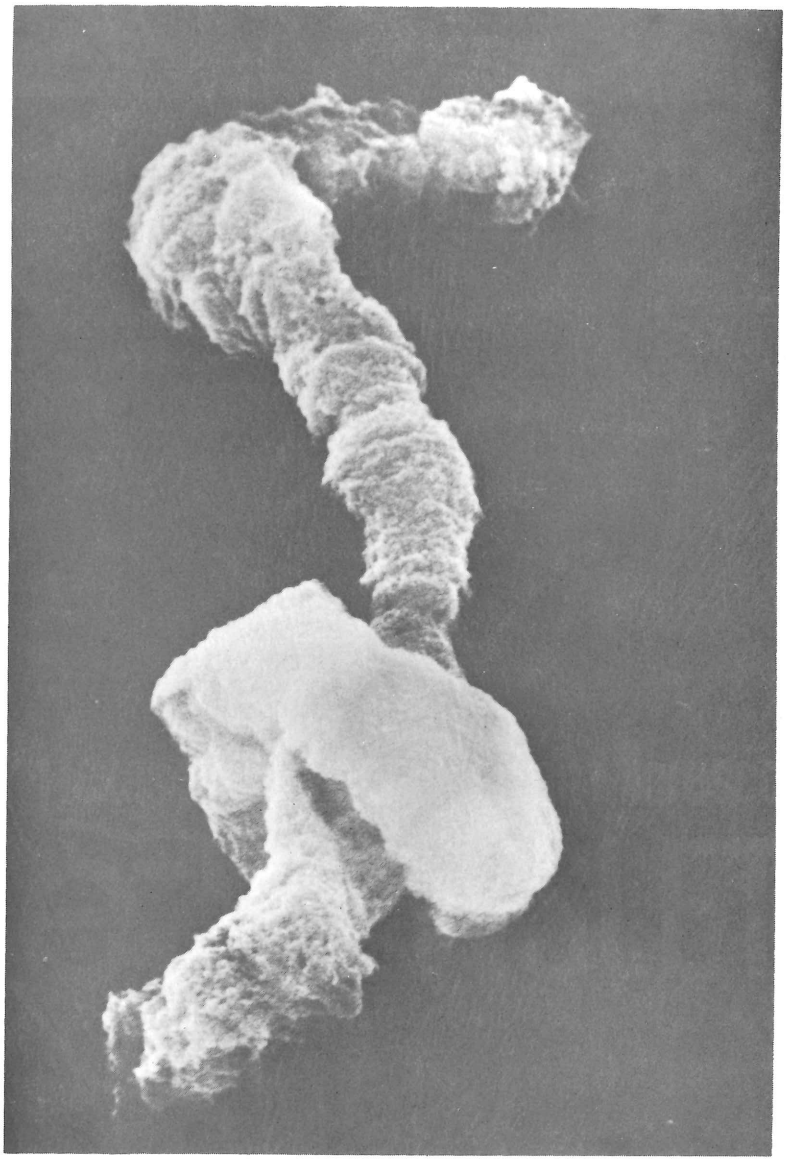
TSEM Texas Society for Electron Microscopy NEWSLETTER



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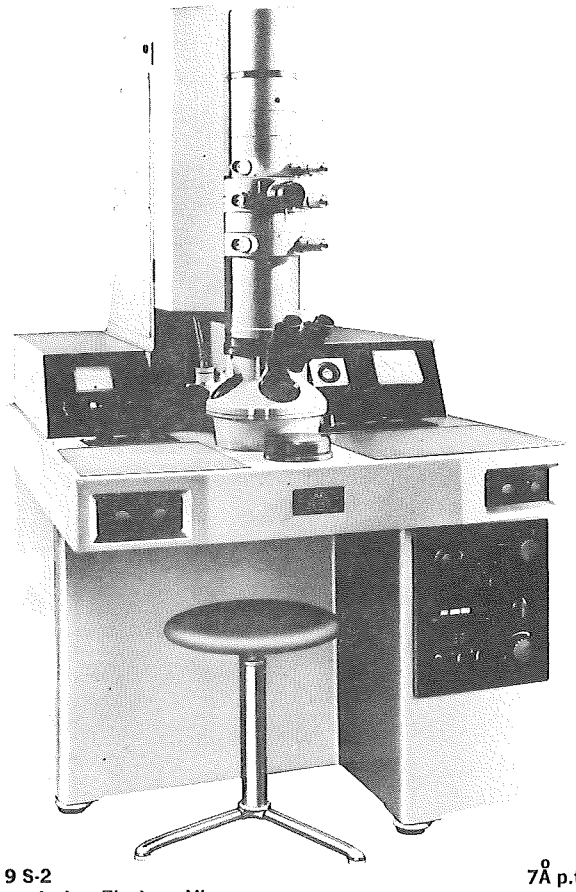

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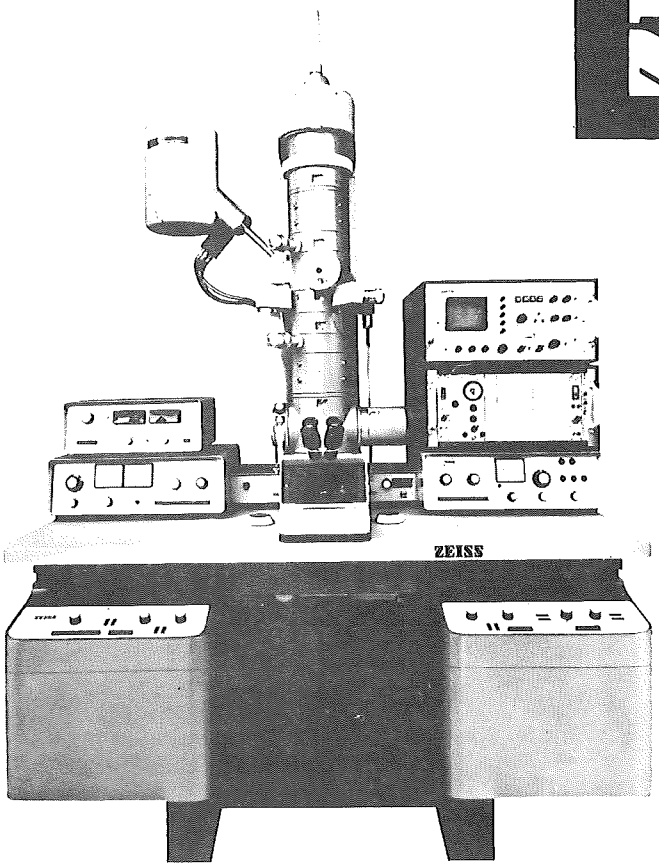

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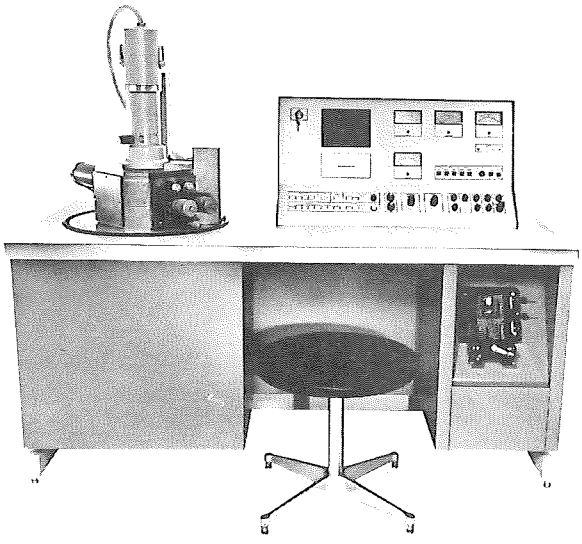

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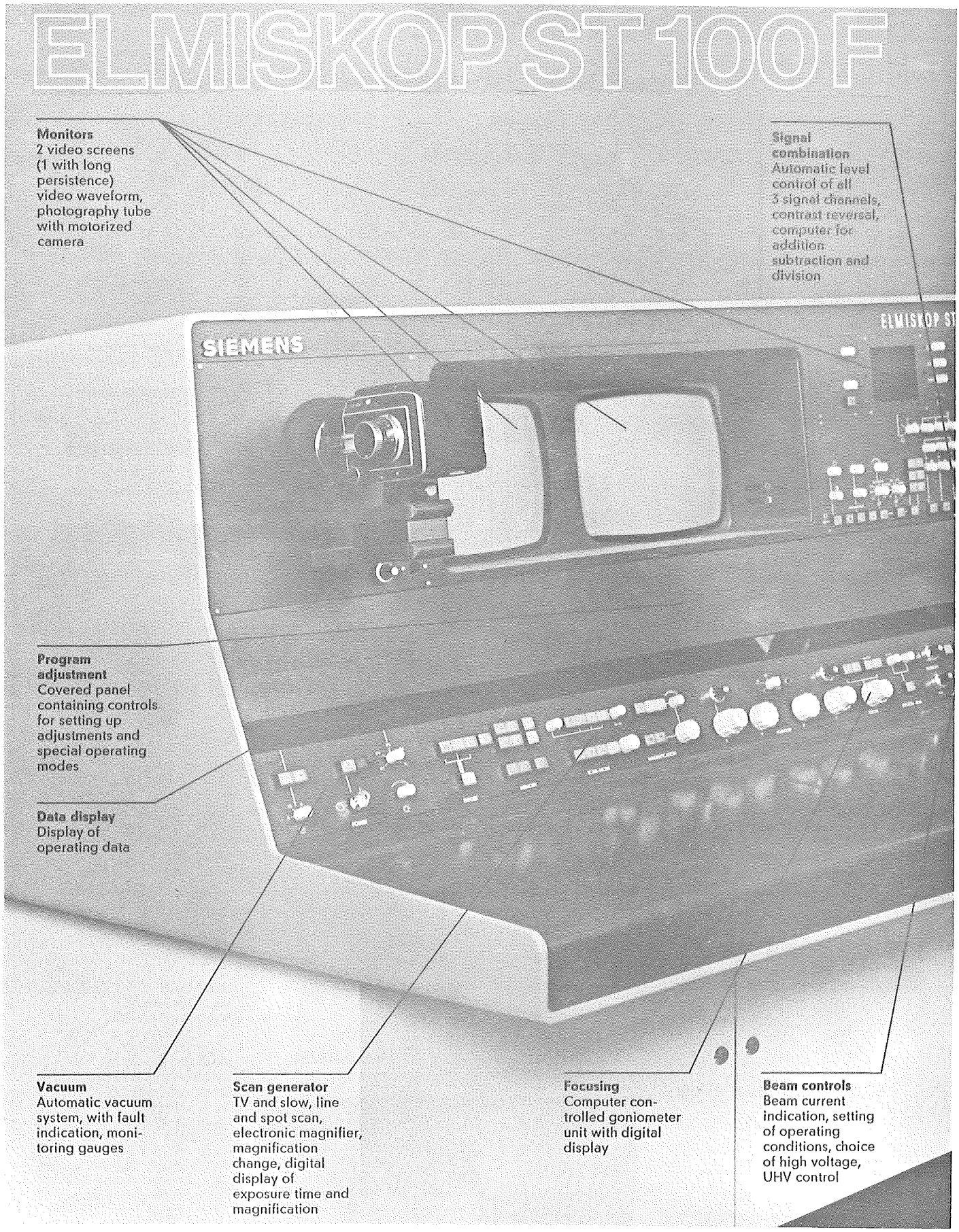

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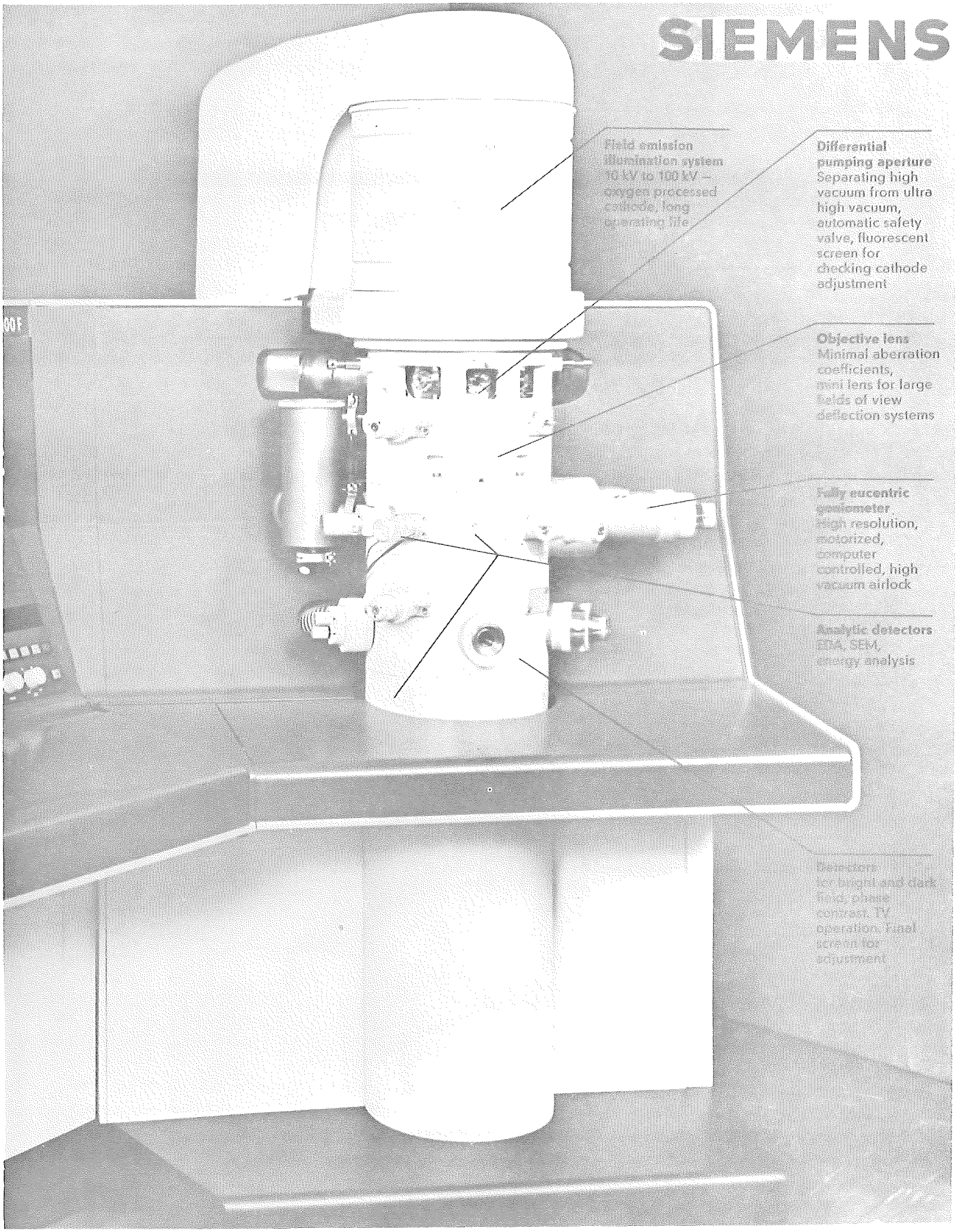
Beam controls
Beam current indication, setting of operating conditions, choice of high voltage, UHV control

