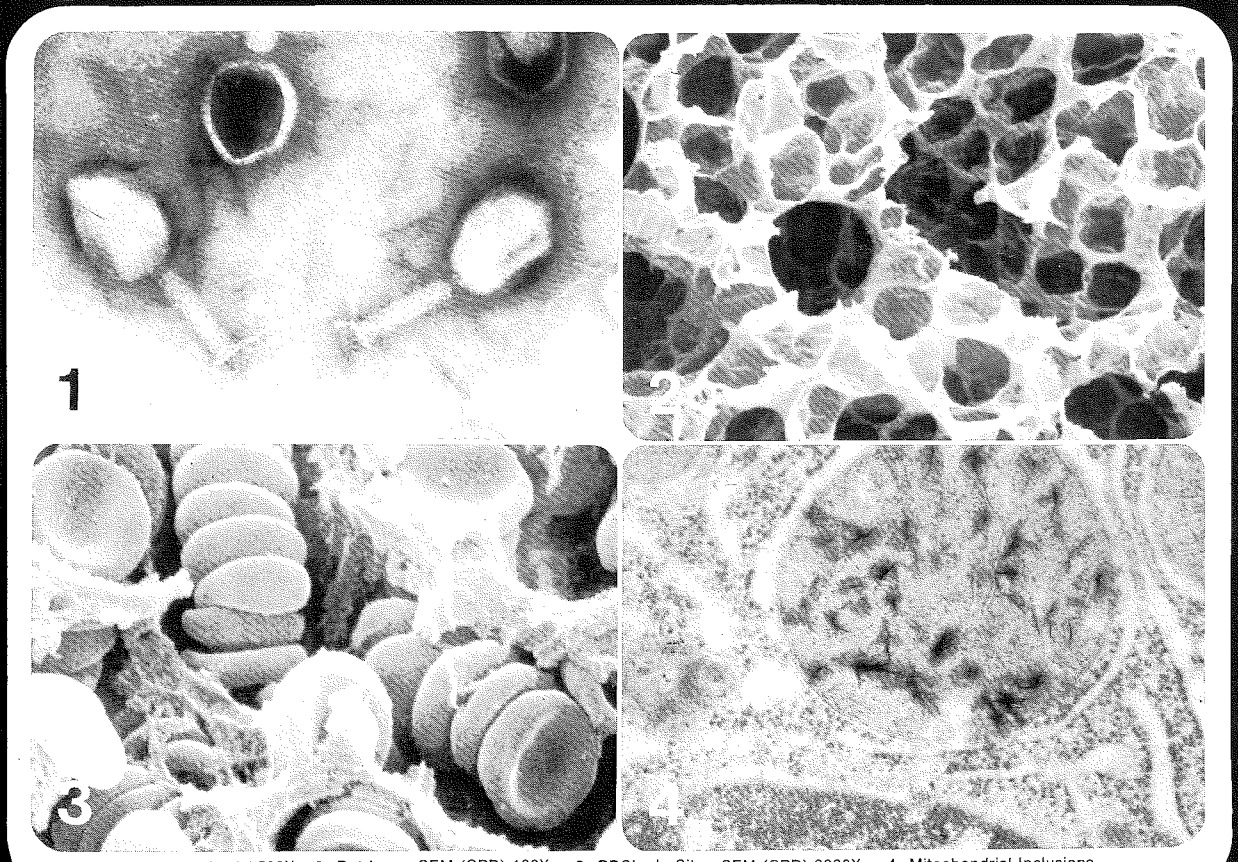
TSEM

Texas Society for Electron Microscopy

NEWSLETTER
VOLUME 10 NUMBER 4
WINTER 1979

Analytical Research Services



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Volume 10, Number 1

Winter, 1979

Texas Society for Electron Microscopy
"For the purpose of dissemination of research with the electron microscope"

ON THE COVER

Freeze-fracture replica of large nexus region between two ventricular cells. The fracture plane reveals the densely packed particles of the P face of one cell and the pitted membrane E face of the opposing cell. X80,000. W. B. Van Winkle, Department of Medicine, Section of Cardiovascular Sciences, Baylor College of Medicine, Houston.

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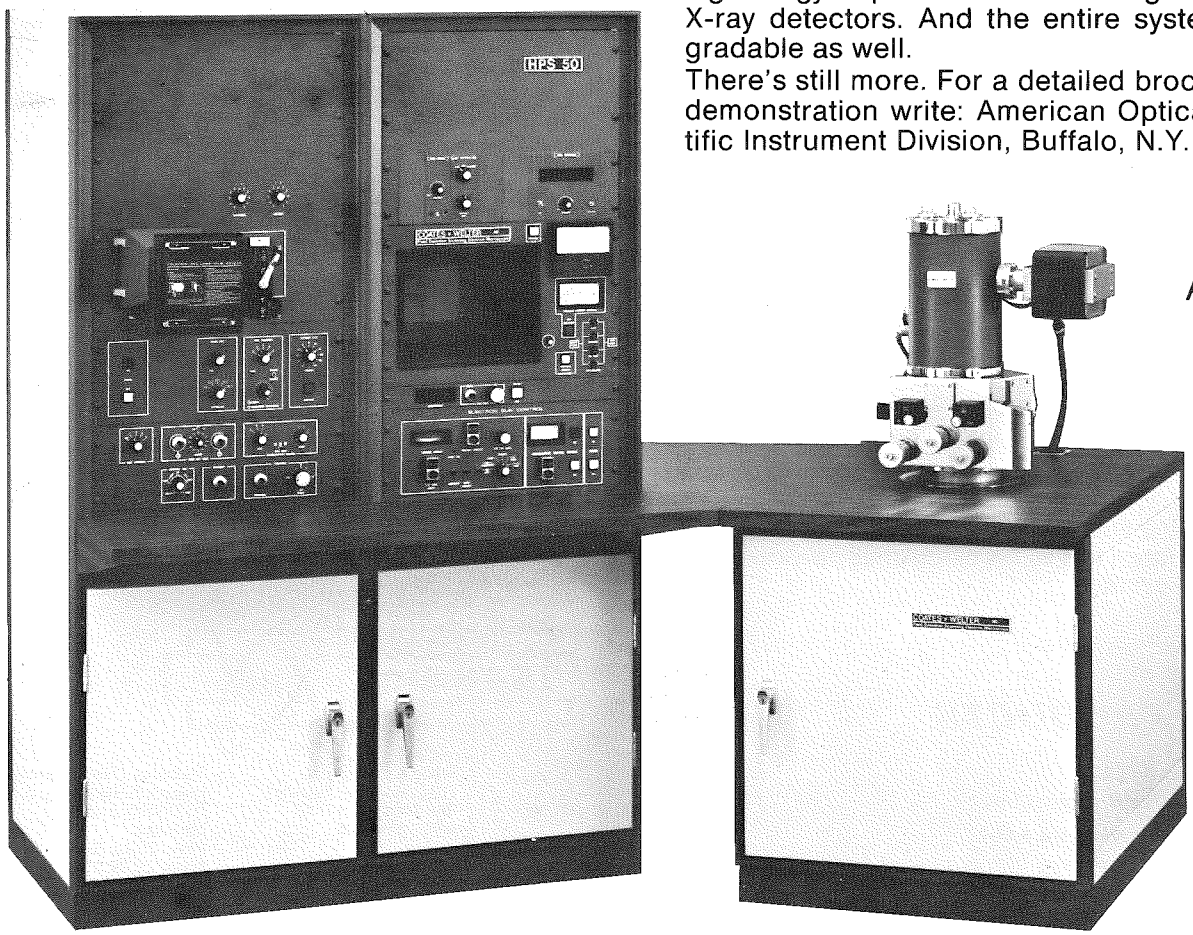

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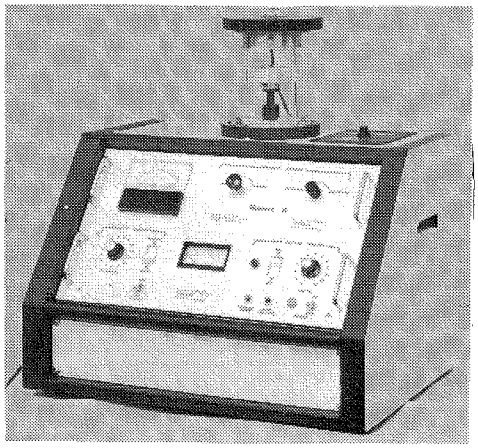

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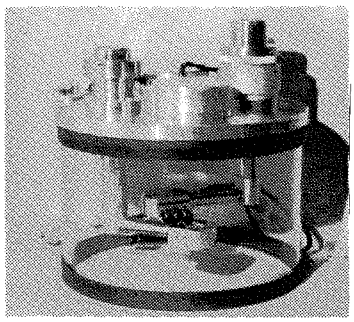

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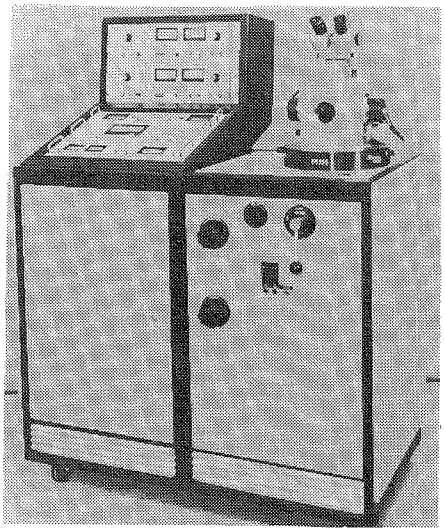

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President's Message

It is a pleasure for TSEM members to accept the warm hospitality of the Louisiana Society for Electron Microscopy at our Eighth Annual Joint Symposium. The scientific exchange and the exposure to new electron microscopic information and instrumentation make these joint meetings of great value to all of our members. Our corporate members are keenly aware of the opportunities that these joint meetings provide for direct communication with their best customers. Although the attendance at the joint meetings has grown over the years, these meetings are still small enough to permit the close exchange of ideas and information that we desire. Let's be straight forward; we Texicans love your Cresent City. If it were just a little bigger we might be interested in buying the outfit.

The TSEM spring meeting is to be held in Dallas with Leonard Seelig and Jerry Shay working on arrangements. Some outstanding guest speakers and programs will soon be announced.

The next EMSA meet will be in San Antonio, August 13-17, 1979. TSEM president-elect Bill McCombs is in charge of local arrangements. Our regular TSEM meeting will be held jointly with this EMSA meeting.

In response to a request from LSEM members, our 1980 joint symposia will be held in Houston.

It seems to me that the development of new equipment and new preparative procedures for specimens makes the field of electron microscopy more exciting than ever. It takes little imagination to foresee quantum jumps in information that electron microscopy will provide in the near future. I think we would all agree that research with electron microscopes is progressing at an exponential rate. Many of us are excited by these prospects.

Ivan Cameron
President

TSEM FINANCIAL REPORT
Period Ending September 20, 1978

Total Assets (5/1/78)		$ 5,064.86
Certificate of Deposit (University National Bank)		2,000.00
Certificate of Deposit (University Bank)		1,309.83
Certificate of Deposit (Fannin Bank)		1,000.00
Savings Account		305.57
Balance in Checking Account as of 5/1/78		449.46
RECEIPTS:		
Income from Registration (Lubbock)	$ 1,065.50	
1978 Dues	258.50	
Interest on Cert. of Dep. No. 17864	92.68	
San Antonio Delinquent Payments	1,100.00	
EMSA	500.00	
Newsletter Editor (Turner) Revenue	885.75	
San Antonio Revenue (Harvey Thomas)	1,200.00	
Total Income	5,102.43	
Subtotal		(+) 5,551.89
DISBURSEMENTS:		
(Lubbock Meeting Expenses)		
Bartender	$ 15.00	
Bill McCombs	6.75	
Music West	100.00	
South Park Inn	582.47	
Randy Brackeen	244.19	
Presidents Award	24.60	
Secretary's Expense (McCombs)	100.00	
Postal Service	260.00	
Secretary's Expense (Hansen)	600.00	
Registration Returns (Overcharges)	30.00	
San Antonio Residual Expenses	485.00	
Student Travel Lubbock	300.00	
Newsletter Expenses (Ann Goldstein)	1,900.00	
Awards (EMSA/TSEM)	400.00	
Nacogdoches Expenses (Charles Mims)	150.00	
Total Disbursements		(-) 5,198.08
Balance in Checking Account as of 9/20/78		$ 353.34
Certificate of Deposit (University National Bank No. 1099)		2,000.00
Certificate of Deposit (University Bank No. 4470)		1,329.62
Certificate of Deposit (Fannin Bank No. 17864)		1,000.00
Savings Account No. 01-7420-3 (U.N.B.)		309.26
TOTAL ASSETS as of 9/20/78		**$ 4,992.22**

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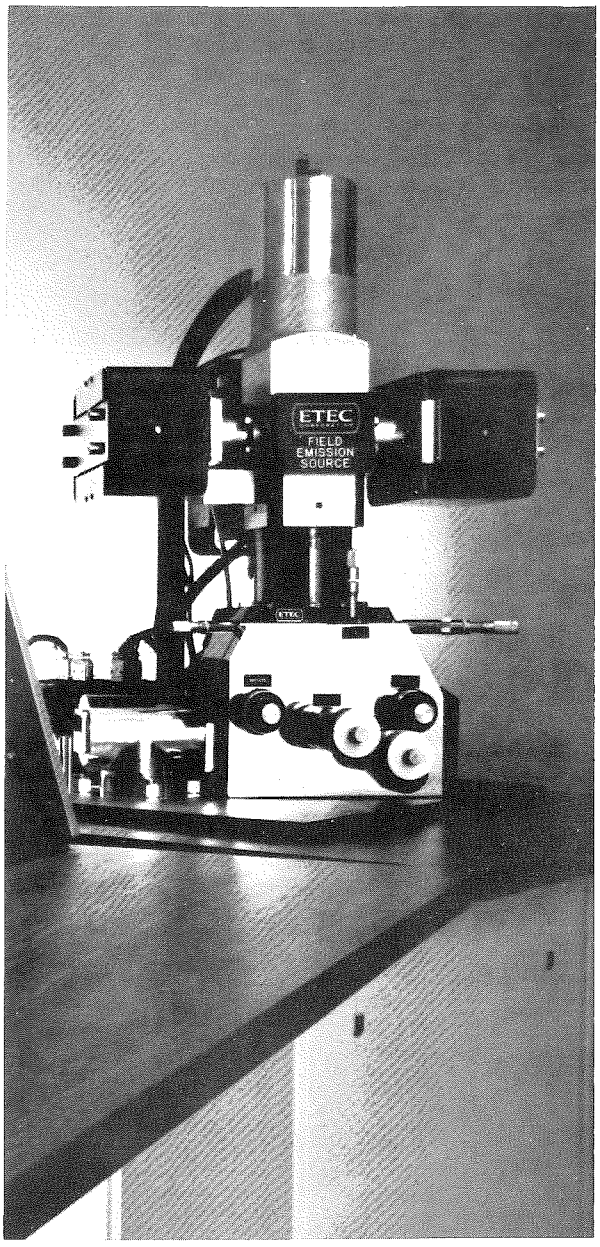

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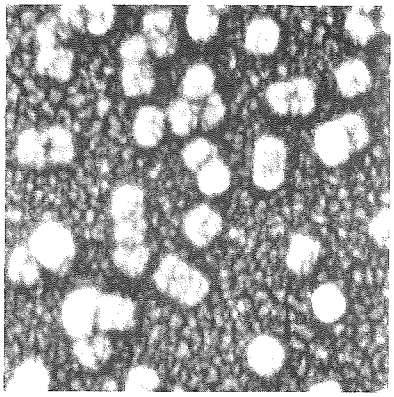

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Editor's Comments

My job is made so much easier by our corporate members and other advertisers. Without them the newsletter would not be the same. My special thanks to those of you who meet the deadlines early, who make helpful suggestions and most of all to those of you who have agreed to buy ads for all three issues in 1979.

The response to our joint meeting has been good, in fact so good that Dr. Diane Smith has made an alphabetical listing for the numerous abstracts submitted. We have included this in our newsletter.

I have received Reader Questionnairs from all members of your Executive Council. They have set a good example. A number of you have already responded, but we have not yet reached the fifty mark. The return of fifty questionnaires still represents less than 10% of the membership. Try filing it out before you leave the meeting and give it to me or leave at the registration desk. That way you save yourself the hassle of an envelope and the cost of a stamp.

Two outstanding feature articles have been submitted for this issue. They reflect the exciting new developments of which the president speaks. Let me remind you that the fall issue which coincides with the EMSA Meeting will contain a number of short articles and will not contain the abstracts. This is a marvelous opportunity for those of you who keep saying "Next time I will submit an article." We have a special request for articles from people in the materials sciences. Surely, this is a good time for an article on the ion probe or scanning EM, etc.

Dr. Charlotte Ownby, president of the Oklahoma Society for Electron Microscopy, writes that their fall meeting was held on Friday, November 3, 1978 in Stillwater. Thirteen talks were given by members and a special address was given by Dr. Daniel Pease. The next meeting for OK-SEM will be the Second Annual Workshop and Symposium in March, 1979 in Oklahoma City.

Dr. Tom McKee of Arizona State University writes on behalf of the Arizona Society for Electron Microscopy and Microbeam Analysis that a second issue of their newsletter was published November 14, 1978. Their biggest news is that the EMSA Council accepted them as a local affiliate and Dr. Ward Kischer presented preliminary plans for the February meeting of ASEMMA in Tucson which coincides with the EMSA Council meeting. ASEMMA now has 90 members.

Your colleagues across the state want to know your news and our regional editors are faithful in submitting it. So please let us hear from you, too . . . next issue.

Have a happy new year.

Ann Goldstein
Editor

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Position Desired — I am a Research Microbiologist. I am seeking employment in the field of electron microscopy and/or microbiology. Curriculum Vitae, containing details of my educational background, research interests, and a list of publications, is available upon request. Demetrios J. Politis, Research Microbiologist, Department of Plant Pathology, University of Missouri-Columbia, 108 Waters Hall, Columbia, Missouri 65201. 65201.

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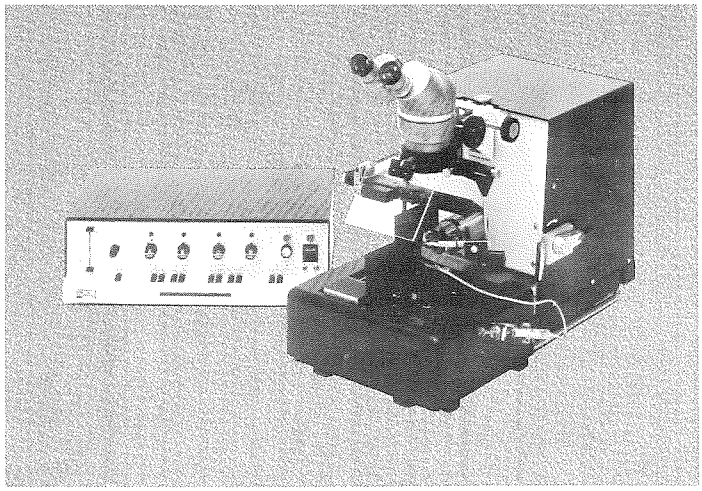

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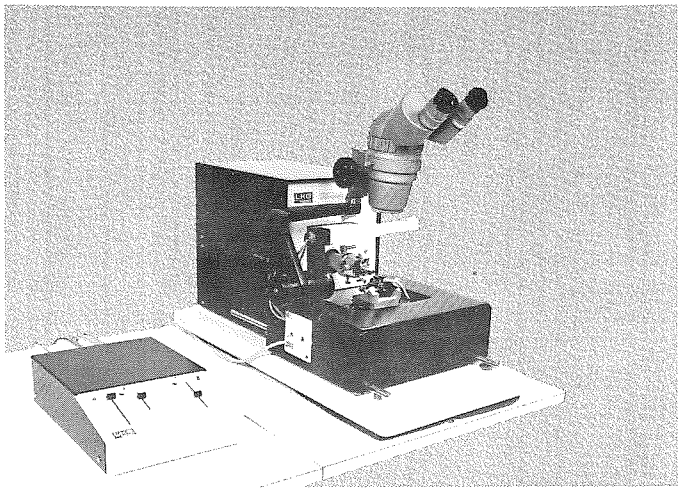

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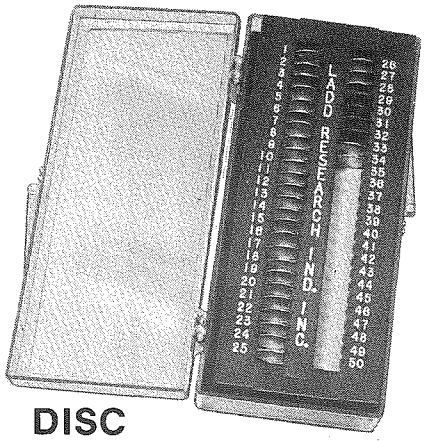

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Mitotic Spindle Ultrastructure And Chromosome Movement: A Mini-Review

B. R. Brinkley and J. Bryan
Department of Cell Biology
Baylor College of Medicine
Houston, Texas

"Development and growth, be they normal or abnormal, are intimately bound to the process of mitosis, and a successful analysis of its basic mechanism is as important to the student of embryology as to the specialist who is trying to solve the riddle of malignant growth"
—Professor Franz Schrader, 1949.

INTRODUCTION

The mitotic spindle is a motile apparatus which has evolved in eukaryotic cells for the express purpose of facilitating the systematic distribution of genes from one cell generation to the next. The spindle is a transient structure which appears only briefly in the cell cycle. It is assembled at the onset of mitosis, functions to achieve precise alignment and segregation of replicated chromosomes, and is dismantled when mitosis is over. An error in spindle structure and function can lead to grave genetic and developmental consequences — yet errors are infrequent. In normal cells, the spindle functions flawlessly for millions of cell generations to insure precise genetic continuity. In spite of its precision and importance however, less is known about the mitotic spindle than any other motile apparatus in the cell. The purpose of this report will be to present a concise but hopefully not cursory, overview of mitosis with special emphasis on the ultrastructure of the mitotic spindle and to review current models for chromosome movement in eukaryotic cells.

DYNAMICS OF SPINDLE ARCHITECTURE

Although a few early investigators believed the mitotic spindle to be an artifact of fixation, most concluded that the spindle was composed of fibrous elements which in some way provided the forces necessary for chromosome movement (see Schrader, 1949 and Mazia 1961 for early reviews). In 1949 Ostergren proposed a dynamic equilibrium model for chromosome movement in which "spindle fibers" were assumed to be liquid crystal-like aggregates to which molecular subunits were added or removed to lengthen or shorten the fibers. This view was supported and extended by the pioneering studies of Inoue (1964) who demonstrated by polarization microscopy that spindle fibers were highly labile in living cell. He not only proved that the spindle was a real structure in living cells but provided convincing evidence through birefringence retardation measurement for a dynamic monomer-polymer equilibrium existing between pools of cytoplasmic subunits and polymerized spindle fibers. According to Inoue, the polymerization reaction involved a small change of free energy ($\Delta G = .07$ Kcal/mol of subunit polymerized), was endothermic ($+\Delta H = 30\text{-}40$ Kcal/mol) and was driven by entropy change ($+\Delta S = 100\text{-}200$ Eu). As will be discussed later, Inoue and coworkers (1967, 1975) have concluded that the birefringent fibers provide the force for chromosome movement through a dynamic equilibrium associated with the assembly-disassembly of subunits in the spindle fiber.

Electron microscopy has provided important information on the structure and organization of the metaphase spindle and has given some insight into the mechanisms of chromosome movement. Early studies by Harris (1962) and Roth and Daniels (1962) confirmed the fibrous nature of the spindle although fixation in osmium tetroxide alone inadequately preserved the individual components of the spindle fiber. As fixation procedures improved and

especially when glutaraldehyde became available as a primary fixative (Sabatini **et al.**, 1963), the basic elements of the spindle fiber were identified as hollow microtbules 240 A in diameter and several microns long. Not only were microtubules found in the mitotic apparatus but they were identified as ubiquitous structures throughout the cytoplasm of nondividing cells in most eukaryotic organisms (Figs. 1 and 2).

Biochemical studies over the past decade have led to the conclusion that microtubules are composed of 13 globular protein subunits called tubulin which exist in a dynamic equilibrium with the soluble tubulin subunits of the cytoplasm surrounding the microtubule. Thus the birefringent elements of the mitotic spindle indentified in early studies by Inoue (1964) correspond very well to the distributions of microtubules which are identified by electron microscopy. The dynamic nature of the spindle explains why fixation of mitotic cells in the cold (0-4°C) results in the dissolution of most spindle microtubules. Thus for good microtubule preservation, fixation must be carried out at higher temperatures (28-37°C).

Most electron microscopic studies show the microtubules to be the most conspicuous structural component of the mitotic spindle. They attach to chromosomes by way of a specialized structure called the **kinetochore.** In some species the kinetochore is positioned at a single locus on the metaphase chromosome while in others this structure extends the entire length of the chromosome and is said to be **diffuse.**

Micromanipulation experiments have argued that the force which produces chromosome movement in mitosis acts at the kinetochores (Nicklas, 1971). Therefore, a knowledge of its structure may be useful in understanding the mechanism of chromosome movement. Although there is considerable variation among species of eukaryotic organisms, the kinetochore of most animal cells is a plate-like structure which is attached to the metaphase chromosome at the centromere or primary constriction (Fig. 3). A diagram based on a three-dimensional reconstruction of serial sections of the kinetochore of a mammalian cell is shown in Fig. 4. The structure consists of three distinct layers with microtubules firmly embedded in the outer layer. A central space of about 400 A thickness separates the outer layer from an inner layer. The latter is firmly attached to adjacent chromatin fibers. The size or surface area of the kinetochore varies considerably with the size of the chromosome. Large chromosomes may have kinetochores which are several microns in length and width, whereas small chromosomes have very small kinetochores of a micron or less in their greatest dimension. The tiny microchromosomes of birds have no visible kinetochores and the microtubules appear to attach directly to the chromatin threads (Brinkley **et. al.**, 1974). A similar association is seen in the small chromosomes of yeasts (Peterson and Ris, 1976).

Kinetochores "turn on" in the sense that they are associated with microtubules and begin directed movements at prometaphase and "turn off" at telophase when movement stops. Concommitant with the initiation of chromosome movement is the appearance of microtubules associated with the kinetochore. Several studies suggest that kinetochores initiate microtubule polymerization and therefore serve as one of several microtubule organizing centers (MTOCs) of the cell. Tubulin subunits could be added to either end of a kinetochore microtubule with growth occurring from the kinetochore toward the poles.

Other investigators have opposed the view that kinetochores function as MTOCs. On the basis of electron microscopic studies of the diatom spindle, Pickett-Heaps and Tippit (1978) concluded that kinetochores "capture" the distal ends of microtubules which grow out from the spindle poles. Obviously such interpretations are interesting and worthy of more careful investigation. In our opinion, however, this view is not compatible with experimental evidence involving the assembly of exogenous tubulin from kinetochores of extracted or isolated metaphase chromosomes (McGill and Brinkley, 1975; Telzer **et al.**, 1975; and Borisy and Gould, 1977).

The poles of the mitotic spindle are themselves both interesting and highly variable in structure. Spindle poles are often characterized by their tubule distributions which consist of the convergence of both kinetochore and non-kinetochore microtubules including those from the interzone and asters. The poles may also contain a highly structured organelle such as a centriole pair as is the case for most higher animal cells (Fig. 5), or simply a loose series of membraneous vesicles and amorphous densities as in the case of higher plant spindles.

The precise pattern of centriole replication and distribution in animal cells and the fact that they serve as the focal point for spindle microtubules has led to the conclusion that centrioles are important organelles which function as pole determinants in mitosis (Brinkley and Stubblefield, 1070; Mazia, 1961). However, in view of the fact that centrioles are absent in many cell types such as higher plants and early cleavage stages of the fertilized mammalian zygote, many investigators have taken a more cautious view of their importance in spindle function. In some cells, centrioles can be experimentally eliminated from the spindle without serious pertubation of the mitotic process (Deitz, 1964). Pickett-Heaps (1969) has suggested that centrioles have no direct role in the mitotic spindle, but are benign passengers which utilize the spidle as a handy conveyance to get from one cell generation to the next. In this way the spindle insures that progeny cells receive a ready supply of basal bodies. There is considerable new evidence, however, that the centrioles serve an important role as MTOCs for both cytoplasmic and spindle microtubules. Moreover, drugs such as ethidium bromide, which specifically alters centriole structure, also increase the incidence of multipolar spindles in cultured cells (McGill **et al.**, 1976). This evidence does not support the view that centrioles are passive organelles in mitosis, but argues that they may play a more active role as pole determinants in those cells which contain them. In those cells which have no centrioles, it is concluded that a centriole-equivalent such as membraneous vesicles or amorphous plaques can carry out the same function.

Has electon microscopy revealed the complete structure of the mitotic spindle? Probably not. With conven-

tional glutaraldehyde-osmium tetroxide fixation procedures, microtubules appear to be the primary structural component of the spindle fiber. Yet evidence extending from microbeam irradiation experiments by Forer (1966) argued that force production arises from structures other than microtubules. This has led to the present day search for actomyosin-like structures within the spindle. Recently, actin filaments have been resolved in the mitotic spindle of various animal and plant cells (Forer, 1976). Thin filaments which can be "decorated" with skeletal muscle heavy meromyosin (HMM) have been observed in both thin sections of spindles as well as negatively stained preparations (Forer, 1976). Although it can be argued that these are artifacts which arise from the glycerination steps required to decorate with HMM, newer evidence has made the observation more acceptable. Antibodies to skeletal muscle actin specifically decorate regions of the mitotic spindle (Cande **et al.**, 1977). Moreover, immunofluorescence studies using anti-myosin have also indicated that myosin-like molecules are in the spindle (Fjuiwara and Pollard, 1976). Whether or not these contractile proteins are functional components of the spindles and provide the force for chromosome movement must await further studies.

