TSEM
Texas Society for Electron Microscopy
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WINTER, 1982
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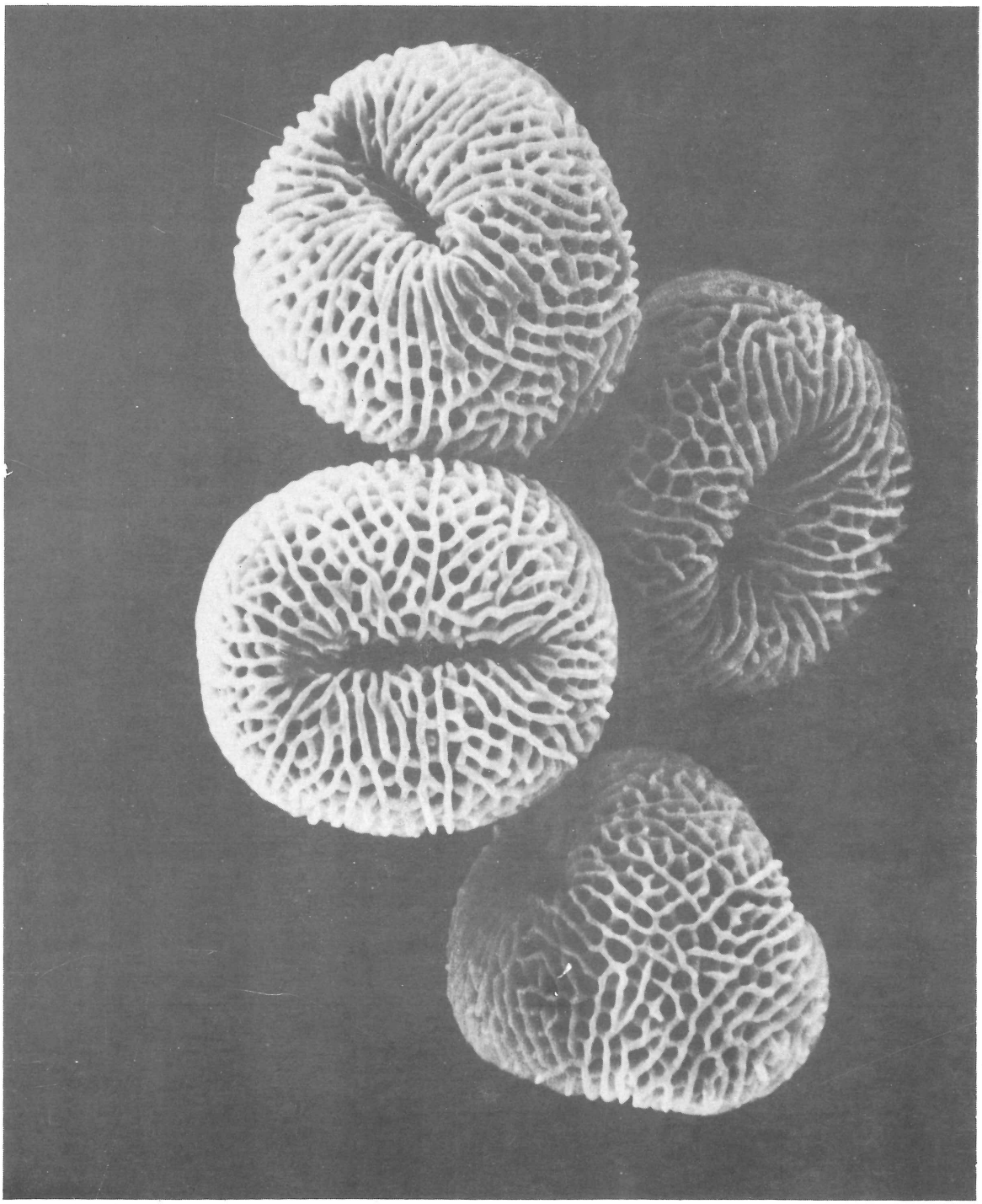

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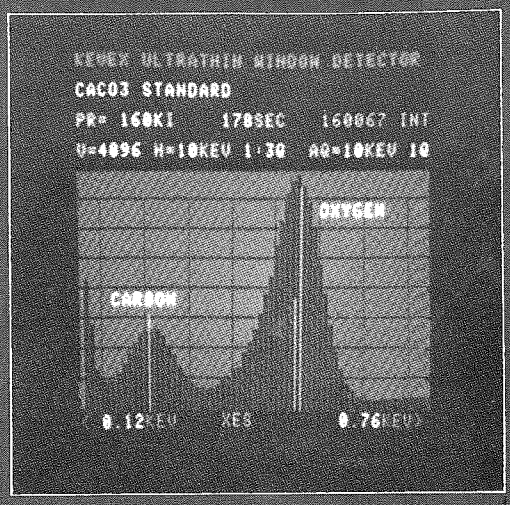