SIEMENS



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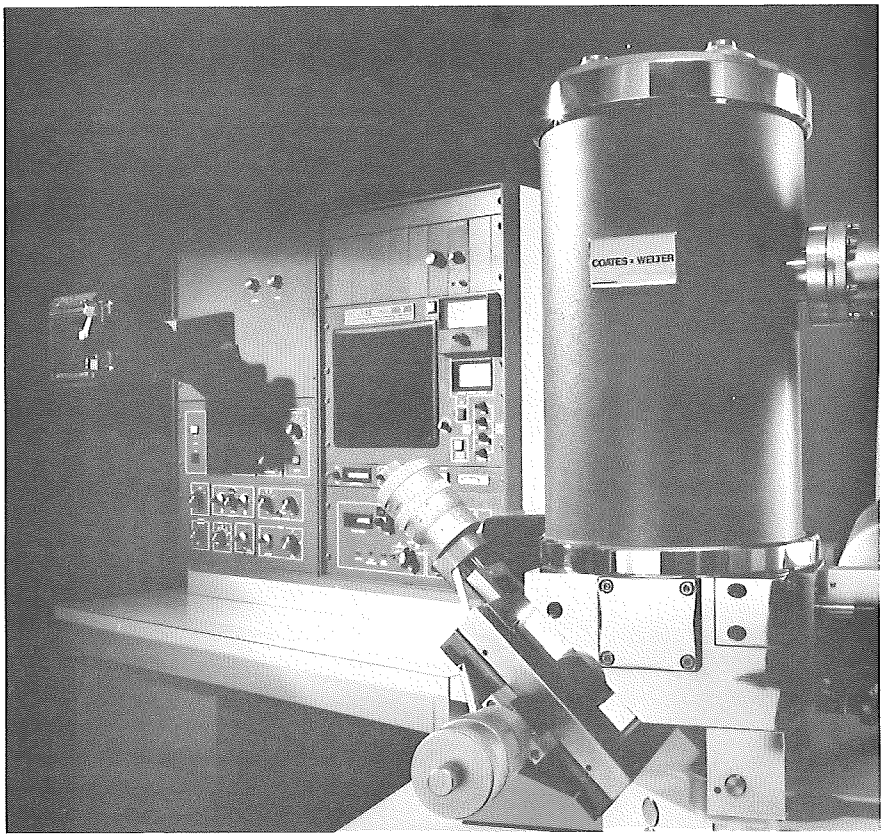

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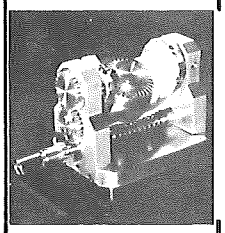

TENSILE SUB-STAGE
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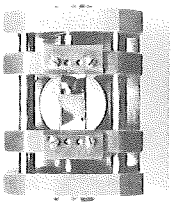

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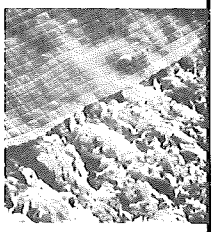
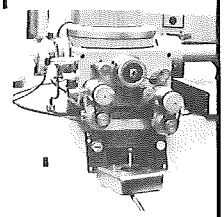


SEM JOYSTICK STAGE CONTROL
CAT.#1191



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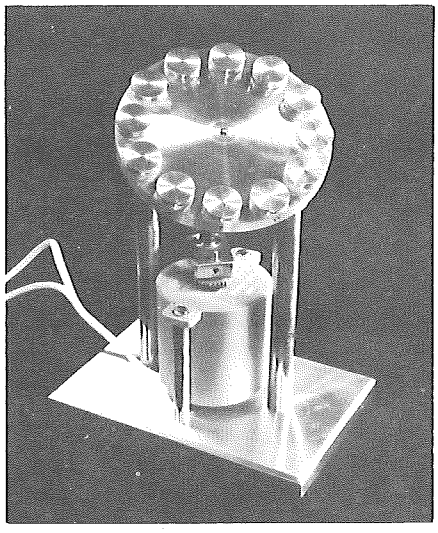

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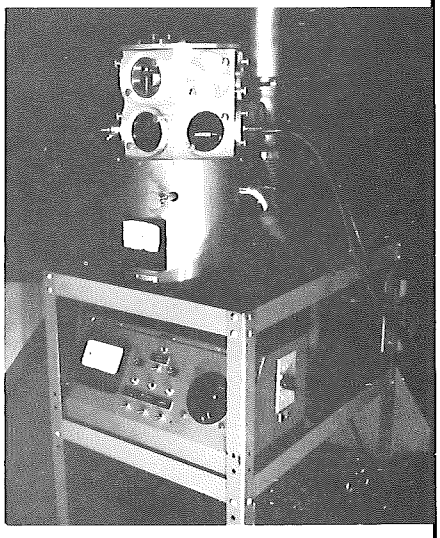

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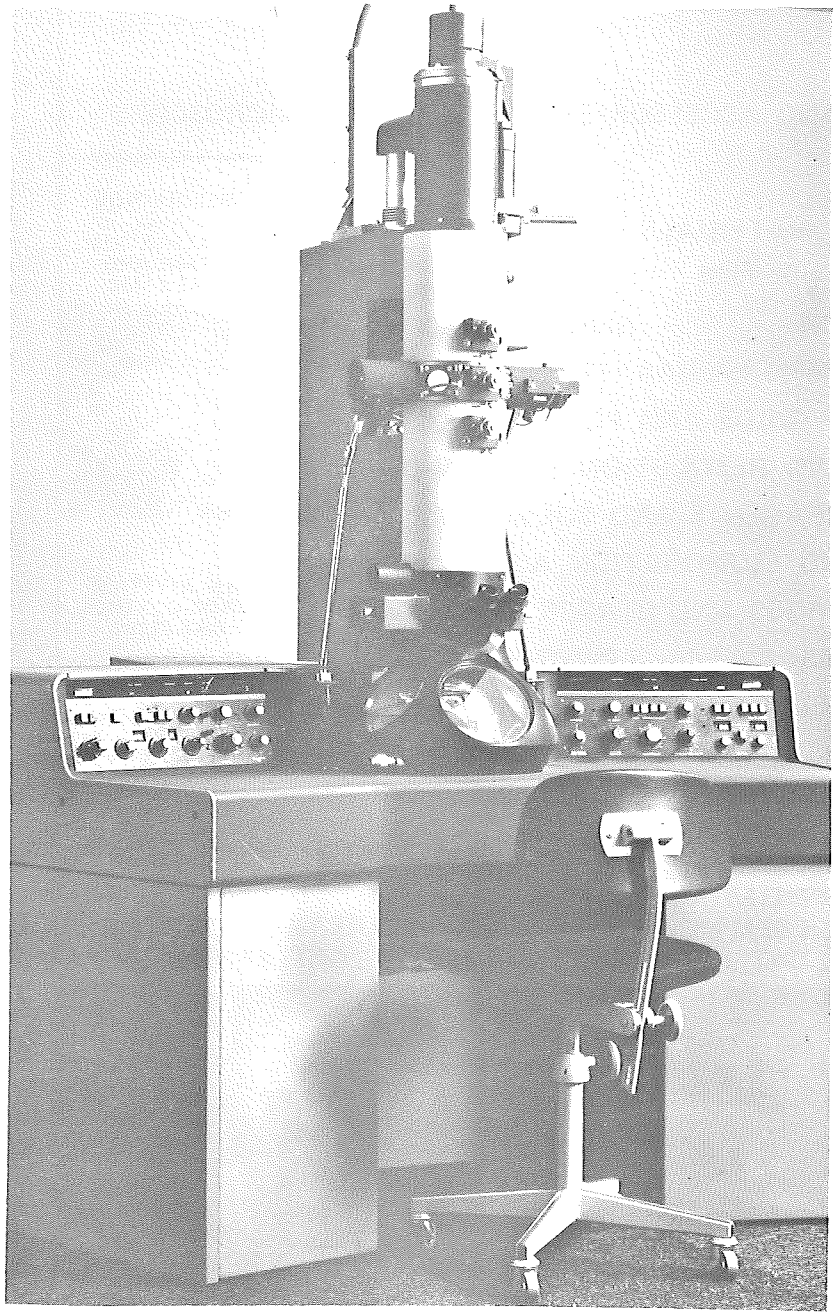

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Horatius at the Bridge

The other day I received a letter from a former college roommate of mine who included a newspaper article about my *alma mater,* Iowa State University. The headline read: "Revolutionary Student Brigade Demands New Dorms at ISU"! Oh God! I thought. Armageddon has arrived. Nothing *ever* happened at *this* school. Everyone was too busy studying. The wildest thing used to be an annual march down the main street backing up traffic 15 miles to the next town and which culminated in the burning of a few cardboard boxes in front of the Ames-town Police Station. Afterwards, the police finished their card game of "pitch" and everybody went back to the Greek houses. Nobody every really knew what, if anything, was being protested.

Reading on I found that the "Brigade" was composed of 14 members! They describe themselves as a Communist organization and they want a roll-back of increased room and board rates, an end to tax-free dormitory construction bonds and unlimited admissions with no future tuition raises. Their leader says tax-free bonds are just a way "the super-rich prey upon workers and middle-income taxpayers of the country". The regents say taxable bonds would have to be sold at a higher interest rate and would result in higher rates to students.

There persists a student element in our institutions who are so incredibly naive, and so incredibly dependent, it defies adequate description. The character of this small population (but increasing in numbers) of students has been derived largely from the '60s activist groups. However, instead of burning down ROTC buildings and occupying Presidents' offices they have found that the *threat* of such, or similar, actions works even better. For example, I recently heard a group of students addressing a faculty meeting saying if such and such did not occur. . . "we won't be responsible for what might happen!" Thus, many of these students find administrators who are easily intimidated, and not only do they get what they want, but they also get an apology along with it.

Where these students find resolute faculty and administrators they take their protests to Judicial Administrators (judges of the courts) who now are accepting cases brought by students against universities claiming they did not get out of a course what they should have! Oh yes, it is happening! And the pitiable thing about it is that these cases are being settled out of court in favor of damages to the plaintiffs because the universities do not have the funds to fight it out in court.

Scholarship at virtually all levels of education is gradually becoming equated with attendance. Many students appear to act out the role of computers - whatever goes in comes out in essentially the same way. One dare not challenge a student to explore or inquire independently because the student must be told what is important and what is not! What is more, that which is important is what will appear on the examinations!

Attempts to restore academic discipline are being met with administrative reservation at best and outright censure or dismissal of faculty at worst. At a time when basic mental discipline needs restoration and reinforcement in academia what we get is administrative default, Deans who make no value judgments and curricular "experts" who magically define relevancy based on the number and constancy of complaints by students at their door.

Basic literacy is not popular at the student or administrative level. It is easier, for example, for the revolutionary brigade at ISU to rail at the Regent's door about tax-free construction bonds than to retire to their study cubicles and bone-up on some simple economics. Why is this so? The answer is because the students feel the implied threat

of failure. This particular type of student has been fawned over and virtually carried every step of his way towards development. He has also been instructed that failure is bad and By God! he must succeed in virtually everything. Spawned and nurtured by affluent parents who provided a sterile environment during his most inquisitive years, sustained by an early educational system which sponsors the "do your own thing" progressive learning approach (John Dewey again!) these students know no other way to deal with their fears or their ignorance but to make someone else responsible for their real and potential faults.

It is also easier for some administrators to receive idiopathic complaints from certain students and appear understanding and benevolent, following this up with some expedient device to remove the complaints (like removal of faculty) than providing academic shovels and rubbing liniment to the students.

Where then does this leave our conscientious faculty? John Miles writing in National Review (27:772, 1975) said it:

> "One is forced then to an ugly choice. Either he will deliver lectures as if they were understood, when he knows they are not, and correct papers as if they were written in standard English, when he can see that they are not - or he will teach what needs to be learned and resign himself to early severance. The University does not want to know how bad things are. It cannot, accordingly, admit that teaching basic literacy is a reasonable - indeed often the only reasonable - course of action."

It is not easy to hold fast to the mission amidst ever lowering SAT scores, rampant violence in the schools, a system whose symbol is now not the mortar board but the bus, student voting membership on faculty committees, and sophistic Deans who delight in a kind of false statesmanship (it's patronization, really) which never reaches the real root of a problem.

For those in the profession who will maintain their resolve I am afraid only history can now be their reward. The world has not forgotten how Horatius stood before the bridge. In the academic sense there are many who today stand before that bridge. Will the "Brigade" pass?

Ward Kischer
President

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COMMENTS FROM THE EDITOR

With this edition of the Newsletter of the Texas Society for Electron Microscopy, I am resigning as the Editor. I have enjoyed my tenure as Editor of this monograph, and will continue to serve the new Editor, and the Society in any manner possible.

As the Editor of the Newsletter I have become more familiar with the real workings of the Society, and now can truly appreciate all the work done in the past by those faithful servants of the Society. Also I have come to know many of the members of the Society who have worked from behind the scenes in very quiet manners. These persons are to be commended for their work, for it is this kind of work that keeps the Society alive and well. Many of you have given little to the Society and yet have received the benefits of the Society. If we could "mobilize" all the persons on the membership list who claim membership, but never contribute to the Society in any manner, we would have a really working force, but then again, beneficence, it must be given.

One segment of our membership that cannot be thanked enough is the Corporate Members. Without their support, we could not have enjoyed many of the remunerative experiences that we have encountered. Their support *is* the life of the Newsletter. Also their support makes our meetings more enjoyable socially, and their exhibits have given us exposure to many of the recent advances in technology.

The Newsletter of the Texas Society for Electron Microscopy is one of the most successful monographs of its kind. From its beginning as a mimeographed and stapled handout, it has continued to grow to a sizeable monograph. Those who have served as Editors in the past are to be commended for their work in establishing the Newsletter and setting a high level of excellence. I hope that I have at least continued this excellence, and maybe even added a little. The membership of TSEM has a viable, creditable monograph at its disposal, and should take advantage of it on every occasion.

Finally, a big thank you to all the *participating* Regional Editors who have submitted works to the Newsletter, and have actively solicited materials from their respective institutions. Also, a bouquet to the fast and nimble fingers of Barbara Haberman and all the persons at the Word Processing Center who have been responsible for the composition of this and all previous Newsletters while I have been Editor.

Ron Gruener
Newsletter Editor

Texas Society For Electron Microscopy

be it known that

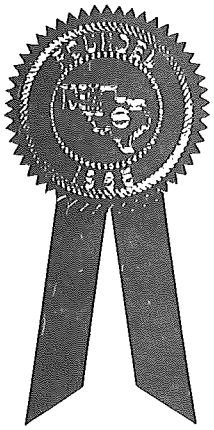
Donald Duncan

having made significant contributions and given outstanding service to the field of electron microscopy and this society is hereby recognized as an

Honorary Member
in this Society

with all rights and privileges thereof.

Founded May 14, 1965



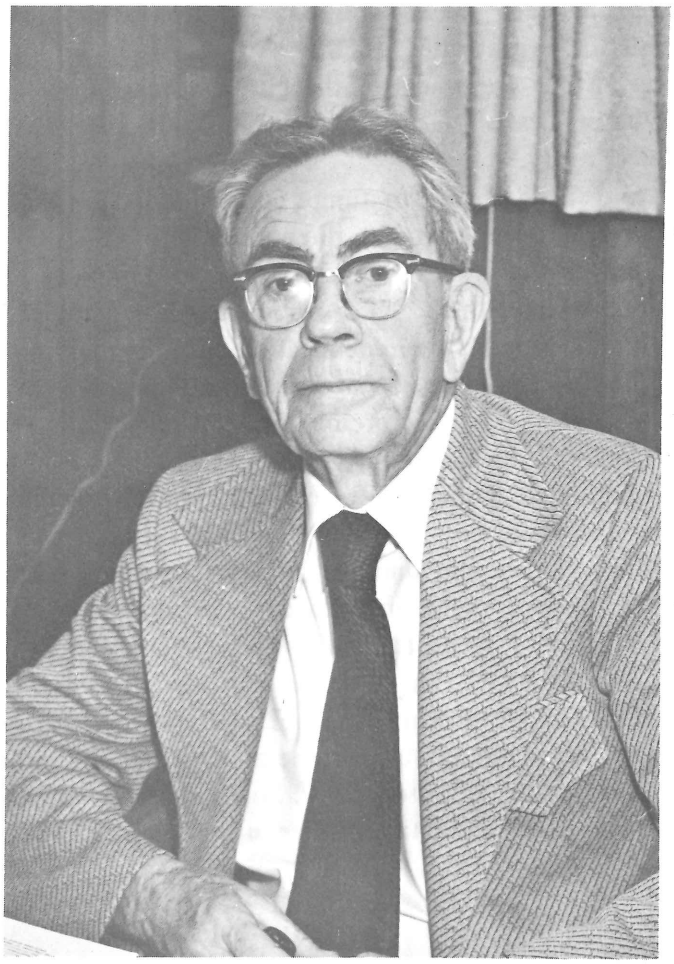
C. Ward Fischer
President

J. Richard Hillman
Secretary

Dr. Donald Duncan was formally inducted into the Texas Society for Electron Microscopy as our second honorary member during our joint TSEM - LSEM meeting in San Antonio last February. He is presently Ashbell Smith Professor of Anatomy at The University of Texas Medical Branch where he served as Chairman of the Department of Anatomy for 22 years.