In addition to contractile proteins, it is likely that other components such as regulator molecules have not been detected by conventional electron microscopic studies and will be revealed by techniques such as immunofluorescence or immunoelectron microscopy. Indeed this is the case for the calcium binding protein, calmodulin, which has recently been identified as a component of the spindle by immunofluorescence technique (Welsh **et al.**, 1978). In summary, while we have a working knowledge of the most prominent structural components of the mitotic spindle based on light, electron microscopic and biochemical studies, additional structural and regulatory components are still being identified. The assumption is often made, however, that we have identified the most important functional components. It is therefore possible to speculate, and more importantly, to design experiments to determine how these components bring about chromosome movement in mitosis.

SPINDLE STRUCTURE AND CHROMOSOME MOVEMENT

Several models for spindle function and chromosome movement have been proposed which generally fall into two categories; those in which the force for movement is derived from microtubules and those where movement is initiated largely by contractile proteins or microfilaments. The latter models do not exclude the possible interaction between microtubules and microfilaments. The essential features of each model are described below.

MICROTUBULE POLYMERIZATION—DEPOLYMERIZATION

As previously mentioned, Inoue and coworkers (1964, 1967, 1975) have described a dynamic equilibrium between assembled structures (microtubules) in the spindle fiber and soluble subunits (tubulin) in the cytoplasm. According to their model, the force needed to move chromosomes is provided by the localized disassembly of spindle microtubules at the poles. As subunits are removed, the kinetochore-associated microtubules shorten and chromosomes move toward the poles. Subsequent spindle elongation occurs by the addition of tubulin subunits to the existing interpolar microtubules which serve to push the poles apart. This model supported by experiments with microtubule inhibitors such as colchicine, cold temperatures and hydrostatic pressure (6000 PSI), agents which have been used to slowly remove tubulin from assembled microtubules in the spindle and results in directed chromosome movement (1975). Some features of this model are obviously correct. From all indications, microtubule depolymerization must occur before chromosome movement can take place (Brinkley **et al.**, 1978). The recent discovery of a calcium binding protein, calmodulin (CDR) at the spindle poles (Welch **et al.**, 1978), and the finding that CDR can modulate Ca++ sensitivity of microtubules at physiological concentrations **in vitro** (Marcum **et al.**, 1978) suggest that localized microtubule depolymerization can be regulated by Ca++. Nevertheless, this model fails to explain how removeal of microtubule subunits at the polar ends of microtubules can move chromosomes without interacting with a second system. A recent model proposed by Margolis **et al.** (1978) provided a clue as to how this might occur.

CHROMOSOME MOVEMENT BY INTINSIC MICROTUBULE BEHAVIOR

Margolis and Wilson (1978) provided evidence that the assembly of tubulin **in vitro** is a steady state summation of two similar but separate reactions. Using p^{32} labeled GTP they found that tubulin is added at one end of the microtubule and subsequently removed at the same rate at the opposite end of the tubule. This dynamic state results in the slow movement of tubulin subunits along the tubule in a polarized direction. Utilizing this information, they proposed a model for mitosis in which force generation results from a combination of the movement of tubulin subunits along the tubule and sliding of adjacent microtubules (Margolis **et al.**, 1978). This model differs from that of Inoue in several ways. Tubulin subunits must be added to the kinetochore end at the kinetochore-associated microtubules and to the interzonal end of the non-kinetochore microtubules whose free ends overlap in the interzone. The overlapping interzonal microtubules are presumed to be antiparallel and to produce opposing forces on each other by a sliding mechanism. In this model, anaphase movements occurs by blocking the assembly end of the microtubules at the kinetochore. Linkage of the non-fluxing kinetochore tubules to the interzone tubules which continue to cycle generates force. Sister chromatid linkages are broken by this force. Kinetochore-associated microtubules depolymerize at the pole region as antiparallel microtubules lengthen and slide apart, causing spidle elongation. This is an exciting model based on recent experimental evidence of microtubule assemby — disassembly. Thus far, however, the data have been derived entirely from **in vitro** steady state assembly systems and have not been tested in living cells.

SLIDING TUBULE MODEL

McIntosh and coworkers (1969) utilized ultrastructural

observations showing cross-bridges between adjacent microtubules and knowledge of tubule assembly equilibrium to propose a model for mitosis in which force production was provided by the sliding or shearing of adjacent antiparallel microtubules. Accordingly, the cross-bridges were proposed to be mechanochemical couplings which forced antiparallel microtubules to slide apart, pushing chromosomes to the poles and causing spindle elongation. Although a working model can be constructed from knowledge of spindle microtubule polarity, many types of chromosome movement cannot be adequately explained by this model. For example, the spindle organization of some species, such as the diatom, in which kinetochore microtubules and those which form the central spindle are widely separated, will not conform to this general sliding mechanism. Furthermore, the data on microtubule distribution in mammalian and plant cells fail to support the simple sliding model (Brinkley and Cartwright, 1971; Jensen and Bajer, 1973). However, some features of the model are appealing and computer "tracking" studies of microtubules in serial sections suggests that sliding of microtubules may occur in the interzone or midbody (1975).

The bending motion of cilia and flagella involves a sliding tubule mechanism which has been well characterized by electron microscopy and molecular studies (Satir, 1968; Gibbons, 1977). An ATPase molecule called dynein forms a series of short arms which are positioned at regular intervals along the A subfiver of the outer doublet. The energy for sliding is provided by the hydrolysis of ATP by dynein through a mechanochemical coupling to adjacent doublets. If microtublues of the spindle interact in a manner analogous to those of cilia, an energy source such as dynein must be present in mitotic cells. Indeed, recent evidence from several sources indicate that a spidle dynein exists in mitotic cells. Salmon and Jenkins (1977) identified a dynein-like molecule in isolated spindles of sea urchins. Sakai and coworkers (1976) reported that anaphase motion in extracted spindles was arrested by the addition of antibodies to dynein but not to myosin. Vanadate, a specific inhibitor of ciliary dynein ATPase, has been found to inhibit anaphase chromosome movement in lysed mammalian tissue culture cells (1978). Taken collectively, these studies provide strong evidence for a spindle dynein whose function might well be to initiate inter-microtubule sliding. Obviously, other mechanochemical mechanisms can be envisioned and more studies are needed before the true function of spindle dynein can be defined.

THE ZIPPER MODEL

Bajer and coworkers (1975) have presented clear evidence for the lateral interaction of adjacent microtubules in plant spindles and have proposed a "zipper" hypothesis for force production in mitosis. Chromosome movement occurs when various spindle tubules zip together and unzip as chromosomes approach the pole. Careful electron microscopic studies along with high resolution polarizing microscopy have provided structural support for this model. Aside from microscopic data, however, the proposal has received little experimental support. Moreover, since many protists have only one microtubule per kinetochore,it is difficult to visualize zipping in these types of spindles.

ACTIN-MYOSIN CONTRACTILE MODEL

From his early UV microbeam irradiation experiments on the spindles of cranefly spermatocytes, Forer (1966) concluded that a "traction fiber" other than the microtubule was responsible for chromosome movement. Evidence has now come from several sources which supports the notion that contractile proteins such as actin and myosin exist in the spindle and may be involved in chromosome movement. Such movement may require an actin-activated myosin ATPase, in much the same way as muscle contraction. It should be noted, however, that just because actin and myosin can be localized in the spindle by several techniques, they need not be functional. Clearly these proteins, especially actin, are rather widely distributed in the cytoplasm of most cells and may be trapped in the spindle during fixation or extraction. Indeed, microinjection of antibodies to myosin failed to arrest anaphase chromosome movement in marine oocytes as might be expected if actomyosin interaction were necessary for movement (Kiehert **et. al.**, 1976). In the same experiment, however, antimyosin blocked cytokinesis, a process which is known to require contractile proteins.

If actin and myosin are functional components of the spindle, it is strange that microfilaments are so rarely observed in the spindle region by conventional electron microscopy using techniques which clearly demonstrate them in other parts of the cell (La Fountain, 1975).

SUMMARY

Studies of mitotic cells by electron microscopy have provided fundamental information of spindle ultrastructure which has been useful in defining the mechanism of chromosome movement. Clearly, microtubules are the most prominent spindle structure and indeed several models for spindle motility are based upon intrinsic properties of these organelles. There is considerable evidence, however, that important contractile proteins exist in the spindle which are not readily seen by conventional techniques for electron microscopy. Special labeling techniques such as HMM decoration of actin filaments and immunofluorescence microscopy have indicated that actin and perhaps myosin are functional components of the spindle. If a muscle-like contractile mechanism is responsible for chromosome movement, a series of regulatory molecules also would likely be associated with the spindle. One such protein, calmodulin, has already been found, although at present its function appears to be more related to microtubules than microfilaments. The mitotic spindle is a complicated apparatus and future studies will probably reveal additional components which must be defined before the molecular basis of chromosome movement can fully be understood.

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Description for Photos at Right

Figure 1 — Survey electron micrograph of prometaphase spindle of rat kangaroo PTK1 cell. C, centriole; CH, chromosome; K, Kinetochores. X 7,500. Figure 2 — Diagram of mitotic spindle similar to the one shown in Fig. 1. Figure 3 — Gigh magnification of kinetochore (K) showing microtubules associated with outer layer of the kinetochore. X 37,000. Figure 4 — Diagram of metaphase chromosome showing details of kinetochore structure. The diagram in the left-hand corner shows the structure of the kinetochore after treatment with colcemid. (Modified from a deagram in Biology of the Cell, Stephen L. Wolfe, Wadsworth Publishing Co., Inc., Belmont, Calif., P. 330. Figure 5 — Centriole pair in dividing chinese hamster cell. Parent centriole (C) appears in cross section and daughter centriole (C') is in longitudinal profile. X 75,000.

TSEM e-

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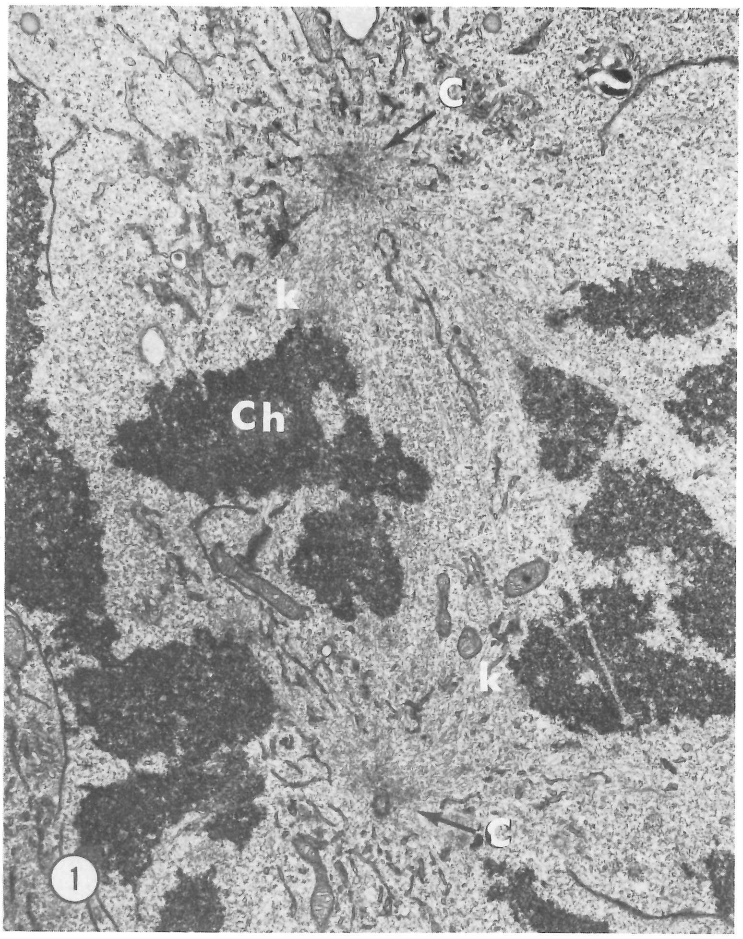
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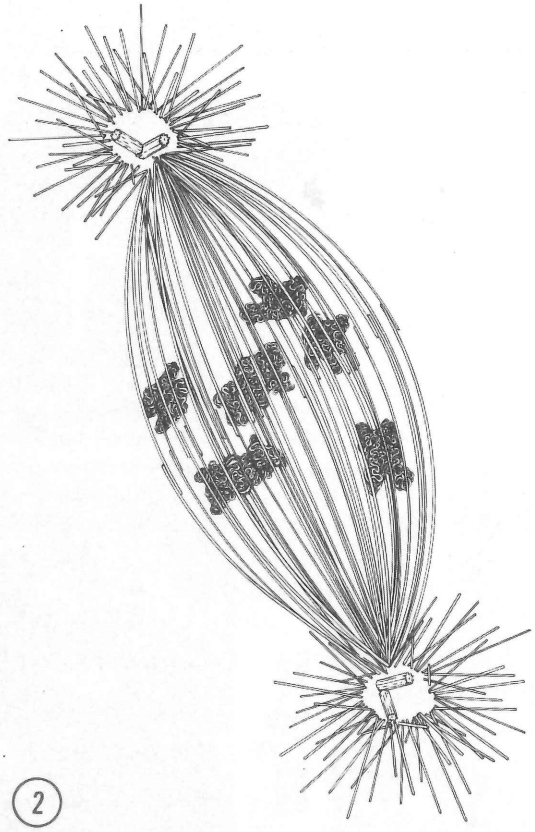
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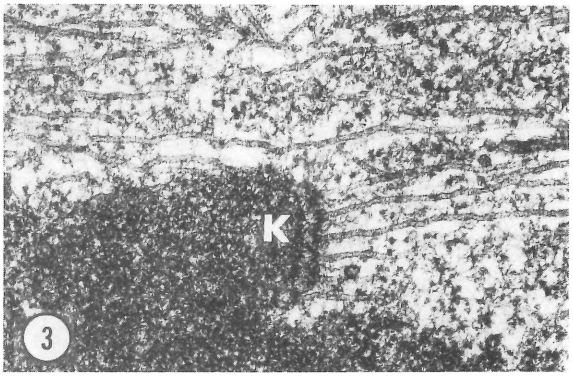
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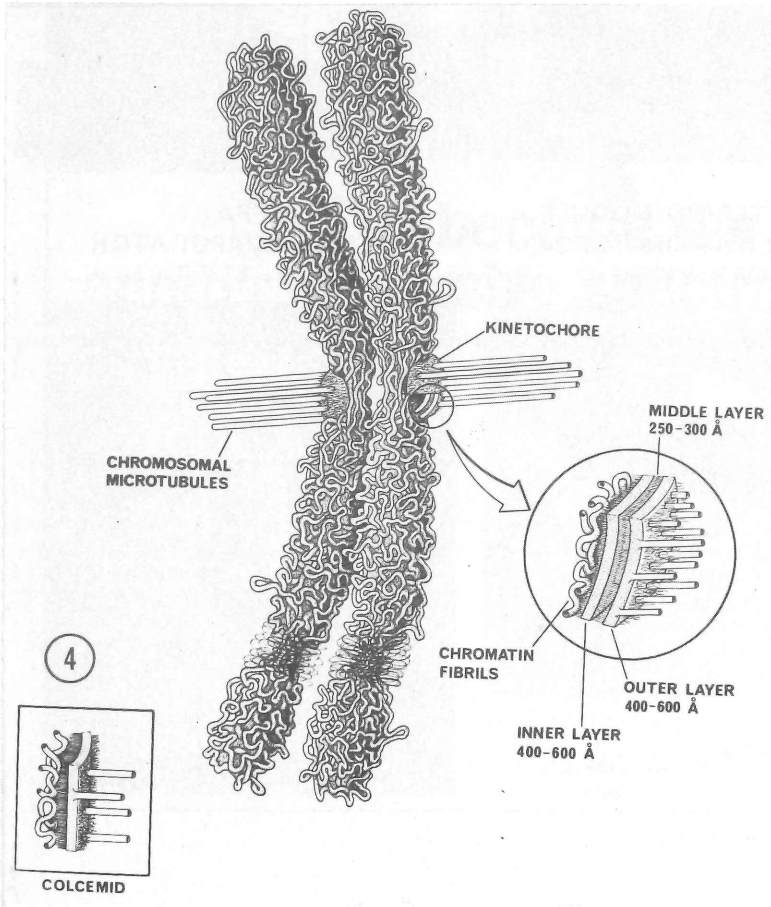
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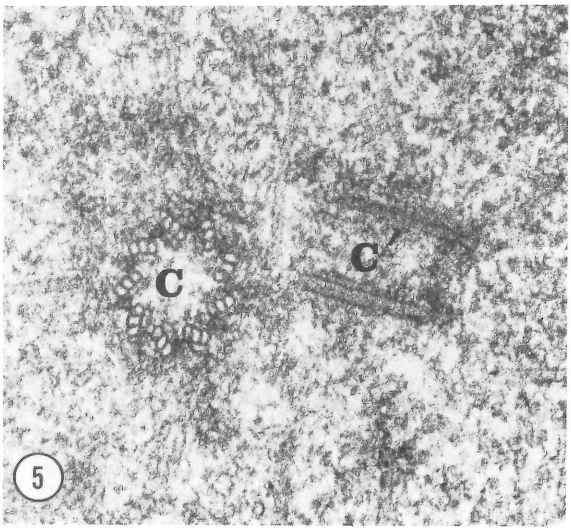
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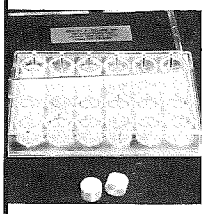

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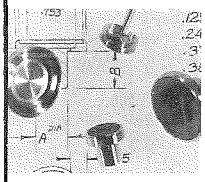

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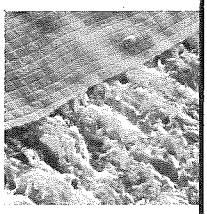

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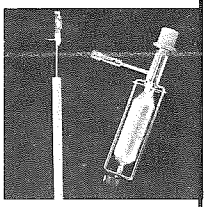

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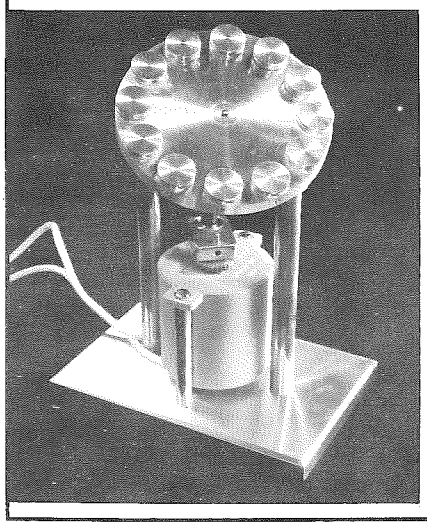

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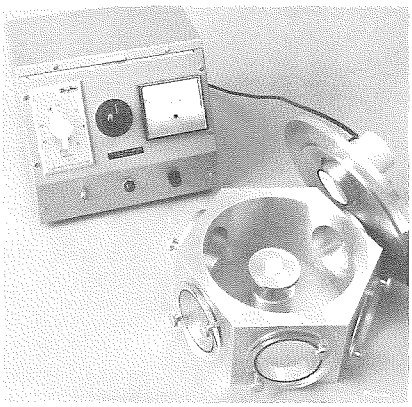

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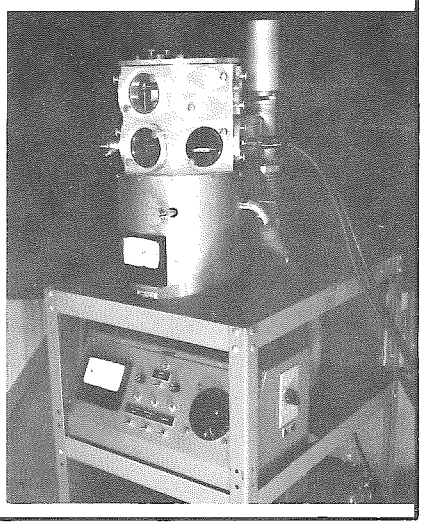

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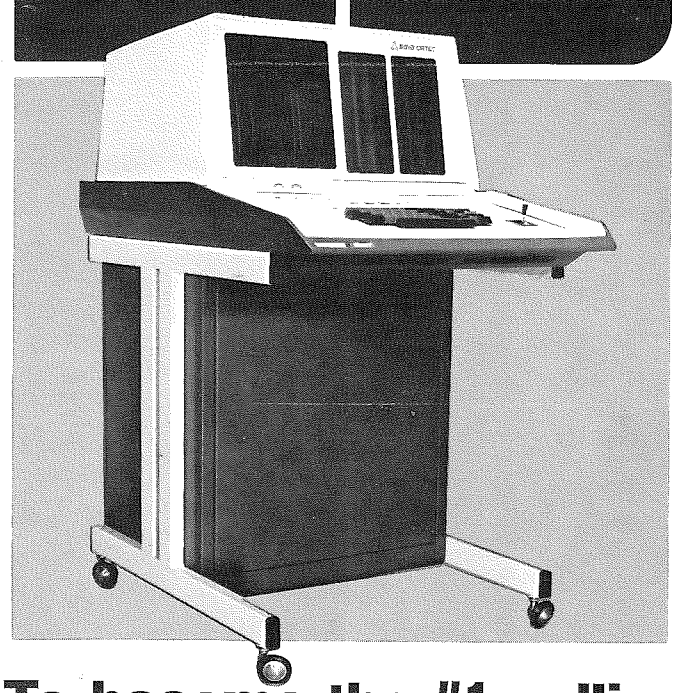

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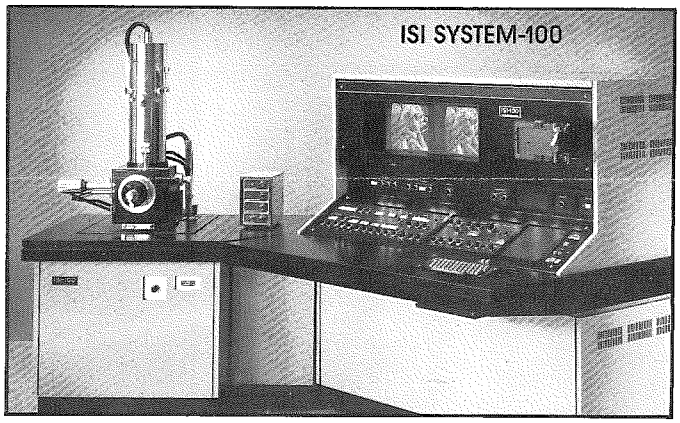

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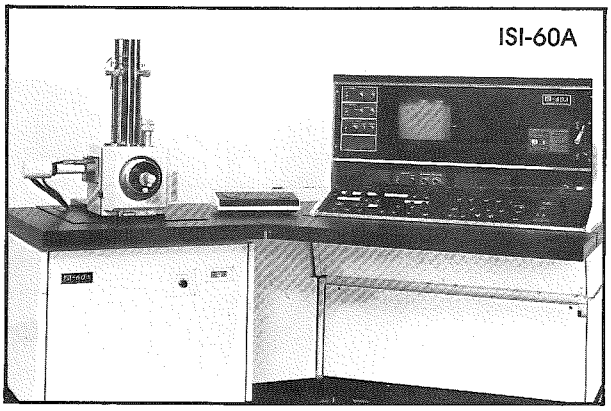

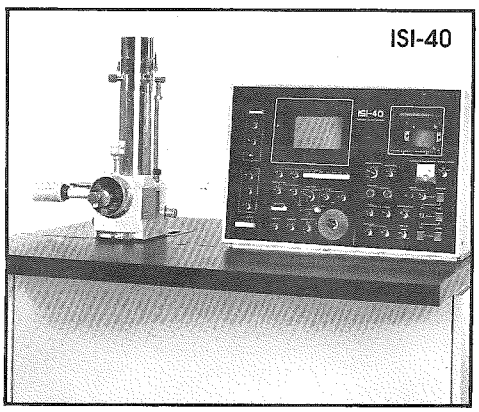

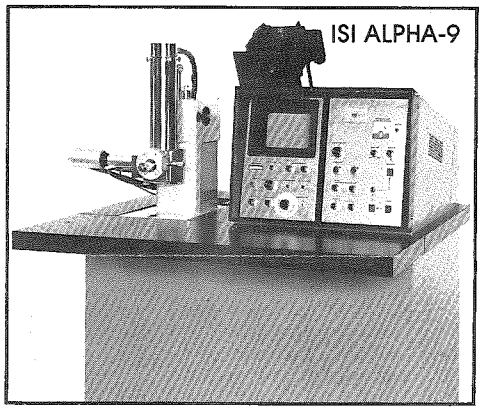

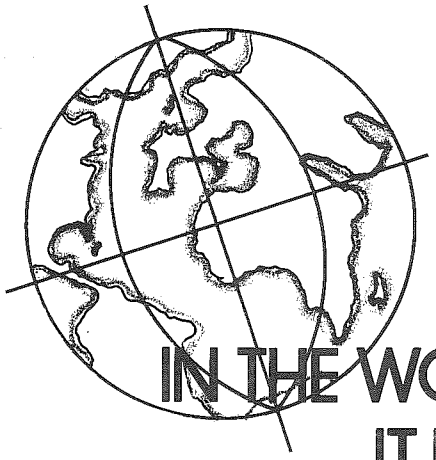

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Freeze-Fracture Electron Microscopy

by
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Department of Medicine
Section of Cardiovascular Sciences
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Houston, Texas 77030

INTRODUCTION

Since its first application to biological materials twenty years ago by Steere (Figure1), freeze-fracture of specimens for electron microscopy has become one of the most useful techniques not only in studies of subcellular organization but, perhaps more importantly, in elucidation of the macromolecular organization of biological membranes. A complete treatise on the theories, and applications of this procedure is beyond the scope of this review. However, interested readers are referred to the list of selected references for further, more extensive, treatment of various aspects of freeze-fracture.

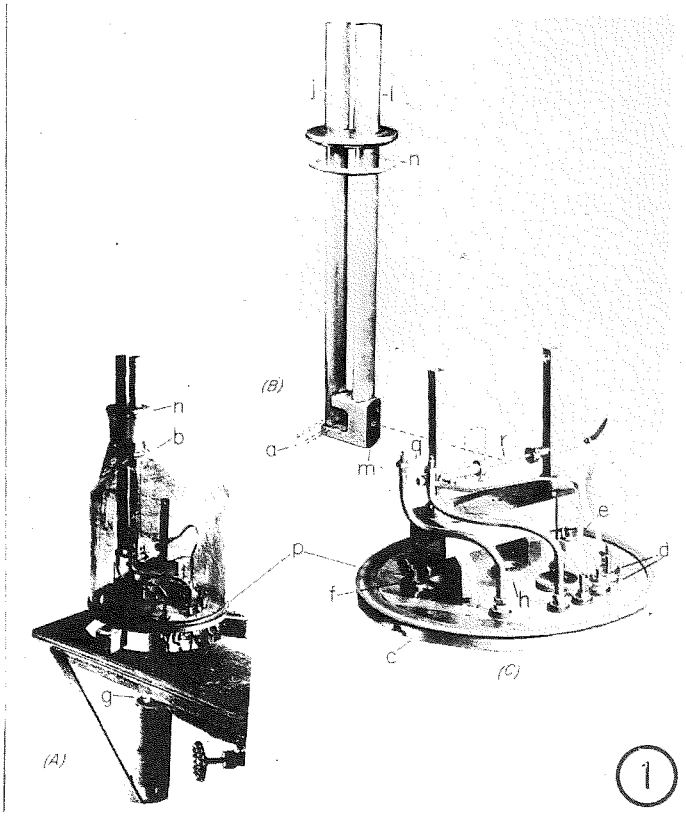

figure 1 — Early unit used by Steere in 1957 employing vacuum and replication systems for freeze replication studies of viral particles. Figure (A) shows entire apparatus while (B) and (C) are details of cooled specimen mount and evaporation equipment, respectively. This picture reproduced by permission of Dr. Russell L. Steere and the Rockerfeller University Press. (J. Biophys. Biochem. Cytol., 3:45-60, 1957)

It is unfortunate and confusing that the terms —— freeze-fracture" and "freeze-etching" have been used interchangeably by many authors. Freeze-fracture, **per se,** describes that process in which a frozen specimen is cleaved and the resulting surface immediately replicated. In "freeze-etching", the sample is fractured, then the surrounding medium is allowed to sublime or "etch" to reveal surfaces not previously fractured. Replication of both the fractured and the "etched" areas is then accomplished in the usual manner. A separate technique, "freeze-drying" is also gaining popularity. This technique employs only sublimation of frozen medium to reveal surface structure prior to replication (Nermut).

BASIC PRINCIPLES

The important advantage of freeze-fracture over conventional fixation-dehydration-embedding-sectioning-staining techniques is that it allows the three dimensional examination of specimens in their fully hydrated state. The specimens are not subjected to various fixatives, dehydrants and metal stains although brief fixation in glutaraldehyde is often used to prevent membrane protein movement. Furthermore, the time spent from preparation of the specimen to viewing of its replica in the electron microscope is usually much shorter than the conventional sectioning procedure — a definite advantage when electron microscopy is used as an integral portion of membrane biochemical studies. Although, as will be pointed out later, biological membranes yield considerably more topographical information when examined after freeze-fracture, numerous techniques such as cytochemistry and autoradiography seem more suited to sectioned material. Furthermore, at the current state of the art, sectioning remains the choice of microscopists when studying such areas as contractile protein organization and disposi-

tion and organization of smaller non-membrane cellular components such as microtubules, cytoplasmic filaments, ribosomes, chromatin and glycogen.