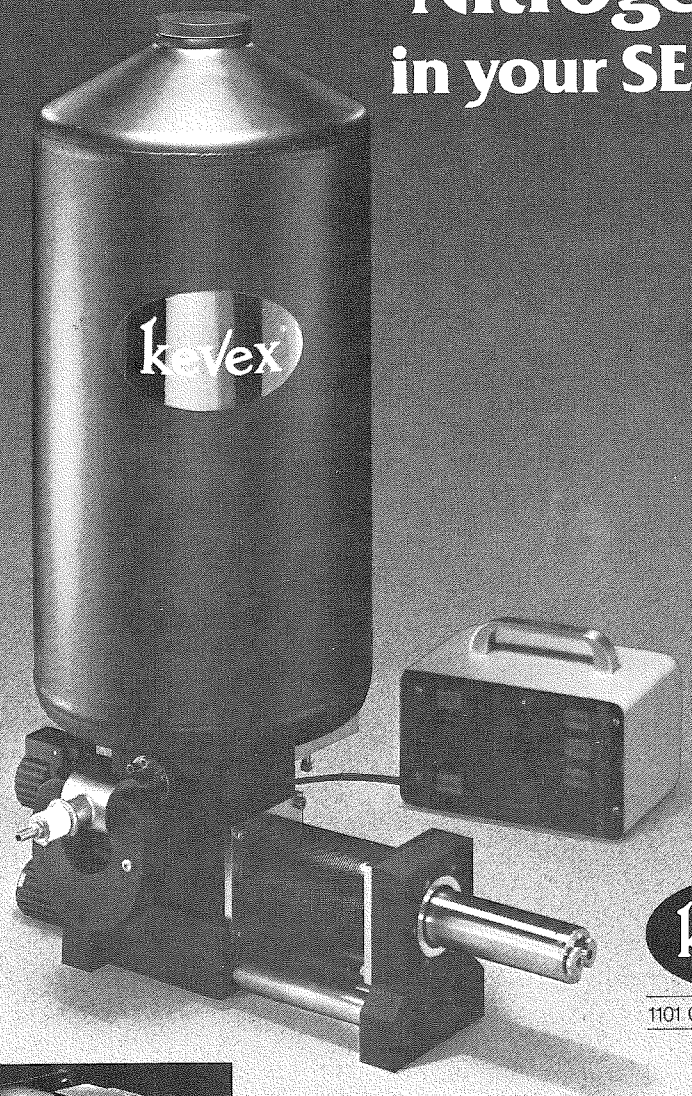

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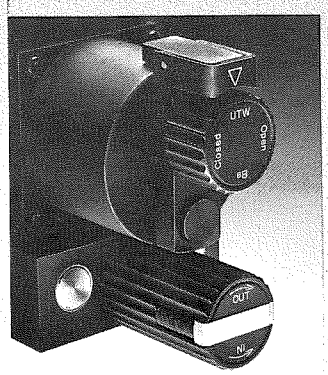

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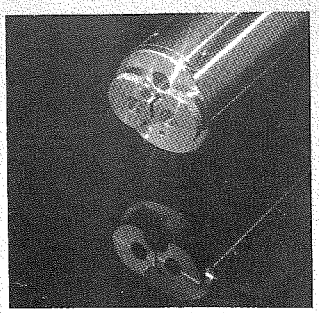

A small, 1⅜" outside diameter and tight system geometry minimize the distance to the specimen. The resulting larger solid angle and increased countrates are of overwhelming significance in light element detection.

Contents

Volume 13, Number 1
Winter, 1982
ISSN 0196-5662

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

EDITORIAL POLICY

Feature articles, news, letters to the editor, and micrographs may be submitted. Feature articles should be 3-10 typewritten pages, double spaced, with figures, tables and electron micrographs mounted for an 8-1/2x11 inch format. Three types of articles are solicited: 1) reviews 2) research reports 3) techniques papers. Reviews provide background material on a given research problem and often are condensed versions of review sections from current grant proposals. Research reports are short summaries of work published in part or in full in other journals but presented for a diverse audience with an interest in electron microscopy and allied technical approaches. Techniques papers describe new or rediscovered methods for improving or adding to existing techniques and give examples of the results obtained with these methods.

News items should be submitted through the regional editor in your area and conform to the standard format used by the regional editors. Letters to the editor are printed as they are received in the order of their arrival. These letters reflect the opinion of the individual members and do not necessarily reflect the opinions of the editor or the society. Electron micrographs to be used for cover photos are welcome and should be selected with some attention to aesthetic appeal as well as excellence both in technique and in scientific information content.

ON THE COVER

SEM showing four pollen grains from the common house plant geranium **(Pelargonium sp.)**. X1000. Submitted by Tyrrel C. Grohman, Department of Biology, Stephen F. Austin University, Nacogdoches, Texas.

President's Message

Dear Fellow Members,

I hope those of you who attended the Fall Meeting enjoyed the meeting at Corpus Christi as much as I did. Our Spring Meeting in Denton, April 1-3, 1982 promises to be a good one and I hope that many of you will be able to attend. Marilyn Smith and her committee are already hard at work. The accommodations are on campus and are very reasonable. The call for abstracts will be coming before you know it, so plan now.

Those of us involved in planning the joint meeting with the Texas Chapter of the Biological Photographic Association found it a pleasure to work with them. One thing we learned for sure was the correct name for each other's society. I have heard from several members already about how much they enjoyed our joint meeting. Let the Council know if you have any thoughts or comments about past or future meetings. We are open to suggestions. My thanks again to all of you who worked so hard on our October meeting.

Remember to send in your articles to the TSEM Journal. We have expanded to four issues per year. Encourage the people in your laboratory to hone their writing skills by submitting short articles directed at a general electron microscopy audience. Our special thanks to Elaine McCoy for her fine work as editor of the TSEM Journal.

Have a happy holiday season.

Sincerely,

Ann Goldstein, Ph.D.
TSEM President

Editor's Message

In this my last issue as editor, I want to express to each of you my gratitude. I thank those of you who have regularly sent in news items, and I especially thank those who have contributed scientific articles at my request. The program chairmen I worked with, Leon McGraw and Hilton Mollenhauer, were terrific. Our advertisers have been very cooperative, making the business part of this job almost easy for me. A great spirit of helpfulness prevails among members of this society, and I am glad to have been a recipient of your good will. Thank you all for allowing me this learning experience.

Great plans have been made for expanding the scope of this publication. Dr. Paul Baur will now assume the job of editor, and he has already started appointing a staff to assist him. I know all of you will want to be participants in the growth of this Journal, so start planning your contributions now.

I hope the New Year is happy and prosperous for all of you.

Elaine McCoy
Editor 1981

Dear TSEM Member:

Pursuant with the directives of TSEM's executive council, I will assume editorship of the TSEM Journal in January of 1982. Soon after this transition, the quarterly issued Journal will revise some of its operational policies and format. The changes will include: 1) the appointment of an Editorial Review Panel for review of all submitted contributions, except abstracts, 2) the acceptance of proffered papers in addition to the current practice of publishing mini-topical review articles, 3) the revitalization of the regional editors, and a change in the format of regional news section, 4) the cessation of an issue publication prior to each meeting (those deadlines are too costly and difficult to meet), 5) an incorporation of a "technical notes" section in each journal, 6) the termination of advertisers' discounts, 7) an increase in the number of libraries to which the Journal will be sent (we'll give them the first year's subscription and sell them the ensuing issues) and, 8) the appointment of an advertising editor.

All of these changes have been brought before the executive council and have been approved by that panel. Additional and even more exciting changes in the Journal are envisioned in the coming years; they will unfold as our Journal settles in its new mold of operation. Of course all future changes will be approved by the Journal's review board initially and the TSEM council subsequently. Naturally, we'd like your suggestions on the future course and content of our publication.