Dr. Duncan has been a member of the Texas Society for Electron Microscopy since 1966. He has contributed significantly to our meetings, often co-authoring abstracts with Ricardo Morales, Robert Yates and Joe Mascorro. He has encouraged support of TSEM as witnessed by the service in the Society rendered by several members of his department, many of them having served as officers. He has been one of the true pioneers of electron microscopy in Texas, and instrumental in support of the activities of TSEM within the state of Texas. He has acquired funds for the purchases of EM installations within his own department and encouraged similar ventures in others.

In 1972 Dr. Duncan wrote a supplement to an issue of the TSEM Newsletter describing the early work in electron microscopy in Texas. It also very effectively speaks for his dedication to the progress of EM work in the state. It is reprinted with this issue for the benefit of the more recent members of TSEM, and as a tribute to his endeavors toward electron microscopy as an investigational tool.



A SHORT HISTORY

Reprinted from TSEM Newsletter, Spring 1972.

The following is an account of what I know, heard and remember about early electron microscopes in Texas.

Either in 1941 or early in 1942 I proposed and recommended that the Medical Branch acquire an electron microscope. A decade earlier I had good reason to believe that there were nerve fibers too small to be visible with the light microscope. Naturally, I wanted to see them.

In June of 1942 I left UTMB and did not return until February 1, 1946. By that time it was rumored that there was an electron microscope at the Brooke Army Hospital in San Antonio and there may have been two 'scopes in the possession of and used by industrial firms. It was about 1946, '47 or '48 that there was a microscope in Port Arthur and another in Freeport.

The first University of Texas microscope was purchased in 1947 or 1948. It was acquired by a special legislative appropriation and was assigned to the Electrical Engineering Department on the Austin Campus. The tale I heard was that this was due largely to a young member of the legislature who was also a student at the main campus. World War II produced a number of these.

Upon my return to the Medical Branch I again recommended purchase of an electron microscope. Doctor Chauncey Leake's response to this, after some delay, was that he had consulted with the authorities of the day and they had expressed exceedingly pessimistic opinions on the usefulness of the electron microscope as a biological research tool. They told Doctor Leake that what little could be garnered with this instrument had been harvested already.

During 1947 and 1948 I sort of fumed and fumbled on this topic. I did propose to the American Optical Company that they make a modification of their microtome by reducing the pitch of the advancing screw so as to permit at least the possibility of sections less than one micron thick. Their response was it couldn't be done despite the fact that it had been done long before in connection with the making of diffraction gratings.

In 1948 I learned of the invention of Pease and Baker, a simple device that reduced the unit advance of the Spencer Microtome from 1u to 0.1u. I acquuired one of these wedges as soon as possible and began to work with it. After much struggle it seemed fairly certain that I had some sections that were considerably less than 1u thick. Remember this was done with a steel knife and no trough to receive the sections. Furthermore, material was doubly embedded in celloidin and paraffin. As to where and how I learned about and acquired grids to mount them on I have no memory.

With sections in hand I went to Austin to use the electron microscope. It was in the charge of Mr. Leland Antes, an electrical engineer. Between us, but mostly Antes, we produced the first EM pictures in Texas of biological specimens. After much effort a little paper on the ultrastructure of peripheral nerves was published in 1950.

(Editor's Note: Duncan and Antes, 1950. Some electron microscope observations on the structure of myelin sheath and axis cylinder in thin sections. Texas Reports on Biology and Medicine, 8:329-340.)

In the meantime, Francis O. Schmitt, Fernandez-Moran and F. Sjostrand had beaten me to the draw.

Antes was succeeded for a brief period by a young lady whose name I have forgotten. After leaving Texas she was for a time the EM technician for Doctor Farquhar and that is the last I know about her. She was followed by Hilton Mollenhauer. Hilton at that time was an electrical engineer. Subsequently, he developed into one of the truly distinguished members among the biologically oriented electron microscopists. Many and many were the days when I was on the road to Austin by 4 a.m. and back in Galveston at 11 p.m. Hilton and I spent many very happy but nearly always frustrating hours together. Each visit was always preceded by weeks of effort here at the Medical Branch. Somehow I did manage to put out another paper, this one on the posterior lobe of the chicken pituitary. Our chief problem was focus. In retrospect I cannot understand why this was so; but at least 9 pictures out of 10 were hopelessly fuzzy.

One amusing incident occurred on a fine spring day when Hilton and I were working together. Close to noon a redheaded fireball of a young woman burst into the E.M. lab where we were working. (By the way this was the first air-conditioned facility on the Austin campus). Without introduction or explanation this lady said, "Are you the people who work with the electron microscope?" We gently allowed that we were. The next blast was "I don't want my boss to know I am here, so I'll not tell you where I come from". More or less glaring at Hilton she said, "Can you cut thin sections?" His answer was no, that is Dr. Duncan's department. So it was my turn, "Where are you from, how do you do it, etc. etc?" My response was "You are from Houston, aren't you?" She wanted to know how I knew that and I said it was fairly obvious. "You won't tell my boss, will you?" "Of course not, but if you will come to Galveston a week from today (Saturday) with some of your blocks I shall try to help you". The story behind this is to the effect that Dr. R. Lee Clark requested funds from the Regents to buy an electron microscope and was turned down. So he went down Fannin or Main Street a few blocks and came back with the money to buy the best available.

(Editor's Note: This must have occurred in 1954 or 1955. The Houston Post reported the installation on December 13th, 1955. The microscope was installed for use by Dr. Leon Dmochowski.)

The Medical Branch did not have an electron microscope until Doctor Leake left and the institution was essentially managed by Doctor Blocker. This was carefully disguised as the infamous "Interim Committee", but that is another story. Truman Blocker secured the necessary money for the first 'scope from the Brown-Lupton Foundation. This must have been in 1955. The previous year I received an NIH grant for the development of biological electron microscopy in Texas, a grant that has been approved until 1974.

The initial instrument was the old RCA EML which is still functioning at Lamar University in Beaumont.

Charlie Moore of Dallas uncrated it and put it together. He, more than anyone I know, might have facts and figures on the first ten microscopes in Texas.

Prior to the one at the Medical Branch, first came the one in the Electrical Engineering Department at Austin. Texas A & M University received one at about the same time but this was put in the hands of a faculty committee and as a result was virtually sterile. Then came the one at M. D. Anderson (Editor's Note: see note below concerning Rice

University), and following the Medical Branch another EML found residence at Southwestern Medical School in Dallas. Not too long after the installation at Southwestern I was invited to bring specimens and demonstrate its potentialities. Fortunately I had some very good sections of the *pars intermedia* of the rat pituitary and took those with me. The Dean (a pathologist by trade) was very interested. Cell boundaries, mitochondria and Golgi apparatus seemed all according to Hoyle. Next we looked at the nucleus and especially the nucleolus. He said, "Do you mean to say that the nucleolus is not a solid round dot?" My response was that the electron microscope says no. He said, "I give up" and left. Subsequently, this microscope was used with ever so great effectiveness by Hilton Mollenhauer.

Probably the third electron microscope in an educational institution came to Rice University under the control of Doctor Milligan. It was a Phillips 100. I spent one delightful day with him and the new microscope. Although he was the perfect host and put all else aside for the day, it was obvious without asking that the biologists at Rice or from anywhere else were not to have access to that particular microscope.

(Editor's Note: This electron microscope was installed at Rice University early in the spring of 1951. Dr. Milligan states that, "We ordered the projected electrostatic electron microscope to be made by General Electric in approximately 1943, but none were ever produced, and the order was later cancelled.")

In summary, the first working microscopes in Texas belonged to industry; now twenty years later the same can be said of the electron probe.

--Donald Duncan

P.S. Our RCA service men were in succession, Charley Moore, Mr. Lubker, Pat Tyner and Bob Shorthose.

Financial Report
Period ending April 3, 1976

Total assets December 29, 1975		4,692.64
Certificate of deposit (University Bank #4470)		1,144.47
Certificate of deposit (Fannin Bank #17864)		1,000.00
Savings account (Fannin Bank #12-0900043)		2,000.00
Balance in checking account December 29, 1975		548.17
Receipts		
Corporate dues	250.00	
Member dues	224.00	
Registration (winter meeting)	2,584.50	
Corporate (exhibit space)	1,111.50	
Corporate contributions	775.00	
EMSA	500.00	
Total income	5,445.00	5,445.00
		5,993.17
Disbursements		
Secretarial expenses	274.87	
Reprints	64.73	
San Antonio Meeting	3,106.53	
Presidential expenses	32.52	
Total expenses	3,478.65	-3,478.65
		2,514.52
Transferred to savings account (Fannin Bank #12-0900043)		-1,000.00
Balance in checking account		1,514.52
Savings Accounts		
Certificate of deposit (University Bank #4470)		1,161.76
Certificate of deposit (Fannin Bank #17864)		1,000.00
Savings account (Fannin Bank #12-0900043)		3,058.16
Total assets of TSEM (April 3, 1976)		6,734.44

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

PLACEMENT SERVICE

PERSONS AVAILABLE

D.C. Ochoa-Guerrero - M.S. from Texas A & M University. M.D. from La Universidad del Zulia, Maracaibo-Venezuel. Desires position beginning this summer; experience in ultrathin sectioning, and transmission and scanning electron microscopy.

P. O. Box 119
College Station, Texas 77840

Rodney Keith Nelson - Ph.D. Candidate, University of Southern Mississippi. Extensive training in transmission and scanning electronmicroscopy, and associated areas. Research interests are ultrastructural and biochemical events associated with and controlling morphogenesis, with particular interest in the lower plant groups. Seeks involvement in an academic situation which would include general biology, general botany, cell biology, plant taxonomy, or biochemistry.

500 Cahal #45
Hattiesburg, Miss. 39401
601/593-2034

Elsie Mae Sorenson - Ph.D. The University of Texas at Austin. Research interests in Piscine physiology; factors affecting heavy metal toxicity and accumulation, ultrastructure of changes following heavy metal accumulation, and thermal effects on metabolism. Teaching interests in physiology, anatomy, cytology, scientific techniques, and microbiology.

Department of Zoology
The University of Texas
Austin, Texas 78712

Frank E. Summers, Jr. - B.A. Nine years experience in all phases of electron microscopy. Desires part-time employment. Available immediately.

3411 Greenbriar
Houston, Texas 77098
713/523-9117

Mary Lou Percy - M.S. Texas A & M University. Desires position in scanning and transmission electron microscopy. Available about January 1, 1977. Experience in light microscopy and histology. Research in muscle fibers and connective tissue with publications in press.

1602 S.W. Pkwy. Apt. 714
College Station, Texas 77840
713/693-3239

Person seeking career in sales. M.S. in Biology (electron microscopy) 1973. Experience in TEM since 1968. Business and sales experience. For information contact Editor of TSEM Newsletter.

Tapan K. Chatterjee - Ph.D. Syracuse University, Materials Science Interested in a research or teaching position. Experience EM and TED studies of heavy metal intoxication in brain tissue.

New Slocum Heights
Bldg. A-7, Apt. 4
Syracuse, New York 13210
315/446-0246

William C. Sanford - Ph.D. University of California at Davis.
Ten years experience in various preparation and imaging techniques including: electron diffraction, Balzers freeze-etch, critical point drying, lang-muir trough. High vacuum and electronic industrial experience. Six years university faculty experience teaching cell-molecular and developmental biology. Desire teaching and/or research position to pursue independent and team approach research. Available May 15, 1976.

School of Biological Sciences
Oklahoma State University
Stillwater, Oklahoma 74074
405/372-6211, ext. 6049

POSITIONS AVAILABLE

Electron Microscopy Technician I
Prefer someone with training in electron microscopy and histology. Extensive experience not required. Contact:

Randy Brackeen, Coordinator of Electron Microscopy Lab
Department of Anatomy
P. O. Box 4569
Texas Tech University School of Medicine
Lubbock, Texas 79409

Electron Microscopy Technician
Possible summer employment; June to August, inclusive. Student or graduate. Should know good darkroom tecnique. Prefer some experience in electron microscopy. Apply to:

Dr. C. Ward Kischer
Department of Anatomy
The University of Texas Medical Branch
Galveston, Texas
713/765-1293

Electron Microscopy Technician
General Schedule of the Federal Civil Service - depending upon education and experience - GS 5 - GS 9. Required are U.S. Citizenship and previous experience

of the incumbent in tissue handling of biopsies and ultrathin sectioning techniques. Contact:

Dr. B.E.F. Reimann
Department of Pathology
WILLIAM BEAUMONT ARMY MEDICAL CENTER
El Paso, Texas 79920
915/568-2403

REGIONAL NEWS

Dallas

THE UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL SCHOOL

Department of Cell Biology

A meeting was held on February 24th to determine if those people utilizing electron microscopy as a research tool at The University of Texas Health Science Center at Dallas would be interested in meeting informally every few months to discuss topics of mutual interest. More than thirty-five people attended, and it was generally agreed that informal meetings would establish a beneficial medium for the flow of technical information. A number of the people in attendance also indicated an interest in joining T.S.E.M. A noon meeting will be held on April 20th when a representative from Eastman Kodak will discuss the use of photographic films in electron microscopy. The meetings were organized by Donna Rainey, Mary Tobleman, and Dr. Jerry Shay.

Galveston

THE UNIVERSITY OF TEXAS MEDICAL BRANCH

Department of Anatomy

Dr. Ward Kischer received a foundation grant for work on the hypertrophic scar. For this project Elizabeth Gasch, formerly with Baylor Medical, has begun work in his laboratory.

Dr. Donald Duncan, Ashbell Smith Professor of Anatomy, was formally inducted into TSEM as an honorary member at the joint TSEM - LSEM meeting in San Antonio last February.

Recent Publications: Cannon, M.S. and J.R. Hostetler. The anatomy of the parotid gland in *Bufonidae* with some histochemical findings. II. *Bufo alvarius*. J. Morphology (In Press). Cannon, M.S., J.R. Hostetler and W.D. Belt. Crystalloid configurations in the adrenal cortex of the Siamese Tree shrew (*T. Glis*). Anat. Rec. (In Press).

Department of Pathology

Dr. Edward S. Reynolds, previously from Peter Bent Brigham Hospital (Harvard University), originator of the lead citrate stain for electron microscopy, has been named Chairman of the Department of Pathology. He will assume his full-time duties beginning July 1st, 1976.

Houston

THE UNIVERSITY OF TEXAS HEALTH SCIENCE CENTER AT HOUSTON

Department of Neurobiology and Anatomy

Dr. Richard G. Peterson has presented recent advances in the Toxicology and Pharmacology of Abused Inhalants to the National Institute for Drug Abuse. Dr. Peterson also presented work at the American Society for Neurochemistry meeting in Vancouver, which was entitled "The Effect of Triton X-100 on Protein and Electron Microscopy of PNS Myelin," and was co-authored by Ron Gruener.