Although any water-containing material will freeze, it is only when freezing is controlled that usable replicas are obtained. Ice crystal formation takes place no matter how rapidly freezing is accomplished. Intracellular ice crystals, which can become quite large during slow freezing (<10°C/sec), must be kept minimal if any real detail is to be preserved. Minimizing ice crystal damage may be accomplished by several techniques: (1) Since freezing in liquid nitrogen at -196°C produces an initial insulating nitrogen gas barrier around the specimen, (hence slower freezing of the specimen), an intermediate agent, most popularly Freon 13 or 22, cooled by liquid nitrogen, is used. Cooled liquid propane, nitrogen slush and liquid helium have also been used for rapid freezing. (2) Although applicable only to small samples, isolated cells or fractions, the spray-freezing technique described by Bachmann and Schmidt gives excellent tissue preparation. The small droplets sprayed into the freezing bath result in very high freezing rates (50,000 C°/sec). (3) A rapid-freeze device has been employed by Heuser and his co-workers in which a plunger presses the sample against a liquid helium-cooled block. (4) Most popular of all techniques to reduce ice crystal damage is the use of cryoprotective agents prior to fracturing. Glycerol or dimethyl sulfoxide bind water molecules and lower the freezing point of the cytoplasmic and extracellular water. Since structural changes in certain membranes have been observed in non-fixed glycerinated tissues, many workers employ a brief glutaraldehyde treatment prior to glycerination. Recently, certain polymers such as polyvinyl pyrillodone (PVP) have been used successfully as cryoprotective agents with little or no detectable damage to the specimen.

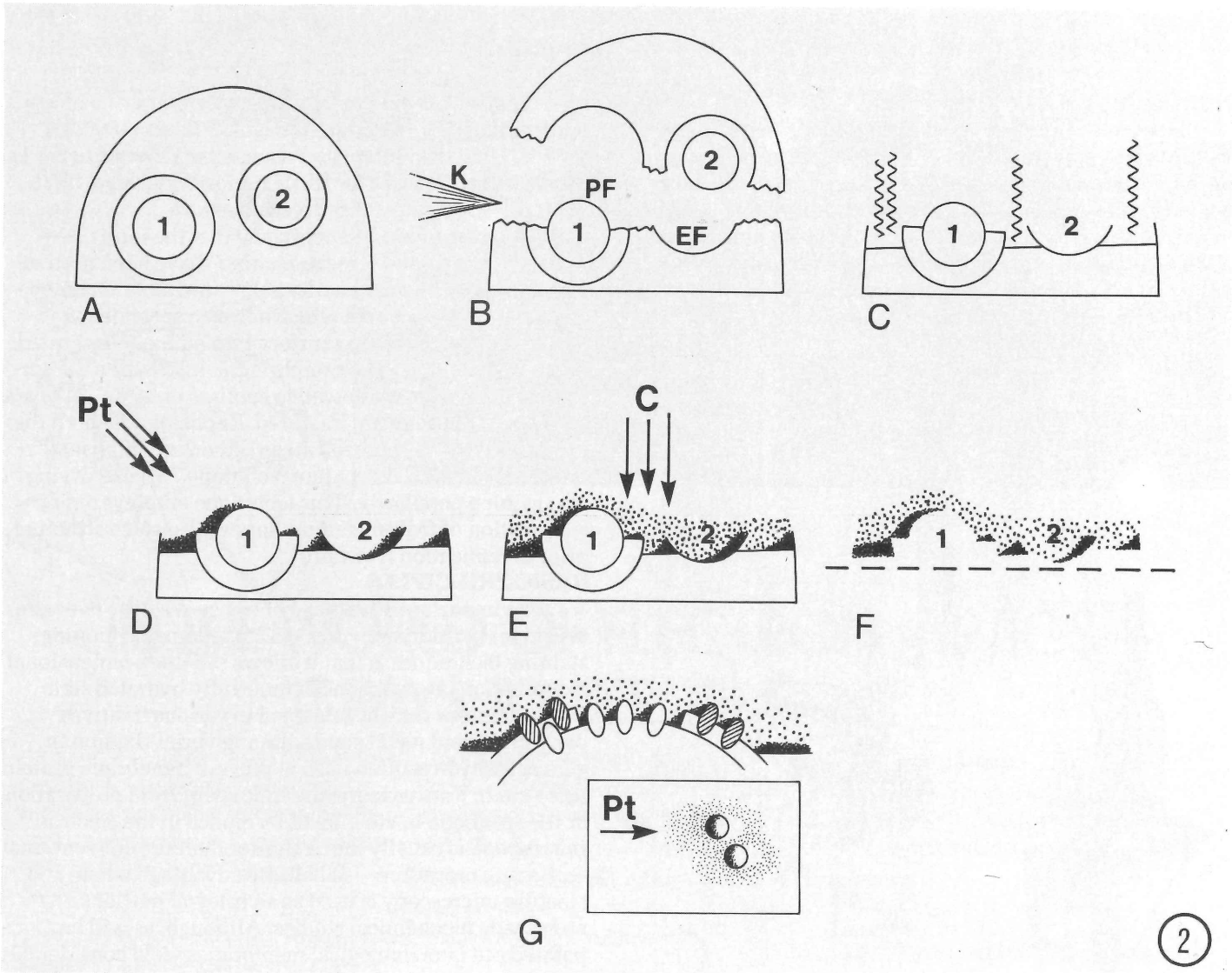

Figure 2 — Diagrammatic representation of fracturing, etching and replication process. Two bilayer-bound vesicles are shown in A in a frozen state. The fracture, initiated by the liquid nitrogen cooled knife (K) in B splits the bilayer revealing the PF face of vesicle 1 and the EF face of vesicle 2. In C the specimen temperature is held at -100°C resulting in sublimation of the ice medium. Step D represents the shadowing of the specimen surface of 45° angle by evaporated platinum. To stabilize the shadowed surface, carbon is evaporated in E. Following degestion of the specimen this replica is picked up on a copper grid and examined in the microscope. The membrane represented in Figure 2 illustrates protein particles embedded in the two halves of the bilayer. The shaded particles are revealed on the ES only after etching whereas the clear particles are revealed after the initial fracture. The boxed area shows how "white" whadows are cast by the particles.

INSTRUMENTATION

Most freeze-fracture devices consist of four main components: (1) a vacuum system, usually a rotary mechanical pump and a high vacuum oil-diffusion or turbomolecular pump (2) a freezing system for maintaining the specimens at low temperature, usually via controlled liquid nitrogen cooling, (3) a fracturing device to cleave the frozen specimens and (4) an evaporative system for the initial heavy metal (usually platinum) shadowing and the subsequent carbon evaporation for replica stabilization. Two major freeze-fracture instrument, Denton*, and Balzers, have evolved from the original devices of Steere and Moor, respectively. Several other commercial devices as well as those built by individual investigators are also in use.

Fracturing devices range from the liquid nitrogen-cooled, motor driven microtome of the Balzers instrument to "chipping" devices such as that found on the Denton instrument. Although the Balzers liquid nitrogen microtome permits repeated fractures to "smooth" the fractured surface, the knife edge frequently causes "knive marks" and areas of frictional melting that may obscure fine detail. Fracturing devices typically yield replicas of only one surface, i.e. the one which remains on the specimen stage following the fracture. Special specimen holders are now available which permit retrival of replicas from both fracture faces enabling investigators to more accurately study protein disposition in complimentary halves of the bilayer.

Recently, freeze-fracture of tissue culture monolayers has been possible by growing cells on special coverslips, freezing the coverslips on special specimen stubs and fracturing by "lifting" or "peeling" the coverslip up inside the vacuum chamber.

The frozen specimens may be fractured by several methods, depending on the instrument and particular modifications as described in the preceding two paragraphs. Following the fracture (see Figure 1 for illustration of procedure) the specimen may be etched, usually by raising the specimen to -100°C and maintaining suitable high vacuum. Under these conditions, and with a suitable liquid nitrogen-cooled cold trap or surface to reduce water condensation on the specimen, sublimation of water occurs at ~100 nm/min. The surface of the specimens is then replicated, first by shadowing with platinum or other heavy metal at an angle of 45° followed by deposition of carbon to reinforce the replica. The amount of platinum deposited may be precisely monitored in the newer Balzers instruments equipped with a quartz crystal monitor which controls a shutter to terminate evaporation. A recent development by Steere, and now marketed for the Denton apparatus, also uses a monitoring system permitting better reproduceability of replicas. The replica with adhering specimen is cleaned by several reagents, usually sodium hypochlorite (Clorox bleach) or chromic acid, picked up on standard grids and examined in the electron microscope.

ARTIFACTS

Artifacts, the bane of electron microscopist no matter what preparative technique is employed, are no strangers to freeze-fracture electron microscopists. The importance in the recognition (and, hopefully, prevention of misinterpretation) or artifacts, has prompted Balzers Corporation to devote a large illustrated pamphlet to the description of various artifacts which plague this technique

The use of commercial names does not imply endorsement nor partiality by the author.

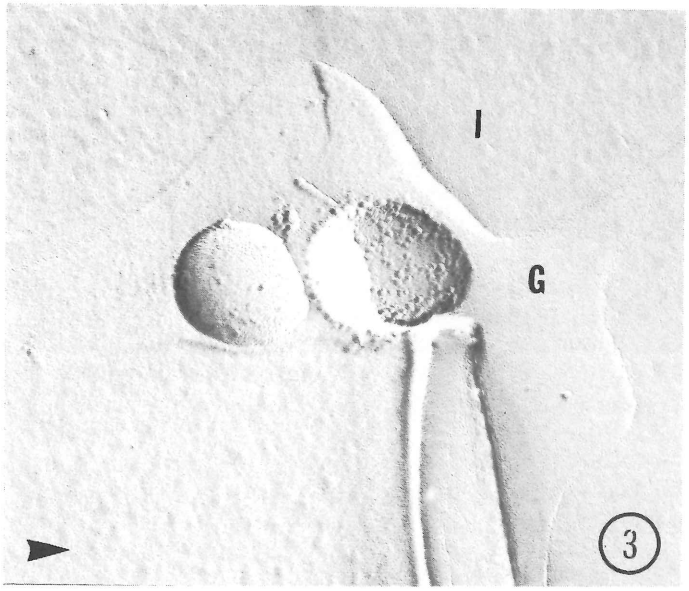

figure 3 — Platinum carbon replica of two isolated cardiac sarcoplasmic reticulum vesicles oriented and fractured as in Figure 2. Note how the dark platinum region is on opposite sides of the vesicles due to their convex and concave faces. Ice and glycerol segregate (I&G) during freezing. 75,000X

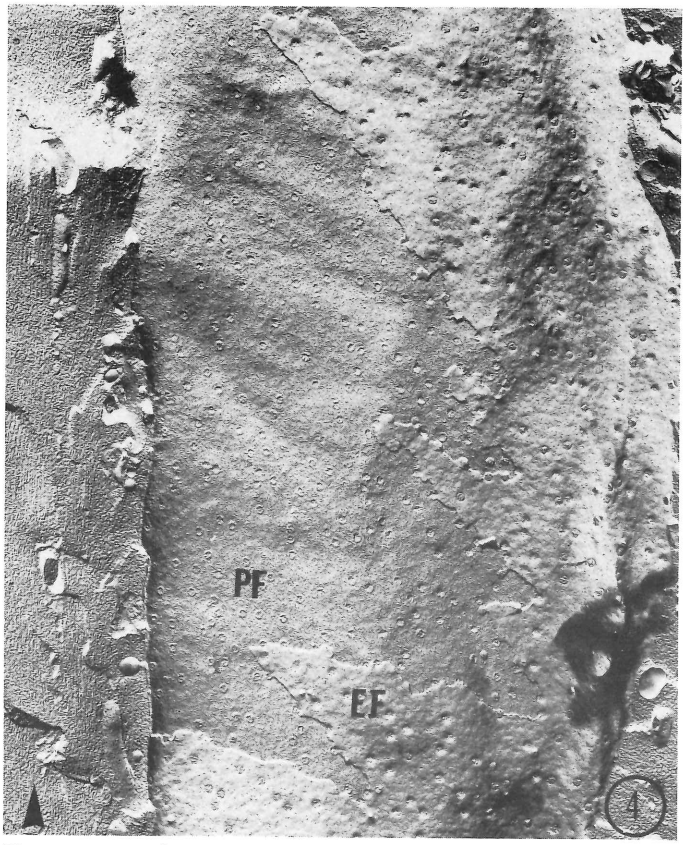

Figure 4— Two fracture faces of two membranes in dog heart nuclear envelope. Shadow direction shown by arrow. PF face of inner membrane is physically "lower" than EF face of outer membrane. However, turning the micrograph 180° may temporarily give the opposite effect. 30,000X

(Bohler). In addition to several review of freeze-fracture artifacts, a symposium on this topic was held at the American Society for Cell Biology meetings in San Antonio.

One of the most persistent artifacts or source of less than optimal replicas is the presence of water vapor in the specimen chamber. Although more efficient vacuum systems and cold traps have helped reduce surface contamination, the specimen itself may be the source of its own contamination via liberation of water molecules and their subsequent condensation back upon the freshly cleaved surface. Specimen chips adhering to the Balzers microtome blade may also be a prime source of water contamination. Water contamination produces "wartiness" of membrane particles and an "orange-peel" effect on phospholipids, while heavier water condensation on the fractured surface may actually obscure membrane protein particles entirely.

During the fracturing process it is possible that structures, i.e. membrane proteins and layer structures, may be "stretched" resulting in the artifact known as plastic deformation. Unlike other artifacts, however, it is not well understood nor is its solution at hand.

The replication process itself, by depostion of platinum, may lead to false interpretations. It should be remembered that platinum enlarges particle size while diminishing corresponding "pits" or depressions.

Although not a true artifact, problems often occur in interpretation of freeze-fracture micrographs. To avoid problems in topographical orientation, especially since "white" shadows are very deceiving, it is often suggested that micrographs in publications be oriented such that the shadowing direction is from the bottom. To illustrate the problem, examination of the nucleus in Fig. 4 (in which the shadow is from the bottom) reveals that the more particulate fracture face of the inner nuclear membrane (left side, labelled PF) is lower than the other membrane fracture face (right side, labelled EF). However, turing the micrograph upside down will, to most observers, reverse the perception of which membrane is truly inner or outer. As a further attempt to clarify inner or outer or concave or convex faces, use of internegatives or a direct film reversal process, both of which result in "dark" shadows, has been used. Proponents of the"black" shadow method maintain that the brain can more easily perceive and assimilate information from such micrographs while proponents of the "white" shadow argue that the true nature of the replica (white being electron transparent due to lack of platinum) is maintained. Hand lens examination of membrane particles in micrographs to determine shadow direction is most helpful to trained observers but those inexperienced in freeze-fracture procedures and observation still find interpretation difficult.

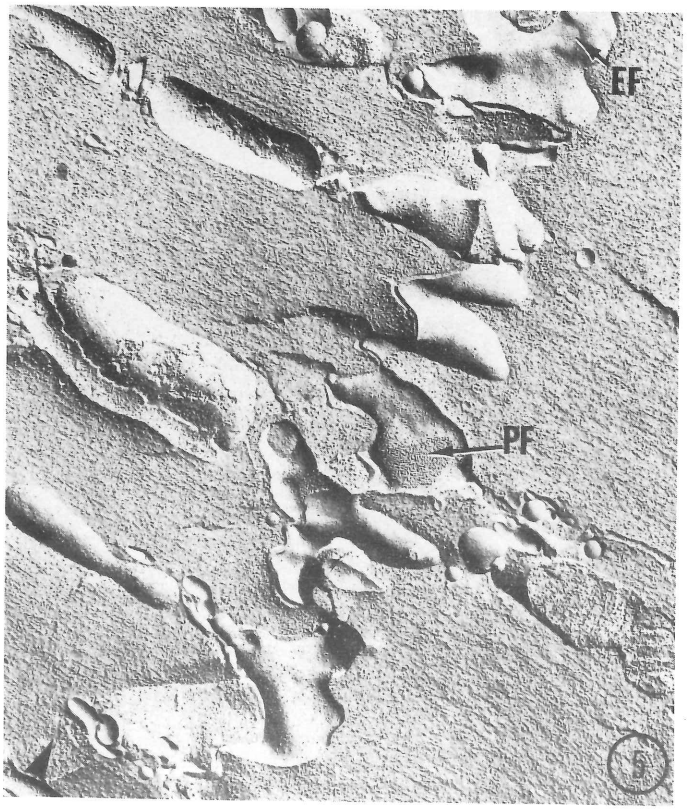

Figure 5 — Intercalated disc from cat right ventricular papillary muscle showing aggregates of particles (nexus) in PF face and corresponding "pits" in EF face. 24,000X

MEMBRANES AND FREEZE-FRACTURE

It is apparent that no scientific discipline has gained more with the advent of freeze-fracture electron microscopy than has membrane biology. Although the composition of biological membranes has been known for some years, the general macromolecular organization of membrane phospholipids and proteins prior to freeze-fracture observations was not clearly understood.

By revealing **en face** views or large areas of membrane, freeze-fracture has revealed new structures involving discrete regions of membrane specialization such as putative acetylcholine receptors, cell junctions and the "zipper" region of certain sperm, to name only a few.

Freeze-fracture now provides direct visual correlation with physical observation of the dynamic state of membranes and has given support to the current fluid mosaic model of biological membranes proposed by Singer and Nicholson.

Although initial freeze-fracture studies indicated that the fracture plane exposed the true outer or inner face of membrane bilayer, results of subsequent studies supported the now-accepted image of a fracture plane which travels through the hydrophobic core of the bilayer (See Figure 2g). Thus the particles actually observed on fracture faces represent the "rear ends" of those particles which face either the outside or inside of the cell or organelle. Only by etching (Figure 2c) is the true outer or inner surface of the membrane revealed. Combined fracture-etch procedure therefore may yield four separate views of one membrane. Designation of the "faces" has been confusing, if not to those associated with the field, certainly to those in other aspects of cell biology.

Based on the standarized nomenclature for the four possible faces of each membrane, (Branton, et al) the fracture face associated with the protoplasmic matrix (or the matrix of the nucleus, mitochondrion or chloroplast) is designated PF. That face in contact with the extracellular space (or the spaces inside the endoplasmic reticulm, between the double membranes or organelles) is designated EF. The true surfaces (visible only after etching) of the

above faces are the PS and ES, respectively. These terms are meant to supercede such designations as A,B,C,D, and numerous other less-descriptive terms.

There is substantial evidence from numerous studies that the particles observed in fracture faces represent proteins intercalated in the smoother lipid layer, although the possibility that some adhering phospholipids may also contribute to the shape of the particles needs further clarification. The development and use of devices to recover replicas from both halves of the bilayer has aided in showing, in some cases, that particles and various aggregates such as those in the nexus (See Fig. 5) epithelial tight junctions and arrays in muscle sarcolemma do, in fact, leave imprints or complimentary "pits" on the opposite fracture face. However, the precise nature of such particles, resolution of subunits, possible splitting of transmembrane proteins and problems in plastic deformation of proteins during the fracturing must still be resolved.

Reversible changes in protein particle distribution may be observed in membranes following alteration in PH or temperature. Thermotropic partitioning of proteins due to phospholipid transition from sol to gel phases may be easily observed by freeze-fracture techniques in artificial as well as native systems (Fig. 6). Correlative studies of differential scanning calorimetry and changes in energies of activation of intercalated enzymes may be used with freeze-fracture techniques.

Comparative freeze-fracture data of number, distribution and surface labelling of particles has provided visual correlation of such membrane activities as active cation transport and surface receptors. Further developments which slow promise relate changes in membrane protein distribution to overall membrane structure in numerous pathological situations such as Duchenne muscular dystrophy, and renal and myocardial ischemia. Elegant application of stereological techniques to freeze-fracture such as that shown by Weibel and his co-workers should provide basic quantitation for many comparative studies. Additional new applications such as rotary shadowing, computer-aided quantitation and paired stereo images of freeze-fractured replicas are but a few of the recent developments in freeze-fracture that hold promise for this procedure in elucidation of macromolecular cytology.

ACKNOWLEDGEMENTS

The author expresses his thanks to Dr. Bill R. Brinkley for the use of the Balzers 301 in his laboratory. This work is supported by grants from the Jerry Lewis Neuromuscular Disease Research Center, the National Heart and Blood Vessel Research and Demonstration Center, and the National Institutes of Health.

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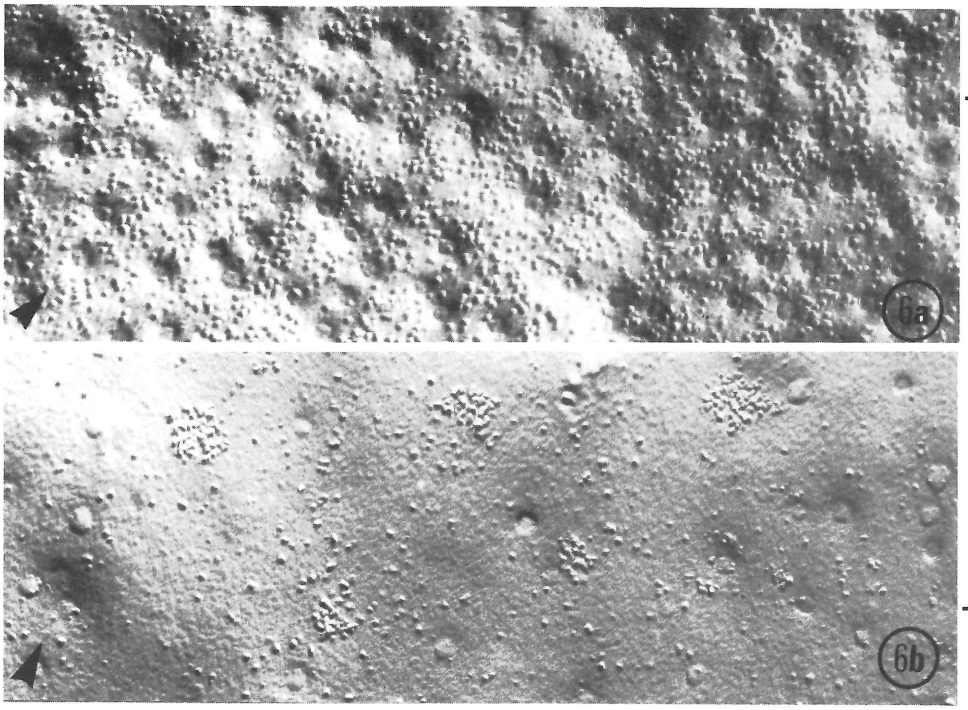

Figure 6a, b — Cappilary plasma membrane luminal PF fracture faces incubated at 36°C (upper) and 4° (lower) prior to fixation and rapid freezing. 64,000X

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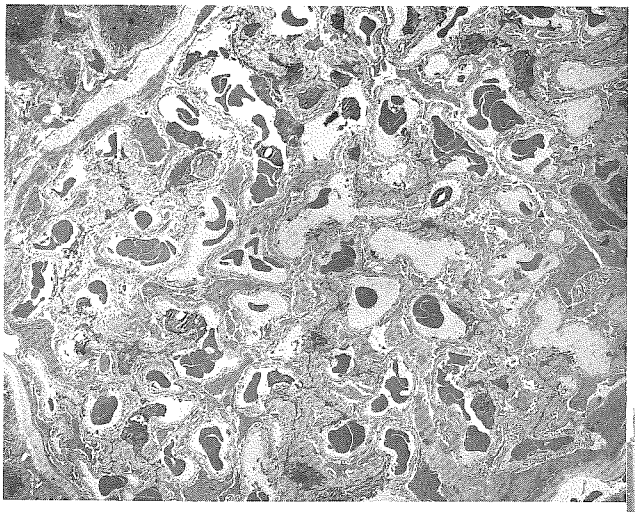

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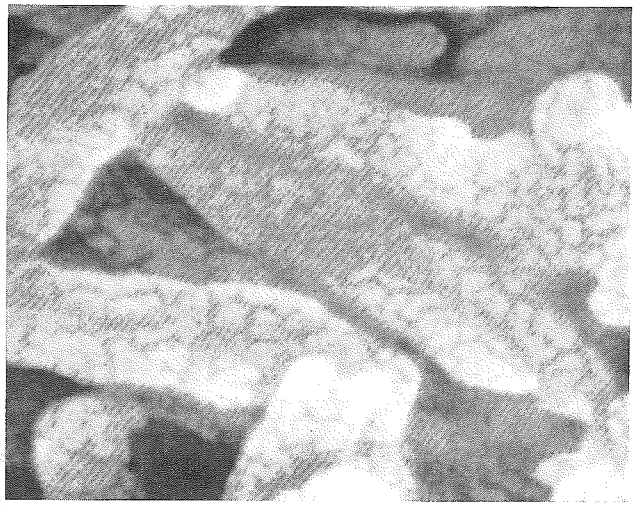

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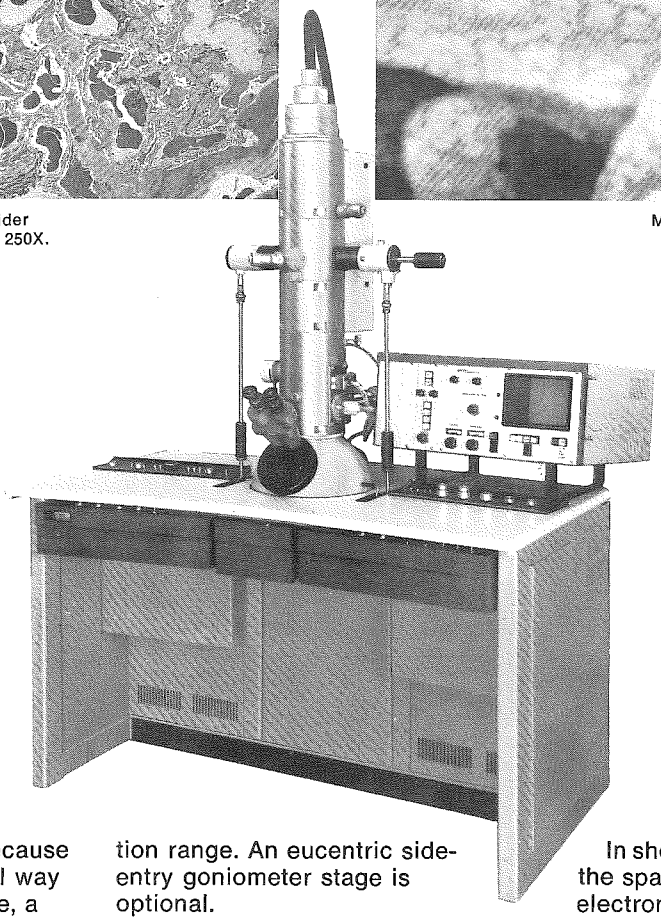

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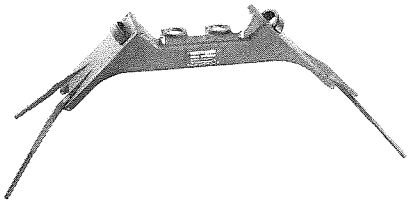

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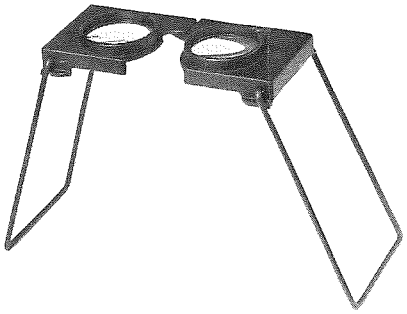

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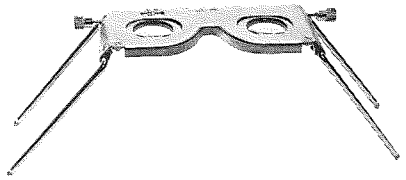

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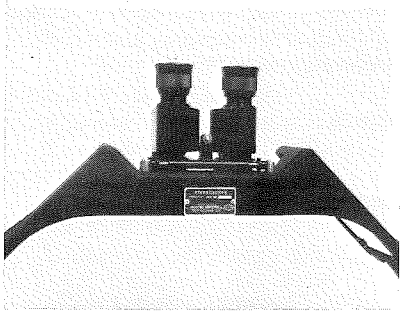

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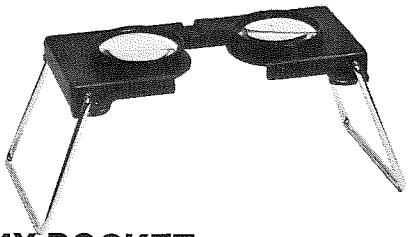

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Abstracts

* Poster Presentation

MYOGENESIS IN DYSTROPHIC CHICKEN MUSCLE GROWN IN VITRO. E. Raworth Allen and James F. May, Department of Anatomy LSU Medical Center, New Orleans, Louisiana 70119.

New Hampshire chickens, homozygous for inherited muscular distrophy, display clinical manifestations at two weeks of age. Recent studies from the same strain have shown abnormalities in muscle at an early embryonic age. Tissue was taken from the pectoralis muscle of twelve day control and diseased embryos and grown identically and simultaneously in tissue culture. Growth patterns were monitored with an inverted phase contrast microscope prior to fixation for transmission electron microscopy.

