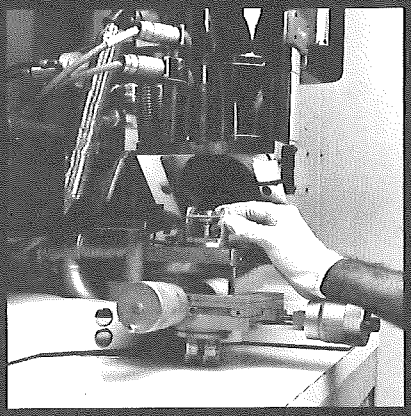

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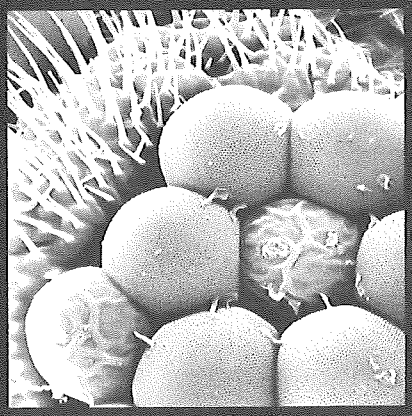

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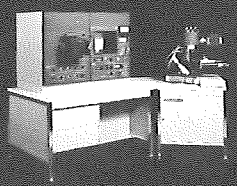

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