The department was represented at the Houston Neurological Symposium by Drs. Joe G. Wood and Dianna A. Redburn. Dr. Redburn served as moderator for General Aspects of Basic and Clinical Aspects of Neurotransmitter Function, and presented work she has done concerning "A Model System for *In Vitro* Analysis of Stimulis Secretion Coupling." Dr. Wood served as moderator for the session on Behavioral Disorders, and presented "Cytochemical Correlates of Synaptic Agents" during the session concerning Visualization of the Synapse. The Symposium was attended by researchers from around the world, and featured such distinguished speakers as U.S. von Euler, Karolinska Institute, Stockholm, and D.R. Curtis from the John Curtin School of Medical Research, Canberra.

New faculty member Sam Enna will be joining the department soon. Also Richard Wiggins, John De France, and Ante Padjen have recently become members of the department. Ms. Leslie Arwin has joined the department as Medical Illustrator, and Randall Spallinger has joined the technical staff in Dr. Wood's lab.

Neurobiology and Anatomy graduate student Randall Stovall has a recent publication in the Journal of Herpetology entitled "Observations on the Micro- and Ultrastructure of the Visual Cells of Certain Snakes."

Lubbock

TEXAS TECH UNIVERSITY

Department of Biological Sciences

Meetings:

Dr. Jerry Berlin contributed a paper entitled "An autoradiographic study of the outer epidermis of the cotton ovule" to the Beltwide Cotton Production Research Conferences held in Las Vegas during January 1976.

Publications:

Pizzolato, T. D., G. Smutzer, and J. D. Berlin. 1975. An *in vivo* demonstration of pollen tube growth for elementary botany laboratories. American Biology Teacher 37: 503-504.

Smutzer, G. and J. Berlin. 1976. The use of Nomarski interference contrast microscopy as an alternative to the staining of Epon sections following autoradiography. Trans. Amer. Micro. Soc. 95: 109-112.

Ramsey, J. C. and J. D. Berlin. 1976. Ultrastructure of early stages of cotton fiber differentiation. Botanical Gazette, accepted for publication.

Burbano, J. L., T. D. Pizzolato, P. R. Morey and J. D. Berlin. 1976. An application of the Prussian blue technique to a light microscope study of water movement in transpiring leaves of cotton (*Gossypium hirsutum* L.). J. Exptl. Botany, accepted for publication.

Pizzolato, T. D., J. L. Burbano, J. D. Berlin, P. R. Morey, and R. W. Pease. 1976. An electron microscope study of the path of water movement in transpiring leaves of cotton (*Gossypium hirsutum* L.). J. Exptl. Botany, accepted for publication.

Vollet, J. J. and J. D. Berlin. 1976. Effects of X-irradiation on certain phosphatase enzymes in human testes. An electron microscopic cytochemical study. Cytobiologie, accepted for publication.

Pease, R. W. and J. F. Bailey. 1975. Thin polymer films as non-charging surfaces for scanning electron microscopy. J. Microscopy 104: 281-285.

TEXAS TECH UNIVERSITY SCHOOL OF MEDICINE

Department of Anatomy

Workshop

Dr. Donald L. Wilbur attended a symposium-workshop on Radioimmunoassay sponsored by the Endocrine Society. Dr. Wilbur was in San Diego, California March 7th through the 13th for the workshop.

Lectures

Dr. Roger Markwald was invited to present a seminar at George Washington University on January 14, 1976 in the Department of Anatomy. The topic of discussion was "The Role of the Extracellular Matrix in Cardiac Development". Dr. Markwald was also invited to lecture at the Veterans Administration Hospital, Department of Medicine in Big Spring, Texas on February 24. The title of his seminar was "The Relationship of Extracellular Macromolecules to Congenital Heart Disease".

Several members of the department attended the national meeting of the American Association of Anatomists April 18-22, 1976 in Louisville, Kentucky. Those presenting papers at these meetings included Drs. Patrick R. Sterrett, Donald L. Wilbur, John A. Yee, Robert L. Casady, Roger R. Markwald, Mr. Don Cameron and Ms. Judy Land.

Dr. Wilbur has also been invited to present a paper at the annual meeting of the Endocrine Society on June 24, 1976. His paper is entitled "Ultrastructural observation of anterior pituitary somatotrophs following hypophyseal portal vessel infusion of a new growth hormone-releasing hormone".

Publications

Wilbur, D. L., W. C. Worthington and R. R. Markwald. 1975. An ultrastructural and radioimmunoassay study of anterior pituitary somatotrophs following pituitary portal vessel infusion of growth hormone-releasing hormone. Neuroendocrinology. 196:12-27.

Yee, J. A., D. B. Kimmel and W. S. S. Jee. 1976. Periodontal ligament cell kinetics following orthodontic tooth movement. Cell Tissue Kinet. (In Press).

San Antonio

TRINITY UNIVERSITY

At the recent Collegiate Academy meetings in College Station (meeting jointly with the Texas Academy of Science) 5 research papers were presented by Trinity undergraduates.

1. Birgit Landgrebe, "A Survery of Rat Spleen Ultrastructure"

2. James Kells & Todd Holman, "Preliminary Ultrastructural Study of Hamster Retina"

3. Christina Ng, "Effect of 5-Hydroxytryptamine on Synaptic Ultrastructure in the Visual Cortex of Hamster"

4. Carol Kendall & Sarah Cochran, "Fine Structure of Microwave Treated Adrenal Gland"

5. John Ord & Chris Johns, "Ultrastructural Analysis of the Cercaria and the Metacercarial Cyst Wall of the Trematode, *Philophthalmus sp.*"

THE UNIVERSITY OF TEXAS HEALTH SCIENCE CENTER AT SAN ANTONIO

Department of Anatomy

Visiting Scientist

Dr. Arthur M. Zimmerman, Professor of Zoology, University of Toronto, is a Visiting Professor in the Department of Anatomy and is doing research on the structural and functional effects of marijuana on cells with Dr. Ivan L. Cameron. Dr. Zimmerman is the President-Elect of the Canadian Cell Biologists and Treasurer of the American Society for Cell Biology. He is currently serving a term on the Research Council of Canada and is an advisor to the National Drug Abuse Panel in Washington. He will be working at the Health Science Center for two months.

New Equipment

The Department of Anatomy has recently purchased and has installed a JEOL 35 scanning electron microscope as well as a nuclear semi-conductor X-ray energy dispersive microprobe and a Tracor Northern computer for analysis of data. The department also obtained a stereo-image analysis system all of which are now functional. Dr. Lawrence Thurston has presented a formal course in the use of the scanning electron microscope and sixteen individuals have been trained for "hands on" operation of the instrument. Dr. Ivan L. Cameron is coordinator of this new laboratory facility and Dr. Nancy Smith, a new member of TSEM, is directly responsible for the laboratory.

Publications

Bowie, E. P. and Herbert, D. C. (in press) Immunocytochemical evidence for the presence of arginine vasotocin in the rat pineal gland. *Nature.*

Cameron, I. L. and Bols, N. (1975) Effect of cell-population density on G_2 arrest in Tetrahymena. J Cell Biol. *67*:518.

Dung, H. C. (1975) Evidence of prolactin cell deficiency in connection with low reproductive efficiency of female torpid mice. J. Reprod. Fert. *45*:91-99.

Hansen, J. T. and Yates, R. D. (1975) Light, fluorescence and electron microscopic studies of rabbit glomera. Amer. J. Anat. *144*:477-490.

Hansen, J. T., Yates, R. D. and Chen, I-L. (1975) An electron microscopic study of the effects of reserpine on the subclavian glomera of the rabbit. Amer. J. Anat. *144*:491-502.

Yates, R. D., Mascorro, J. A., Hansen, J. T. and Chen, I-L. (in press) Comparison of the structure of carotid and subclavian bodies and abnormal paraglanglia. *In*: NIH Symposium of SIF Cells (ed. O. Eranko). Washington, D. C.

Seminar

Dr. G. Niswender from Colorado State University, Ft. Collins, Colorado, entitled "The Physiological Regulation of Ovarian Function".

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ULTRASTRUCTURAL MORPHOLOGY OF TWO SENSILLA PLACODEA ON THE ANTENNA OF A PARASITIC WASP

Margaret R. Barlin

Department of Entomology, Texas A&M University, College Station, TX 77843

Females of the wasp Tetrastichus hagenowii (Hymenoptera: Chalcidoidea) lay their eggs in cockroach egg cases, the young hatch and feed on the cockroach eggs. Prior to egg deposition the parasites follow a complex behavioral sequence, including tapping their antennal tips on the egg case, thus enabling them to detect various mechanical and chemical properties of the cockroach egg case and determine its suitability for oviposition (unpublished data).

Sensory sensilla occur along the entire length of the female antenna (Fig. 1). Two of the more prominent sensilla were studied by scanning and transmission electron microscopy, these are both sensilla placodea (P1 and P2 in Figs. 1 and 2) which are chemoreceptors (1). Numerous hair-like sensilla (H) and a few bulb-like sensilla (B) are also present (Figs. 1 and 2). Sensilla placodea occur frequently on hymenopteran antennae (1), but the occurrence of two types on the same insect has not been reported previously.

The main difference between these two sensilla placodea is that one possesses a thin cuticular wall with numerous pores (Fig. 3) and the other possesses a thick cuticular wall with few pores (Fig. 4). Each segment has a ring of both sensilla types, in segments 3 to 5 the thick-walled sensilla occur on the proximal portion of the segment and the thin-walled sensilla, on the distal portion. The three distal segments (segments 6 to 8) of the antenna are shortened to form a club and on these segments the two sensilla alternate in a ring (Fig. 2).

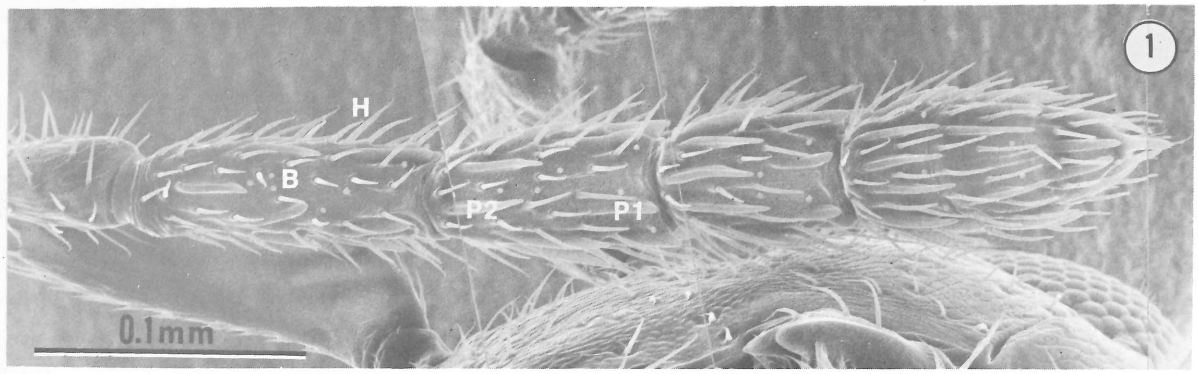
In cross-section the placoid sensilla consist of a tormogen cell (TO) and a trichogen cell (TR), the surface of the latter bearing numerous lamellae and mitochondria (Figs. 3 and 4). There are two invaginations (IN) of the cuticular wall on the lateral sides of each sensillum. The dendrites of the nerve cells enter at the base or proximal region of the sensillum in the ciliary region (C) and from this point the dendritic branches are enclosed in a cuticular sheath (S). After they enter the upper region of the sensillum, the dendritic branches turn and pass along its long axis. These dendritic branches contain neurotubules and come in close contact with the pores (PO) in the cuticular wall.

Sensory sensilla on the antennae of the insect detect specific chemical compounds which stimulate or deter oviposition. It is through the cuticular pores in the sensory sensilla that molecules are allowed access to the nerve endings. It may be speculated that the sensilla placodea are able to perceive specific chemical signals and therefore play a vital role in the discrimination of viable cockroach egg cases.

The author gratefully acknowledges the cooperation of Drs. G. W. Frankie, S. B. Vinson and G. L. Piper. This research was sponsored by the Environmental Protection Agency, grant number US EPA R-803068-01.

Reference

1. Slifer, E. H.: Annu. Rev. Entomol. 15, 121 (1970).



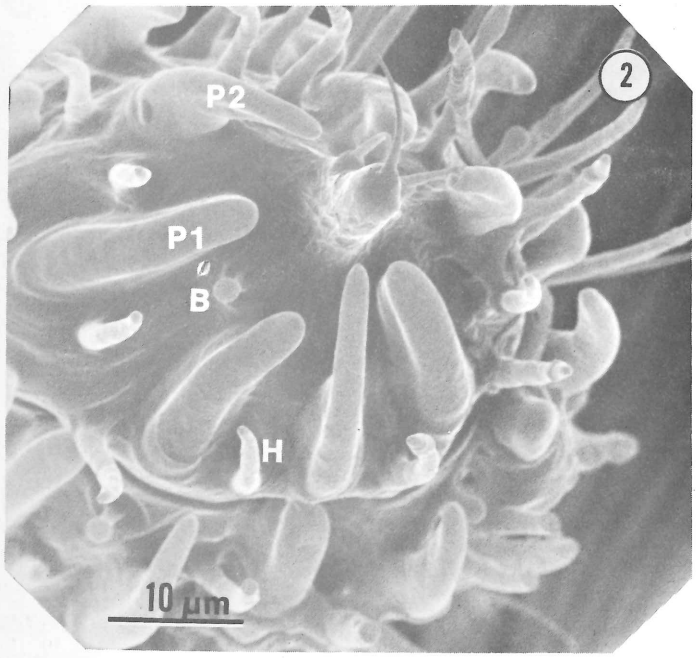
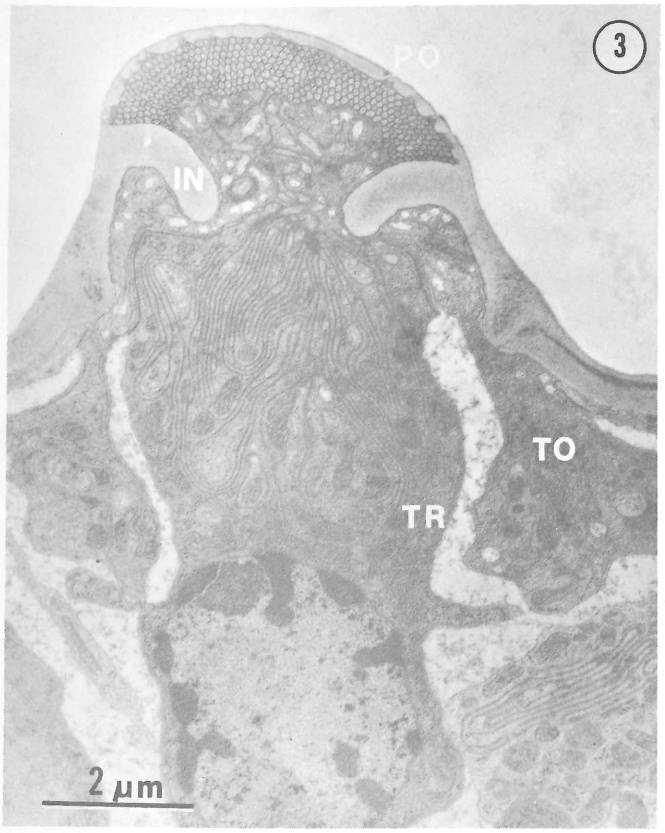


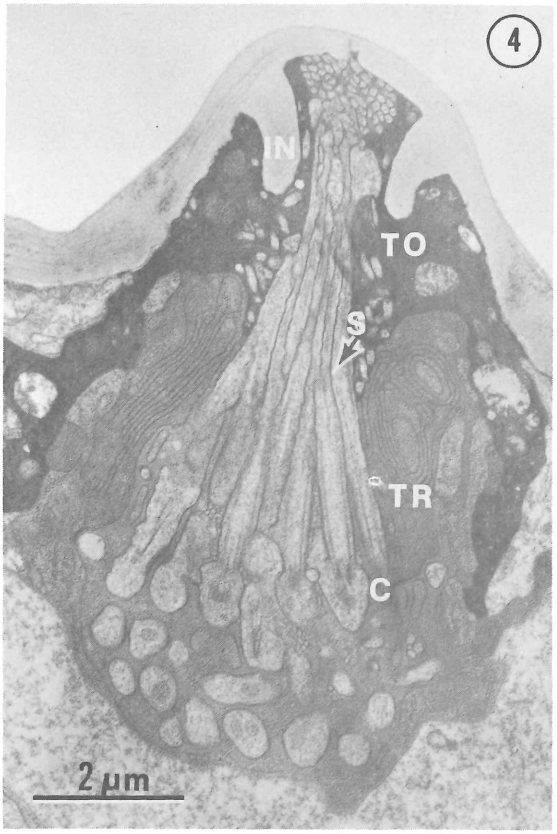
Fig. 1. Antenna of Tetrastichus hagenowii.