Now, why are we going to this trouble? First of all, contributing to the Journal is very time consuming and does not count professionally if the contributions are not critically reviewed. Secondly, the Journal should have some control over the standards of the articles published therein. Thirdly, our Journal has grown up, whether we like it or not. My shelf collection of TSEM Newsletters and Journals is incredible. It shows growth from a few mimeographed sheets of paper to a Journal of exceptional quality. This growth and maturity has happened because of the hard work and money we've all put into the effort. The changes we now make will mark the Journal's final transition to that of a full-fledged, citable scientific publication.

I want to assure you that the Journal will **never** become inaccessible to the TSEM membership. It will remain a forum for an exchange of ideas, knowledge, techniques and news between TSEM members everywhere. Furthermore, our review panel will be comprised of our most active members (TSEM Journal abstracts and reviews) to insure that the Journal's best interests are in good hands and under our control.

Please let me hear from you concerning your ideas about our publication and start writing papers for our Journal.

Best Wishes,

Paul S. Baur, Jr., Ph.D.
Editor, TSEM Journal
c/o Division of Cell Biology
Shriners Burns Institute
610 Texas Avenue
Galveston, TX 77550

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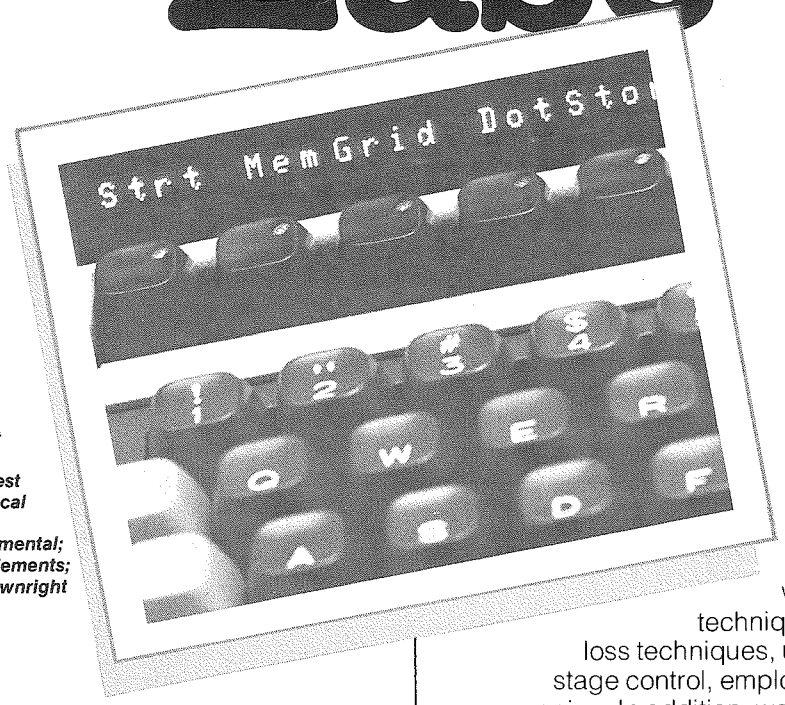

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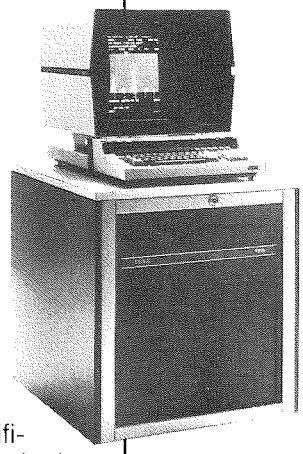

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Letters to the Editor

After returning from the recent TSEM meeting in Corpus Christi, I thought it might be interesting to attempt to identify some of the various factors that contribute to a successful meeting of our society. Although there are undoubtably a number of intangible factors involved, my list eventually came to include the following tangible factors: (1) location of the meeting (2) time of year (3) special invited speakers (4) workshops or demonstrations (5) commercial exhibits and (6) contributed papers. Although each of these factors probably has a different importance value for each of us, I personally kept returning to the fact that for me the contributed papers portion of a program really "makes or breaks" the meeting. With this in mind I thought it would be interesting to examine the contributed papers portion of our meetings for the last few years in an attempt to determine where support for this part of our meetings is coming from. After accumulating the data it occurred to me that the information might be of interest to the general membership of the society. I am therefore submitting the information to you for possible inclusion in the TSEM Journal.

The information shown in Tables 1-3 was accumulated by me and I alone am responsible for any errors. The data were obtained by referring to the published abstracts of papers and poster sessions contained in the TSEM Journal. In the case of joint meetings, only contributions by authors from Texas laboratories were considered. In the case of joint authorship, only the senior author was considered. Special invited papers were not counted.

Table 1 shows the total number of papers and poster sessions presented at our last 13 meetings beginning with the winter meeting of 1977. Table 2 shows the total number of papers coming from a particular laboratory or university. Some of these laboratories were combined simply because of the joint affiliations of certain workers. The decision to cut off at eight papers was an arbitrary one.

In closing I believe that it is quite apparent that our Society is a strong one with broad support. Papers and posters for the meetings listed in Table 1 came from over forty different institutions in the state, many with multiple E.M. facilities. We cannot, however, rest on our laurels and I hope that each member of the society will help to recruit new members. If there are individuals who should be in TSEM but who are not, then they should be encouraged to join and give papers. With the cost of attending national meetings at an all time high I think a local society such as ours has a unique opportunity to prosper. Perhaps some of the information included in Tables 1-2 can be used to convince other electron microscopists in the state that our society is an active one and that they also need to be involved.