After only two days in culture, distrophic could readily be differentiated from control tissue. Both cell types spread out in an attached monolayer of elongate cells growing on a glass surface. Dystrophic myoblasts appear as elongate cells polarized in their orientation, in a sheet like fashion. The control myoblasts, however, tend to become more closely associated in small non-polarized clusters. After four days in culture, multinucleated muscle straps accumulate in the control cells whereas many myoblasts do not fuse but form pseudostraps in dystrophic cultures. Myofilaments are randomly scattered within the sarcoplasm of both cell types, and a few crude, non-striated myofibrils are seen in control myotubes. Cross striations are evident with phase contrast microscopy and a few spontaneous contractions occur in control myotubes after six days in culture. Typical banding patterns are seen within these myofibrils observed in electron microscopy. In dystrophic muscle, many unorganized myofilaments and rare myofibrils occur. It appears that myogenesis in dystrophic is similar to normal muscle but less organized and delayed. The results to these preliminary studies indicate a factor present of unknown etiology but intrinsic to the muscle tissue to cause a retardation in histogenesis prior to degenerative changes.

ULTRASTRUCTURE OF GREGARINES PARASITIC IN THE INTESTINE OF TENEBRIO MOLITOR LARVAE. Mary Barham, Department of Biology, Stephen F. Austin State University, Nacogdoches, Texas 75962.

The mealworm **Tenetrio molitor** is often infected with several species of gregarine intestinal parasites (Protozoa, Sporozoa). The life cycle of these gregarines involves only sexual reproduction, with the formation of gametocysts within which spores are produced. Infective sporozoites, released from ingested spores, attach to the epithelium of the host intestine, after which they undergo a period of growth and development. Subsequently, they detach and are free in the intestinal lumen.

The adult gregarine is characterized by longitudinally oriented epicytic ridges. The pellicle is composed of three membranes, of which the middle and inner are in close proximity. Electron dense fibrils are adjacent to the inner pellicular membrane near the ridge tips. The cytoplasm contains numerous characteristic cellular organelles. A septum divides the organism into an anterior region, the protomerite, and a posterior region, the deutomerite. The protomerite often has dense granules concentrated near the anterior end. The nucleus is always located in the deutomerite.

Certain ultrastructural modifications appear during the early development of the attached trophozoite stage. The attachment organelle, or epimerite, is electron transparent near its outer edges, with organelles clustered in its center. The formation of the epimerite is followed by the appearance of the septum and the development of ridges at the posterior end of the organism.

FIBROCLASTS IN HUMAN SCARS. Paul S. Baur, Jr. George F. Barratt, and Gary M. Brown, Department of Human Biological Chemistry and Genetics, University of Texas Medical Branch and Shriners Burns Institute, Galveston, Texas 77550

Osteoclasts and chondroclasts are cells involved in the degradation of bone and cartilage, respectively. Both cell types enzymatically mediate the breakdown of collagen and mucopolysaccharide ground substances such as chondroitin 4 & 6 sulfate and keratin sulfate. We have observed by light and transmission electron microscopy cells of seemingly similar function within human scar tissues. These cells are most prominent in tissues showing the greatest degree of remodeling activity. Scanning electron microscopic studies of these same tissues have shown that over a period of time the remodeling processes convert solidified masses (nodules) of scar tissue into nearly normal configurations of collagen fibers. The ultrastructural aspects of these cells, which we propose to call **Fibroclasts**, are comparable to those reported for osteoclasts. The cells appear to be similar in size to fibroblasts and are likewise filled with rough endoplasmic reticulum, mitochondria, and numerous vesicles.

GASTRIC MUCOSA IN GERMFREE AND CONVENTIONAL RATS. M.V. Benson, M.L. Zimney, Department of Anatomy, and F.C. Nance, Department of Surgery, LSU Medical Center, New Orleans, Louisiana 70112.

Germfree rats are protected from restraint-induced gastric ulceration as shown by Nance et al. This investigation compares the surface mucous cells (SMC) of gastric mucosa in nonstressed conventional and germfree rats.

Specimens prepared for transmission electron microscopy (TEM) were fixed in 3% glutaraldehyde buffered with cacodylate, pH 7.4. Subsequently they were postfixed in osmium, dehydrated with alcohol, and embedded in a mixture of Epon and Araldite. Thin sections were stained with uranyl acetate and lead citrate. Specimens for scanning electron microscopy (SEM) were similarly fixed, and then dehydrated in alcohol. After critical point drying with liquid CO_2, they were coated with gold palladium.

With TEM surface mucous cells of conventional rats contained many apical mucous granules, fine strands of rough endoplasmic reticulum (RER), few lysosomal-like bodies, and a small interstitial space. SMC of germfree rats had fewer apical mucous granules and a vacuolated apical membrane — suggestive of granule loss. These SMC also had fine RER and some lysosomal-like bodies, but the interstitial space was larger.

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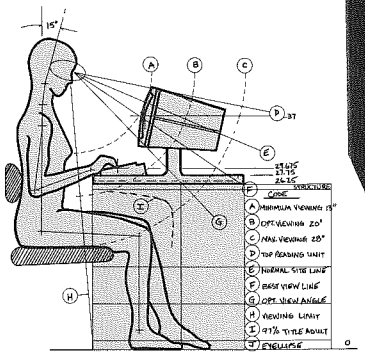

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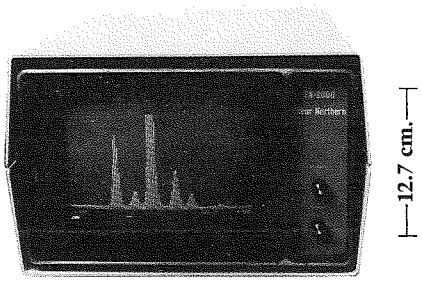

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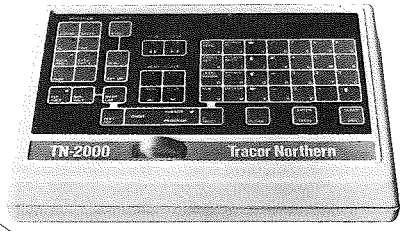

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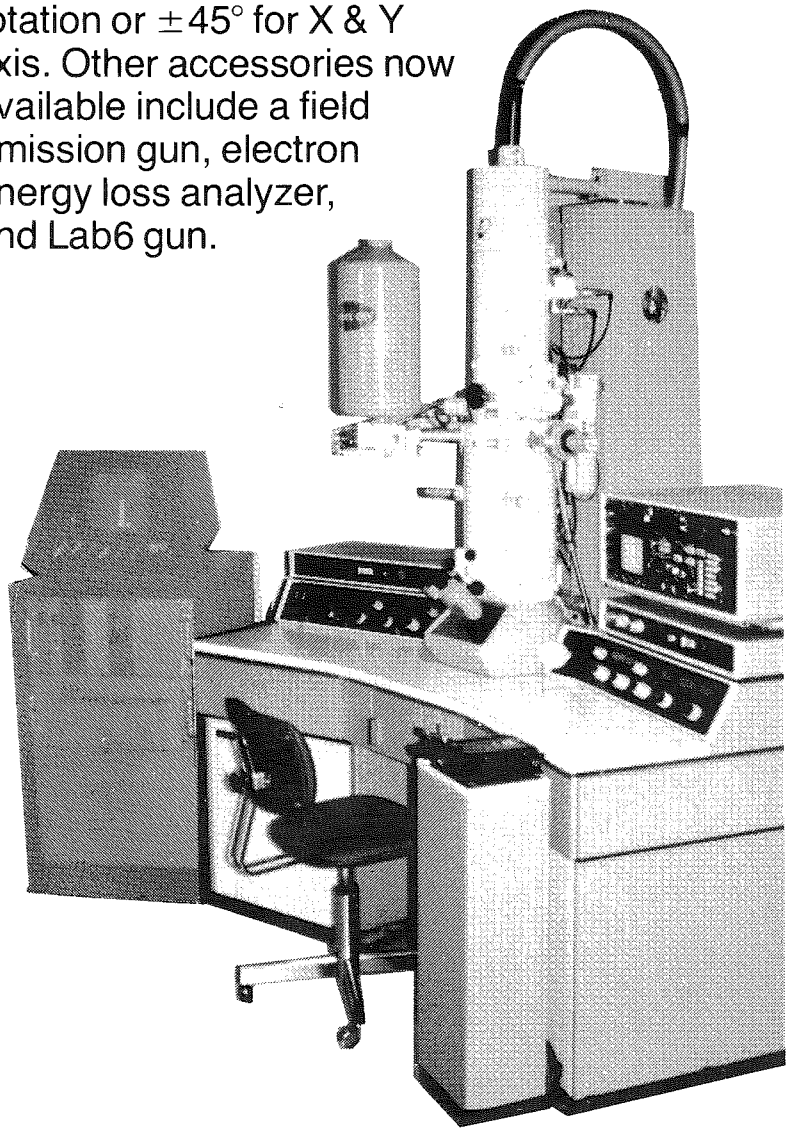

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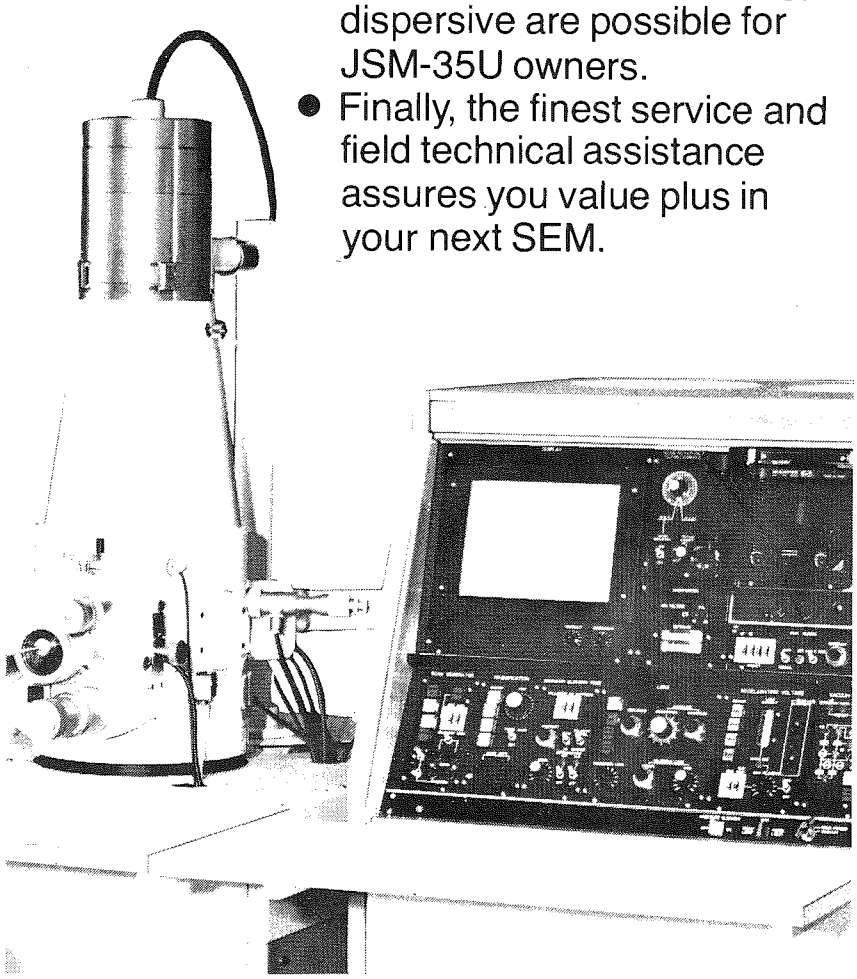

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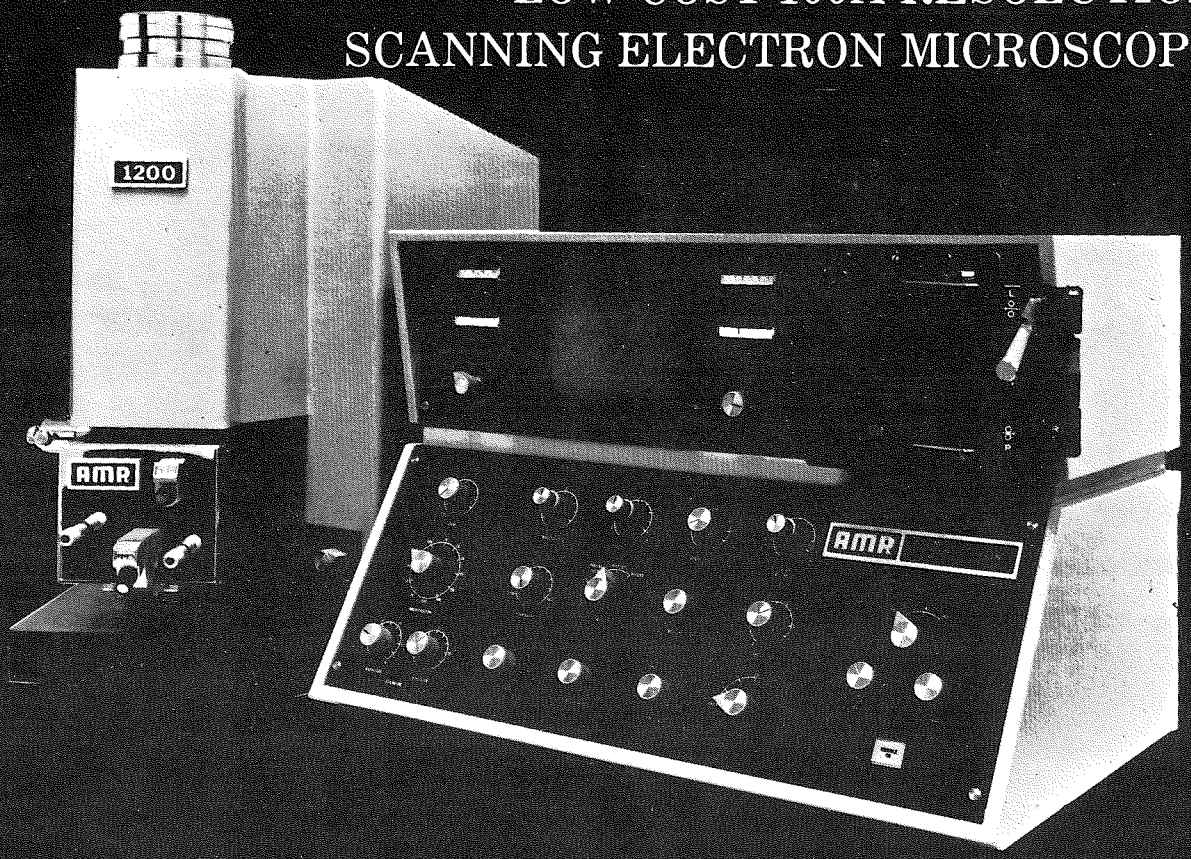

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STEREOLOGICAL ANALYSIS OF COTTON FIBERS. Jerry D. Berlin and Franklin Bailey, Department of Biological Sciences, Texas Tech University, Lubbock, Texas 79409.

Cotton fibers are individual cells that arise from the outer epidermal layer of the cotton ovule. The fibers first initiate elongation on the day of anthesis and continue elongating for 15-20 days resulting in a 3,000 fold increase in cell size. Transmission electron micrographs of cells in the outer epidermis and of developing fibers were quantitatively analyzed and the relative areas of several organelles correlated with other studies, e.g., autoradiographic studies, to compile a dynamic profile of fiber development. For example, the 10 fold increase in nucleolar size in fibers at one and two days post-anthesis was accompanied by ^{3}H-uridine incorporation, presumably into rRNA. By six days postanthesis the nucleolus had decreased in size and uridine was no longer incorporated suggesting that ribosomes required during later stages of fiber development were preformed by six days postanthesis. Also, the number of lint and fuzz fibers on mature cotton seeds of a number of commercial, as well as unusual, cultivars was determined by scanning electron microscopy. Statisitical analysis showed significant differences between cultivars for the number of fibers per seed, but no difference for different locations on the same seed.

The generous support of the State Organized Research Funds, College of Arts and Sciences, Texas Tech University and Cotton Incorporated is acknowledged.

A SYSTEMATIC SCANNING ELECTRON MICROSCOPIC EXAMINATION OF THE SOLIDAGO SEMPERVIRENS L. COMPLEX (COMPOSITEA). Roger Boettcher, Department of Botany, Louisiana State University, Baton Rouge, Louisiana 70803.

Solidago (Compositae) is a worldwide genus of about 100 species with its greatest diversity in temperate North America. The treatment of this genus has often been found to be taxonomically difficult and has resulted in confusion in the literature. The group is composed of many species complexes which may at first appear distinct but upon closer examination are found to intergrade in many respects and even to hybridize. The most useful taxonomic treatments of this group consider all known characters in an attempt to show overall relationships among the taxa. The present study is an examination of the **Solidago sempervirens** L. complex using the scanning electron microscope. Features examined include style branches, phyllaries, epidermal surfaces, trichomes, stomata and achenes. The information obtained with the scanning electron microscope adds to our overall knowledge of the genus and helps to clarify relationships among the taxa. Hopefully, through consideration of scanning electron microscope characters future taxonomic treatments will more closely reflect phylogenetic affinities within this group.

ULTRASTRUCTURE OF THE SPERMOGONIAL STAGE OF THE RUST FUNGUS GYMNOSPORANGIUM CLAVIPES: SPERMATIUM FORMATION AND HOST-PATHOGEN RELATIONSHIP. Janice Borland, Department of Biology, Stephen F. Austin State University, Nacogdoches, Texas 75962.

The spermogonial stage of **Gymnosporangium clavipes** is produced on the leaves of hawthorne (Craetagus). Basidiospores of the fungus infect the leaves, giving rise to a limited mycelium consisting of intercellular hyphae. These hyphae give rise to absorbing organs called haustoria which penetrate the wall of the host and invaginate the host plasma membrane. These haustoria are hyphal-like in appearance, and a single septum is located at the proximal end of the haustorial apparatus in the spermogonial stage of **G. clavipes** is not as morphologically complex as that reported in the uredial and telial stages of other rust fungi.

The spermogonium is a small flask-shaped structure produced in an infected leaf. It contains the male and female sex organs of the fungus. The male sex organs are termed spermatia. Spermatium formation in **G. clavipes** is similar to phialidic conidiogenesis in the deuteromycetes. The spermatiophore is of fixed length and possesses a thickened neck region and a distinct collarette of wall material at its distal end. Each Spermatium initially possesses a thin wall which is attached to the inner surface of the spermatiophore wall in the region of the collarette. Spermatia are blown out the tip of the spermatiophore in a basiseptal fashion, delimited by a centripetally forming septum, and pushed into the spermogonial cavity by the next spermatium initial. Mature spermatia possess a single layer of thin wall material, a single U-shaped nucleus, and numerous lipids, mitochondria, and ribosomes.

SEM OBSERVATIONS ON THE ORIGIN AND DEVELOPMENT OF THE CORK WINGS OF EUONYMUS ALATUS. William R. Bowen, Department of Biology, University of Arkansas at Little Rock, Little Rock, Arkansas 72204.

Preliminary SEM observations on the origin and development of cork wings of the burning bush, **Euonymus alatus,** will be correlated with previous light microscopical observations. The first phase of development involves the delimitation of grooves or depressions in the surface of the primary stem. The epidermal cells within this groove region are distinct from those of the surrounding stem surface. The second phase involves the differentiation of a localized phellogen beneath, and within, the confines of this groove. Activity of this phellogen results in the rupture of the epidermis at the margins of the groove and subsequent emergence of the cork wing. Continued phellogen activity, as delimited by the groove, accompanies development of the cork wing.

CYTOPLASMIC TUBULES IN EARLY SPORE WALL FORMATION IN A MOSS (MUSCI), DITRICHUM PALLIDUM. Roy Curtiss Brown and Betty E. Lemmon, Department of Biology, University of Southwestern Louisiana, Lafayette, Louisiana 70504.

The earliest indication of spore wall formation in the moss, **Ditrichum pallidum**, occurs in the newly separated tetrad immediately following meiosis. An amorphous thickening accumulates along the periphery of the incipient spores within the original sporocyte wall. This dense layer is underlain by parallel ranks of cytoplasmic tubules. We suggest that the initiation of the moss spore wall is a function of the sporocyte-spore cytoplasm and not, as has been supposed for other mosses, a heterogenous contribution of tapetum or tapetal-like cells.

A MECHANICAL KNIFE BREAKER AND KNIFE INSPECTION DEVICE FOR RALPH-TYPE GLASS KNIVES. James K. Butler, Ultrastructure Research Laboratory, Department of Biology, The University of Texas at Arlington, Arlington, Texas 76019.

Two devices, a knife breaker and an inspection stand, that greatly simplify the production and selection of Ralph-type knives are discussed. Routine production of knives with usable edge lengths of from one to two and a half centimeters is commonplace. These knives are particularly suited to cutting large face-blocks of water miscable-plastic embedded specimens in the 0.5 to 4 um thickness range. These sections are well adapted to use for both light microscope morphological and cytochemical surveys preliminary to electron microscopical studies. The inspection stand is usable without modification for inspecting either Ralph or Latta-Hartmann knives. A simple means of mounting Ralph knives that eliminates the use of molten wax is also described.

CORRELATION OF INTRACELLULAR ELEMENT CONCENTRATION AND THE CESSATION OF CELL PROLIFERATION DURING THE MATURATION OF CARDIAC MYOCYTES IN MICE. Ivan L. Cameron and Nancy K. R. Smith, Department of Anatomy, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284.

To determine the percentage of ventricular myocytes with a nucleus labled with tritiated thymidine (^{3}H-T) as a function of age after birth, mice were given three injections of ^{3}H-T (1 μCi/gm body weight) spaced eight hours apart and the mice were killed eight hours after the last injection. The ventricles 3 mm above the apex were cut off for histological and radioautographic preparations. There was a rapid decline in the labeling index (a measure of cell proliferation) during the first two weeks after birth. A low percentage of cells were labeling up until 50 days after birth, but no cells were labeled in older mice. A correlative study using x-ray microanalysis to measure intracellular concentration of elements was done in collaboration with S. G. Dykes. The data show that Na, Cl, K and P all decrease in concentration as the myocytle matures. Thus, the decrease in Na, Cl, K and P concentration can be related to the control of cell proliferation in this and several other **in vivo** mammalian cell populations. This relationship between element concentration and cell proliferation control **in vivo** will be presented as a theory.

THE BLOOD LEUKOCYTES OF ICTALURUS PUNCTATUS. M. S. Cannon[1], T. E. Eurell[2], H. H. Mollenhauer[3], D. H. Lewis[2], C. TOMPKINS[4] and A. M. Cannon[1], Departments of [1]Anatomy, [2]Veterinary Microbiology, [3]Pathology [4]College of Medicine, Texas A&M University, College Station, Texas 77843 and [3]Veterinary Toxicology and Entomology Research Laboratory, AR/SEA, USDA, P.O. Drawer GE, College Station, Texas 77840.

The blood leukocytes of the channel catfish **(Ictalurus punctatus)** were determined from Leishman-Giemsa stained smears, supravital blood films visualized on phase contrast microscopy, and by electron microscopy. Blood cells were also subjected to the periodic acid-thiosemicarbazide-silver proteinate (PA-TSC-SP) reaction for glycogen and certain glycoproteins. The present study agrees with several previous investigations that obvious errors in fish blood leukocyte identification, particularly regarding granulocytes, can result from observations derived exclusively from fixed, stained blood films.

Several distinct types of blood leukocytes occur in the channel catfish; i.e., small lymphocytes, medium and large lymphocytes, neutrophils (hetrophils),monocytes, thrombocytes and macrophages. Transitional forms between monocytes and macrophages are also present. Neither blood eosinophils nor basophils appear to be present. Thrombocytes and small lymphocytes are the most abundant leukocytes, while characteristically present near the elongated ends of thrombocytes. Small to moderate amounts of glycogen are present in small lymphocytes, while large numbers of glycogen granules are present in neutrophils. Monocytes possess more RER, more free ribosomes, fewer granules, and much less glycogen, than neutrophils. Detailed morphology of each cell type is given.

ULTRASTRUCTURE OF CEPHALEUROS (CHLOROPHYTA) GAMETES AND THE QUESTION OF SYMMETRY IN ALGAL MONADS. Russell L. Chapman, Department of Botany, Louisiana State University, Baton Rouge, LA 70803.

Examination of gametangia in natural **(i.e.** foliicolous) **Cephaleuros** thalli has revealed that nearly mature, unreleased gametes bear two isokont "keeled" (or "winged") flagella similar to those present in zoospores of **Cephaleuros** and in zoospores and/or gametes of **Phycopeltis** and **Trentepohlia.** Two multilayered structures (MLS) and component microtubular splines are present in each cell and are positioned lateral to, rather than beneath, the basal bodies. The two basal bodies are parallel, overlapping, side by side, and inserted from opposite sides of the apical papilla. A single prominent nucleus is located in the anterior region on the gamete directly beneath the flagellar apparatus. The arrangement of basal bodies and MSL appears to be similar, but not identical to that proposed for the "motile cells" of **Trentepohlia.** In discussions of the arrangement of subcellular components (especially the flagellar apparatus) in algal monads,the terms "symmetrical" and "asymmetrical" are employed despite clear disagreement among investigators over the denotations of the terms. The external morphology of **Cephaleuros** motile cells as seen with the light microscope and scanning electron microscope appears to be bilaterally symmetrical; however, consideration of the internal morphology as seen with the transmission electron microscope indicates that the motile cells cannot be divided by a median plane to form simple mirror images. Such median planes separate **reversed** mirror images. Thus, the gametes can be described as having "reversed bilateral symmetry" or "balanced asymmetry." The same semantic question arises in discussion of the biflagellate and quadriflagellate cells of other chroolepidaceous taxa and of other algae in general.

MORPHOLOGICAL OBSERVATIONS ON Y-1 CELLS. Mike A. Clark, Bill Brown and Jerry W. Shay, Department of Cell Biology, The University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

The Y-1 cell line originally derived from a murine adrenal tumor has retained the ability to secrete steroids in response to ACTH treatment. These cells and their response to ACTH were observed using the SEM, TEM and indirect immunofluorescence in an effort to help elucidate the mechanism of steroid secretion.

As observed in the SEM, prior to ACTH treatment, Y-1 cells are epithelioid in shape and their surfaces are populated by few microvilli and blebs. As observed in the TEM the cells have few microtubules, but many cholesterol crystals. When these cells are stained with antitubulin antibody and observed in the fluorescent microscope, many bright fluorescing granules may be seen.

Teatment of the cell line with ACTH for 30 minutes results in the cells rounding up and a 10-20 fold increase in steroid secretion. In the SEM these cells, after ACTH treatment, are now covered by many blebs. As seen in the TEM, the cells now have many microtubules and the cholesterol crystals now contain electron lucent areas. In addition, the bright fluorescing granules which were stained with antitubulin antibody in control cells have largely disappeared. We also investigated the effects of colchicine and cytochalasin B which are known to affect microtules and microfilaments, respectively. Both cytochalasin B and colchicine were found to disrupt the granules and also initiate steroid secretion. Thus, our preliminary data suggests that tubulin may be important in the regulation of steroid secretion.

CORRELATION OF BIOCHEMICAL EVIDENCE OF LIPID PEROXIDATION WITH MORPHOLOGIC CHANGES SEEN IN RAT LIVERS FOLLOWING THE ADMINISTRATION OF DIQUAT TO SELENIUM-DEFICIENT ANIMALS. Jane H. Dees, Department of Biochemistry, University of Texas Health Science Center, Dallas, Texas 75235, and Raymond F. Burk, Departments of Medicine and Biochemistry, LSU Medical Center, Shreveport, Louisiana 71130.

Lipid peroxidation can be assayed by the production of

malonaldehyde in cell homogenates, but until recently no reliable method has been available for its detection in intact animals. It has now been shown that ethane production in expired air as measured by gas chromatography correlates with malonaldehyde formation and may be used to follow lipid peroxidation in intact animals. Serum glutamic pyruvic transaminase (SGPT) levels reflect the extent of liver damage.

During the testing of ethane production following treatment with various compounds in control, Vitamin E-deficient, or selenium-deficient rats , it was observed that a low dose of Diquat, a bipyridylium herbicide, leads to high levels of ethane production as well as high SGPT levels, suggesting lipid peroxidation and liver necrosis respectively.

In order to correlate morphologic changes with the above biochemical data, livers were obtained from Diquat treated (19.5 μmoles/kg, i.p.) animals at 0 time, 30, 60, 90, 120 and 165 (time of death) minutes, and processed for light and electron microscopy. Evidence of cell injury in these livers is clear cut at 60 minutes, just as ethane and malonaldehyde levels are rising. At the time of death the livers are congested and necrotic correlating with elevated SGPT levels. Light and electron micrographs of the livers at various time intervals will be related to the biochemical data at corresponding times.

Poster Session.

APPLICATION OF A MODIFIED GOLGI METHOD TO STUDY SUBSTANTIA GELATINOSA FINE STRUCTURE. Donald Duncan and Ricardo Morales, Department of Anatomy, The University of Texas Medical Branch at Galveston.

A modification of the Golgi method described recently by Fairen, Peters and Saldanha was used to study the fine structure of the substantia gelatinosa in the spinal cord of the cat. The essential feature of this method is replacement of the silver chromate deposited with the Golgi method by metallic gold particles. The gold particles tag individual cells in such a way that all processes can be identified without recourse to reconstruction from serial sections. The major results so far have been to confirm observations and inferences obtained by use of other methods. Of these the most important was to supply positive visual evidence in support of studies indicating that a nerve cell in the substantia gelatinosa receives input from many dorsal root fibers and that individual dorsal root ganglion cells communicate directly with many neurons in this part of the spinal cord.