Fig. 2. Tip of antenna showing sensillum types.

Fig. 3. Cross-section of thin-walled sensillum placodeum.

Fig. 4. Cross-section of thick-walled sensillum placodeum in region of nerve entry.





STUDY OF THE ATTACHMENT OF A CHEMOAUTOTROPHIC AND THERMOPHILLIC MICROORGANISM ON MINERAL SURFACES

V.K. Berry

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Biological mineral transformation is common to all cellular life, but quantitatively significant transformations are restricted to a few groups of organisms. *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans* belong to this group of microorganisms and play an important role in the bacterial oxidation of mineral sulfides and elemental sulfur to water soluble sulfates and ferrous iron to ferric state. These microorganisms are chemosynthetic aerobic autotrophs. A thermophile[1] designated *Caldariella*[2] and having the same characteristics as *Thiobacilli* is used in this study. The necessity of physical contact or attachment of bacteria to mineral surfaces during oxidation reaction is not fully established yet. Schaeffer, et al.[3] demonstrated that physical contact or arrachment of *T. thiooxidans* is essential for oxidation of sulfur. This was also shown by Baldensperger, et al.[4] on colloidal sulfur. Silverman[5] proposed a direct contact mechanism for sulfide minerals. Though it was not verified by direct evidence but it was demonstrated by observations of bacterial acceleration of the rate of oxidation of iron free sulfide minerals such as covellite (CuS), chalcocite (Cu_2S), molybdenite (MoS_2), etc. This mechanism was difficult for him to demonstrate with iron containing sulfide minerals such as pyrite (FeS_2), chalcopyrite ($CuFeS_2$), etc. In this study direct observation of bacterial attachment to pyrite and chalcopyrite in the oxidation process is reported.

Waste ore sections of pyrite and chalcopyrite ranging in size from 3-6 mm in length and 1-3 mm thick were polished on two flat sides. The slices were washed in distilled water in an ultrasonic cleaner to remove debris and then air dried. Erlenmeyer flasks, each containing culture medium[6] with 0.02% yeast extract[1] (final concentration) and pH adjusted to ~2.5 were covered with aluminum foil and sterlized in steam under pressure. Four specimen slices of each kind were transferred to each of the two flasks on cooling. The flasks were then inoculated with *Caldariella* from stock culture and incubated in water bath at 60°C for four weeks. After four weeks the samples were removed, rinsed lightly in distilled water and allowed to dry in air for 24 hrs. The samples were then mounted on standard aluminum stubs with silver paste and coated with 400-500A° 60/40 Au/Pd in a sputtering unit. The specimens were then examined in a Hitachi-Perkin Elmer HHS-2R scanning electron microscope fitted with an Ortec energy dispersive X-ray analysis system and operated at 25KV in the secondary electron emission mode.

The results of this study show that the bacteria attach selectively to pyrite and chalcopyrite phases. Pyrite and chalcopyrite phases were identified by energy dispersive X-ray analysis by monitoring X-ray maps of the area and selecting Cu-Fe-S for chalcopyrite and Fe-S for pyrite as the region of interest in the energy spectrum. Figure 1 shows these observations. Figure 1(a) shows bacterial attachment somewhat systematically over the entire $CuFeS_2$ phase region but not on the silicate matrix. Figure 1(c) shows the same selective attachment on pyrite surface. Again no attachment on the silicate martix is seen. Figure 1(b) and (d) are magnified views of attached bacteria. Different cell sizes can be seen and the cells have collapsed and deformed morphologically due to a lack of cell wall[1] rigidity.

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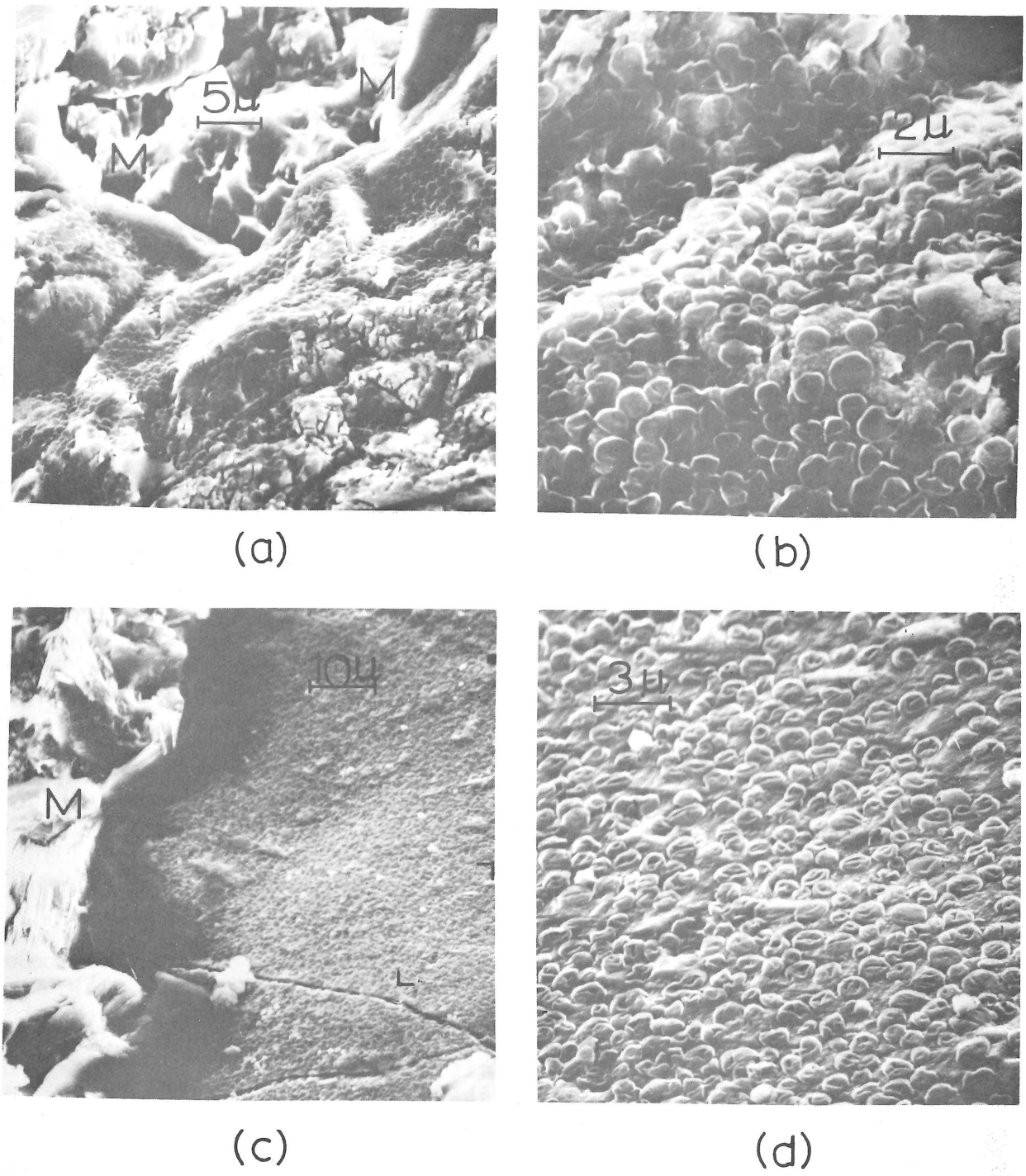

Fig. 1: (a) *Caldariella* on chalcopyrite, the attachment is only on the chalcopyrite region, silicate matrix shown marked M. (b) Magnified view of of bacteria on chalcopyrite region. (c) *Caldariella* on pyrite. Silicate matrix seen on the left marked M with no bacteria. (d) Magnified view of the portion marked in (c).

SPECIES-SPECIFICITY OF ECHINOID FERTILIZATION

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The species-specificity of echinoid fertilization has been previously investigated, but a definitive explanation of this phenomenon has not been accomplished. Our ultrastructural observations have identified an event, primary gamete binding, which exhibits species-specificity of occurrence.

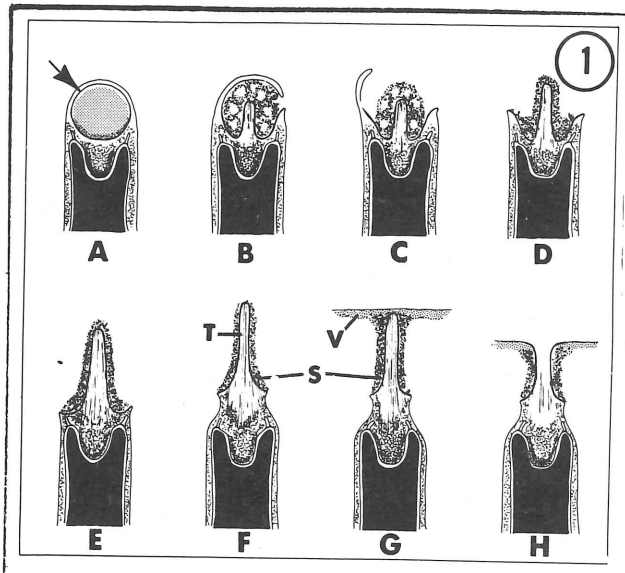
Early fertilization comprises four sequential events: penetration of egg jelly by sperm, acrosome reaction of sperm, primary gamete binding, and gamete membrane fusion (Fig. 1). The acrosome of the unreacted sperm contains the acrosomal vesicle (Fig. 1A). In response to egg jelly, the acrosome reaction is initiated by fusion and loss of the acrosomal vesicle membrane and the overlying sperm plasma membrane around the equator of the vesicle (Fig. 1B,C). The remaining basal portion of vesicle membrane is everted to form the acrosomal tubule (Fig. 1B-F). The vesicle contents are not dispersed, but remain adherent to the tubule membrane forming a persistant extracellular coat on the surface of the reacted sperm. Large numbers of reacted sperm become bound to the egg surface via a morphologically identifiable association of gamete extracellular coats (the coat on the reacted sperm and the vitelline envelope of the egg, Figs. 1C, 2). We have termed this reversible event, primary gamete binding. Subsequently, cytoplasmic confluence between the fertilizing sperm and the egg is established by gamete membrane fusion (Fig. 1H). Ultrastructural observation also indicates that the fertilizing sperm has previously undergone primary gamete binding.

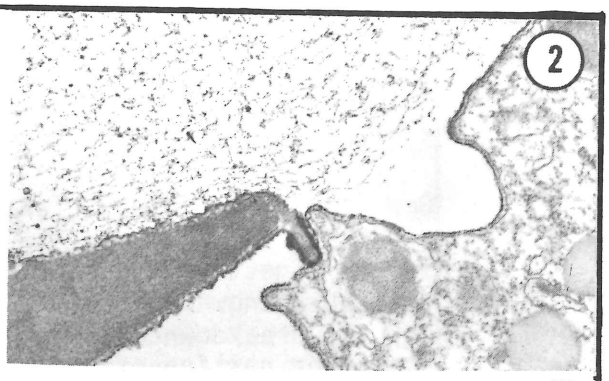
Homologous and heterolgous crosses were examined ultrastructurally to determine if one or more of these events exhibited species-specificity of occurrence (Fig. 3). Four species of sympatric echinoids with simultaneous breeding seasons and low percentages of cross-fertilization were used. In each homologous cross, primary gamete binding was frequently observed; in some instances, the fertilizing sperm was identified. However, although sperm are able to penetrate and undergo a normal acrosome reaction in heterologous egg jelly (such sperm were often seen in close proximity to the vitelline envelope, Fig. 5), primary binding of heterologous gametes was never observed.

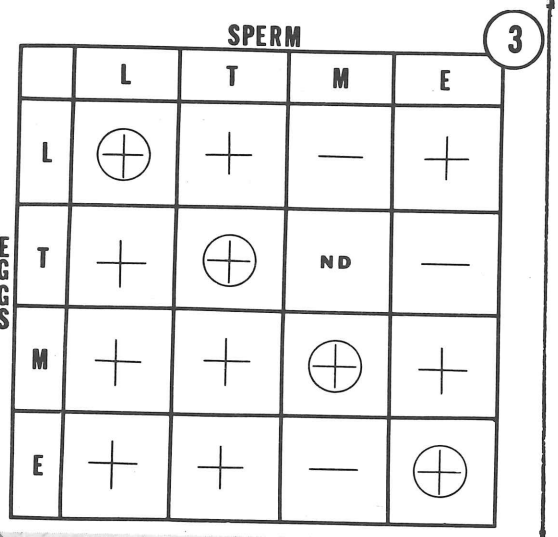
Quantitative data on homologous and heterologous gamete binding was obtained. The results of a representative experiment are shown in Fig. 4. In each homologous cross, large numbers of sperm underwent reversible binding. However, comparable binding of heterologous sperm did not occur.

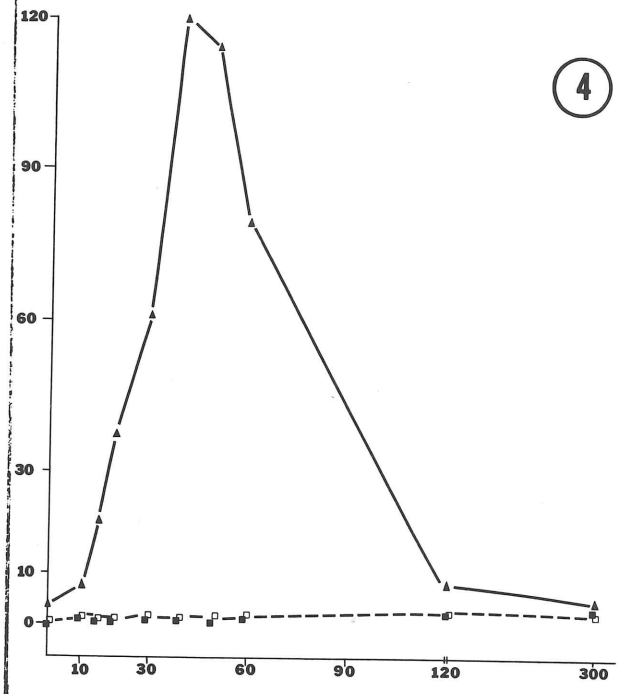
It is suggested that specific recognition and binding of gamete extracellular coats stabilizes initial gamete contact and facilitates homologous gamete membrane fusion. Failure of heterologous gametes to undergo primary binding, may result in unstable contact and exclusion of heterologous sperm from further gamete interaction.

This investigation was supported in part by a Bermuda Biological Station S.L. Wright Fellowship to B.L. Hylander and a University of Maine Faculty Research Award to Dr. R.G. Summers.