Sincerely,

Charles W. Mims

TABLE 1

Meeting, Date and Location	Number of contributed papers and posters presented by Texas authors
Winter 1977 - New Orleans, LA. Joint Meeting of TSEM, LSEM, SSEM	26
Spring 1977 - Austin, TX	21
Fall 1977 - Arlington, TX	9
Winter 1978 - San Antonio, TX, Joint Meeting of TSEM and LSEM	58
Spring 1978 - Lubbock, TX	20
Fall 1978 - Nacogdoches, TX	19
Winter 1979 - New Orleans, LA., Joint Meeting of TSEM and LSEM	26
Spring 1979 - Dallas, TX	16
Winter 1980 - Houston, TX, Joint Meeting of TSEM and LSEM	40
Spring 1980 - Waco, TX	15
Fall 1980 - College Station, TX	25
Spring 1981 - Forth Worth, TX	24
Fall 1981 - Corpus Christi, TX	36

TABLE 2

Sources of Papers	Number of papers and posters presented at the meetings listed in Table 1.
VA Medical Center and U.T. Health Science Center at Dallas	50
Texas A&M University	48
U.T. Health Science Center at San Antonio	30
M.D. Anderson Hospital and Tumor Institute	27
Stephen F. Austin State University	21
Shriner's Burns Institute and U.T. Medical Branch, Galveston	20
U.T. Arlington	15
Baylor University	13
U.T. Austin	12
USDA, ARS, VTERL, College Station	11
Texas Tech School of Medicine	10
Baylor College of Medicine	9
Texas Tech University	9
Scott and White Clinic	8

Information for Author's

PURPOSE: The goal of the TSEM Journal is to inform members of the society and the Journal's readers of significant advances in electron microscopy, research, education, and technology. Original articles on any aspect of electron microscopy are invited for publication however, the TSEM Journal is biologically oriented and articles along those lines will be preferred. Guidelines for submission of these articles are given below. The view expressed in the articles, editorials, and letters represent the opinions of the author(s) and do not reflect the official policy of the institution with which the author is affiliated or the Texas Society for Electron Microscopy. Acceptance by this Journal of advertisements for products or services does not imply endorsement. Manuscripts and related correspondence should be addressed to Paul S. Baur, Jr., Ph.D., Editor, TEXAS SOCIETY FOR ELECTRON MICROSCOPY JOURNAL, Division of Cell Biology, Shriners Burns Institute, 610 Texas Avenue, Galveston, Texas 77550.

GUIDELINES: Manuscripts written in English will be considered for publication in the form of original articles, historical and current reviews, case reports and descriptions of new and innovative EM techniques. It is understood that the submitted papers will not have been previously published. Accepted manuscripts become the full property of the TEXAS SOCIETY FOR ELECTRON MICROSCOPY JOURNAL and may not be published elsewhere without written consent of the Editor. The author should retain one complete copy of the manuscript. The JOURNAL is not responsible for loss of the manuscript in the mail.

GALLEY PROOFS & REPRINTS: The author(s) will receive a page proof for review and will be responsible for the content of the article, including copy-editing changes. Page proofs should be carefully read, corrected, and returned to the Editor within 48 hours of receipt. The author(s) should sign the page proofs indicating approval. Reprints may be ordered when page proofs are received, and a table showing the cost of reprints will be enclosed with the proofs. Reprints may also be ordered from the printer.

MANUSCRIPT PREPARATION. Manuscripts should be submitted in conformance with the following guidelines:

FORMAT. Submit an original and two copies of the entire manuscript, typed, double-spaced, on 8-1/2 x 11 white paper, leaving ample margins. Number each page and identify the article by placing, at the top left of the page, a shortened form of the title, followed by the last name of the first author.

TITLE PAGE. Include:

a. Full title of the article
b. Initials and last names of all authors
c. Current positions of each author (title, department, institution, city)
d. Full name, telephone number and address of the author to whom reprint requests are to be sent.

SECTIONS. The text of each article should be divided into four major sections entitled INTRODUCTION; METHODS AND MATERIALS; RESULTS; and DISCUSSION.

ABSTRACT. Summarize the article in no more than 150 words. This takes the place of a final summary paragraph.

REFERENCES to other work should be consecutively numbered in the text using parentheses and listed at the end, as in the following examples:

(1) A. Glauert, Practical Methods in Electron Microscopy, Vol. 2 (North-Holland, Amsterdam, 1974) 82-88
(2) P.S. Baur, Jr., G.F. Barratt, G.M. Brown, and D.H. Parks, Ultrastructural Evidence for the Presence of "Fibroclasts" and "Myofibroclasts" in Wound Healing Tissues. J. of Trauma. 19 (1979) 744-756.
(3) D. Gabor, Information Theory in Electron Microscopy, in: Quantitative Electron Microscopy, Eds. G.F. Bahr and E. Zeitler (Williams and Wilkins, Baltimore, 1965) 63-68.

NOTE: Authors are responsible for the accuracy of references.

TABLES

a. Type, double-spaced, each table on a separate sheet.
b. Number in order in which they are referred to in the text.

ILLUSTRATIONS

a. Submit three complete sets of illustrations. Indicate which is the original photograph or illustration.
b. Number the figures in the order in which they are referred to in the text.
c. For black and white illustrations, submit sharply focused, glossy prints, or line drawings, 1.5 times larger than they are to appear in print (1/4 or 1/2 page). Scale should be drawn on the photograph itself, not below.
d. For color illustrations, if needed, submit positive 35-mm color transparencies (not prints) for the original (prints may be used for the two copies). Authors will bear the cost of color reproductions.
e. Identify all illustrations (author, title of paper, and number) by a gummed label on the back of each. Do not mount the illustrations, write on the backs of them, clip them, nor staple them.
f. Illustrations taken from other publications require reprint permission and must be submitted in the form described above.

NOMENCLATURE AND ABBREVIATIONS. Journal abbreviations used should be those listed by the "Index Medicus." Nomenclature abbreviations should be similarly standardized.

ACKNOWLEDGEMENTS and research funding should appear as a footnote which will appear at the beginning of the article.

Industrial News

Your Epoxy Embedding Procedure is the Second Most Important Factor in Obtaining Beautiful Electron Micrographs of Biological Specimens

John E. Johnson, Jr., Ph.D.
Hitachi Scientific Instruments
Rockville, Maryland

National Institute on Aging, NIH
Baltimore, Maryland

Department of Neurology
John Hopkins School of Medicine
Baltimore, Maryland

If you have read the title of this article, then I must answer your mental question before proceeding; What is the **most** important factor in biological electron microscopy? **Fixation.** This of course refers to straight forward structural studies of cell membranes and organelles. For analytical electron microscopy, fixation with chemicals may impede data acquisition, but the specimen must still be fixed, at least by freezing and, perhaps, then by drying.