A number of unexplained artifactual changed induced in fixed tissues by the method will be presented for discussion.

COMPARATIVE FREEZE-ETCH STUDY OF THE BACTERIUM AZOTOBACTER VINELANDII. Carol S. Fuqua, Karen S. Howard, and M.D. Socolofsky, Department of Microbiology, LSU, Baton Rouge, Louisiana 70803.

The technique of freeze-etch has been used in a comparative study of vegetative cells of the Gram negative soil microorganism **Azotobacter vinelandii.** Replicas of cells untreated prior to rapid freezing were compared with those of cells cryoprotected with glycerol, or fixed with glutaraldehyde, or both glutaraldehyde fixed and glycerol cryoprotected. The outer surface of etched untreated cells is characterized by a tetragonal arrangement of subunits (S) along with numerous flagella. This surface is not visable when cells are cryoprotected. The major fracture plane appears through the outer membrane (OM) in all preparations, exposing both a concave face (OMF) and the opposing convex face (OMF). A secondary fracture plane frequently appears through the inner membrane (IM) yielding a concave face (IMF) and a convex face (IMF). All four fracture faces are studded with numerous intramembranous particles (IMP), although particle distribution and size vary. The most external face, OMF, and the most internal face, IMF, show the greatest IMP densities, while the largest IMP are found in the OMF. There is no variation seen in the fracture planes of cells treated with glutaraldehyde; i.e., no secondary fracture planes are seen. The information gained from this study has enabled development of a topographical freeze-etch model of untreated vegetative cells of **Azotobacter vinelandii.**

A MORPHOMETRIC ANALYSIS OF ISCHEMIC MYOCARDIUM. M. A. Goldstein, D. L. Murphy, Dept. of Medicine, Baylor College of Medicine, Houston, Texas 77030 and A. Schwartz, Dept. of Pharmacology, Univ. of Cincinnati Medical School, Cincinnati, Ohio 45267.

Progressive morphological changes in ischemic (left posterior papillary muscle, LLP) and control (left anterior papillary muscle, LAP) regions of canine hearts were studied. Muscle strips removed immediately after a 5 or 10 minute occlusion of the left circumflex artery were compared to those removed after reperfusion for 20 or 60 minutes following the 10 minutes of occlusion and to those removed immediately from normal dogs. Fibers which showed no apparent damage and no severe shortening at the light microscope level were examined by electron microscopy for subtle changes. Decreased glycogen content and a variety of mitochondrial changes were observed in fibers from ischemic regions as early as 5 minutes after ligation. These changes were further assessed by morphometric analysis of cells with and without glycogen depletion both in ischemic and control regions. Volume fractions of mitochondria and number of lipid droplets per unit area were compared in LAP and LPP in the five groups of animals - normal, 5, 10, 10-20, and 10-60. The ultrastructural changes in this study are believed to be transient adjustments which correlate with the biochemical changes at these same time periods.

OPTICAL RECONSTRUCTIONS OF THE CARDIAC Z LATTICE. M. A. Goldstein, Depts. of Medicine and Cell Biology, Baylor College of Medicine, Houston, Texas 77030, J. P. Schroeter, Dept. of Chem. Engineering, and R. L. Sass, Depts. of Biology and Chemistry, Rice University, Houston, Texas 77001.

Laser optical diffraction and image reconstruction techniques have been used to analyze electron micrographs of normal Z bands in canine cardiac muscle. Both longitudinal and cross sections show a three dimensional repeating structure partially obscured by coexisting amorphous matrix material. Images of the ordered lattice structure have been obtained through optical reconstruction of each projected EM image filtered at the Fourier transform plane. Filters were constructed to pass only the central beam and the first few orders of diffraction spots. Reconstructed images of cross sections clearly showed the presence of two lattice forms. Both forms were routinely observed in the reconstructed image from a single Z band. Both forms could be generated from either of two distinctly different optical diffraction patterns. These images suggest that the two lattice forms coexist, interconvert or represent two different aspects of the same detail within the lattice subunit. Reconstructed images from longitudinal sections show connecting filaments at each 38 nm axial repeat in an array consistent with the cross sectional data. A three dimensional model of the Z lattice based on diffraction and reconstruction data from cardiac Z bands is presented. Projections of the three dimensional model of the Z band are compared with optically reconstructed EM images.

A STUDY OF LOASACEAE TRICHOMES BY SEM. Rachel Goss and Clarence B. Sinclair, Department of Biology, University of Arkansas at Little Rock, Little Rock, Arkansas 72204.

Gross morphological features and trichome types have commonly been utilized to distinguish members of the family Loasaceae. The best descriptions of the trichomes separate them

into unicellular and multicellular forms, and then further categorize them under a total of seven types.

Herbarium materials were used to make specimens for scanning electron microscope study. Ten (10) genera and a total of thirty-three (33) species were investigated. Our electron microscope studies of these materials indicated that trichomes of the family might better be segregated into twelve (12) types, with some of the types divided further. It also seems probable the genera can be separated by means of trichome types. Further investingation will be needed to substantiate the latter possibility.

CALCIUM LOCALIZATION AND ITS POSSIBLE ROLE IN SECRETION IN THE CAROTID AND AORTIC BODY. John Hansen and Nancy Smith, Department of Anatomy, The University of Texas Health Science Center, San Antonio, Texas 78284.

Chief cells of the carotid and aortic body chemoreceptors are characterized by the presence of abundant cytoplasmic vesicles. These vesicles measure 90-120 nm in diameter, exhibit a central electron-dense core and are known to store catecholamines. The mode of secretion in most cells which store secretory products in membrane-bound vesicles or granules is by the energy and calcium dependent process of exocytosis. We have observed oxocytosis of these dense-core vesicles in both thin section and freeze-fracture preparations. Oschman and Wall (JCB **55**: 58, 1972) have suggested that calcium-containing fixatives may enhance the ultrastructural identification of calcium binding sites intracellularly. Following vascular perfusion of rabbit carotid and aortic bodies with a fixative containing 50mM $CaCl_2$, the depostion of a 20-30 nm electron-dense particle (EDP) is observed in some of the chief cell vesicles. The EDP is usually eccentrically located within the vesicle and appears randomly oriented with respect to the plasmalemma. Approximately 44% of the carotid body and 16% of the aortic body vesicles contain an EDP in any single cross-section. The presence of calcium in the chief cells was verified by electron probe X-ray microanalysis. The EDP probably represents the foci for calcium binding in these catecholaminergic vesicles and may indicate the sites where calcium acts preceding the secretion coupling event. (Supported by a Grant-in-Aid from the American Heart Association (77 630) and with funds contributed in part by the Texas Affiliate).

SCANNING ELECTRON MICROSCOPY OF THE PRE-INFECTION ACTIVITIES OF HELMINTHOSPORIUM ORYZAE ON RESISTANT AND SUSCEPTIBLE RICE VARIETIES. F. C. Hau and M. C. Rush, Department of Plant Pathology, Louisiana State University, Baton Rouge, Louisiana 70803.

Scanning electron microscopy (SEM) achieves greater resolution and depth of focus than light microscopy. It is a powerful tool for studying the microflora in situ on aerial plant surfaces. Under SEM, four stages were recognised after rice leaf surfaces were inoculated with spores of **H. oryzae.** They were 1) spore germination, 2) germ tube elongation, 3) formation of appressoria, and 4) the formation of secondary hyphae.

Extracellular sheaths were consistently associated with hyphae and appressoria of the fungus. The sheaths had a high affinity for the papillae, which are waxy epidermal projections on the leaf surfaces. Under SEM, the wax crystals on the leaf surface were found attached to the underside of the sheath. An imprint was found after hyphae were removed from the leaf surface. Extracellular fungal sheaths were also observed on polystyrene leaf replicas. These observations suggested that the fungal sheaths were secretions of the pathogen and served to facilitate infection by attaching the fungus to the leaf surface.

ULTRASTRUCTURAL OBSERVATIONS ON THE HEART OF THE OYSTER, CRASSOSTREA VIRGINICA (GMELIN). W. E. Hawkins, H. D. Howse, and T. G. Sarphie, Department of Anatomy, University of South Alabama, Mobile AL 36688, and the Gulf Coast Research Laboratory, Ocean Springs, MS 39564.

The heart of the oyster consists of two atria and a ventricle suspended in a pericardial cavity. Both the atria and the ventricle consist of a trabeculated myocardium and an epicardium that is a single cell layer thick and rests of a collagenous basement membrane. The epicardium is studded with microvilli. The atrial epicardium is deeply folded while the ventricular surface is relatively smooth. Both the atrial and ventricular epicardial cells have few organelles and are connected by desmosomes and septate junctions. In the atrial epicardium, but not in the ventricular, are cells that have fenestrated plasmalemmae. Processes of these cells extend between the epicardial cells and the basement membrane. Since the pericardial fluid in molluscs has been shown to be an ultrafiltrate of the blood, the presence of these fenestrated cells that closely resemble mammalian podocytes may indicate that the atrium is the site of ultrafiltration in the oyster heart.

The myocardial trabeculae of the atria and ventricle tissue. Since there is no true endothelium, many myocardial cells have only a thin ruthenium red staining coat separating them from the blood. The myofibers have thick (35 nm diameter) and thin (8.5 nm diameter) myofilaments and are joined end-to-end by a primitive type of intercalated disc that includes a gap junction. Mitochondria of the myofibers frequently contain paracrystalline arrays such as prismatic cristae, helices, and lattices.

Poster Session.

AN ULTRASTRUCTURAL STUDY OF GRISEOFULVIN-INDUCED MICROTUBULAR DISARRAY AND MITOTIC DELAY IN PHYSARUM POLYCEPHALUM. Charles D. Hebert, John J. Wille, and W. L. Steffens, Department of Zoology and Physiology, Louisiana State University, Baton Rouge, Louisiana 70803.

The fungal metabolite griseofulvin has been shown to be an effective agent in delaying plasmodial mitosis in **Physarum polycephalum.** Previous studies on the drug **in vitro** (Weber, Wehland, and Herzog, 1976) and **in vivo** have shown that one effect of griseofulvin is to prevent normal polymerization of microtubular subunits into complete functional microtubules. We have examined the effect of griseofulvin on nucleolar migration and its possible mode of action of spindle microtubule assembly by electron microscopy. Studies show that nuclei of surface plasmodia treated with low doses of the drug (40 μg/ml) more than 90 minutes prior to control mitosis undergo a mitotic delay which is dependent on the time of application of the drug. Nuclei of plasmodia treated within 90 minutes of control mitosis suffer little delay, but experience an abnormal "ring-metaphase", similar to that for mammalian cells treated with colcemid. Electron microscopic observation of these ring-metaphase nuclei revealed a severe disorganization of microtubules, but retention of kinetochores and kinetochore fiber associations. The findings implicate the microtubule-organizer center as the primary site of action of griseofulvin in delaying the onset of mitosis.

CHARACTERIZATION OF THE ENCYSTMENT PROCESS OF THE BACTERIUM AZOTOBACTER VINELANDII BY SCANNING ELECTRON MICROSCOPY. Karen S. Howard and M. D. Socolofsky, Department of Microbiology, Louisiana State University, Baton Rouge, Louisiana 70803.

The soil microbe **Azotobacter vinelandii** has long been an

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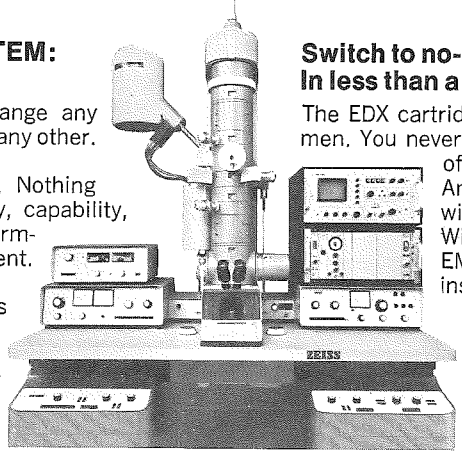

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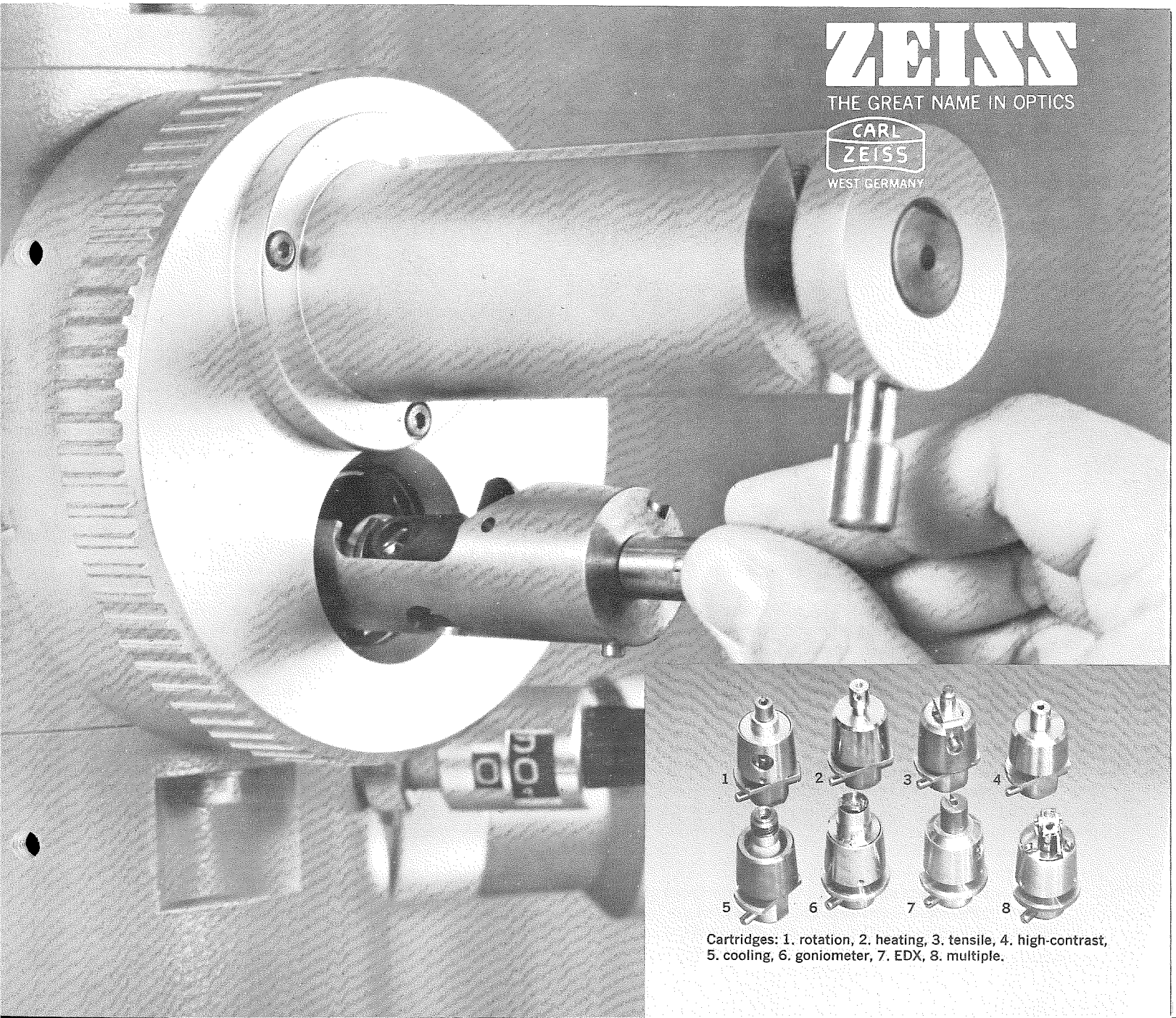

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object of investigation due to its unusual developmental cycle as well as its ability to fix nitrogen aerobically. The plemorphic forms of its life cycle have been observed at both the light and transmission electron microscope levels. Scanning electron microscopy was used in this investigation of the encystment procedure to produce information on a three dimensional level with resolution and depth of field superior to that of the light microscope. In addition, three dimensional analysis was aided by photographing images in stereo pairs.

Poster Session.

SURFACE FEATURES AND PHASE MICROSCOPY OF FRESHLY ISOLATED AND CULTURED NEONATAL RAT HEART CELLS. Frederick H. Kasten, Department of Anatomy, LSU Medical Center, New Orleans, Louisiana 70119, and Marilyn G. Kilgen, Department of Biological Sciences, Nicholls State University, Thibodaux, Louisiana 70301.

Heart cells were isolated from ventricles of 3- to 4-day rats using pieces of heart exposed to cold trypsin overnight and then digested sequentially with collagenase-hyaluronidase. Cardiac cells were separated from endothelial cells using Kasten's Differential Attachment Technique (90 mins. adhesion). Freshly isolated cells were fixed in 1% glutaraldehyde in PBS, bound to polylysine-coated coverslips, and prepared for SEM by critical point drying. Other cells were cultured and fixed after a period of 2 hrs. to 33 days **in vitro.** Spontaneous contractions and development of synchronized networks were observed by time-lapse cinematography. Film records demonstrated irregular contractions of myocytes at 15 hrs. and well-defined beating at 24 hrs. Formation of synchronized networks, binucleation, myofibrils, and migrating endothelial cells were also seen. SEM observations showed most freshly isolated myocytes to be rounded with convoluted surfaces. Cells attached and developed membranes on their lower surfaces at 2 hrs., leaving a dome shaped nuclear region on top. Membranes spread underneath the dome for 16 more hours. Then the cell became spindle-shaped, showed thick nuclear regions, binucleation (light spheres), and thick myopodia which connected to other cells. Isolated endothelial cells were rounded with fine surface processes. These cells attached in minutes, remained flat, and had numerous filopodia. Mixed cell cultures showed multilayering and lack of contact inhibition.

CINEMATOGRAPHY AND SCANNING ELECTRON MICROSCOPY OF ISOLATED ADULT MAMMALIAN MYOCYTES. Marilyn B. Kilgen, Department of Biological Sciences, Nicholls State University, Thibodaux, Louisiana 70301, and Frederick H. Kasten, Department of Anatomy, Louisiana State University Medical Center, New Orleans, Louisiana 70119.

Contractility and morphology of adult rat and dog myocytes were characterized using phase cinematography and scanning EM. Cells were isolated with collagenase/hyaluronidase techniques and stained with trypan blue. Fractions with the highest viabilities were fixed in glutaraldehyde and critically point dried for SEM. Freshly isolated cells had trypan blue viabilities of 50 - 80%. Unstained cells exhibited spontaneous contractions while stained cells did not contract. Beating cells slowed as many developed surface vesicles and eventually degenerated into flattened or rounded cells and died. Few survived more than several hours at 25° or 37°C, and none were able to live in cell culture conditions which support neonatal rat heart cells. With SEM, healthy isolated cells exhibited typical branching and cross-striations with mitochondria bulging between Z lines on the cell surface. T tubule openings and raised T tubules were seen at the level of the Z lines. Intercalated discs could be seen at the ends of the cells and cell branches. Cell damage seen with SEM included fine holes in the sarcolemma, pocked-marked surface vesicles and spherical mitochondria protruding between loose degenerating bundles of myofibrils. It was concluded that although adult cardiac cells isolated by conventional enzymatic procedures become irreversibly injured, a high percentage of them do exhibit contractions before they undergo morphological degeneration and die.

Poster Session.

ULTRASTRUCTURAL STUDIES OF SUBFORNICAL ORGAN AND THE AREA POSTREMA IN SPONTANEOUSLY HYPERTENSIVE RATS. P. M. Klara and E. D. Frohlich, Department of Anatomy, Tulane Medical School and Division of Hypertension Research, Ochsner Foundation Hospital, New Orleans, Louisiana 70112.

While the etiology of essential hypertension remains obscure, most investigators feel that the central nervous systems (CNS) is involved. Two CNS structures, the area postrema (AP) and the subfornical organ (SFO), are sensitive to the pressor agent, angiotensin and may play a role in the initiation of maintenance of the hypertensive state. With this in mind, ultrastructural studies of the SFO and AP in the spontaneously hypertensive rat (SHR), one of the best models of human essential hypertension, were undertaken. Results of transmission electron microscopy indicate that neuronal and perivascular pathology is present in the parenchyma of the AP and adjacent structures in the SHR. Similar pathology has not been demonstrated in the SFO or surrounding structures. Surface ultrastructure of the AP shows no significant alterations as compared to controls. However, the SFO appears to show increased numbers of type II supraependymal cells on SEM examination. The alteration in morphology observed in the SHR, AP and SFO may be the result of ischemia and reflect concommitant physiological changes. Changes in the physiological response of angiotensin sensitive areas of the CNS could thus play a significant role in the etiology of hypertension.

IDIOPATHIC ORTHOSTATIC HYPOTENSION: A PHYSIOLOGICAL, BIOCHEMICAL AND ULTRASTRUCTURAL STUDY. Richard L. Klein, Asa Thureson-Klein, Jack McC. Baggett and Herbert G. Langford, Departments of Pharmacology and Medicine, Univ. Miss. Med. Ctr., Jackson MS 39216.

Idiopathic orthostatic hypotension is a relatively rare syndrome manifested by dizziness, a marked fall in blood pressure and syncope upon standing. Usual therapeutic measures, such as blood volume expansion with salt and corticosteroids, are largely unsatisfactory. The etiology of the disease and the specific underlying defects in noradrenergic funtion are unknown. It is suggested that patients can be divided into two general classifications (Zeigler, Lake and Kopin, 1977): (1) Those with documented CNS defects and 'apparent' peripheral autonomic nervous systen dysfunction (Shy-Drager type) who are reported to have normal circulating catecholamines and low dopamine β-hydroxylse. (2) Those with autonomic nervous system deficiency but without CNS defects ('peripheral' type) who are reported to have abnormally low levels of circulating catecholamines and low dopamine β-hydroxylase. In a Clinical Research Center Project, a protocol was developed to test for possible defects in sympathetic function and to perform correlative ultrastructural examination of saphenous vein biopsies. Based on the results from the above, a number of possible therapeutic regimens could be considered which include direct and indirect sympathomimetic stimulation and blocking of presynaptic inhibitory feedback mechanisms to improve autonomic dysfunction. Comparisons of five patients (3 reported

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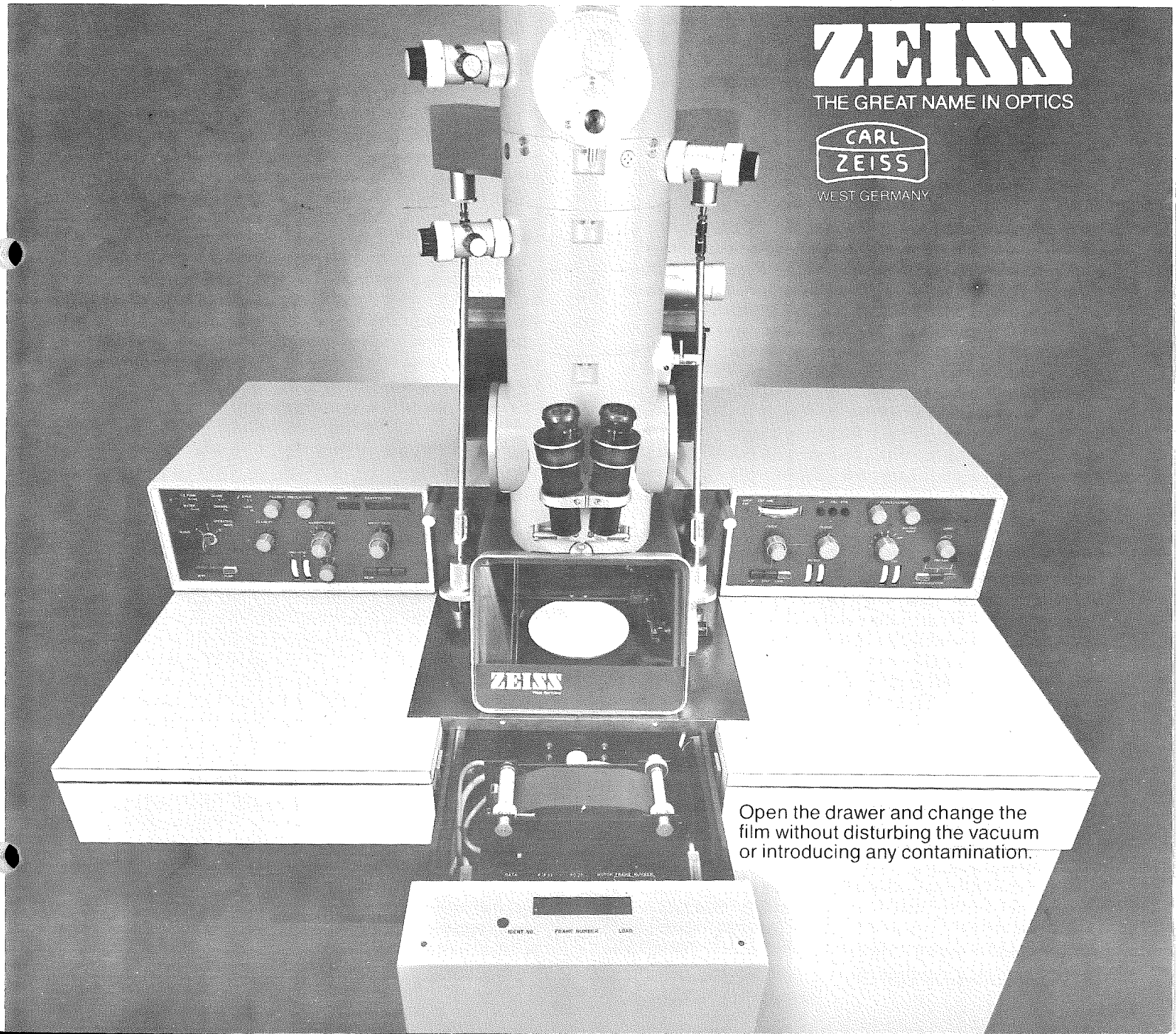

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with symptoms suggestive of idiopathic orthostatic hypotension reveal pronounced differences in circulating catecholamine, blood pressure and pulse rate responses to postion change, as well as differences in the ultrastructural characteristics of the sympathetic innervation and smooth muscle of saphenous veins.
Supported by GM 15490 and RR 00626 USPHS.

Poster Session.

AGE-RELATED ALTERATIONS IN STRUCTURAL, HISTOCHEMICAL AND PERMEABILITY CHARACTERISTICS OF RAT CEREBROVASCULATURE. Craig A. Knox and Robert D. Yates, Department of Anatomy, Tulane University School of Medicine, New Orleans, Louisiana 70112.

Most investigations of the morphological changes which occur in the aging central nervous cystem have centered on structural alterations which occur in neurons and glia. Although many reports have briefly mentioned vascular abnormalities in the aging brain, a systematic ultrastructural evaluation of age-related changes in the arterioles, venules and capillaries of the cerebral cortex is absent from the literature. In our studies, we are attempting to elucidate the role of cerebrovascular pathology in aging of the brain. Since chronic hypertension is postulated to induce premature aging phenomena in various tissues, we are comparing the ultrastructural, histochemical and permeability characteristics in the frontal cortex of aged Wistar-Kyoto (WKY) and Spontaneously Hypertensive Rats (SHR).

Our preliminary findings suggest that qualitatively similar changes occur in the cerebral blood vessels of aged SHR and WKY. These alterations include: (1) extensive collagen deposits in the subendothelial space, adventitia and in the neuropil surrounding blood vessels; (2) abnormal deposition of basal lamina in the neuropil, with focal increases in the thickness of the subendothelial basal lamina; (3) large vesicular inclusions within the endothelium; (4) inter-endothelial tight junctions appear morphologically intact and remain impermeable to exogenous (i.v.) horseradish peroxidase; (5) fragmentation and vesicular degeneration of smooth muscle cells in cortical arterioles; and (6) deposits of flocculent material in the adventitia of cortical arterioles. In addition to these findings, the endothelium of cerebral blood vessels in SHR is characterized by an increase in the numbers of intermediate (10 nm diameter) filaments and increased activity of magnesium-activated adenosine triphosphatase in the luminal plasmalemma. Widespread alterations in the blood-brain barrier to horseradish peroxidase in aged WKY have not been demonstrated. Vascular permeability in aged SHR will also be discussed.

ENDOMYOCARDIAL BIOPSY IN ANTHRACYCLINE CARDIOMYOPATHY. Bruce Mackay, M.D., Robert S. Benjamin, M.D. and Sidney Wallace, M. D., Departments of Pathology, Developmental Therapeutics, and Radiology, The University of Texas System Cancer Center, Houston, Texas, 77030.

Anthracycline antibiotics, notably adriamycin, have been found to have a broad range of activity in a number of human solid tumors. Their most significant side effect is irreversible and progressive cardiomyopathy. The toxicity is dose related, but individuals vary in their susceptibility, and monitoring procedures to detect the development of cardiac dysfunction are necessary in the management of patients receiving these drugs. Assessments of the effectiveness of various methods that have been used, including electrocardiograms and echocardiograms, have yielded conflicting data.

There is accumulating evidence that structural alterations in the myocardial muscle fibers can provide an indication of the presence and severity of anthracycline toxicity. Tissue is obtained from the right ventricular septum using the technique of percutaneous transvenous endomyocardial biopsy. The biotome is guided by fluoroscopy, and three bites are usually taken, providing material for 6 to 10 E.M. tissue blocks. One micron sections usually reveal an admixture of fat, and contracture artifact is common and must be taken into account as the biopsies are studied. Severe toxic changes can be detected by light microscopy, but ultrastructural study is essential for the detection of early changes and for an accurate evaluation of the extent and severity of the morphologic alterations. Since scattered fibers are affected in the earlier stages of toxicity, all the biopsy material must be be evaluated. The two main types of myocyte damage that occur are drop-out of myofibrils and distension of the sarcoplasmic reticulum. Mitochondrial changes may also be observed, but their significance is uncertain at the present time. Histopathologic assessment of endomyocardial biopsies appears to be an accurate method to detect and quantitate anthracycline cardiomyopathy.