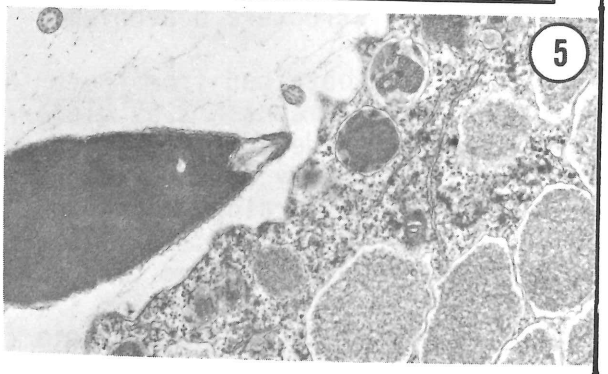


Fig. 1. Early fertilization. A. The unreacted acrosome, acrosomal vesicle (arrow). B-F. The acrosome reaction results in the formation of an extracellular coat (S) on the acrosomal tubule (T). G. Primary gamete binding, an association of extracellular coats- the vitelline envelope (V) of the egg and that of the reacted sperm (S). H. Gamete membrane fusion.

Fig. 2. Primary gamete binding.

Fig. 3. Acrosome reaction and primary gamete binding in homologous and heterologous crosses. L, Lytechinus; T, Tripneustes; M, Mellita; E, Echinometra. (+) Acrosome reaction, (⊕) Primary gamete binding, (-) No reaction observed, (ND) No data.

Fig. 4. Primary gamete binding of homologous and heterologous sperm to T eggs. Abcissa: seconds post-insemination; Ordinate: number of sperm bound per egg. T♂, ●—● ; E♂, ■---■ ; L♂, □---□ .

Fig. 5. A sperm which has penetrated and undergone a normal reaction in heterologous egg jelly. T♂ x L♀ .

FIXATION OF THE CRYSTALLINE STYLE OF BIVALVE MOLLUSCS (ANODONTA SP.) FOR SCANNING ELECTRON MICROSCOPY

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In the digestive tract of certain bivalves such as oysters and mussels, a structure known as the crystalline style functions as a grinding mechanism for the breakdown of large food particles. The style is located in the posterior portion of the bivalve stomach where it is extruded from the style sac and slowly rotated across the interior of the stomach. It grinds against a hardened portion of the stomach wall known as the gastric shield. The rotation of the style serves to draw the contents of the stomach through this organ, thus taking the place of any musculature that would otherwise be necessary. In several of the species that produce this structure, the style is re-absorbed when the bivalve is not feeding. This has lead to the proposal that the style serves as a nutrient source in starvation condition as well as an organ for maceration. The style is extruded again when food is available, incorporating in its structure the first food particles to enter the stomach.

The needle shaped flexible style is usually several centimeters long but varies in length and width dependant on the size of the bivalve. The style consists of 87% water, 12% proteins and organic materials such as mucin, globulins and albumin and 1% inorganic materials. The large percentage of water present as well as the mucoid nature of the style makes fixation of this structure a problem.

Styles obtained from Anodonta species, a freshwater mussel, were fixed by several means: 2.5% glutaraldehyde followed by 1% OsO_4 (Fig. 1); 2.5% glutaraldehyde with 0.05% (w/v) ruthenium red (Fig. 2); 2.5% glutaraldehyde followed by 1% OsO_4 with 0.05% ruthenium red added to the OsO_4 (Fig. 3,4); 2.5% glutaraldehyde without a post fix (Fig. 5,6). All fixations were buffered by 1% cacodylate, pH 7.4 with all other physical parameters such as temperature and time, critical point drying, mounting, remaining the same for each fixation. In each type of fixation, mucoid strands (M), food particles (F) and bacteria (B) can be observed on the style surface.

Due to the greater amounts of shrinkage which occurred whenever OsO_4 was used, the cracks are most prominent in these fixations (Fig. 1,3). Addition of ruthenium red also produced some cracks, but never to the extent of the OsO_4 (compare Fig. 1 with 2 and 5). The addition of ruthenium red to any fixation increased contrast, probably due to deposition of the dye around any projection from the style surface (compare Fig. 2,3,4 with 5 and 6). The use of OsO_4 and ruthenium red together appear to produce even greater shrinkage artefacts. This shrinkage with the added effect of the dye reveals areas where many mucoid strands stand out, making it hard to distinguish strands from bacteria.

Glutaraldehyde without post-fixation gave the best overall results. No cracking or shrinkage was obvious. Bacteria on the surface are readily recognizable (compare Fig. 4 with 6). The only difficulty with this fixation may be the faint transparency (T) of objects on the style surface, most likely due to the fact that gluteraldehyde is not a good secondary electron emitter.

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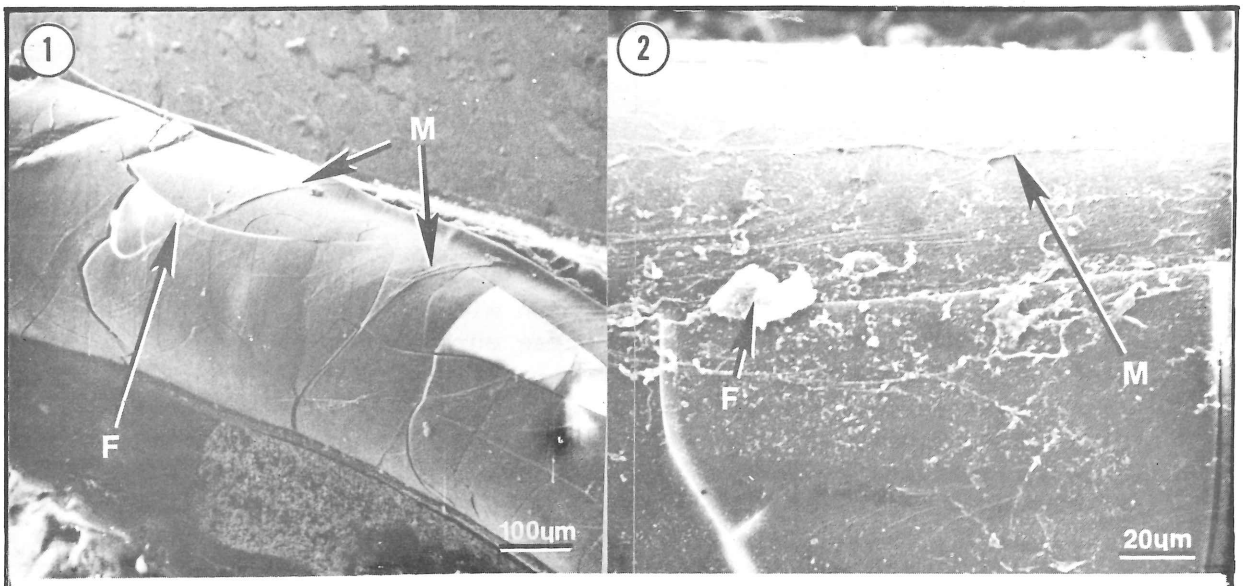
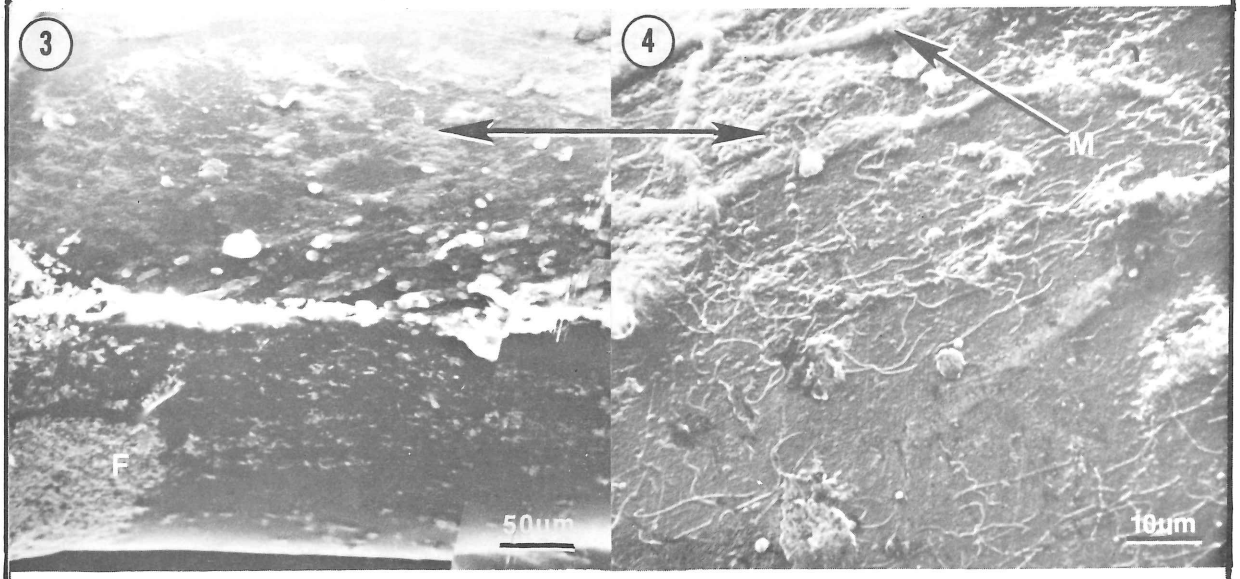
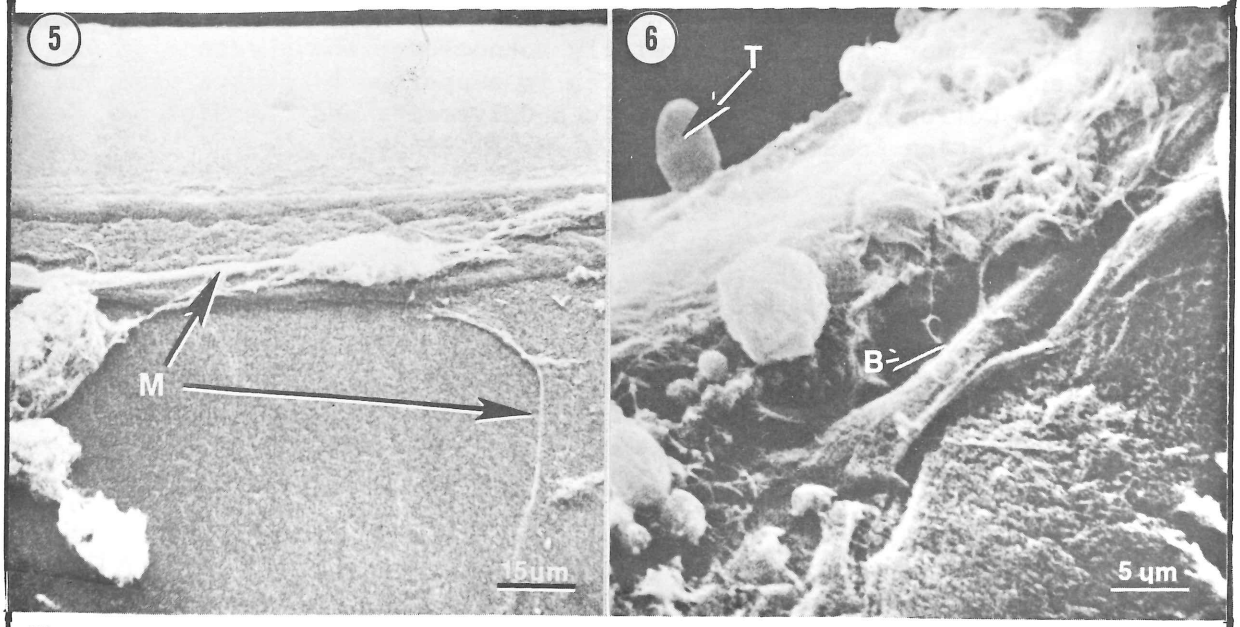


Glutaraldehyde and OsO_4.

Glutaraldehyde with OsO_4, ruthenium.



Glutaraldehyde with OsO_4 and ruthenium red.



Glutaraldehyde without post fix.

ULTRASTRUCTURAL STUDIES OF ISOLATED POLYTENE CHROMOSOMES BY SCANNING ELECTRON MICROSCOPY

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Scanning and transmission electron microscopy studies of mitotic chromosomes have demonstrated that these chromosomes are composed of single intricately coiled and folded chromatin fibers 200-300 A in diameter (1). Transmission electron microscopy studies (2) have shown similar fibers in dipteran polytene chromosomes. It has been proposed that these fibers are extended in the less densely appearing interband regions and become more tightly coiled or folded at specific sites to produce the densely appearing banded regions (3). With the scanning electron microscope it is now possible to observe the characteristic appearance of the chromatin fibers in the band and interband regions of isolated intact polytene chromosomes. Furthermore, the morphology of the nucleolus and Balbiani rings can be observed as specialized structures in the chromosome.

Salivary glands were dissected from the fourth instar larvae of Chironomus stigmaterus. The glands were briefly fixed in 3:1 ethanol:acetic acid and individual chromosomes were isolated in 45% acetic acid. The isolated chromosomes were critical point dried and coated with a thin layer of gold. This technique preserved the morphology of the chromosomes and permitted observation of the ultrastructure under the scanning electron microscope.

Figures 1 and 2 demonstrate the presence of the chromatin fibers within t chromosome. Figure 1 shows chromosome I with its distinct Balbiana ring. In interband regions it is possible to observe single chromatin fibers extending linearly in the chromosome. In banded regions, these fibers appear to be tightly coiled or folded which gives the band its dense globular appearance. This is especially evident in figure 2. In figure 3 a higher magnification of the Balbiani ring is shown. Both fibrillar and granular elements are observed in this structure. Figure 4 shows the nucleolus of chromosome II encircling the chromosome.

The author wishes to gratefully acknowledge the guidance of Dr. T. E. Brady and J. F. Baily. This research is supported by grants from The Institute for College Research, Texas Tech University and the National Science Foundation #BMS-75-10974 to Dr. T. E. Brady.

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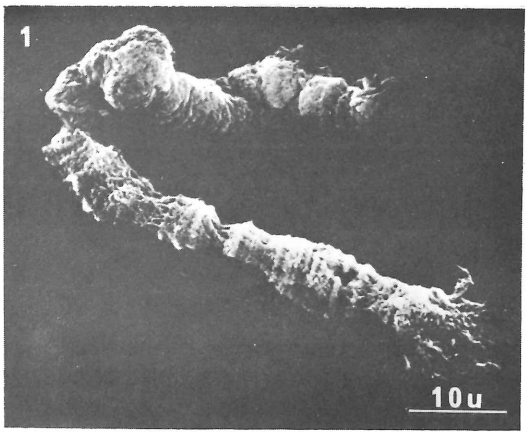


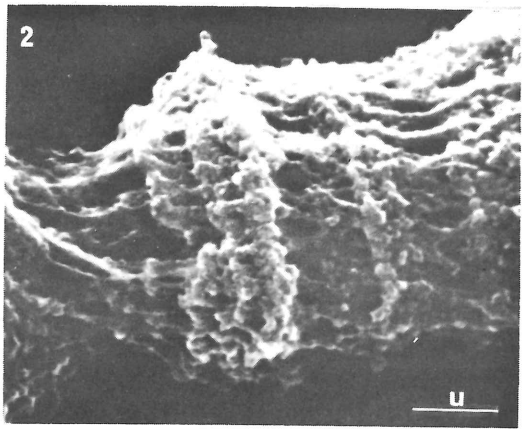
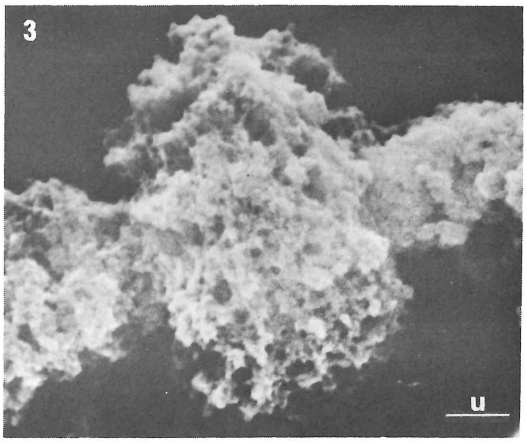


Figure 1. Chromosome I with its distinct Balbiani ring.