Following fixation, the details of which are discussed in a current text (Johnson, 1981), the specimen is usually post-fixed in osmium, dehydrated and, then, embedded. Embedding, usually in an epoxy of one sort or another, has received only cursory coverage in the literature compared to fixation and staining. The procedures for perfusion fixation require an adroit surgical hand, and the neophyte is faced with a redoubtable skill to acquire. The results are erratic even when the skill is learned. Embedding, however, is not subject to the emotional vagaries of the surgeon, the animal being too light or too deep in anesthesia, fixative too cold or too warm. It is a simple procedure that produces reproducible results if certain steps are followed; steps I will attempt to proselytize you to since they are inconvenient and go against dogmas.

The first dogma we must dismiss is that embedding of any reasonably sized block can be done overnight. That is to say, done overnight, properly. I had for years, embedded in a matter of hours, with the last step, overnight. My colleagues did this too. The results were always unpredictable; chatter, holes in the thin sections, blocks never consistently the same hardness. Did your heart ever pound when you asked your technician how that critically important block thin sectioned that morning? Me too. Did you ever wonder if putting votive candles next to the microtome would help? Me either. Normally, I would refrain from levity in an article but it brings home one of the most frustrating problems and bottlenecks in each of our EM laboratories; thin sectioning.

Although they make nice excuses, the truth of the matter is that bad thin sections are probably not the fault of the microtome or microtomist but, rather, the block itself. That is not to say that we can ignore the precepts of care in trimming the block or having a clean knife edge, but, other than this, a good block should section well, routinely. The goal is to produce a **good block, routinely.** Assuming that the specimen is well fixed, the key to good thin sections, in my opinion, is extended dehydration and infiltration times. I also find that rinsing the specimens in buffer for several days between aldehyde fixation and osmium treatment significantly improves membrane preservation. In most instances there is no reason to rush the processing, and our tendency to get the specimens embedded in as short a time as possible is not so much a result of being impatient but simply because we are inured to doing it this way.

Basically, my own protocol for dehydration and embedding takes several days. After treatment with osmium the specimens are rinsed in three changes of saline at least 30 minutes each. This is followed by 3 hours in 70% alcohol (methanol or ethanol) 3 hours in 95% alcohol, one or two days in absolute alcohol (three changes) and one or two days in acetone or propylene oxide (three changes). Contrary to dogma, extended immersion in these solvents does not make the specimens too brittle nor does it appear to ruin cell membranes. Two days in a mixture of 50/50 acetone and epoxy are then followed by several hours in 100% epoxy. The specimen bottles should be on a rotator all the way from osmium through 100% epoxy. The blocks are then embedded in Beem capsules. **The extended time in the 50/50 is extremely important.** Leaving the specimen in 50/50 for a short time and in the 100% epoxy for a long time does not produce the same results.

Now to the choice of the epoxy formula. After testing over forty different formulas, I have settled on three. They are herein listed in order of decreasing viscosity.

1. **EA #5**
30 gm EPON 812 (or equivalent)
10 gm Araldite 502
18 gm NMA
18 gm DDSA
8 gm DMP-30

2. **EALLV #39**
20 gm vinyl cyclohexene dioxide (VCD)
42 gm Hexenyl succinic anhydride (HXSA; Ladd Research Industries, Burlington, VT.)
6 gm DER-736
30 gm EPON 812 (or equivalent)
10 gm Araldite 502
32 gm NMA (for softer block use 18 gm NMA, 18 gm DDSA)
2.5 gm DMP-30

3. **Quetol #34**
30 gm Quetol 651 (Ted Pella, Inc., Tustin, CA)
50 gm NSA
8 gm NMA
2 gm DMP-30
A slightly softer block is produced by a formula consisting of one part Quetol 651, two parts NSA and 2%-2.5% DMP-30 by weight.

At present, I use the quetol almost exclusively. It has low viscosity, is water soluble, has high contrast, stains well and thin sections easily. Its one drawback relates to the infiltration time. For some reason, in spite of low viscosity (which results in a long pot life) small holes will be found in the sections unless the blocks are infiltrated in the 50/50 for the time specified above. It is suggested that this may be a result of being water soluble since any remaining moisture may preferentially mix with the Quetol component rather than the anhydrides. Two days in 50/50 allows the mixture to be evenly distributed. However, blocks embedded in EPON are also more consistent using extended schedules.

Figures 1-2 illustrate examples of specimens embedded in Quetol using the suggested protocol. High contrast, uniform embedding and lack of chatter, even between cell processes, are characteristic features.

REFERENCES

Johnson, J.E., Jr., 1981, Transmission and scanning Electron Microscopy, In: Current Trends in Morphological Techniques, Vol. I (J.E. Johnson, Jr., Ed.), CRC Press, Boca Raton.

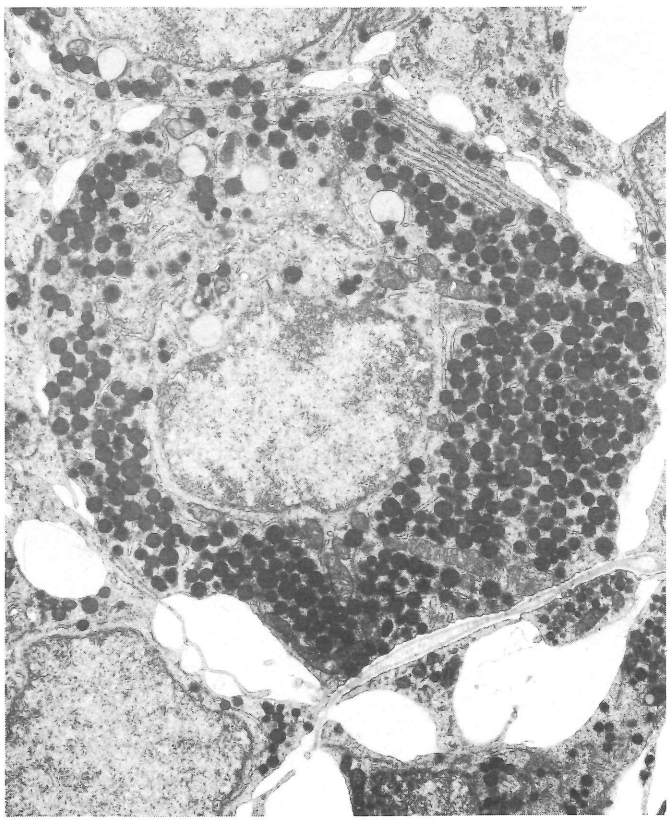

FIGURE 1. This micrograph illustrates an adenohypophyseal cell from a rat pituitary gland. The specimen was embedded in Quetol as described. The cell organelles are well preserved and the sectioning quality is smooth even through lipid droplets and extracellular spaces. X7,000.