ULTRASTRUCTURE OF THE CYST WALL OF SARCOCYSTIS SP. IN THE RAT. James R. Maleckar and Paul C. Beaver, Department of Tropical Medicine Tulane University, New Orleans, Louisiana 70112.

The rat, **Rattus norvegius,** was infected with sporocysts from the feces of the Malaysian Reticulated Python (**Python reticulatus**). Findings from light microscopy of the cysts which developed in the skeletal muscles revealed the presence of three distinct species. Ultrastructural studies showed that one species lacked villi, but contained irregular mushroom-shaped processes. Another species, previously described by Zaman and Colley (Z Parasitenk 47:169-185, 1975) as **S. singaporensis,** contained large villi situated on stalks which projected out from the cyst wall. The third species showed structures, a type of microvilli, previously not described for **Sarcocystis.** Bottle-shaped villi projected outwardly from the surface of the cyst wall. The microvilli were found arising from the surface of the cyst and the surface of the villi. In the apical region of the villi, large numbers of vesicles were seen. An accumulation of host cell mitochondria was found near the villi. No cellular breakdown of pathological changes were observed.

THE RELATIONSHIP OF CUTICULAR WAXES TO RESISTANCE TO THE RICE SHEATH BLIGHT DISEASE, D. S. Marshall and M. C. Rush, Dept. of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, LA 70803.

Rice plants of resistant, intermediate and susceptible varieties were grown in the greenhouse under identical conditions. Eight weeks after planting, some of the plants were excised and processed for scanning electron microscopy. Others were subjected to several treatments, then inoculated with **Rhizoctonia solani,** the causal organism of rice sheath blight. The treatments used were, (1) untreated; (2) plant tissue drawn through a pad of dry cotton, (3) plant tissue drawn through a pad of cotton saturated with chloroform; (4) plant tissue drawn through a pad of cotton moistened with water. Several treatments were used in order to determine their first effect on the reaction of the fungus to the host. Forty-eight hours after inoculation, the plant tissue of all treatments was fixed and prepared for scanning electron microscopy. It was found that the fungus was attached to all of the varieties under all treatments and that it grew along the tissue following the lines of junction of underlying epidermal cells. However formation of infection cushions differed for the different treatments. The results suggest that inpermeable waxes deposited onto the cuticle surface can be removed thereby allowing infection cushions to form on resistant varieties, making them susceptible.

HISTOLOGICAL AND ULTRASTRUCTURAL OBSERVATIONS OF TISSUES EMBEDDED IN LX112*, A NEW EMBEDDING MEDIUM FOR ELECTRON MICROSCOPY. Joe A. Mascorro and Craig A. Knox, Department of Anatomy, Tulane University School of Medicine, New Orleans, Louisiana 70112.

LX112, an epoxy resin similar in chemical and physical characteristics to Epon 812, has been employed successfully for embedding various biological tissues. This plastic **replaces** Epon 812 in the usual formulas and can be used according to the individual investigator's routine methodology. Utilizing LX112 with a WPE value of 145, a 50 gram mixture was prepared as follows: 25.39 grams LX112, 8.44 grams DDSA, 16.18 grams NMA and 1.0 gram DMP-30. The resulting medium proves to be a close facsimile to Epon 812 as regards viscosity, curing time, consistency, cutting qualities and beam stability. LX112 is thoroughly miscible with ethanol and propylene oxide. Following dehydration, tissues are passed to a 1:1 mixture of resin and propylene oxide or ethanol (1 hour) and full resin (3 hours) for final infiltration. Infiltration is best when tissues remain in full resin overnight, but final results are acceptable when the tissues infiltrate for the shorter time period. Polymerization is readily achieved at 70°C for 16-24 hours. The medium displays very satisfactory sectioning qualities. Thick and thin sections are very receptive to toluidine blue, lead citrate and uranyl acetate stains. Thin sections on unsupported grids can withstand a cross over beam without noticeable damage to the plastic and/or tissue. The relatively low viscosity, ease of infiltration, stainability and section strength make this plastic a useful alternative for electron microscopy embedding.

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AXON SPROUTING INTO SEGMENTS OF RAT SPINAL CORD ADJACENT TO THE SITE OF A PREVIOUS TRANSECTION. M. A. Matthews, M. F. St. Onge, C. L. Faciane, and J. B. Gelderd, Department of Anatomy, LSU Medical Center, New Orleans, Louisiana 70119.

Transection of the spinal cord in adult Long-Evans Hooded rats is followed by formation of a connective tissue matrix in the lesion site and the rapid erosion of the neural elements above and below this zone, particularly within the dorsal white columns. In the period between 15-45 days postoperative two significant events begin to occur. First, the injured surfaces of the divided cord become invested by a glial limiting membrane (glia limitans) and, concomitantly, large numbers of axons ensheathed by Schwann cells sprout into the scar matrix and along the eroded column region. The injured surface of the spinal cord is highly irregular with deep, collagen-filled rifts into which the sprouting axons may probe and penetrate into the adjacent normal neuropil. Electron microscopic examination generally reveals the interposed glia limitans and that these fibers are usually restricted to the peripheral enviroment. However, as some axons approach the reconstituting glia limitans, they are enveloped by an astrocytic cytoplasmic process which may either displace the Schwann cell or encompass it together with the enclosed axons. This last phenomenon appears to precede the entry of some axons into the neuropil and suggests that the glia limitans may not necessarily represent an impenetrable barrier to the passage of regenerating axons into the CNS. Apparent maintenance of most of these fibers for periods of up to three months may suggest that viable, functional synapses were established upon available neuronal elements, but clear evidence of this could not be documented.

Supported by USPHS Grants NS 14699-01, RR 05376-11 and the Edward G. Schlieder Educational Foundation.

Poster Session.

SPINAL CORD TRANSECTION: A QUANTITATIVE ANALYSIS OF ELEMENTS OF THE CONNECTIVE TISSUE MATRIX FORMED WITHIN THE SITE OF LESION FOLLOWING ADMINISTRATION OF PIROMEN, CYTOXAN OR TRYPSIN. M. A. Matthews, M. F. St. Onge, C. L. Faciane, and J. B. Gelderd, Department of Anatomy, LSU Medical Center, New Orleans, Louisiana 70119.

Long-Evans hooded rats were cordotomized at the T-5 level and given: 1) cyclophosphamide, (Cytoxan) an immunosuppressive; 2) Piromen, a bacterial polysaccharide-nucleic acid complex; 3) topical and systemic trypsin; 4) no further specific treatment. Because of past and present controversy surrounding the proposed ability of these agents to promote spinal cord regeneration, a systemic study, employing light and electron microscopy, and quantitative methodology in a single animal model, was done in order to re-evaluate the effects of such treatment upon the connective tissue matrix which forms in the defect left by transection.

After an initial inflammatory reaction during the first week following surgery, the lesion zone is characterized either by areas of dense collagenous connective tissue with occasional fibroblasts and macrophages, or a loose, areolar tissue with numerous sheets and cords of mesodermal cellular elements but minimal collagen. By 45 days postoperative (dpo), axons supported by Schwann cells invade and become entangled in the loose connective tissue matrix. With longer postoperative survival, cysts appear cranial and caudal to the lesion and erode much of the scar together with viable neural tissue. Administration of Cytoxan or Piromen did not result in any qualitative alteration of the scar matrix as evidenced by electron microscopy. Quantitative analysis revealed a slight reduction in the fibrous connective tissue component of the scar at 45-90 dpo but this proved to be a transient occurrence as longer postoperative periods were studied. The use of trypsin resulted in significant reduction of the amount of fibrous connective tissue with a concomitant increase in loose connective tissue and the appearance of a few distinctive, compact bundles of unmyelinated axons lacking Schwann cells.

Consistent behavioral modifications were not observed in any of the treatment groups which would significantly distinguish them from controls. Our results appear to contradict the findings of Matinian and Andreasian (1976) who reported return of normal sensori-motor function in 80% of their animals treated with topical and systemic trypsin.

It is concluded that a major impediment to whatever long term regenerative potential exists within the spinal cord is the lack of axonal guiding elements within the scar, but more importantly, the severe erosion of the remaining spinal cord due to cyst enlargement.

Supported by USPHS Grants NS 14699-01, RR 05376-11 and a grant from the Edward G. Schlieder Educational Foundation.

Poster Session.

ULTRASTRUCTURAL OBSERVATIONS OF EPIPLEXUS MACROPHAGES FROM NORMAL AND BCG-CHALLENGED DOGS. Dr. Randall E. Merchant, Department of Anatomy, Louisiana State University Medical Center, New Orleans, Louisiana 70119.

Kolmer first described macrophage-like, free cells resting upon the choroid plexus ependyma of mammals (Anat. Anz.,**54**:15,1921). More recent studies, employing modern techniques of scanning and transmission electron microscopy (SEM,TEM), confirmed Kolmer's conclusion that indeed epiplexus free cells possessed a morphology consistent with that of macrophages found on free surfaces in other locations of the body. The present investigation examines the morphological characteristics of epiplexus macrophages responding to the pre-

sence of bacillus Calmette-Guerin (BCG) in the cerebrospinal fluid. Mongrel dogs were anesthetized, a 19-gauge spinal tap needle was inserted into the cisterna magna and 0.5 ml of BCG (0.5-4.0 x 10^8 viable microorganisms) was injected. Three days following injection, animals were anesthetized and perfused with buffered aldehydes. The choroid plexus of each cerebral ventricle was removed, post-fixed in buffered 2% osmium tetroxide and routinely prepared for SEM and TEM. Choroid plexuses from normal animals (no BCG injection) were similarly prepared. At all ventricular levels, experimental animals expressed a ten-fold increase in their epiplexus macrophage populations. Many cells demonstrated pleomorphic shapes and smooth cell surfaces not unlike those found in normal animals. However, the majority of macrophages presented extensive blebbing and ruffling of their plasmalemmas. These alterations of the normal cell surface may reflect a state of activation of these cells due to the presence of the BCG antigen(Merchant and Low, J. Comp. Neur.,**172**:381,1977). Intimate cell associations mediated through cell surface microappendages may also reflect the initiation and/or maintenance of the cellular immune response to BCG (Merchant, et al., J. Reticuloendothel. Soc., **22**:199, 1977). The increase in population and non-specific activation of epiplexus macrophages by intrathecal injection of BCG may prove of clinical significance as in the control of meningitis caused by certain intracellular bacterial parasites or as a member of chemotherapeutic regimes combating central nervous system malignancies. (This work was supported by United States Public Health Service Grant NS 09363 from the Institute of Neurological and Communicative Disorders and Stroke.

Poster Session.

ULTRASTRUCTURE OF BASIDIOSPORE FORMATION IN THE FUNGUS PISOLITHUS TINCTORIUS. Charles W. Mims, Department of Biology, Stephen F. Austin State University, Nacogdoches, Texas 75962.

Pisolithus tinctorius is an important mycorrhizal fungus belonging to the class Basidiomycetes. For the present study, fresh basidiocarps of the fungus were collected in nature and returned to the laboratory where they were sectioned vertically to reveal the small pea-shaped chambers (peridioles) containing the basidia and basidiospores. Peridioles of various ages were removed from the basidiocarp and prepared for study with transmission electron microscopy.

Basidia of **P. tinctorius** develop from terminal hyphal cells. Ribosomes and mitochondria accumulate at the apex of the basidium along with the nucleus of the cell. Following meiosis basidiospore initials develop on the surface of the basidium. A large vacuole develops in the basal portion of the basidium and appears to push the contents of the basidium into the spore initials. Young basidiospores contain numerous mitochondria, ribosomes and strands of rough ER. Spores appear to be binucleate and are at first highly vacuolate. As the spore matures the vacuoles disappear and a droplet thought to be lipid in nature appears within the spore. This droplet increases in size until it almost completely fills the spore. At maturity the spore possesses a multilayered wall and is covered by prominent electron-dense surface markings.

VARIATIONS IN TISSUE PRESERVATION AS RELATED TO FORMULATION OF EPOXY RESIN. Hilton H. Mollenhauer and Robert E. Droleskey, Department of Veterinary Microbiology, Veterinary Toxicology and Entomology Research Laboratory, SEA/AR, USDA, P.O. Drawer GE, College Station, Texas 77840.

Epoxy resins are almost universally used in electron microscopy for embedding tissue samples. Preservation of cell ultrastructure is good and the resins are usually not difficult to section. A particular advantage reported for epoxy resins is a low linear shrinkage of only about 2%. Presumably, the low shrinkage factor contributes to the good tissue preservation. We have found, however, that marked differences exist between various brands and/or formulations of epoxy resins in relation to the electron microscopical appearance of the embedded tissue. The most noticeable difference is, perhaps, related to specimen contrast. However, significant differences also exist in relation to tissue preservation. For example, we have found that cellular organelles such as nuclei and peroxisomes may vary as much as 15% in linear dimension, dependent upon the brand and/or formulations of epoxy resin used. The preservation of other cellular constituents such as endoplasmic reticulum and mitochondria appears to vary also but these differences are difficult to document because of the nonregular form of the constituents. The above observations will be illustrated using aldehyde-osmium tetroxide fixed rat liver embedded in four commonly used epoxy resin mixtures.

GLOMERULAR PERMEABILITY IN THE FETAL RABBIT. Monica L. Monica, Marilyn L. Zimny and Sam G. McClugage, Department of Anatomy LSU Medical Center, New Orleans, Louisiana 70112.

This study was designed to determine fetal age differences in glomerular permeability using a graded series of dextrans, T-10, T-40, and T-11- (10,00;40,000 and 110, 000 M.W., respectively). Fetuses (120) of pregnant white rabbits 20, 22, 24, 28 days and one day old neonates were anesthetized with 20% urethane, a longitudinal incision was made in the abdomen and an incision was placed in the exposed uterus allowing for removal of fetus. Fetuses were injected with .1 - .2 cc of a 1% concentration of the dextran fractions through the superior sagittal dural venous sinus. Circulation time was 5 min. Fixative containing formaldehyde (1.5%) gluteraldehyde (2.5%) OsO_4 (0.66%) and lead citrate (2 - 3 mg/100 ml) in 0.1 M arsenate buffer, pH7.4 was used for dextran demonstration. Control specimens were fixed in 3% gluteraldehyde in 0.1M cacodylate buffer, pH7.2. Tissues were post-fixed in 2% OsO_4 buffered with cacodylate, pH7.2. All fetal ages and one day neonates showed glomerular filtration of T-10 and T-40 dextrans. Particles were visualized within the capillary lumen and Bowman's Space. T-110 fractions displayed an increased permeability with age. Filtration of this fraction was not seen until 28 days gestation and in one day old neonates. Dextran particles appeared on both sides of the glomerular basement mambrane establishing that filtration had occurred. Neither 20 nor 24 day fetuses showed filtration of T-110. Thus, there seems to be an increase in glomerular permeability to large MW particles (110,000 MW) with increasing age in the fetal rabbit kidney.

Poster Session.

GOLGI APPARATUS ALTERATIONS AND RADIOPRECURSOR UPTAKE IN STIMULATED GLANDS OF DROSERA CAPENSIS. Robert L. Outenreath, The Cell Research Institute, The University of Texas at Austin, Austin, Texas 78712.

Drosera capensis, a carnivorous plant native to South Africa, possesses remarkably complex glands which have been studied both developmentally and after stimulation with living insects. The gland cell changes that occur in response to insect capture are the subject of this presentation. By using a variety of techniques including ultrastructural morphology and radioautography at both the light and electron microscopic level, several aspects of stimulated glands revealed a marked proliferation of both the Golgi apparatus and of Golgi-associated vesicles.

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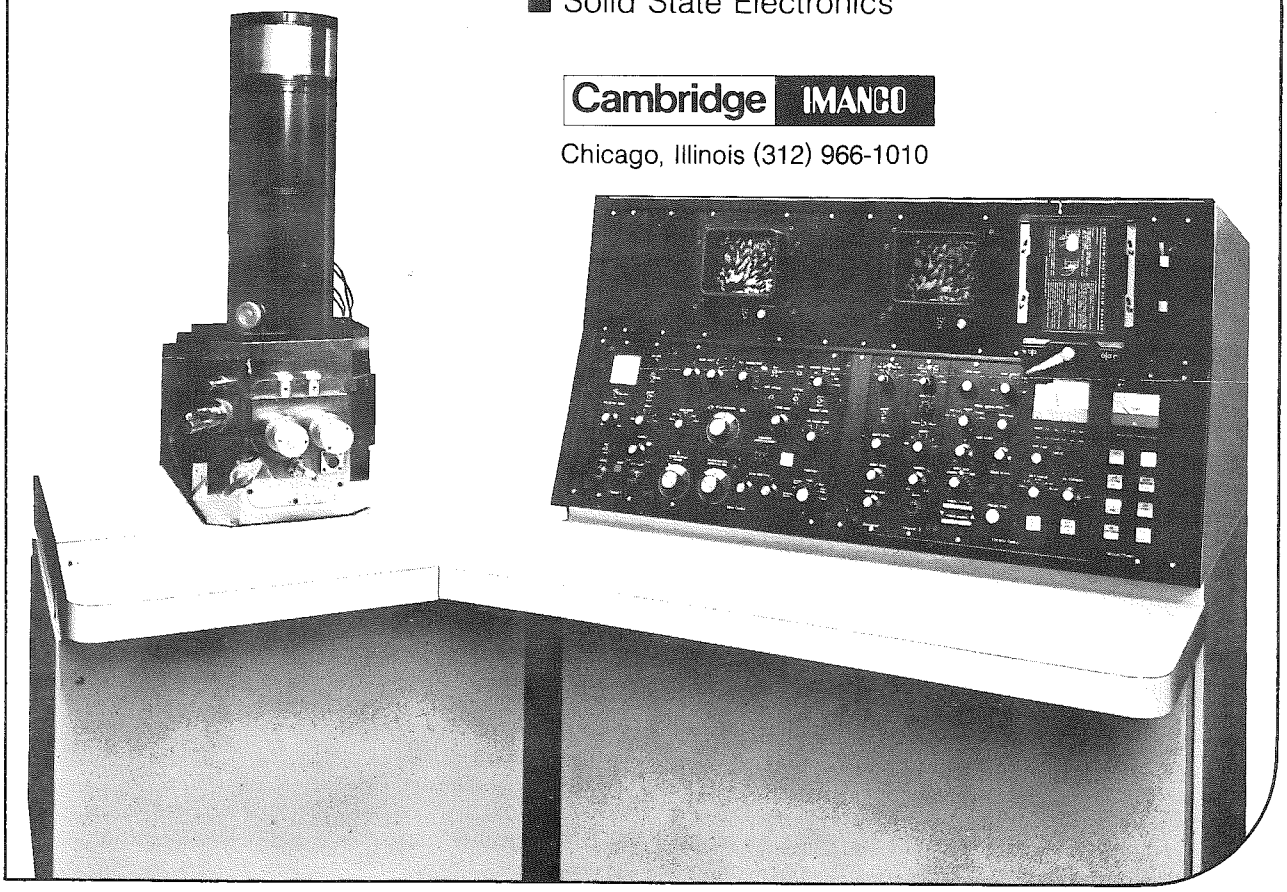


When glands were first stimulated and then allowed to incorporate tritiated galactose, label was observed to be almost exclusively localized over either the Golgi apparatus or over vesicles associated with the Golgi apparatus. Further, light level radioautography demonstrated that concentrated labeling occurred only in those outer digestive gland cells which are located at the upper half of the gland. These results not only establish the role of the Golgi apparatus in mucilage production in stimulated glands, but they also indicate that there exists a functional difference between the various gland cells of the **Drosera capensis** digestive gland.

SCANNING ELECTRON MICROSCOPE STUDY AND ENERGY DISPERSIVE X-RAY MICROANALYSIS OF DEPOSITS ON THE ROOTS OF SPARTINA ALTERNIFLORA. Micheal T. Postek, Department of Botany, and I.A. Mendelssohn, Center for Wetland Resources, Louisiana State University, Baton Rouge, Louisiana 70803.

Spartina alterniflora, salt marsh cord grass, is the dominant angiosperm of a large majority of regularly flooded marshes of the Atlantic and Gulf coasts. In Louisiana, this plant species often occurs in two distinct zones: a more productive streamside site (adjacent to tidal creeks) and a less productive and sparsely populated inland area. Reddish-brown deposits are present in the roots of streamside **Spartina**, while these coatings are visually absent on the inland roots. A scanning electron microscope study, coupled with energy dispersive X-ray microanalysis of streamside roots demonstrated these coatings to be restricted to the cell wall of the outer epidermis and to consist primarily of iron. As demonstrated by scanning electron microscopy, inland roots possess only small amounts of this iron deposition.Dithionite extraction of these root coatings and subsequent atomic absorption spectrophotometric analysis confirmed these deposits to be iron. An order of magnitude more iron was present on streamside roots relative to the inland samples. The adaptive significance of this iron deposition on **Spartina** roots will be discussed.

PREPARATION OF RED/GREEN ANAGLYPHS FOR THE PRESENTATION OF STEROE ELECTRON MICROSCOPY. Micheal T. Postek, Department of Botany, Louisiana State University, Baton Rouge, Louisiana 70803, and W.L. Steffens, Department of Plant Pathology and Crop Physiology and the Department of Zoology and Physiology, Louisiana State University, Baton Rouge, Louisiana 70803.

The use of stereo in the presentation of optical electron microscopic data has become increasingly prevalent in recent years. The standard methods for the display of stereo data have been mounted stereo pairs, the light polarization method, and the red/green anaglyph method. Of the two methods suitable for group presentations, the polarization method, although an excellent technique, presents several distinct disadvantages. In this technique a special lenticular screen and dual projectors are necessary, in addition to special polaroid glasses. Generally the requirement of the lenticular screen is the greatest disadvantage, particularly for large groups, as these screens are usually rather small and not always readily available. Similarly, the standard anaglyph method usually requires stereoscopic or dual projectors and special glasses, but can be seen displayed on a standard screen. In this presentation we describe a simplified method for the preparation of red/green anaglyph slides. This technique provides the distinct advantage of the elimination of the need for a dual projector system, as the image is prepared on a single 35 mm slide.

Poster Session.

APPLICATION OF STEREO SCANNING ELECTRON MICROSCOPY TO FLORAL MORPHOLOGY OF MAGNOLIA GRANDIFLORA. Micheal T. Postek and Shirley C. Tucker, Department of Botany, Louisiana State University, Baton Rouge, Louisiana 70803.

The determination of the floral phyllotaxis and developmental ontogeny of the stamens and carpels of **Magnolia grandiflora** has been facilitated by the use of the scanning electron microscope. The great depth of field obtainable in this microscope imparts an apparent 3-dimensional appearance with resolution capabilities far superior to comparable light microscope techniques. Unfortunately, experimental error can be introduced due to perspective distortion in the 2-dimensional micrograph. Because of the compression, much information in the photo is obscured or unavailable. With the use of stereo scanning techniques, much more of the recorded information can be obtained and the distortion circumvented. This paper describes the application of stereo-anaglyph procedures in the analysis of floral bud micrographs of **Magnolia grandiflora.**

ULTRASTRUCTURE OF THE DEVELOPMENT OF DAUGHTER SPOROCYSTS TO CERCARIA OF SCHISTOSOMA MEKONGI. E.R. Rivera, Department of Biological Sciences, University of Lowell, Lowell, Massachusetts 01854.

Host snails **(Lithoglyphopsis apecta)** infected with miracidia of **Schistosome mekongi** were allowed to develop daughter sporocysts in the digestive gland. When cercaria began to be shed, the snail glands were inspected for ultrastructural development of sporocysts to cercaria. The early stages of sporocysts show highly condensed groups of cells contained within a wall that has fibrillar substructure. The sporocyst then expands producing more cells which become separated from one another. Most of the centrally located cells in the developing sporocyst have rather small amounts of cytoplasm in relation to their nuclei. The cells at the periphery are highly reticulate with large numbers of microvilli and mitochondria suggesting absorptive properties. At the time of sporocyst expansion, many osmiophilic granules develop which often have non-staining central cores. Simiarly staining granules are found in fully-developed cercarial acetabular glands. The muscle cells develop from peripherally located cells in sporocysts that initially show fine fibrillar components in the cytoplasm. These cells increase in size, the fibrils become more evident and the cells become appressed to one another. The oral sucker differentiates about this time and the cercarial cuticle become apparent. As the cercaria mature the cuticle develops a large number of acicular spines with slightly enlarged bases.

ULTRASTRUCTURAL OBSERVATIONS OF THE GELATINOUS MATRIX OF THE CHLOROPHYCEAN ALGA, HAEMATOCOCCUS. Danna Rosell and William R. Bowan, Department of Biology, University of Arkansas at Little Rock, Little Rock, Arkansas 72204.

A prominent gelatinous matrix usually distinguishes the motile species of **Haematococcus** from other unicellular members of the Volvocales. The gelatinous matrix is a jelly-like region that occurs between the cell wall and the plasma membrane of this algae. Ultrastructural observations of the nature, origin and development of the matrix in four species of **Heamatococcus** will be presented.

PHENCYCLIDINE-INDUCED CATECHOLAMINE RELEASE FROM GRANULES IN RAT ADRENAL MEDULLA. John Saer, Joe A. Mascorro, and Robert D. Yates, Department of Anatomy, Tulane University Medical School,

New Orleans, Louisiana 70112.

Phencyclidine (angel dust, PCP) is a much abused and potent schizophrenicomimetic drug. While PCP appears to affect most of the putative neurotransmitters in the central nervous system, little is known about its peripheral effects. The purpose of the present study was to determine if PCP affects a peripheral catecholaminergic (CAM) system such as the adrenal medulla (AM).

Twelve young adult female Sprague-Dawley albino rats were used in this study, in three groups of four. The control group received 0.15 cc. injection of saline, i.p.; one received 15 mg/kg PCP i.p., and the last group received 30 mg/kg PCP i.p. Each animal was perfused through the left ventricle with 3% glutaraldehyde in 0.1M phosphate buffer three hours after injection. Ultrastructural examination of the AMs of these rats indicated a release of CAMs from the granule pool in a dose-related fashion. Both norepinephrine (NE) and epinephrine (E) were affected, although the effects were more striking in the NE cells. E granules in the PCP-treated animals exhibited a slight decrease in granule opacity and number. While the affected E cells were in the minority in the 15 mg/kg group. The Ne granules in the 30 mg/kg series were markedly swollen, and their limiting membranes occasionally fused. Fusions of adjacent granules suggested intracellular channels through which NE might be released from the cells. The NE granules in the 15 mg/kg animals exhibited cores which were intermediate in form between the control and high dose animals. On the basis of altered granule morphology, these results suggest that PCP causes a release of CAMs from the granule pool on the rat AM.

EVIDENCE FOR NEURONAL SYNAPSES ON THE BASEMENT MEMBRANE OF CEREBRAL CAPILLARIES. K.E. Savage, P.S. Baur, J.S. Crawford, H.M. Eisenberg, and R.L. Suddith, Dept. of Human Biological Chemistry & Genetics, Shriners Burn Institute, Div. Neurosurgery, and Marine Biomedical Institute, University of Texas Medical Branch, Galveston, Texas 77550.

While innervation of large cerebral vessels by adrenergic and cholinergic nerve fibers is well documented, similar knowledge of the innervation of the cerebral microvasculature is unknown. In this study isolated cerebral microvessels (rat) were examined using transmission and scanning electron microscopy to determine if neuronal terminals are associated with the cerebral capillaries. Further, the presence of the degradative enzymes for acetylcholine and the biogenic amines in these microvessels were investigated using radioenzymatic assay techniques. Cappillaries were isolated from the cerebral cortex of mature Sprague-Dawley rats by serial filtration and the cerebral cortex of mature Sprague-Dawley rats by serial filtration and differential centrifugation. The isolate contains numerous capillary segments and fewer segments of arterioles and venulae.

Transmission electron microscopy studies reveal that the capillary segments are circumferentially complete with endothelial cells being surrounded by an intact basement membrane. Electron dense areas containing numerous uniform vesicles are found external and adjacent to the basement membrane. Morphologically these areas are similar to neuronal terminals on larger vessels. Further the microvessel isolate contains acetylcholinesterase and monoamine oxidase, the degradative enzymes for acetylcholine and noradrenalin, respectively.

These studies show that the electron dense bodies present on the basement membrane of cerebral capillaries have the appearance of neuronal synapses and that the enzymes for inactivation of released neurotransmitters are present in the cerebral microvessel preparation. Additional experiments are necessary to determine whether these bodies are functional synapses. If they are, their role in the control of cerebral blood flow and control of vasospasm will be investigated.

This work was supported in part by NIH Grants CA18877 and NSO7377.

SCANNING ELECTRON MICROSCOPY OF CERATIOMYXA FRUTICULOSA. Raymond W. Scheetz and Rodney K. Nelson, Department of Biology, University of Southern Mississippi, Hattiesburg, Mississippi 39401.