Figure 2. Chromatin fibers within the chromosome. Banded areas appear to represent fibers which are highly coiled or folded.



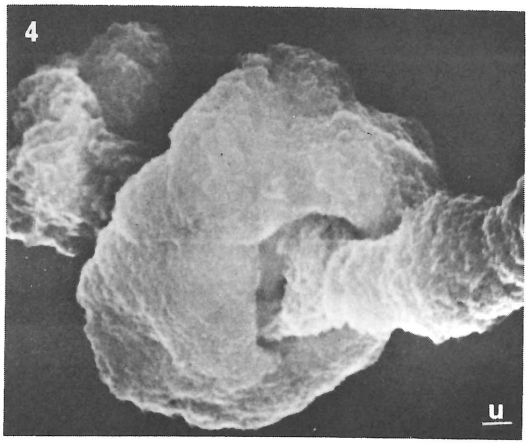


Figure 3. A higher magnification of the Balbiani ring showing the fibrillar and granular elements.

Figure 4. Nucleolus encircling the chromosome.

IMMUNOFLUORESCENCE AND IMMUNOELECTRON MICROSCOPY OF TUBULIN DISTRIBUTION IN MITOTIC MAMMALIAN CELLS

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Monospecific antibody directed against bovine brain tubulin was employed as an immunofluorescent and immunocytochemical probe to study the distribution of tubulin and microtubules in dividing mammalian cells at the light and EM levels.

Immunofluorescence microscopy. Rat kangaroo cells (strain PtK_1) were grown on coverslips in Ham's F-10 medium. The coverslips (with cells attached) were them processed for the indirect immunofluorescent localization of tubulin using the method of Fuller et al (1). After fixation for 20 min with 3% formaldehyde followed by absolute acetone at -10°C for 7 min, the cells were incubated with monospecific rabbit immunoglobulin prepared against bovine brain tubulin. Subsequently, the cells were incubated with fluorescein-tagged goat antiserum against rabbit immunoglobulin G. The preparations were then mounted in glycerol and examined with a Leitz ultraviolet microscope. Control cells were fixed and incubated with the fluorescein-tagged goat antiserum alone or non-immune serum followed by fluorescein-tagged goat antiserum before examination.

Routine electron microscopy. PtK_1 cells, grown as monolayers in 60mm Falcon dishes, were fixed in 3% glutaraldehyde, post-fixed in 1% OsO_4, and embedded according to the procedure of Brinkley et al (2). Sections were obtained with a diamond knife, stained with uranyl acetate followed by lead citrate and examined with a Philips 201 electron microscope.

Immunoelectron microscopy. PtK_1 cells were grown in Falcon dishes as described above and fixed with 3% formaldehyde in phosphate-buffered saline (PBS). The cells were then treated with the rabbit (anti-tubulin) antibody, rinsed with PBS, and incubated with peroxidase-tagged goat antiserum against rabbit immunoglobulin (indirect method). As controls, separate dishes of cells were incubated with the peroxidase-tagged goat antiserum alone (without exposure to rabbit anti-tubulin antibody) and were subsequently treated in a manner identical to the experimental preparation. The immunocytochemical localization of peroxidase-antibody complex was carried out using the diaminobenzidine method of Graham and Karnovsky (3). The cells were then osmicated and flat-embedded for EM (as described above). Serial sections were collected on Formvar-coated slotted grids and placed directly in the Philips 201 electron microscope without further staining.

Results. Figure 1a shows the typical appearance of the glutaraldehyde-fixed mammalian mitotic apparatus at anaphase as seen in routine EM preparations stained with uranyl acetate and lead citrate. The centriole, pole-to-chromosome microtubules, and kinetochores all exhibit characteristic features described by previous investigators. Figure 1c (inset) shows the immunofluorescent tubulin staining pattern of the PtK_1 spindle apparatus when viewed in the ultraviolet microscope. Although the centrioles cannot be resolved by this procedure, the spindle fibers and chromosomes are very apparent. Control preparations failed to exhibit fluorescence in the spindle, and only a very weak non-specific fluorescence was present in the surrounding cytoplasm. Figure 1b illustrates the specific immunocytochemical staining of tubulin in the PtK_1 mitotic spindle as seen at

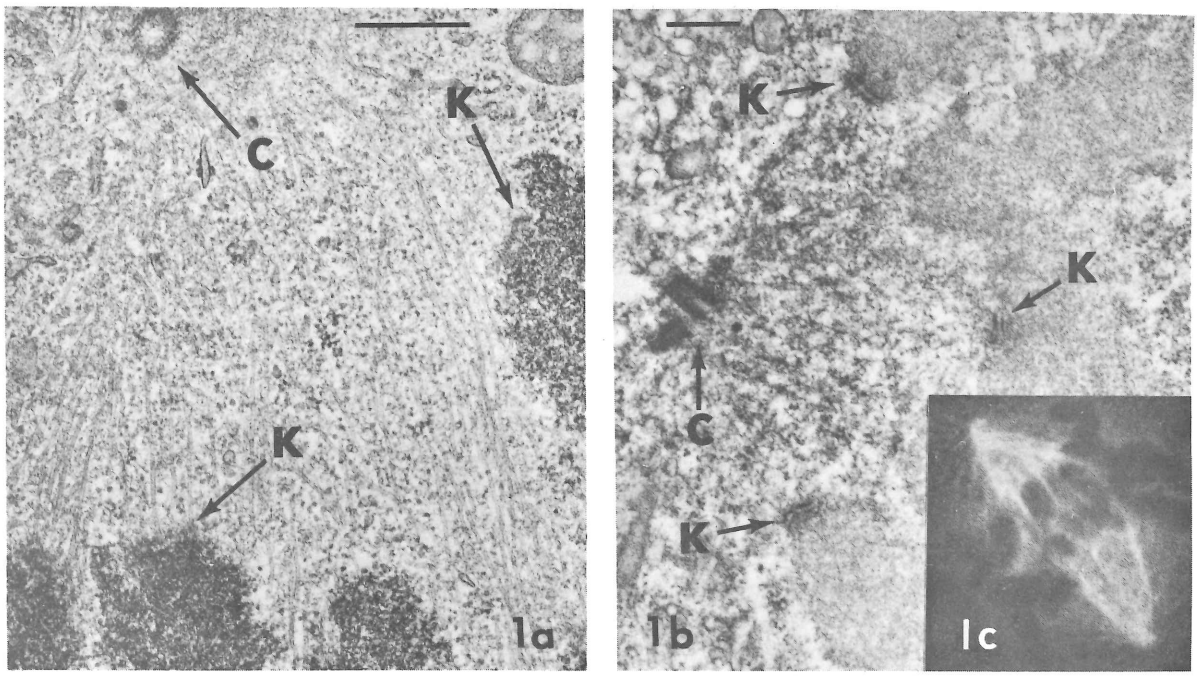

Figure 1. (a) Glutaraldehyde-OsO_4 fixed preparation of a dividing (anaphase) PtK_1 cell showing centrioles (C) and kinetochores (K). Bar is .5μ. (b) Immunoelectron microscopy of a dividing PtK_1 cell showing centrioles (C) and kinetochores (K). Bar is .5μ. (c) Immunofluorescence of the PtK_1 spindle.

the EM level. The centrioles stain very densely with this procedure (as would be expected, since they are composed of microtubules). The pole-to-chromosome microtubules stained with this method exhibited a loosely fibrous structure (compare with Figure 1a) which is probably due to their characteristically poor fixation with formaldehyde (which was necessary to retain antigenicity). The most striking feature of this preparation, however, is the dense staining of the kinetochores on the nonstaining chromosomes. These results strongly suggest that the kinetochores are (at least in part) composed of tubulin, a finding heretofore unreported in the literature. Thus, the kinetochore, a microtubule organizing center (MTOC), may be composed of the protein tubulin which serves to nucleate the assembly of cytoplasmic tubulin into spindle microtubules.

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CILIARY MICROTUBULE DEFORMATION: A PRELIMINARY REPORT

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Various hypotheses for the mechanism of ciliary motility either purport or oppose the concept of microtubule contraction. Recent literature, supporting the Sliding Microtubule Model has established that microtubule doublets move relative to one another during the process of bending. Satir (1968) concluded that there is no change of length associated with the doublet movement during bending of cilia. He based this conclusion upon a close correlation between observed and predicted values of the extension of specific doublets beyond the termination of other doublets in ciliary tip regions. Circular relationships formed the basis for his predicted values. Such an analytical procedure, as applied by him, presupposes that the microtubules remain in a two dimensional configuration throughout the length of the cilium.

An alternate mode of bending exists. The microtubules may rotate or twist slightly about the longitudinal axis of the axoneme as bending proceeds. Under such conditions, determination of the length of a specific doublet, and of variations in length, would be dependent upon three dimensional measurements.

This communication is a preliminary report on a study designed to explore the possibility that twisting may accompany bending in cilia. The study has had two specific goals: (1) to establish an analytical procedure which is independent of curvilinear relationships; and (2) to obtain appropriate micrographic information from thin sections of cilia which can be used in analysis.

The analytical procedure is based on the radial symmetry of an axoneme's cross section. When any three doublets (or two doublets and a central microtubule) are projected onto a plane perpendicular to the cross section, there is a specific ratio formed by the projected distances between the doublets that can be used to determine the angle formed between the plane of projection and a reference plane within the cross section. A thin, longitudinal section through a cilium forms an arbitrary, perpendicular plane through the axoneme's cross section. With the appropriate microtubules present, measurements between the microtubules at a specific longitudinal location can be used to determine the angle between the plane of the thin section and a reference plane of the axoneme's cross section. Comparison of this angle to one determined at a more distal or proximal location establishes whether rotation of the axoneme had occurred at the time of fixation. With this angle determined, it is also possible to establish three dimensional coordinates for points on all of the axenome's microtubules. This provides a means for approximating the length of doublets.

Longitudinal sections of Rana pipiens mouth epithelial cilia are shown in Figure 1. Three cilia in the micrograph were sectioned from the basal body to the tip and have at least three microtubules which can be seen for the full length. Higher magnification micrographs for each cilium have been used in the analysis. Note that each cilium is in a different motile position. The results of the analysis allows comparison of the rotational features as well as the relative amounts of microtubule shortening or elongation between motile positions.

The analytical procedure has been applied to computer analysis in which the basic measurements, trigonometric, rotational, microtubule length and relative length computations have been determined from grid coordinate data input obtained from the micrographs.

Discussion will be limited to describing the type of information gained from micrographs, details of the analytical approach, and results of the analysis.

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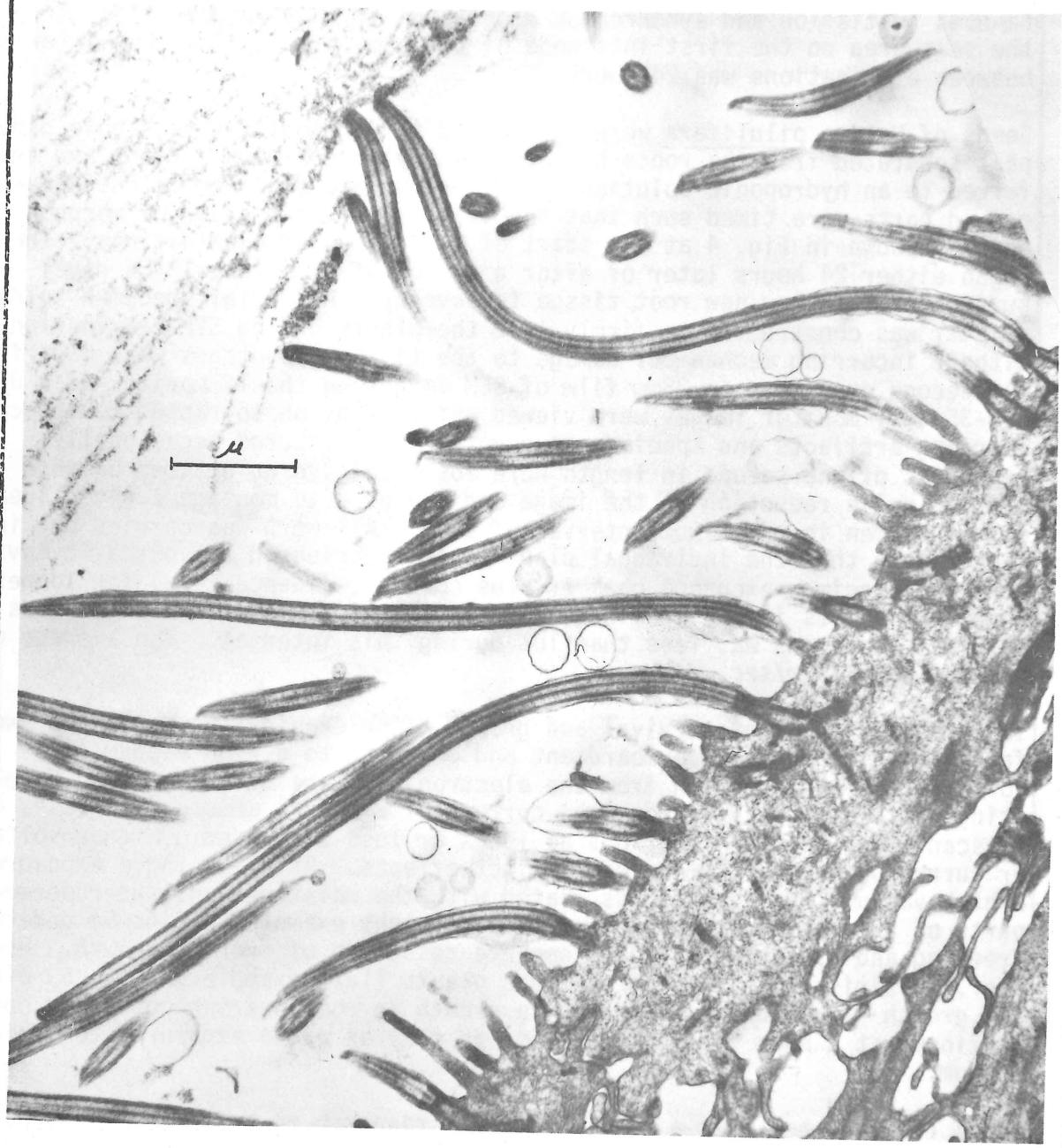

FIGURE 1. Characteristic longitudinal sections of Rana pipiens mouth epithelial cilia used in computer analysis of microtubule deformational features are shown in this micrograph. (X18,500, glutaraldehyde-osmium, R. Redding, Ultrastructure Laboratory, Dept. of Biol. Sciences, Univ. of Texas at El Paso).