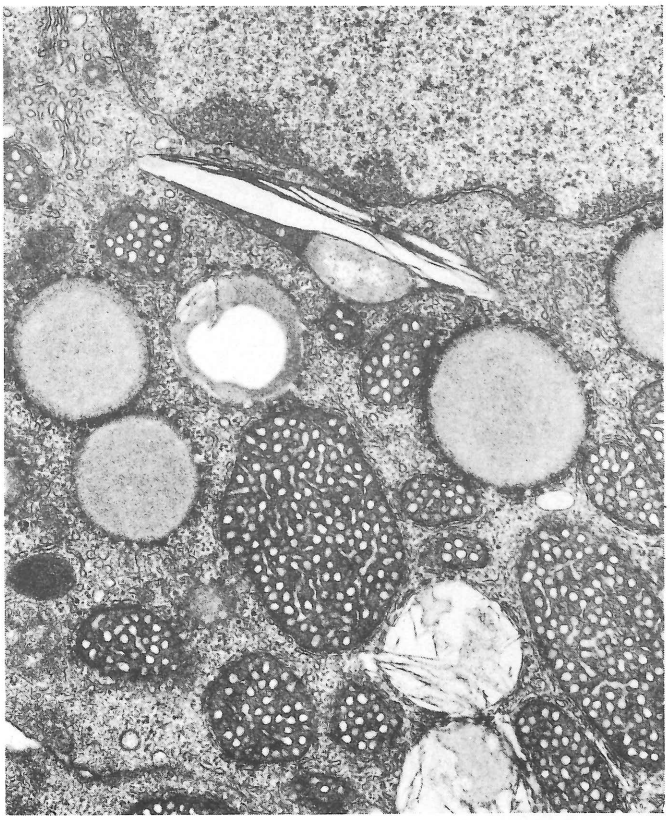

FIGURE 2. The cytoplasm of this rat adrenal cortical cell contains mitochondria, lipid droplets, various granules, and membranous inclusion bodies. The membranes are crisp and well preserved. The specimen was embedded in Quetol as described. 20,000.

Both micrographs were taken on an Hitachi H-600 TEM.

DUPONT BOOKLET IS USEFUL GUIDE TO HANDLING OF DIAMOND KNIVES

A new publication from Du Pont, entitled "Care and Use of the Du Pont Diamond Knife," details the proper handling, storage, use and cleaning of diamond knives. This booklet was prepared as an aid to diamond knife users and provides information on maintaining the fragile diamond knife cutting edge over a long period of time.

This booklet is available free of charge by writing the Du Pont Company, Microtomy Products, Concord Plaza, Quillen Building, Wilmington, DE 19898.

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DENSE-CORE VESICLES IN THE MAMMALIAN PINEALOCYTE AND THEIR RELATION TO SECRETORY PROCESSES

By
Michal Karasek,[1] [3]Thomas S. King,[1] Larry J. Petterborg,[1] John T. Hansen,[1] Andrzej Bartke,[2] and Russel J. Reiter[1]

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The University of Texas Health Science Center
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and

[3]Laboratory of Electron Microscopy,
Department of Pathological Anatomy,
Institute of Pathology,
Medical Academy,
Lodz, Poland

In several recent reviews[5 16 20] the mammalian pineal gland has been characterized biochemically, morphologically and functionally as a highly active secretory organ. Although the pineal gland synthesizes a number of compounds, few if any of these compounds meet all of the classical criteria of a hormone, i.e., a substance being produced in the gland, released to the bloodstream and having a specific functional influence on target organ(s). Nevertheless, several structurally diverse pineal compounds may prove to be hormonal.

Two general categories of putative pineal hormones have been described, namely indoleamines and polypeptides. Among the indoleamines, melatonin has attracted the most attention in the past several years. There is no longer any doubt that melatonin not only is synthesized within the gland but also is secreted from the organ as well.[15] Although numerous biologically active peptidergic fractions have been isolated from the pineal, arginine vasotocin is the only one which has been specifically identified.[1] Considering the large number of endocrine functions influenced by the pineal gland, it is easy to envision that the organ secretes more than one active factor. Therefore, it is quite reasonable to assume that the pineal gland may secrete both categories of putative hormones.

On the basis of ultrastructural studies Pevet and Karasek[12 14] propose the existence of at least two different secretory processes in the mammalian pinealocyte. One of these secretory processes actually was first described in

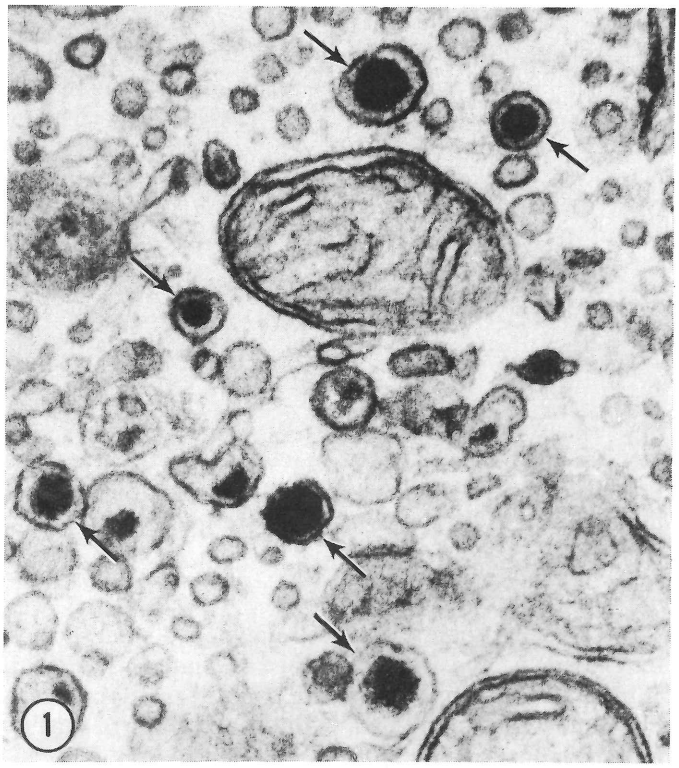

FIGURE 1. Laboratory mouse. Numerous dense-core vesicles (arrows) within a pinealocyte process. X80,000.