The development of the slime mold **Ceratiomyxa fruticulosa** was followed from swarm cell through sporophore development by scanning electron microscopy. Motile stages were fixed with gluteraldehyde, dehydrated, critically point dried, and coated with Au Pd. Delicate sporophores were vapor fixed with osmium tetraoxide, freeze dried, and coated with Au Pd. Swarm cells were found to be uniflagellate when first formed, with a second flagellum becoming plainly visible within 13-1/2 hours after germination. Fructifications at the prespore stage consisted of several layers of prespore cells underlayed by a fibrous network. The column proper was found to be hollow. Spore elevation observed by scanning electron microscopy in large, confirms the sequence described by Famintzin and Woronin. Granules were found at the base of the individual stalked spores. Energy dispersive x-ray spectroscopy indicated the presence of calcium in these granules.

Poster Session.

BIOCHEMICAL AND ULTRASTRUCTURAL STUDIES ON ENUCLEATED AND RECONSTRUCTED Y-1 CELLS. Jerry W. Shay and Mike A. Clark, Department of Cell Biology, The University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

Utilizing cytochalasin B (10 mg/ml) in combination with mild centrifugation (8-10,000 g) it is possible to enucleate mammalian cells growing in monolayer culture. In an effort to gain a better understanding of nucleocytoplasmic interactions in the expression of a cell differentiated function we have biochemically and ultrastructurally studied the enucleated Y-1 cell line for their responsiveness to ACTH. Whole Y-1 cells which were originally derived from murine adrenal tumor respond to 30 minute ACTH treatment by morphologically rounding up and secreting steroids into the tissue culture medium. Enucleated Y-1 cells (cytoplasts) when treated with ACTH more surprisingly also round up and secrete steroids indicating that the nucleus is not necessary for this response. We then reconstructed cells containing the Y-1 nucleus (karyoplast) and a cytoplast from a non-responsive cell (AMT). In addition we have reconstructed cells containing the Y-2 cytoplast and AMT karyoplast. The results of our experiments indicate that the reconstructed cells containing the Y-1 karyoplast initially did not respond to ACTH but after a delay of 10-20 generations became responsive. The reconstructed cells containing the Y-1 cytoplast initially did respond to ACTH but after 4-5 generations became unresponsive. These experiments suggest that the cytoplasm initially controls the phenotypic expression of this differentiated cell function but that ultimately the nucleus dominates.

THE ROLE OF TUBULIN AND 10 NM (INTERMEDIATE) FILAMENTS IN THE SPONTANEOUS TRANSFORMATION OF RAT EPITHELIAL CELLS. Jerry W. Shay, John W. Fuseler and Mary Tobleman, Department of Cell Biology, The University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

A cloned epithelial, diploid cell line (RL-PR-C) was established from three-day-old rat hepatic tissue and grown in culture

for over 150 passages. These cells were characterized as normal, preneoplastic and neoplastic studying their growth in soft agar and by "in vivo" growth in immunocompetent animals. The initial work on these cells by Schaeffer and co-workers indicates that at passage 72 the cells are capable of growth in soft agar but that the intrinsic or spontaneous transformational event probably occurs between passages 35 and 56 based on karyotypic instability. In an attempt to help elucidate the nature of this initial transformation event we have studied these cells with the electron microscope and with monospecific antibodies and techniques of indirect immunofluorescence. Our initial studies utilizing tubulin antibodies indicate that the early passages (20-39) contain full complexes of microtubules that appear to be almost continuous with the outer plasma membrane but that the later passages (97-157) contain a diminished complex of microtubules with limited associations with the plasma membrane. Ultrastructurally we have observed that between passages 35-40 there appears to be an increase in the distribution of 10 nM (intermediate) filaments which is not apparent in the earlier or later passages. It is tempting to speculate between passages 35-40 in these cells that there may be a cause and effect type relationship between the temporary appearance of 10nM filaments and the subsequent permanent dimunition of microtubules associated with the plasma envelope. It can be further speculated that the dissociation of microtubules with the plasma membrane may result in a more fluid state which ultimately permits these cells to progress to a neoplastic condition.

Poster Session.

ULTRASTRUCTURAL CHANGES IN THE FISH LIVER FOLLOWING LONG TERM, NATURAL ARSENIC EXPOSURES. Elsie M. B. Sorensen, Department of Biology, Memphis State University, Memphis, Tennessee 38152.

Underground seepage of arsenic-laden wastewaters from an agricultural chemical manufacturing plant in Bryan, Texas resulted in concentrations of from 3 to 1100 ppm arsenic in the water in lakes adjacent to the plant. An endemic population of green sunfish (**Lepomis cyanellus** Rafinesque) in these lakes was therefore exposed to arsenic for extended periods and accumulated an average 3.4 ppm arsenic in the liver, the target organ in cases of arsenic poisoning. Because experimental exposures show that ultrastructural changes occur in hepatocytes following a one-week exposure to 30 ppm arsenic which results in accumulation of 0.00029 (±0.00017) ppm arsenic, the livers of these naturally-exposed fish were examined for possible morphological changes.

Approximately 2 dozen specimens were collected over a 2-year period for histological and ultrastructural examination and for comparison with untreated control fish. Even though all fish appeared normal externally, the livers of a majority of specimens showed fatty-infiltration and centrilobular necrosis, as previously reported in cases of both **Lepomis** and human arsenic poisoning. In addition, parenchymal hepatocytes of green sunfish displayed a variety of degenerative lesions including a large number of massive autophagic vacuoles and extensive lamellar arrays of rough endoplasmic reticulum within large multivesicular-like bodies. These lamellar arrays have been designated "fingerprints" or "onion bulbs" and are believed to indicate stimulated protein synthesis for repair of liver cells following possible injury from fasting, hepatectomy, and carbon tetrachloride poisoning.

ULTRASTRUCTURAL OBSERVATIONS ON THE GREENING OF THE LEAVES OF PONCIRUS TRIFOLIATA. Bettye Stallings and William R. Bowen, Department of Biology, University of Arkansas at Little Rock, Little Rock, Arkansas 72204.

When infected with fungal growth, seeds of the trifoliate orange, **Poncirus trifoliata,** may yield apparent albino seedlings on germination. Some albino seedlings subsequently undergo greening and become green seedlings capable of normal growth and development. Aspects of chloroplast ultrastructure during the greening process, from the albino to the normal state, will be presented.

SOME ULTRASTRUCTURAL ASPECTS OF ASCOSPORE DELIMINATION IN ELEUTHERASCUS PERUVIANUS (PEZIZALES). Walstine L. Steffens and John P. Jones, Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, Louisiana 70803.

The production of sexually derived spores in this ascomycetous fungus has been studied using both optical and electron microscopy. The surface of the spores is seen to be highly ornamented with spines and an equatorial ridge or brim which circumscribes the spores. Soon after early prospore delimitation by ascus vesicle formation, the brims become visible, and appear to have borders continual with the brims of adjacent spores. The inner epiplasm is thus isolated from the peripheral epiplasm, and during spore wall development, is seen to disappear at a faster rate. At maturity, the brims become discontinuous at some junctions and may overlap, apparently as a result of breakage at the brim junctions. Current research is underway to elucidate the cytological events that accompany or contribute to the precise alignment of the ascospore brims during early development.

ATTACHMENT OF EPIPHYTIC DIATOMS TO SEAGRASSES. Michael J. Sullivan, Department of Biological Sciences and Electron Microscope Center, Mississippi State University, Miss. State, MS 39762.

The application of scanning electron microscopy to the study of diatom morphology has revealed the existence of structural elements either previously unknown or known insufficiently from light microscopy. It is the task of the diatomist to assign functions to these recently resolved structures. Diatom species epiphytic on offshore seagrasses in Mississippi Sound exhibit structural modifications of their largely siliceous cell walls that facilitate their attachment to the leaves of the host. Attachment is accomplished via mucilaginous stalks, pads, or strands secreted through the cell wall by the protoplast. Members of the diatom genera **Fragilaria, Synedra, Opephora, Striatella,** and **Grammatophora** possess apical pore fields at the end of the cell that attaches to the seagrass leaf. It is through these tiny pores rather than the larger valve poroids that mucilage is secreted by the protoplast. Species of the genus **Licmophora** lack an apical pore field and mucilage is secreted through ordinary valve poroids. All diatom genera listed above lack a raphe and are non-motile. Of special interest are those genera that possess a raphe and hence are capable of movement, yet have adopted an epiphytic existence and therefore tend to be non-motile components of the flora. An excellent example is the genus **Mastogloia,** which completely lacks apical pore fields of any kind and instead secretes mucilaginous strands for attachment purposes through large locular pores in the sides of the cell. A series of scanning electron micrographs illustrate the structural modifications of selected diatom species for an epiphytic existence.

Poster Session.

MORPHOLOGICAL OBSERVATIONS ON THE DIFFERENTIATION OF THE TERATOCARCINOMA CELL LINE PCC4AZA1 IN VITRO. R. Triplett and J. W. Shay, Department of Cell Biology, University of Texas Health Science Center, Dallas, Texas 75235.

The murine teratocarcinoma is a valuble model system for studying several aspects of mammalian development and cell differentiation. The PCC4azal is an established teratocarcinoma cell line capable of inducing tumors with a variety of tissues when injected into syngeneic hosts, but retains a homogeneous cell morphology **in vitro** except under special culture conditions. If grown in a bacterial petri dish (untreated for cell adhesion) large cell aggregates develope. When transfered to a standard tissue culture surface, cells of differing morphology are observed emerging from the reattached aggregates. In order to investigate these events, PCC4azal aggregates, before and after reattachment were analysed via scanning and transmission electron microscopy. The six day old aggregate consist of a solid sphere of cells covered by flat, microvilli studded cells which are connected by tight junctions. Cells within the interior of the sphere are round and loosely arranged, maintaining contact with neighboring cells by occasional desmosomes and gap junctions. With respect to organellar development, cells at the surface are more differentiated than cells deeper in the aggregate. Scanning EM confirmed the "epithelial" morphology of the surface cells, with microvilli particularly prominant at the cell borders. Twenty four hours after transfer to a tissue culture surface, the aggregates reattached and spread, retaining a dense core of cells. Rounded cells were more numerous on the aggregate surface. Epithelial and fibroblastic like cells were observed to grow out from the aggregate margins, forming a monolayer in a few days. We are currently investigating several biochemical and morphological features of the PCC4azal system and feel it will be useful in studying various aspects of early cell differentiation.

HEMOLYTIC ANEMIA ASSOCIATED WITH LEPTOSPIROSIS: A MORPHOLOGIC STUDY. A. A. Trowbridge, J. B. Green III, R. A. Turner, W. B. McCombs III, J. C. Stinson, Departments of Medicine and Pathology, Scott and White Clinic, Temple, Texas 76501

The etiology of anemia in human leptospirosis is poorly understood. Anemia of various degrees has been frequently noted in individuals with Wiel's syndrome and ascribed on clinical impression to blood loss, renal failure, and/or an ill-defined hemolytic process. Hemolytic anemia secondary to leptospirosis is well documented in animals, although morphologic findings have not been reported. Hemolysins obtained from culture supernate and sonified cell suspensions have been proposed as the etiology of the hemolytic process in animals by virtue of their red cell lipolytic activitiy. We describe a patient who developed a fulminant hemolytic anemia associated with overwhelming leptospirosis. The peripheral blood smear on light microscopy suggested scattered hemoglobin containing particles. Transmission electron microscopy confirmed the presence of 2 to 4 μ membrane bound hemoglobin particles, i.e., microspherules. The latter were distinct from the projections of the echinocytes on serial selections at 500 A. Scanning electron microscopy demonstrated the hemoglobin particles and marked discocytic-echinocytic transformation of the red cells.

Red cell lipid analysis showed a decrease in sphingomyelin and phosphatidyl ethanolamine with a slight increase in phosphatidyl choline of the patient's cells as compared to normal.

The morphologic and red cell phospholipid findings suggest that the hemolysis observed was secondary to red cell membrane damage resulting from the product(s) of the infecting leptospire. These findings are compatable with the effect of a phospholipase.

ULTRASTRUCTURE OF THE PAVEMENT EPITHELIUM IN UTRICULARIA SP. George Turrentine and E. Laurence Thurston, Department of Biology, Electron Microscopy Center, Texas A&M University, College Station, Texas 77843.

Usually found in nitrogen poor environment, **Utricularia** has evolved bladders or utricles which serve to capture and digest prey. The pavement epithelium is a specialized region of secretory trichomes located on the internal ventral surface of the utricle. The trichomes consist of three cell layers, apical, middle and basal. Apical cells appear to be normal plant secretory cells, possessing numberous dictyosomes, wall infoldings and an abundance of rough endoplasmic reticulm. The middle cells appear to morphologically resemble plant transfer cells which are characterized by cell wall ingrowths and plasmadesmata. The basal cells appear to be collumnar epidermal cells possessing numerous plasmadesmata between adjacent basal cells. The pavement epithelium consist of three morphologically distinct zones. The innermost region possesses large secretory trichomes, that produces a highly thickened cuticle on the apical cells. The middle zone possisses the smallest trichomes, and has been termed the "door-stop" region. Secretory products appear to be absent from this region. The outermost zone possesses large trichomes that possess an exfoliated, membranous product known as a velum. The function of this velum appears to seal the trap door of the utricle.

ANTENNULAR SENSORY HAIRS OF THE BRINE SHRIMP. Greta E. Tyson and Michael L. Sullivan, Electron Microscope Center, Mississippi State University, Mississippi State, MS 39762.

Scanning electron microscopy (SEM) was used to characterize the external morphology of cuticular hairs found on the antennules of adult and larval brine shrimp **(Artemia salina).** The permeability of the hairs was studied by means of Slifer's crystal violet method, a technique that has been used to identify chemoreceptors in insects. Each antennule of an adult **Artemia** possessed a terminal cluster of sensory hairs, and within each cluster there were two kinds of sensilla (designated type 1 and type 2). Type 1 sensilla were longer (43 to 80 μm) and simpler in external morphology. They were widest at the base and gradually tapered to a fine tip; no pores were resolved by SEM. Type 2 sensilla were shorter (12 to 23 μm) and possessed a basal articular specialization, as well as a single terminal pore. Dye penetration experiments revealed that type 2 sensilla were permeable to crystal violet, whereas type 1 sensilla were not. The antennules of the naupliar larva of **Artemia** also bear cuticular hairs. These structures resembled adult type 1 hairs in being impermeable to crystal violet and in lacking a terminal pore and basal articular specialization.

Poster Session.

THICKENING IN BASEMENT MEMBRANES ASSOCIATED WITH CAPILLARIES FROM THE SMALL INTESTINE OF THE KETONURIC DIABETIC CHINESE HAMSTER. Elizabeth A. Weaver and Arthur R. Diani, Dept. of Biology Baylor University, Waco, Texas, 76703.

Capillary basement membranes from the intestinal submucosa of ten diabetic Chinese hamsters, matched with nondiabetic controls, were examined in this study. Initial fixation of tissue was carried out in Karnovsky's medium, with post-fixation in osmium. The tissue was embedded in Epon-Araldite and uniform thin sections (60-90 mμ) were obtained. All sections were stained in uranyl acetate and lead citrate. Ten measurable capillaries were randomly selected from the mucosa of both ketonuric and control animals. In order to assure accurate magnification and measurement of basement membranes, the electron microscope was calibrated daily. Measurements were made at the two thinnest points in the short axis of the capillary (at least 1 cm. apart). Measurements were not made in areas jux-

taposed to pericyte basement membranes unless clear delineation of the capillary basement membrane was possible. The two measurements were averaged, and the mean capillary basement membrane thickness for ten measurements was calculated (A). A parametric t-test for comparison of paired means was used to analyze the data. The data indicated significant thickening in the basement membranes of ketonuric animals compared with controls. An attempt was made to correlate capillary basement membrane thickening with the duration of ketonuria and diabetic subline.

OLFACTORY SENSILLA OF THE ANTENNAL FLAGELLUM OF THE HORN FLY, HAEMATOBIA IRRITANS, (LINNAEUS). Sandy L. White and D. E. Bay, Electron Microscopy Center, Texas A&M University, College Station, TX 77843 and Department of Entomology, Texas A&M University, College Station, TX 77843.

Olfactory sense organs on the flagellum of the antenna of the horn fly, **Haematobia irritans,** were examined by scanning and transmission electron microscopy. Four morphologically different types of olfactory sensilla are interspersed with noninnervated cuticular spines on the funicular surfaces: (1) multiporous thick-walled sensilla; (2) multiporous thin-walled sensilla, subtype 1a and subtype 1b; (3) multiporous thin-walled sensilla, type II; and (4) surface grooved sensilla. In addition, 1 ventral and 3 dorsal pits are populated with sunken multiporous thin-walled sensilla and sunken grooved sinsilla. Numerous depressions at the base of the ventral surface possess multiporous clavate thin-walled pegs. All the multiporous sensilla are characterized by a pore-tubular system; grooved sensilla possess cuticular channels continuous with the peg lumen.

One to four bipolar sensory neurons innervate various flagellar sensilla. Dendrites are characterized by the typical ciliary region with nine pairs of peripheral fibrils, a ciliary collar, basal bodies, and rootlets. No cellular organelles, other than microtubules, are present distal to the ciliary region. A cuticular sheath is present in some, but not all, of the thin-walled sensilla and is always present in surface and sunken grooved sensilla. Dendritic branching occurs only in those receptors possessing a pore-tubule system.

ULTRASTRUCTURE OF CHROMATIN DEPLETED NUCLEAR ENVELOPES OF THE SLIME MOLD, PHYSARUM POLYCEPHALUM. John J. Wille, Jr., and Wallstine F. Steffens, Department of Zoology and Physiology, Louisiana State University, Baton Rouge, Louisiana 70803.

The technique of selective removal of histones and DNA by mild extraction of isolate nuclei with excess heparin sulfate has been applied to study of the nuclear envelope components of S-phase, G2-phase and mitotic phase nuclei. Previous biochemical studies in our laboratory have reported a preferential association of temporally-characteristic replicating DNA with heparin-resistant nuclear envelope components (J. Cell Biol. **79,** 8a, 1978). Here we report on an ultrastructural investigation of residual nuclear envelopes derived from heparin-treated nuclei at many different phases of the mitotic cycle in **P. polycephalum.** In early S nuclei, heparin removes most of the chromatin fibrils, all of the nucleolar material, and most of the nuclear membrane. Instead there is a well-formed perinuclear fibrous lamina which forms a structural framework of the remnant nuclear envelope. Late S nuclei retain an almost complete double membrane, occasionally interrupted by gaps which are enriched with a dense fibrillar network of chromatic fibrils (both thicker 300 A and thinner 100 A fibrils). Additional fibrillar material in the interior form a conspicuous framework interlocked by annular structures (about 600-900 A) and extending to the nuclear membrane. The extent of the fibrillar network increases during the G2 phase, and more numerous connections are seen between the intranuclear matrix and the inner nuclear membrane, occasional tangential sections through the membrane also reveal annular structures associated with chromatin material. In prophase, nuclear envelopes display larger gaps in the nuclear membrane often associated with residual nucleolar material. Condensing chromosomes are seen as foci of chromatic fibrils spanning the interior of the nucleus. We, tentatively, conclude that heparin-mediated selective removal of histones and DNA from isolated nuclei reveals a regular subnuclear architecture consisting of a residual chromatin fibrillar network with many persisting connections to possible ring-like components of the nuclear pore complex.

AN ULTRASTRUCTURAL INVESTIGATION OF THE EFFECTS OF COLCHICINE IN THE GREEN ALGA PLATYMONAS SUBCORDIFORMIS. Fred R. Wolf, Department of Biology, Texas A&M University, College Station, Texas 77843.

A previous investigation of cell division in **P. subcordiformis** has shown that both rhizoplasts (fibrous roots) dissociate at their distal ends during prophase, giving rise to granular regions from which the spindle microtubules originate. During telophase the mitotic spindle breaks down, and each daughter cell regenerates a new pair of rhizoplasts beginning at the basal bodies.

Experiments with colchicine, a drug which prevents the polymerization of microtubule subunits, and hence nuclear division, reinforced the hypothesis that the rhizoplasts contribute part or all of the material comprising the spindle microtubules. In colchicine-treated cells which initiated division, the rhizoplasts dissociated with no subsequent spindle formation. Long-term colchicine treatments demonstrated that rhizoplasts were not regenerated at telophase. These data indicate a two-way interconvertibility exists between the material of the rhizoplasts and microtubules. This structural relationship lends further credibility to the theory that intracellular motility systems are biochemically related.

Other effects of the drug included cell enlargement, loss of motility, production of multiple thecae and eyespots, plastid lobing, and a general proliferation of endoplasmic reticulum. All of these aberrations were shown to be reversible by inoculation of treated cultures into colchicine-free medium.

STRUCTURAL ALTERATIONS OF THE INTERNAL CAROTID ARTERY OF THE SPONTANEOUSLY HYPERTENSIVE RAT. R. D. Yates and I-li Chen, Department of Anatomy, Tulane University School of Medicine, New Orleans, Louisiana 70112.

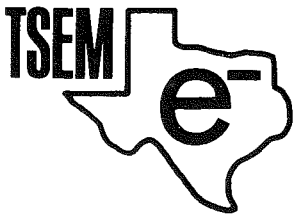
The arterial wall of the rat in the region of baroreceptor nerve terminals has not been studied at the fine structural level. The purpose of this research was to compare the arterial wall structure in control animals (normotensive) with a hereditary strain (spontaneously hypertensive-SHR) which develop hypertension with age.

The area of bifurcation of the common carotid artery into external and internal branches was removed from rats which had been anesthetized and subsequently sacrificed by an intracardia perfusion with 1% glutaraldehyde —1% paraformaldehyde in phosphate buffer (pH 7.1). A short segment of the external and internal carotid arteries were postfixed in osmium tetroxide, dehydrated in ethanols and embedded in Epon 812.

Particular emphasis was given to the internal carotid artery (ICA) in the region of the baroreceptor nerve endings. This region is on the side of the artery opposite the carotid body.

In comparison with normotensive animals the SHR revealed a thickening of the tunica intima of the ICA accom-

panied by an increase in the amount of elastic connective tissue in all tunics. Elongated cells and processes were noted in all three layers of the arterial wall of the SHR but were particularly numerous in the tunica media. Such cells and their processes were very opaque and contained abundant intracelllular filaments which were oriented parallel to the long axis. These cells exhibited the characteristics of both smooth muscle and fibroblasts. Additionally, extracellular material was arranged in a reticular network in the tunica intima and media. The increased number of elongated cells with filaments and the presence of an extensive network of extracellular material in the SHR may be significant in providing added strength to the arterial wall as blood pressure increases.



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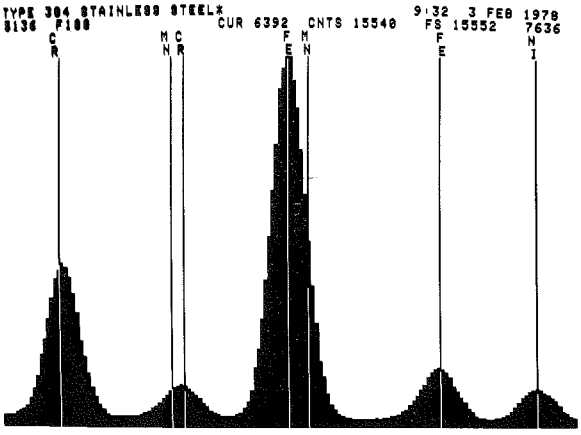

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Regional News

ARLINGTON: UNIVERSITY OF TEXAS, BIOLOGY DEPARTMENT

LECTURES

Dr. Bob Pfister, Chairman of the Microbiology Deparment, Ohio State University presented a seminar "Morphology and Ultrastructure of Mycoplasma Pneumoniae During Surface Growth" and gave two lectures to the Biological Electron Microscopy Class on fixing bacteria and freeze-etch techniques.

NEW FACILITIES AND EQUIPMENT

U.T.A. has added a thirteen room suite to its existing EM facilities. The new addition includes; an office, prep room, light microscope room, a five room EM suite with a negative darkroom, microtome rooms, micrograph analysis room and a print darkroom. The new facility will house an ISI-M7 SEM, a Joel 35 C SEM and Hitachi HU 11B TEM. The Joel 35 C SEM is a newly acquired microscope and will have high resolution back-scatter and STEM detectors.

ELECTRON MICROSCOPY EDUCATION

The graduate level Biological Electron Microscopy course has six students this fall. A graduate level course in scanning electron microscopy is planned for inclusion under the next catalog.

DALLAS: THE UNIVERSITY OF TEXAS, HEALTH SCIENCE CENTER AT DALLAS, DEPARTMENT OF PATHOLOGY

LECTURES

Dr. Herb Hagler presented a paper entitled "Analytical Electron Microscopy of Mitochondrial Inclusions in Infarcted Myocardium: Effect of Tissue Preparation" at the American Heart Association Meeting in Dallas.

NEW STAFF

Dr. Karen Burton Accepted a Post Doctoral Fellowship and will be working in conjunction with the Pathology Department and Dr. L. Max Buja's staff.

Deborah Haun has joined the electron microscopy staff of Dr. Bette Sherrill's lab in Internal Medicine.

Linda Lopez has joined Dr. L. Max Buja's electron microscopy staff.

Mary Tobelman, Research Associate, is now working in collaboration with Dr. Jerry Shay in Cell Biology.

Mark Korte and Gary Lorkowski are new research technicians in Dr. Shay's lab.

Welcome!

RECENT PUBLICATIONS

J.W. Shay, T.T. Peters and J.W. Fuseler: Cytoplasmic Transfer of Microtubules Organizing Centers in Mouse Tissue Culture Cells. Cell 14:835-842, 1978.

J. W. Shay and J. W. Fuseler: Diminished Microtubules in Cells Derived from Inherited Dyotrophic Muscle Explants. Nature. In press.

M. A. Clark, A. H. Crenshaw, and J. W. Shay: Fusion of Mammlian Somatic Cells with Polyethylene Glycol 400 MW. Tissue Culture Manual 4(2):801-804, 1978.

K. P. Burton, H. K. Hagler, G. H. Templeton, J. T. Willerson and L. M. Buja: Lanthanum Probe Studies of Cellular Pathophysiology Induced by Hypoxia in Isolated Cardiac Muscle. Journal of Clinical Investigation.60:1289-1302, 1978.

PERSONAL

Pathology Department Medical Photographer Mrs. Linda Bolding is back at work after gallbladder surgery.

Best get-well wishes are extended to Dr. Rolland C. Reynolds who is recuperating from coronary by-pass surgery.

GALVESTON: UNIVERSITY OF TEXAS MEDICAL BRANCH, DIVISION OF CELL BIOLOGY

GRANTS AWARDED

Dr. Jeffrey P. Chang has received a $55,000.00 renewal grant from Rohm & Haas Company for "Determination of no-effect levels of ethylenethiourea."

PUBLICATIONS

Chang, J. P., An ultracytochemical study of the fate of the Golgi apparatus in mitotic cells with special reference to regenerating liver, In: Studies and Essays in Commemoration of the Golden Jubilee of Academia Sinica, Academia Sinica, Taipei (1978).

Moller, P. C., Chang, J. P. Internalization of cationized ferritin in hepatoma ascites (Poster presentation, 18th annual meeting American Society for Cell Biology, San Antonio, Texas, November 1978).

Lo, J. T., Awasthi, Y., Moller, P. C., Chang, J. P. and Srivastava, A.K.,Enzyme replacement therapy: Distribution of 125I Hexosaminidase A sequestered in liposomes in mice tissues, J. Lab. Clin. Med. (IN PRESS).

PERSONNEL

David J. J. Wang, Visiting Scientist, (and a TSEM member) has recently returned to the Department of Biomorphics at the National Defense Medical College, in Taipei, Taiwan after completing a 1 year fellowship.

DEPARTMENT OF PHYSIOLOGY

PUBLICATIONS

Krauhs, J.M.: Structure of rat aortic baroreceptors and their relationship to connective tissues, J. Neurocytol. (1979) (IN PRESS)

Krauhs, J. M. and Brown, A. M.: Structure of rat aortic baroreceptors. The Physiologist, 21 (4):68 (Abstract).

NEW STAFF MEMBERS

Norman Salinas — EM Technician for Dr. Jane Krauhs.

HOUSTON: UNIVERSITY OF TEXAS MEDICAL SCHOOL AT HOUSTON, DEPARTMENT OF NEUROBIOLOGY & ANATOMY

GRANTS AWARDED

S.J. Enna, Ph.D., Associate Professor, Departments of Neurobiology & Anatomy and Pharmacology, received a United States Public Health Service grant from NINCDS for his project

entitiled "Neurobiology and Pharmacology of GABA Receptors." 3 years

Zehava Gottesfeld, Ph.D., Associate Professor, received a University of Texas Health Science Center Biomedical Research Support Grant for her project entitled "Plasticity in the Adult Mammalian Brain." 9/1/78 to 8/31/79 $5,000.

Gerald Kozlowski, Ph.D., Associate Professor, received an NINCDS grant for his project entitled "Limbic System: Vasopressin and Behavioral Correlates." 9/30/78 to 9/30/81 $83,229.

JoAnn McConnell, Ph.D., Assistant Professor, received a postdoctoral fellowship from NIH for her project entitled "Autonomic innervation of pelvic/perineal viscera." 2 years $28,000.

Jack C. Waymire, Ph.D., Assistant Professor, received a University of Texas Health Science Center Biomedical Research Support Grant for his project entitled "Role of Phosphorylation in Tyrosine Hydroxyase Activation." 9/1/78 to 8/31/79 $5,000.

Jack C. Waymire, Ph.D., has also received an NINCDS grant for his project entitled "Regulation of Adrenergic Metabolism. 12/1/78 to 11/30/81 $49,705.

MEETING, SYMPOSIA AND COLLABORATION

By special invitation from the Society for Neuroscience Richard C. Wiggins, Ph.D., Assistant Professor, attended a conference sponsored by the Society for "Projecting Future Needs of Neuroscience," held in Washington, D.C. on 9/24-27/78.