LIVING PLANTS CONTINUE TO GROW AFTER EXAMINATION BY SCANNING ELECTRON MICROSCOPY

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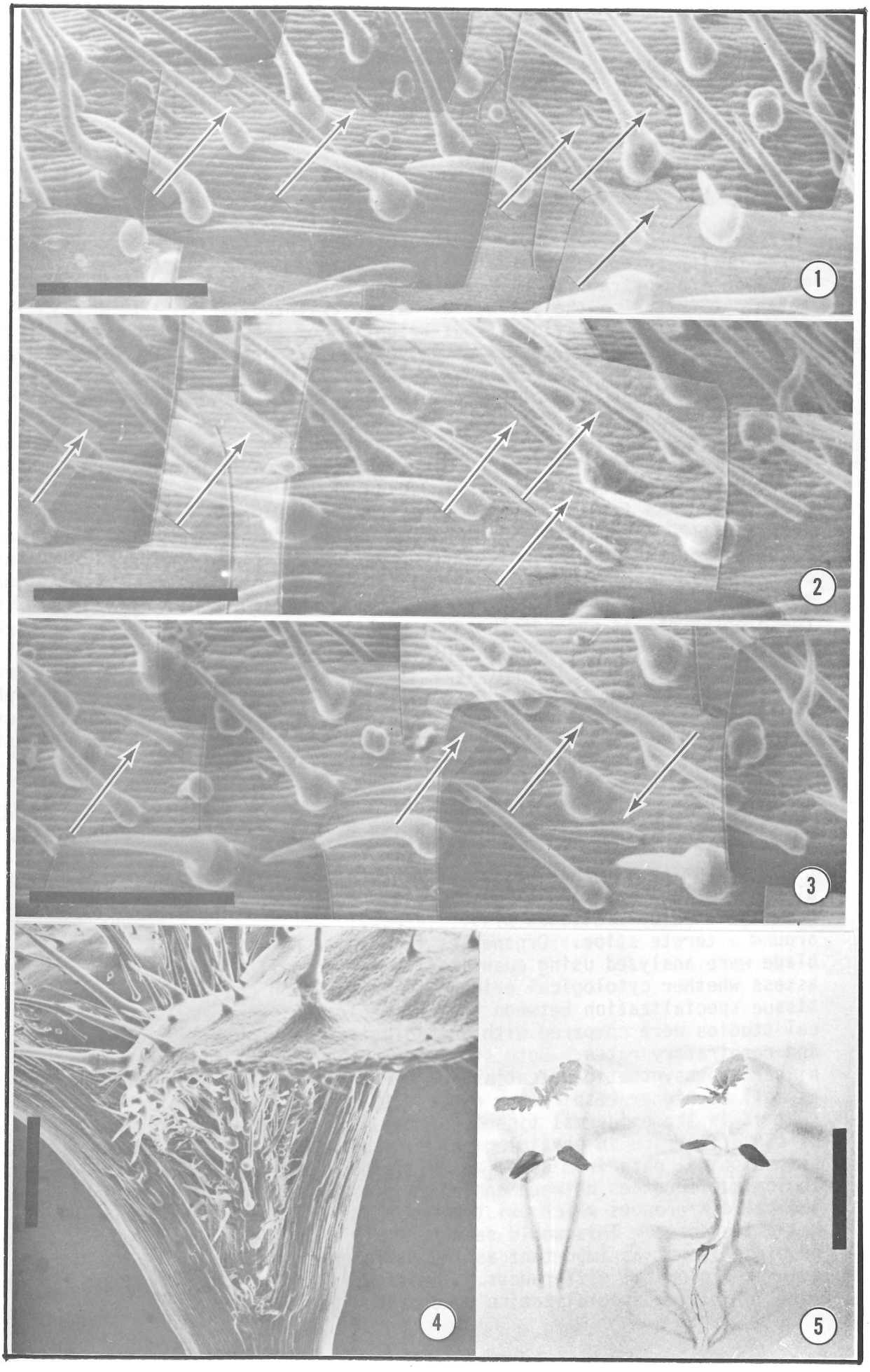
It has been found that, within the constraints and limitations described below, the same individual living plant, Urtica pilulifera (common name - Stinging Nettle), continues to grow after repeated brief (6 minute) examination by conventional scanning electron microscopy. Fig. 1-3 show the simultaneous initiation and synchronous elongation of many epidermal hairs in the same area on the first internode of the same plant. The time interval between examinations was 24 hours.

Seeds of Urtica pilulifera were germinated in vermiculite, the above ground part separated from the roots by a perpendicular cut with a razor and transferred to an hydroponic solution. Germination and transfer of the above ground parts were timed such that the plants were at a stage of morphogenesis shown in Fig. 4 at the start of the daily SEM examinations. They began either 24 hours later or after a period of time (usually 4 days) sufficient to allow new root tissue to develop. A special specimen holding adapter was constructed to firmly hold the plants during SEM examination without incurring mechanical damage to the tissue. Montages were made from one second exposures on 35mm film of SEM images on the TV monitor of a JEOL JSM-35. TV monitor images were viewed while being photographed to detect charging artifacts and specimen movements which occurred occasionally. Exposures of one second in length were found to give an optimum balance between noise reduction in the image and the need to montage a given area on a specimen in a minimum interval of time. All work was carried out in a manner such that the individual plant never experienced a hyperbaric environment (specimen exchange chamber plus column residence time) for longer than six minutes in any examination interval. Average fresh weight loss for rootless specimens was less than 10% during this interval. The average outgassing was 72µgm/sec.

In this species plant survival and growth after examination depend on two factors: electron beam bombardment and exposure to a high vacuum (10^{-4} Torr range). No reaction from the electron beam was detected if an accelerating voltage of 10kv, a specimen current of 2×10^{-8} amps/cm^2 or less, and TV scan rates at magnifications of 1000X or less were used. Higher voltages or currents, however, produced distinct effects. After a single exposure to a high vacuum, the tissues associated with the moistened and water permeable parts of the root in root-bearing plants (right example in Fig. 5) undergo freezing and drying and almost complete cessation of overall growth. However, in similarly sized but rootless plants (left example in Fig. 5) overall growth is reduced as compared to growth in root bearing controls, but continues at a near constant rate for as many as eight exposures to a high vacuum.

These results show that a wide variety of research on surface morphogenesis in many plant systems may be possible without special equipment or elaborate preparations.

Line scales: Figs. 1-3, 200µm; Fig. 4, 1mm; Fig. 5, 3cm.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ABSTRACTS

AGGREGATION AND RANDOM-REDISTRIBUTION OF INTRAMEMBRANE PARTICLES BY POLYCATIONIC FERRITIN BOUND TO THE OUTER SURFACE OF NATIVE ERYTHROCYTE GHOST MEMBRANES

Jeffrey Day and Charles R. Hackenbrock, Dept. of Cell Biology, University of Texas Health Science Center at Dallas, Texas

Using freeze fracture electron microscopy, we have studied the distribution of anionic sites on the surface of native erythrocyte ghost membranes related to the lateral translational redistribution of intramembrane particles (IMP). We have found that low levels of polycationic ferritin (PCF) at pH 7.4 cause aggregation of IMP. In addition, these low levels of PCF were bound in patches on the membrane surface which were restricted to areas over the aggregated IMP (Hackenbrock, Day, Tex. Rpt. Biol. & Med., V.33, No. 2, 1975,p. 347). Subsequent additions of PCF at pH 7.4 resulted in a random-redistribution of previously aggregated IMP and a nearly complete covering of the etched outer surface by PCF. PCF induced aggregation of IMP failed to re-randomize if the patched PCF was first crossbridged by native anionic ferritin. In this case, additional PCF bound extensively to membrane surface areas over IMP-free membrane regions. Thus, anionic sites occur over regions containing IMP and over IMP-free regions. In addition, the PCF induced lateral translational movements of IMP appear to be electrostatic in nature. In agreement with these observations and conclusions, we have recently determined that high concentrations of PCF showed complete coverage of the ghost membrane after aggregation of IMP by low pH(5.5). The surface anionic sites located over IMP-free regions of frozen membranes may be related to electronegative phospholipids, glycolipids, peripheral proteins, or proteins other than major glycoproteins (-the majority of major glycoproteins are currently accounted for by IMP). Supported by National Science Foundation, Grant BMS 75-20141.

A QUANTITATIVE ULTRASTRUCTURAL EVALUATION OF TISSUE SPECIALIZATION IN THE BROWN ALGA SARGASSUM FILIPENDULA. Wayne R. Fagerberg. Dept. of Biology. Univ. of Texas at Arlington.

Sargassum is a member of the order Fucales, an order characterized by complex anatomical and morphological development. Morphologically Sargassum is distinguished by flattened blades laterally attached in a spiral fashion around a terete stipe. Organelles from analogous tissues in the stipe and blade were analyzed using quantitative analytical techniques (stereology) to assess whether cytological evidence existed which could be used to describe tissue specialization between these two organs. The results of the cytological studies were compared with physiological data obtained for photosynthetic and respiratory rates. Both stereological and physiological data suggest a higher photosynthetic potential for the blade tissues than those of the stipe, as well as higher respiratory rates. It appears from cytological evidence that it is the epidermal tissues of the blade which can account for a majority of the difference in physiological data between the two organs. Analysis of stereological data from stipe and blade tissues suggests that there are cytological differences between analogous tissues of these organs and that physiological differences which exist between the two are probably due to cytological differences. This would seem to imply that the morphology of the flattened blade is not as important as the difference in cytology in accounting for such physiological differences. The results of this study suggest a high level of tissue specialization undescribed for algal systems.

HISTOCHEMICAL STUDY OF MICROORGANISMS FOUND IN HUMAN DENTAL PLAQUE

James P. Fancher, The University of Texas Dental Branch at Houston
Introduced by Gwynfryn Hopkins, Department of Histology, UTDB.

An attempt has been made to differentiate ultrastructurally various species of microorganisms in human dental plaque by utilizing horseradish peroxidase as a direct label. Thick sections (1μm) show differential staining with peroxidase-diaminobenzidine complex. Preliminary investigation shows staining to be specific for cocci species. It is thought that the organisms stained may be some of the causative agents involved in dental caries and periodontal disease. This method may be used as a research aid in identification of these organisms.

LIGHT AND ELECTRON MICROSCOPY OF CLOACOGENIC CARCINOMAS

John Gillespie, Bruce Mackay, Richard G. Martin and Robert C. Hickey.
Departments of Pathology and Surgery, M. D. Anderson Hospital and Tumor Institute

Cancers arising in the vicinity of the ano-rectal junction present a variety of histologic patterns. Fewer than 3% are thought to be derived from residual cloacal epithelium and are consequently termed cloacogenic carcinomas. Some resemble basal cell carcinomas of skin by light microscopy, while others appear similar to transitional cell carcinomas of urothelial origin. Light and electron microscopic observations on seven cloacogenic carcinomas will be presented. The two histologic types appear to be variants of the same entity. The tumors will be compared ultrastructurally with normal epithelium from the ano-rectal junctional zone, normal urothelium, and squamous carcinomas of the anal canal, basal cell carcinomas of skin, and transitional cell carcinomas of the urinary tract.

ULTRASTRUCTURE OF THE TRICHOPHYTON MENTAGROPHYTES SEPTUM. Yasuo kitajima*, Takashi Sekiya and Yoshinori Nozawa, Department of Biochemistry, Gifu University School of Medicine, Tsukasamachi-40, Gifu, Japan. *Present address, Department of Botany, University of Texas, Austin, Texas 78712.

A septum-rich fraction was prepared from Trichophyton mentagrophytes, a human pathogenic fungus, and the ultrastructural investigation of septa was carried out by shadowing and thin section electron microscopy before and after enzymatic and alkaline treatments. A tri-lamellar structure, consisting of an electron lucid middle layer and two outer electron dense layers, was observed by thin section electron microscopy. By shadowing electron microscopy, the surface ultrastructure of septa exhibited a randomly oriented microfibrillar network structure, which may correspond to the electron dense outer layers of the septum observed in thin section preparations. However, shadowed septa after papain digestion revealed a spiral arrangement of microfibrils consisting of rodlet-like units, which disappeared during chitinase treatment. This spiral structure may therefore be composed primarily of chitin. It is suggested that this spirally arranged microfibrils may correspond to the electron lucid middle layer observed in thin section preparations.

EWING'S SARCOMA: AN ULTRASTRUCTURAL STUDY

Bruce Mackay, Alberto G. Ayala, and John A. Murray, Departments of Pathology and Surgery, M. D. Anderson Hospital and Tumor Institute

Ewing's sarcoma is a small round cell neoplasm of bone that occurs in children and young adults. By light microscopy, the pathologic diagnosis can be difficult because the tumor cells may appear similar to those of other neoplasms affecting the same age group, including rhabdomyosarcoma, neuroblastoma and lymphomas. Primary soft tissue tumors that resemble Ewing's sarcoma by light microscopy have recently been reported. The findings in a light and electron microscopic study of 35 cases of Ewing's sarcoma will be presented, with emphasis on the range of ultrastructural morphology. The presence of glycogen, often in considerable quantities, is a helpful diagnostic feature in most cases. Although the cell of origin remains an enigma, the possible histogenetic significance of glycogen-rich bone marrow cells will be discussed. The differential diagnosis of Ewing's sarcoma by electron microscopy will be illustrated by comparisons with other small round cell tumors.

IS THERE A QUIESCENT CENTER IN PINE ROOT APEX?

A.J.Mia, Dept. of Life Sciences, Bishop College, Dallas.

Root apices of dormant and germinating pine embryos (Pinus banksiana) were studied using cytochemical and subcellular techniques. Cytochemical observations using the staining techniques for DNA, RNA, total and basic proteins, and carbohydrates showed that a group of apical cells above the root cap initials possessed large lightly-stained nuclei in contrast to that of the surrounding cells. At 96 hrs. of germination, most cells surrounding the apical cells, completed the first cycle of mitotic divisions, yet the apical cells rarely showed divisions. Autoradiographic technique using tritiated water showed little or no incorporation of isotope into the nuclei of the apical cells. Finestructure analysis revealed that the apical cells contained large lightly-stained nuclei compared with the small darkly-stained nuclei.

ULTRASTRUCTURE OF FISH HEPATOCYTES FOLLOWING IN VIVO METAL EXPOSURE.

Elsie M. B. Sorensen, Department of Zoology, The University of Texas, Austin, Texas 78712.

The purpose of this investigation was to determine the qualitative ultrastructural alterations in the hepatocytes of a warm-adapted, fresh water teleost following short-term, acute exposures to three arsenic concentrations. The following changes were observed: the appearance of electron opaque areas in the nuclei, cytoplasm, and lysosomes; proliferation of smooth endoplasmic reticulum; aberrant mitochondria; and enlarged myelin figures.

After osmium fixation and post-staining (using uranyl acetate and Reynolds Lead Stain) the previously-mentioned opaque areas were observed; however, these areas were hardly visible following organic fixation in gluteraldehyde. Preliminary studies using energy-dispersive microprobe analysis shows high osmium and lead concentrations in the opaque areas, possibly indicating that (during tissue preparation) arsenic is removed from organometallic formations and that subsequently these areas are preferentially stained by osmium and lead. Analyses for arsenic following both tissue preparations proved inconclusive.

ELLIPSOIDS OF THE VISUAL CELLS OF SEVERAL SPECIES OF SNAKES

Randall H. Stovall, Department of Neurobiology and Anatomy, The University of Texas Medical Center at Houston, Houston, Texas

This paper presents a survey of the ultrastructure of the inner segments of the visual cells of several species of snakes. Specifically, the ellipsoids are considered on the basis of ultrastructure and histochemical data. Ellipsoids are mitochondrial aggregates containing at least two different classes of mitochondria. Generally the matrices of these mitochondria are more dense than is normally seen. Further, vesicles of varying electron density may occur within the mitochondria located centrally in the ellipsoids. The contents of these vesicles are probably lipoidal in nature. It is suggested that these lipoidal vesicles may be analogous to the oil droplets found within the inner segments of the visual cells of most reptiles. Finally, a brief comparison of snake ellipsoid structure is made with that of other vertebrate groups.

COVER MICROGRAPH

SEM of an isolated polytene chromosome (chromosome I) of Chironomus stigmaterus showing a prominent nucleolus encircling the chromosome.

Contributrd by Mike Payne, Tom Brady, and Franklin Bailey. Department of Biological Sciences, Texas Tech University Lubbock, Texas.

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