lower vertebrate pineal cells. [3 11 19] This neurosecretory-like process is characterized by the formation of dense-core vesicles from the Golgi apparatus. The other process, ependymal-like, consists of an accumulation of proteinaceous material in the dilated cisternae of the granular endoplasmic reticulum or by formation from these cisternae of vacuoles containing a flocculent material.[7 8 12 13 14]

Dense-core vesicles (DCV) are found in the pinealocytes of practically all species examined.[13 20] However, DCV are reported to be scarce in the pinealocytes of most species except those of the hamster, mouse and goldenmole.[13 20] Most of these studies present only qualitative estimates of the number of DCV present in an undetermined number of pinealocytes or unit area of the pineal gland. In our study we have examined by quantitative ultrastructural analysis the number of Golgi profiles (GP) and DCV in nine mammalian species, including rat, cotton rat, Djungarian hamster, laboratory mouse, white-footed mouse, chipmunk, ground squirrel, cat and fox. This paper discusses the potential involvement of DCV in the neurosecretory-like secretory process of the pinealocyte.

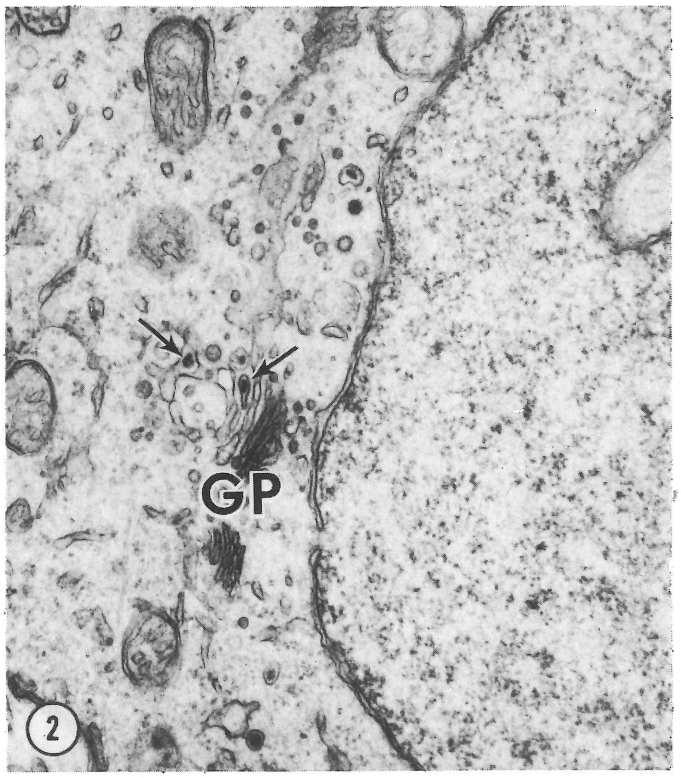

FIGURE 2. Laboratory mouse. Golgi profile (GP) suggestive of the formation of dense-core vesicles (arrows); X28,000.

The Golgi apparatus is well-developed in all species examined and consists of flattened or dilated cisternae (Figs. 2, 3, 4). The number of GP per unit area is comparable in most species studied, with the exception of the fox and the mouse (Table 1).

Dense-core vesicles (Fig. 1) are present in all species examined, although in most of them the number of DCV is relatively low (Table 1) compared to the large number of secretory granules in most neurosecretory cells. Images suggesting the formation of DCV from the Golgi cisternae are observed in all species studied, but they are especially frequent in the mouse (Figs. 2, 3, 4). In the species having the fewest number of DCV, these vesicles are observed with equal frequency in the perikaryon (Fig. 5) and the cell processes (Table 1). In contrast, in those species possessing abundant DCV, the vesicles are localized primarily in the cell processes (Figs. 6, 7; Table 1).

There is no direct evidence that DCV represent secretory products of the pinealocytes. However, DCV appear to originate in the Golgi apparatus, which plays an important role in the secretory process. Images suggesting exocytosis of DCV content have been reported in the rat.[7] Additionally, a substantial amount of experimental evidence strongly suggests a role for DCV in the secretory processes of the mammalian pinealocytes. For example, it has been demonstrated in the hamster that light deprivation, a condition known to enhance the metabolism of the pinealocyte[15 20] is followed by an increase in the number of DCV.[10] An increase in the number of DCV has been demonstrated in the pinealocytes after the addition **in**

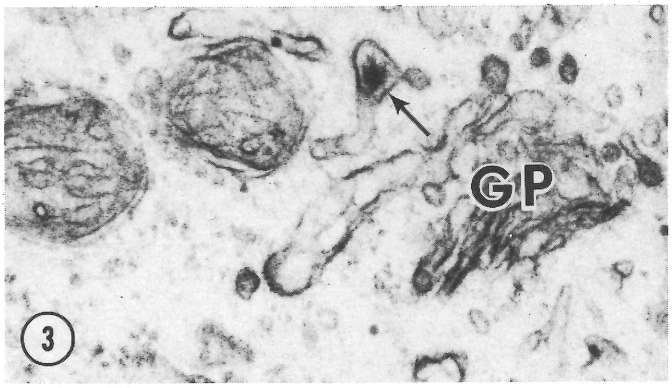

FIGURE 3. Laboratory mouse. Continuity between dense-core vesicle (arrow) and presumptive cisternae of Golgi profile (GP). X56,000.