Jon DeFrance, Ph.D., Associate Professor and Gerald Kozlowski, Ph.D., Associate Professor, by special invitation were asked to participate in a symposium on "Neurotransmitters" at the 8th International Congress of Anatomy in Guadalajara, Mexico on 10/23-29/78.

S. J. Enna, Ph.D., Associate Professor, Departments of Neurobiology & Anatomy and Pharmacology will attend the Annual Meeting of American Colleges of Neuropharmacology in Maui, Hawaii, 12/9-17/78, and present a symposium on "Radioreceptor Assay." Dr. Enna will also travel to Elkhorn, Idaho, 1/20-27/78, to attend the Winter Conference on Brain Research and present a symposium titled "CSF GABA in Neurological Disorder."

The following individuals traveled to St. Louis, Missouri, 11/5-9/78 to attend the 8th Annual Meeting of the Society for Neuroscience and present the following papers: Margaret Bell, Graduate Student, "Effect of Wallerian degeneration on myelin protein synthesis in sciatic nerve of rat."

Marjorie Brown, Research Assistant I, (in collaboration with the Dept. of Anestesiology) "Incremental doses of morphine provide method to identify different patterns of responses recorded from eight brain nuclei": Yvonne C. Clement-Cormier, Ph.D., Assistant Professor, Departments of Neurobiology & Anatomy and Pharmacology, "Characterization of striatal (^{3}H) spiroperidol binding and dopamine sensitive adenylate cyclase utilizing protoberberine alkaloids."

Nachum Dafny, Ph.D., Professor, (in collaboration with the Dept. of Anesthesiology) "Electrophysiological evidence for tolerance to morphine: unit activity recordings from hypothalamus and parafasciculus nucleus in freely moving rats": Jon DeFrance, Ph.D., Associate Professor, "Cholinergic mechanisms and post-tetanic potentiation": S.J. Enna, Ph.D., Associate Professor, Departments of Neurobiology & Anatomy and Pharmocology, "Biochemical Pharmacology: Properties of GABA Receptors."

Gregory Fuller, Graduate Student, "Ontogenic development of tritiated water formation during leucine metabolism in rat brain"; John Haycock, Instructor, "cAMP-dependent activation and phosphorylation of tyrosine hydroxylase"; James Marchand, Graduate Student, "Limbic input into the ventromedial nucleus of the rabbit"; Dianna A. Redburn, Ph.D., Assistant Professor, "Localization of two GABAnergic systems in mammalian retina."

Robert Sikes, Graduate Student, "Regional organization of input and output pathways of the nucleus accumbens septi"; James Stanley, Ph.D., Postdoctoral Fellow, "Potentiation characteristics of hippocampal responses in the acute rabbit";Kathy Taber, Graduate Student, "Valium: a suppressor of hippocampal pyramidal cell excitability"; Dick Yeoman, Ph.D., Postdoctoral Fellow, (in collaboration with the Dept. of Anesthesiology) "Altered caudate nucleus field potentials following sustained stimulation to different substantia nigra regions."

Yvonne C. Clement-Cormier, Ph.D., Departments of Neurobiology & Anatomy and Pharmacology traveled to Washington, D.C., St. Elizabeth's Hospital, 10/24-25/78, to present a seminar entitled "Biochemical characterization of the dopamine receptor in the CNS."

Marilyn Munkres, Teaching Associate, traveled to San Antonio, 11/4-8/78 to attend the American Society for Cell Biology and present a paper entitled "Light causes calcium release in photosensitive irises."

Leonard Aldes, Ph.D., Assistant Professor, 9/16-20/78, traveled to the National Institutes of Health in Bethesda, Maryland to collaborate with colleagues and visit nearby institutions.

S. J. Enna, Ph.D., Associate Professor, Departments of Neurobiology & Anatomy and Pharmacology, 11/28/78 to 12/1/78, consulted with colleagues at Johns Hopkins University in Baltimore Maryland. Dr. Enna then participated in a site visit as a consultant for NIH on a grant application at the Medical College of Virginia in Richmond, Virginia.

Dianna A. Redburn, Ph.D., Assistant Professor, traveled to Boston, Massachusetts, 8/31/78-9/1/78, to participate in a site visit at Harvard University for the National Eye Institute.

NEW FACULTY AND STAFF

Philip N. Patsalos, Ph.D., Postdoctoral Fellow Hilary Hyatt-Fischer, Research Assistant I.

GENERAL NEWS

The department is especially glad to announce the graduation of its first Ph.D. student, Robert E. McClung. Bob was the 2nd graduate student to join the department. Since joining the department Bob has been doing research with Dr. Dafny on pain and morphine, the last two years as an NIDA predoctoral fellow. Bob's Ph.D. dissertation was entitled "Evoked field potentials and single unit recordings from naive and morphine dependent rats."

Among the new Cajal Club members of 1978 is Jo Ann McConnell, Ph.D., Assistant Progessor. The club is supplementary to the American Association of Anatomists and is comprised of a select group of neuoanatomists with keen interests in the nervous system.

All faculty in the Department of Neurobiology and Anatomy plus faculty with joint appointments from other within the Medical and Dental School joined together for a "faculty retreat" on september 14, 15, and 16 at The University of Texas Cancer System Scientific Park in Buescher State Park near Bastrop, Texas. The major objectives of this retreat were to discuss:

a) short and long term goals of the department
b) department organization
c) teaching programs for the present and future
d) research programs and funding

For recreation and relaxation there was a traditional Texas Bar-B-Que on Friday night, with entertainment consisting of a guitar and sing-a-long provided by talented faculty members of the department.

The faculty and graduate students participated in a "faculty/graduate student retreat conference" on Saturday, 21 October in the lounge and upper terrace area of the Prudential Building. This retreat was held to discuss future plans for the graduate students in the department.

SAN ANTONIO: THE UNIVERSITY OF TEXAS, HEALTH SCIENCE CENTER AT SAN ANTONIO, DEPARTMENT OF ANATOMY

GRANTS AWARDED

Dr. Thomas B. Pool, Institutional Grant, "Elemental Regulation of Cell Division in Normal and Transformed Human Cell Lines."

Dr. Ivan L. Cameron, Institutional Grant, for purchase of a model ISI-40 scanning electron microsope. This instrument has been added to the institutional SEM facility and is operated under the direction of Dr. Nancy Smith.

LECTURES

Dr. Thomas B. Pool, invited speaker, Tri Beta National Biology Honor Society Research Symposium, San Houston State University Outdoor Life Sciences Facility, Lake Conroe, Texas, October, 1978.

PUBLICATIONS

Berry, V.K., and L.E. Murr, 1978. Direct observations of bacteria and quantitative studies of their catalytic role in the leaching of low-grade, copper-bearing waste. **Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenoma,** L. E. Murr, A. E. Torma and J. A. Brierley (Eds), Academic Press, New York, Chapter 6, 103-134.

Mehta, A., A. E. Torma, L. E. Murr, and V. K. Berry, 1978. An SEM characterization of biodegradation of aluminum-bearing rocks by fungi. **SEM/1978,** Vol. I, 171-176.

Berry, V. K., L. E. Murr, and B. E. F. Reimann, 1978. An SEM study of morphological and leaching characteristics of thermophilic micro-organisms isolated from an experimental ore body. **SEM/1978,** Vol. I, 177-184.

Berry, V. K., L. E. Murr, and B. E. Reimann, 1978. An ultrastructure comparison of bacteria in relation to their catalytic function in leaching of sulfide minerals. **Proc. Ninth Int. Congress on Electron Microscopy,** Toronto, J. M. Sturgess (Ed), Vol. II, 684 (Abstract).

Berry, V. K., L. E. Murr and J. B. Hiskey, 1978. Galvanic interaction between chalcopyrite and pyrite during bacterial leaching of low-grade waste. **Hydrometallurgy, 3** : 309-326.

Murr, L. E., and V. K. Berry, 1978. Observations of natural thermophilic microorganism in the leaching of a large, experimental, copper-bearing waste body. **Met. Trans.,**(in press).

Cameron, I. L., N. K. R. Smith and T. B. Pool (in press) Element concentration changes in mitotically active and post-mitotic enterocytes; an x-ray microanalysis study. J. Cell Biol.

Kaster, A. G. and I. L. Cameron, 1978. Selective habitation of bacteria on "intermediate soft" keratin surfaces of the rat tongue. J. Dent. Res. **57**: 139-145.

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NEW EQUIPMENT AND FACILITIES

New facility — The Morphology Core has been established as a service laboratory to investigators associated with a center grant funded by NIH for the study of reproductive biology. The Principal Investigator is Dr. Carl J. Pauerstein, Professor of Obstetrics and Gynecology. The core is being directed by Drs. Edward G. Rennels and Damon C. Herbert of the Department of Anatomy and is staffed by Jeanette Zeagler, Senior Research Associate and Sandra Harrison, EM Technician II. The purpose of the core will be to provide a service facility for any investigator within the institution who is conducting research in the area of reproductive biology and desires to employ TEM and/or SEM in his (her) research. New equipment in the facility include an MT2-B Sorvall ultramicrotome, Polaron E5100 sputter coater and an Autosandri Tousimis critical point dryer.

Thomas B. Pool, Nikon Optiphot photomicroscope, Zeiss inverted phase photomicroscope.

NEWS BRIEFS

Dr. Nancy Smith is currently teaching a graduate course in scanning electron microscopy and electron probe x-ray microanalysis. Several members of the Department of Anatomy contributed papers to the recent American Society for Cell Biology annual meeting held in San Antonio. Drs. Ivan L. Cameron and John T. Hansen served as session co-chairmen during the meetings.

HOUSTON: BAYLOR COLLEGE OF MEDICINE, DEPARTMENT OF CELL BIOLOGY

MEETINGS

The Electron Microscopy Core Laboratory actively participated in the American Society of Cell Biology meetings held in San Antonio on Nov. 4-8. The following papers were presented in platform sessions and as posters: **Role of Polyamines in Cytokinesis of Mammalian Cells.** Prasad S. Sunkara, Portu N. Rao, Kenji Nishioka, and B. R. Brinkley; **Effects of Griseofulvin on Mitosis and Microtubules in Mammalian Cells In Vitro.** Susan M. Cox, Peter M. Redding, and B. R. Brinkley.

Modulation of Concanavalin A-Mediated Agglutination of Normal and Neoplastic Mouse Epithelial Cells. Bonnie B. Asch, Daniel Medina, Myles L. Mace, Jr., and B. R. Brinkley; **Immunoelectron Microscopic Localization of Fibrinogen in Rat Kidney.** Chin-Tarng lin, and Pien-Ying Huang; **Involvement of Calcium Dependent Regulator Protein in the Control of Microtubule Assembly-Disassembly.** J. M. Marcum, J. R. Dedman, B. R. Brinkley, and A. R. Means.

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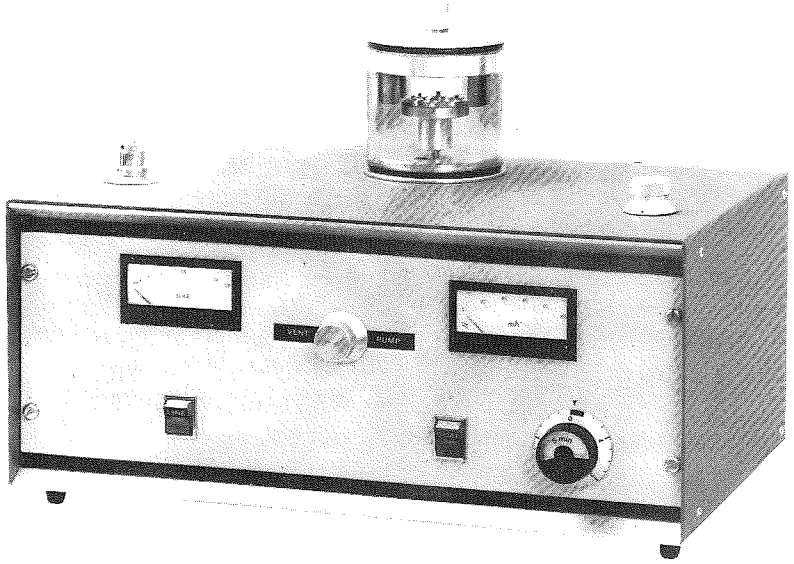

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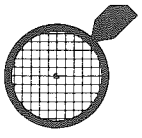

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Involvement of Calcium Dependent Regulator Protein in the Cytoplasmic Microtuble Assembly and Distribution in Enucleated Mammalian Cells. R. Brown and B. R. Brinkley; **Prenatal Effect of Estrogen on Male Embryonic Gonads Development and Function.** C. S. Teng and C. T. Teng.

NEWS BRIEFS

Dr. B. R. Brinkley, Professor of Cell Biology and Director of the Division of Cell Biology, was named president-elect of the American Society of Cell Biology at the San Antonio meetings. Dr. Brinkley will succeed Dr. David Sabotini as president of the 4,000-member society of 1980.

In April, Mr. Randy Spalinger, Photography Core Director, received the Award of Excellence at the photography exhibition sponsored by the Medical Illustration-Medical Photography Department.

NEW FACULTY AND/OR STAFF MEMBERS

Dr. Cynthia Jensen, from the University of Auckland (Department of Anatomy), Auckland, New Zealand, is visiting our EM facility during latter november and early December. Dr. Jensen will be collaborating with Dr. Brinkley on EM studies of mitosis in mammalian cell hybrids.

Dr. Robert Pardue joined Dr. Brinkley's group as a post-doctoral fellow in May. He will be working on immunocytology and the biochemistry of the cytoskeleton in normal and neoplastic cells.

Ms. Debra Hodges was appointed E.M. Core Technical Director in June. Debbie has worked with Dr. Brinkley for more than two years as his E.M. technician, until the Core position was vacated.

Mrs. Donna Turner came on board in August as an E.M. technician. Donna will be working with Dr. Brinkley on E.M. studies of normal and neoplastic cell cytoskeletons.

In September, Dr. Thomas Lin joined the E.M. laboratory as a research associate. Dr. Lin received his PhD. degree with Dr. Jeffery Chang at U.T.M.B. (Galveston) before joining the faculty at the National Taiwan Univerity Hospital (Department of Pathology). Dr. Lin will be working on immunoelectron microscopic localization of structural and regulatory proteins of the cytoskeleton and the mitotic spindle.

Ms. Jo Long, from U.T.M.B. (Galveston), has joined Dr. Claire Huckins as a research associate, as of December 1. She and Dr. Huckins will be studying the relationships between germ cells and Sertoli cells in the male reproductive system.

DEPARTMENT OF MICROBIOLOGY

LECTURES

Dr. Heather D. Mayor presented at The Slow Virus Symposium at N.I.H. in Bethesda, Md. in July 1978; at the 4th International Congress for Virology at the Hague, Holland in October, 1978; at the 4th Biennial Conference on diseases of the vulva and vagina at Baylor College of Medicine in October 1978; at the University of Southern California Medical School in Nov., 1978.

TULANE: TULANE MEDICAL SCHOOL, DEPARTMENT OF ANATOMY

LECTURES

Dr. Antti Hervonen from Finland presented a seminar entitled "Histo chemistry and fine structure of human paraganglia." Dr. Hervonen is Professor of Anatomy at the University of Tampere Medical School as well as President of the Finnish Society of Anatomists.

Dr. Jack Davies, Professor and Chairman, Vanderbilt University School of Medicine, Department of Anatomy, presented a lecture concerning "The embryological basis of the diethylstibesterol problem."

Dr. Murray Matthews from the LSU Medical School presented a seminar on "Quantitative studies in spinal cord injuries and subsequent reactive events."

FACULTY NOTES

Joe A. Mascorro, Instructor of Anatomy, recently was named to serve on the EMSA Technician Certification Board for the certification of electron microscopy technicians.

Joe and his boss, Dr. Robert D. Yates, have received invitations to present papers at the III International symposium on Nervous Transmission to be held in Helsinki in 1979.

PRESENTATIONS

Presentations to the greater New Orleans Chapter of The Society for Neuroscience: **Kenneth R. Brizzee.** Morphometric studies on aging changes in visual cortex and hippocampus in the Rhesus monkey.

Joe A. Mascorro. Histological and Ultrastructural comparisons between small granule containing (SIF) cells and extraadrenal chromaffin (Paraganglion)cells.

John Saer and **Joe A. Mascorro.** Phencyclidine induced catecholamine release from the granule pool in rat adrenal medulla.

Craig A. Knox and **Robert D. Yates.** Age-related alterations in structural histochemical and permeability characteristics of rat cerebrovasculature.

LUBBOCK, TEXAS TECH UNIVERSITY SCHOOL OF MEDICINE, DEPT. OF ANATOMY

GRANTS AWARDED

Coinvestigator Dr. Roger Rudy Markwald

1) NIH grant HD06362 — Nucleotide Metabolism in the Deciduate Uterus — 1978-1981. $263,870.

2)NIH grant — Androgen Action in Cultured Chick Comb Fibroblasts

Dr. James C. Hutson "Diabetes and Reproductive Function" American Diabetes Association (North Texas Affiliate). James C. Hutson, Michael D. Stocco and Surendra K. Varma — T-TUSM $10,000.

Dr. Robert L. Casady "Electron Microscopic Examination of Limb Regeneration in the Adult Salamander"$1,000.00.

Dr. Penelope W. Coates "Sheep Third Ventricle, LH and Gonadal Steroids" from NIH (NICH-HD).

LECTURES

Invitational lecture to the Southwestern Regional Society for Experimental Biology & Medicine. Nov. 1, 1978 entitled "Glycosaminoglycans: Potential Determinants in Cardiogenesis by Roger R. Markwald

Dr. James C. Hutson presented a paper at the annual meeting of the American Society for Cell Biology held Nov. 5-9, 1978. The paper was entitled "Specificity of hormone-induced responses of testicular cells in culture."

PUBLICATIONS

Markwald, R. R., T. P. Fitzharris, D. L. Bolender & D. H. Bernanke. 1979. Cell: Matrix Interaction During Atrioventricular Cushion Tissue Morphogenesis. Develop. Biol. In Press — April issue.

Casady, R. L., G. T. Kitten, I. M. Bradley and P. R. Sterrett. 1978. **Sites of Cerebrovascular Injury Induced by Radiographic Contrast Media.** Am. J. Anat. 153(3):477-482.

"Demonstration of neurosecretory substance in previously scanned specimens: adaptation of the Gomori — aldehyde-

fuchsin method for correlative SEM/TEM/LM histochemistry." P. W. Coates and E. C. Teh. Amer. J. Anat. **153**:469-475, 1978.

"Scanning electron microscopy of cloned astrocytic lines derived from ethylnitrosourea-induced rat gliomas." A. M. Spence and P. W. Coates. Virchows Arch. B. Cell Path. **28**:77-85, 1978.

NEW EQUIPMENT AND/OR FACILITIES

The Department of Anatomy has moved into the recently completed facilities in the new Health Sciences Center. These include three electron microscopy suites, tissue culture, autoradiography and multi functional dark rooms and radioisotope labs in addition to the individual and shared research space.

NEW FACULTY AND/OR MEMBERS

Penelope W. Coates, Associate Professor from the University of Washington in Seattle.

Questionnaire For Readers of TSEM Newsletter

1. What do you read in the TSEM newsletter?

   _____ everything
   _____ advertisements
   _____ a. in general — to see what is available
   _____ b. for specific items such as:
   _____ 1. new accessories for equipment you have
   _____ 2. new equipment by your favorite manufacturer
   _____ 3. new types of equipment
   _____ abstracts
   _____ feature articles(s)
   _____ letters to editor
   _____ regional news
   _____ President's letter
   _____ job descriptions and applications
   _____ by-laws
   _____ notices for future meetings

2. What do you look at in the TSEM newsletter?

   _____ advertisements
   _____ featured electron micrographs
   _____ names of new members or new addresses for current members
   _____ news from EMSA

3. What do you like most about the newsletter?

4. What do you like least?

5. What special features would you like to see in future issues?

6. What items would you like to see eliminated?

7. Do you think we should have a Library of Congress number?

8. If so, do you think the name should be changed to Proceedings of the TSEM?

9. If so, do you think that the personal news items should be sent out as a separate mimeographed sheet?

10. Specific comments you wish to see in print:

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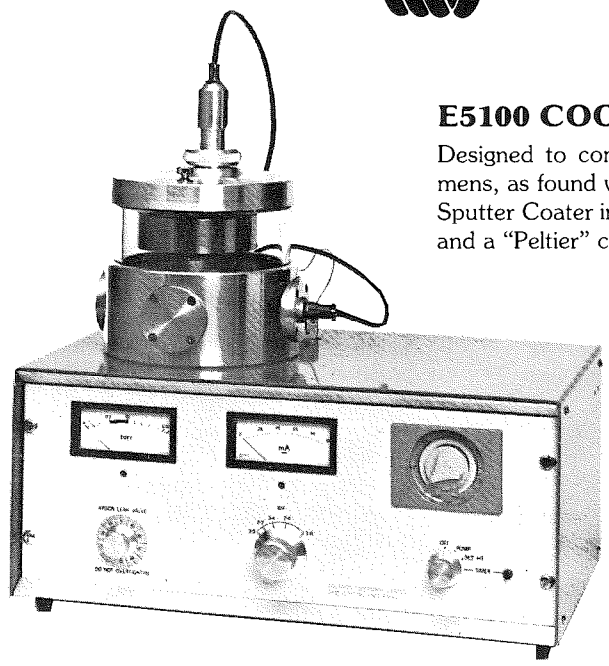

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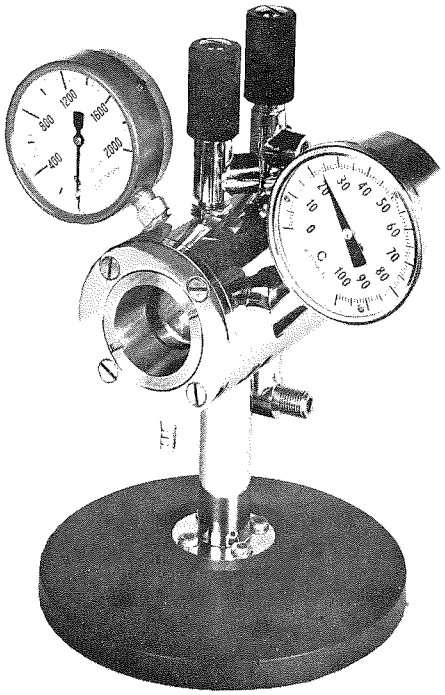

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Action _____

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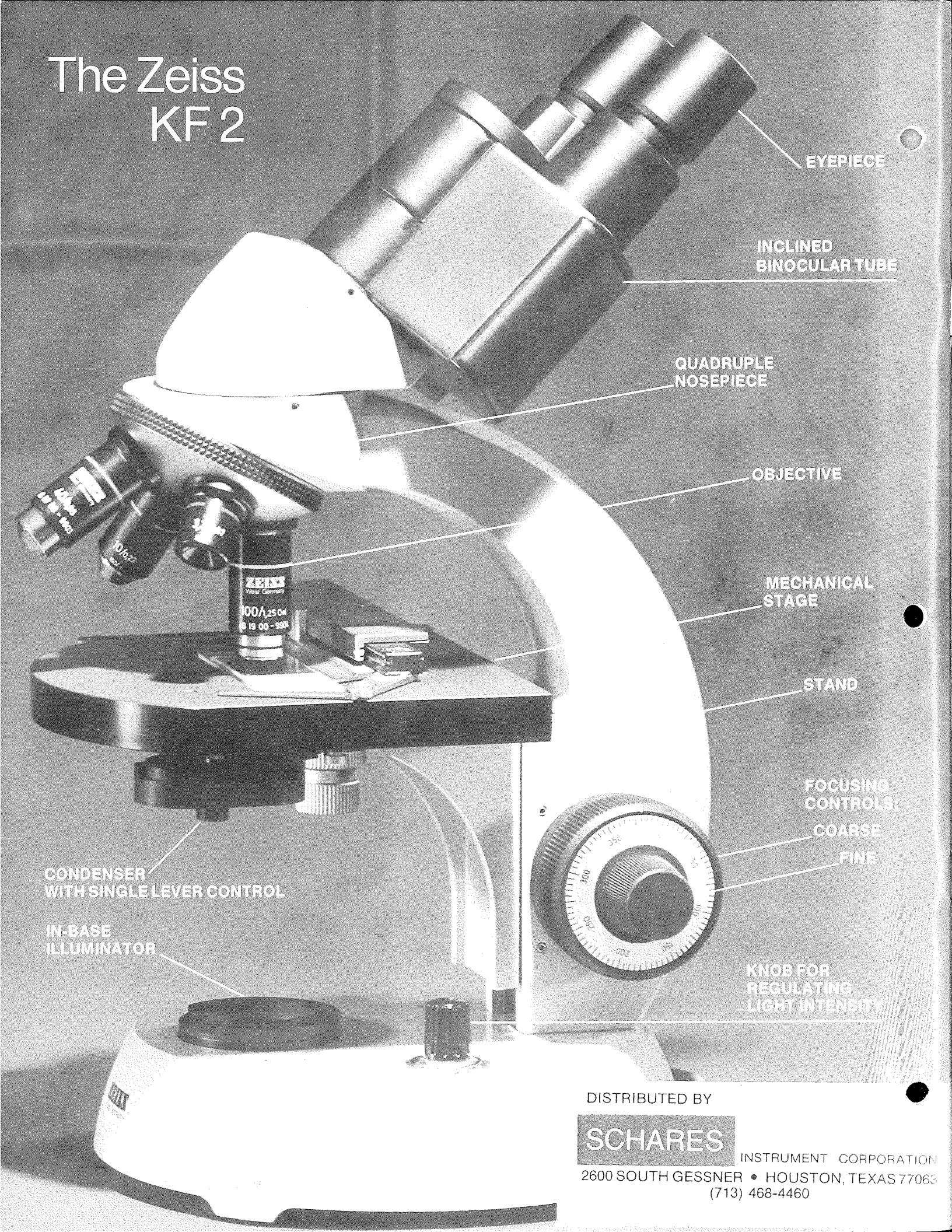

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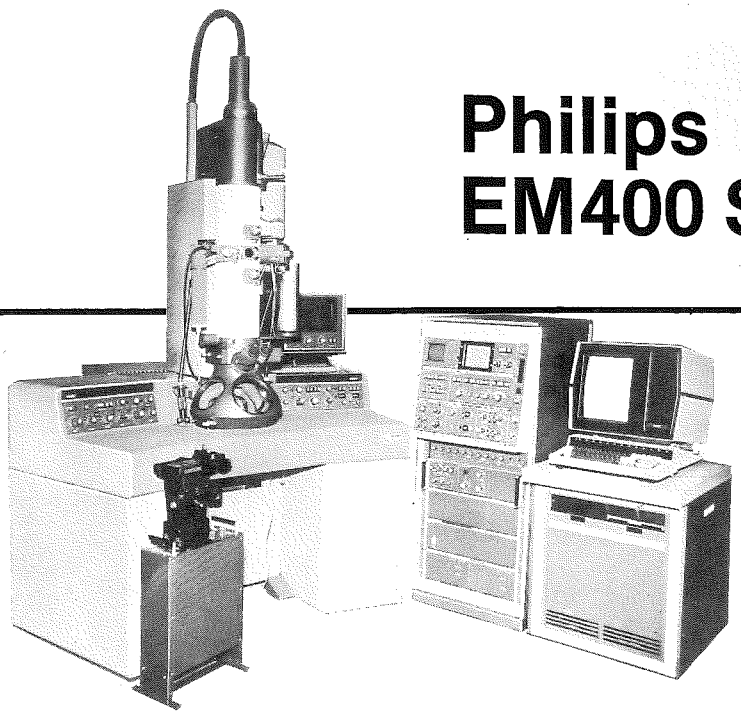

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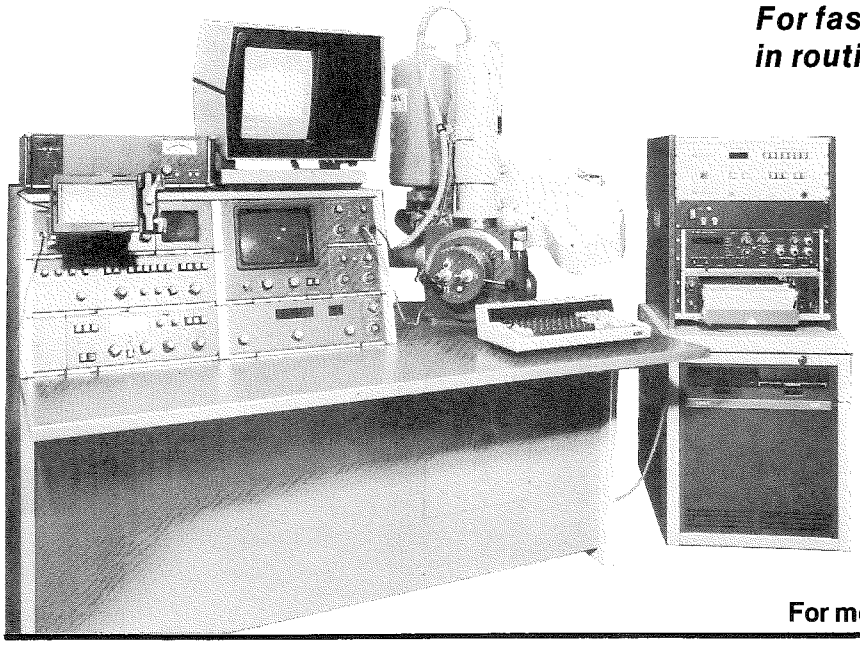

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