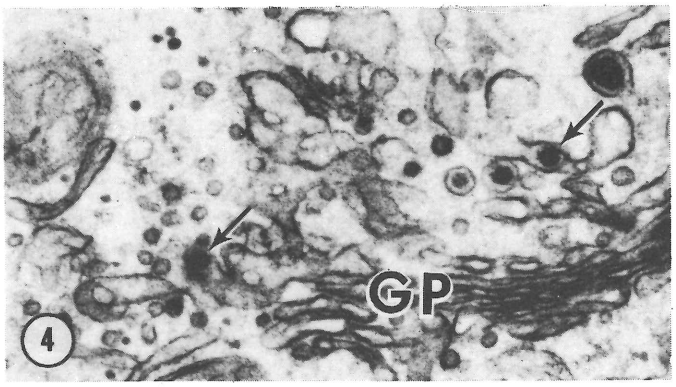

FIGURE 4. Laboratory mouse. Accumulation of dense material (arrows) in the Golgi cisternae (GP) from which the dense-core vesicles is thought to originate. X48,000.

vitro of norepinephrine, which is known to enhance melatonin production.[6 9 17] The administration of gonadotropins also produces an increase in the number of DCV in rat pinealocytes.[8] In contrast, sympathectomy, which depresses pineal secretory activity[15] causes a

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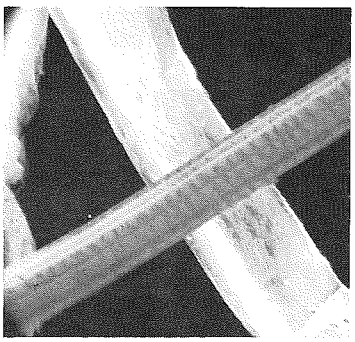

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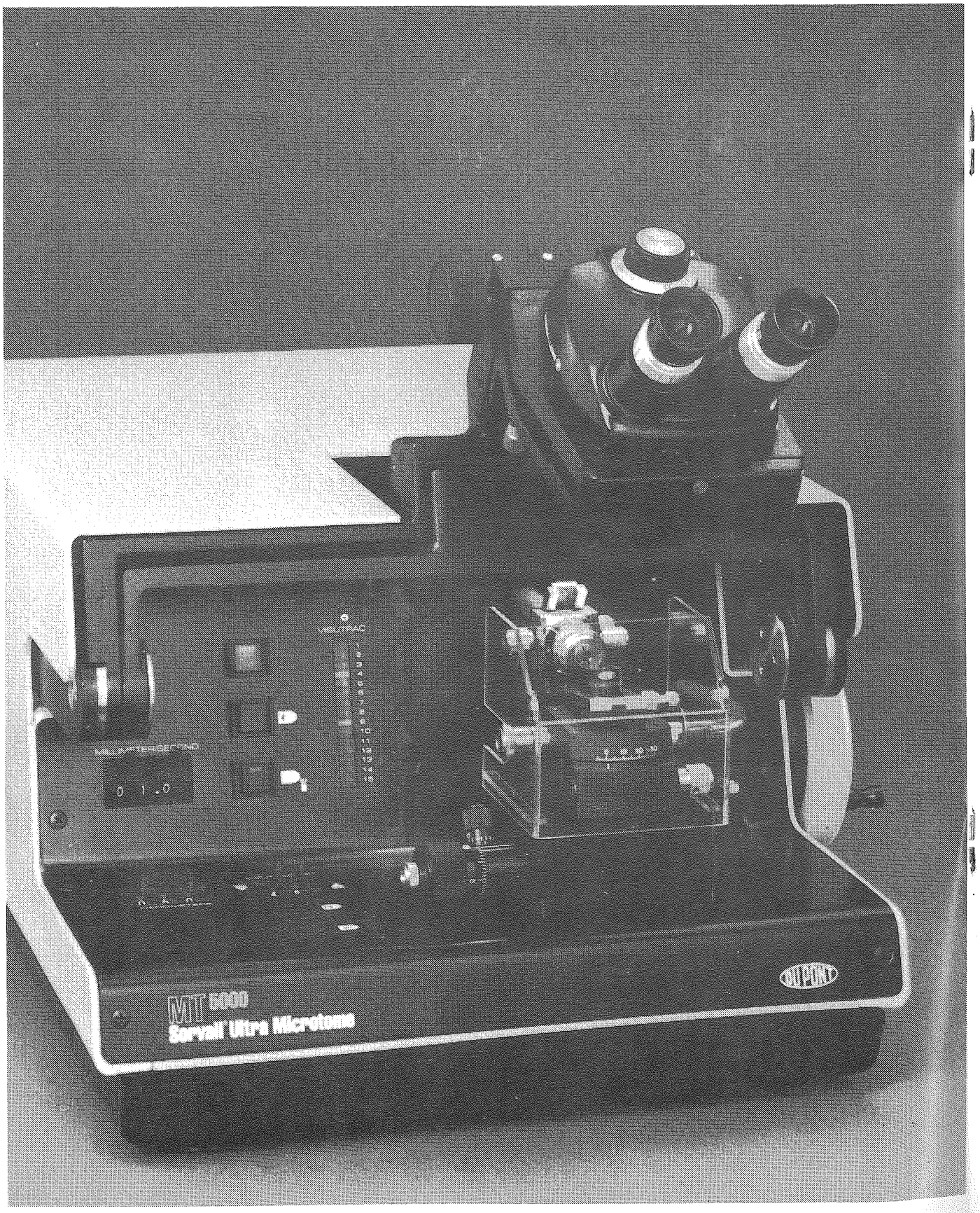

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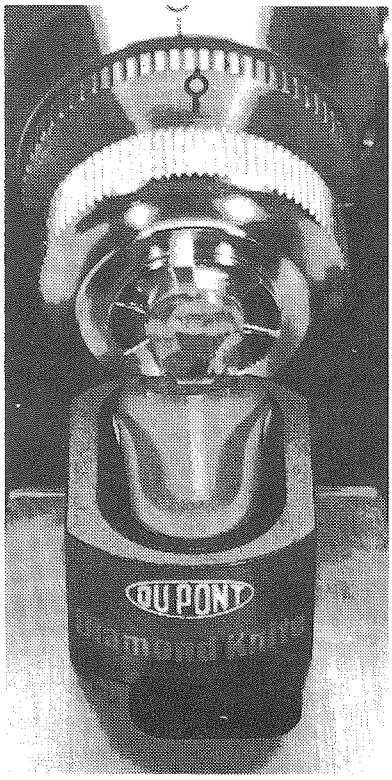

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decrease in the number of DCV in the hamster[10] and the mouse[2] pinealocytes. A circadian rhythm of the number of DCV has been demonstrated in the mouse[2] and rabbit[17] pinealocytes. Maximum levels of DCV are observed during the daytime compared to a minimum level at night. In contrast, the synthesis of melatonin is highest at night.[15] This suggests at least two possibilities: (1) the number of DCV and melatonin synthesis/storage are not directly related, or (2) if related, the nocturnal decrease in the number of DCV is a result of an increased turn-over of these vesicles.

In the pineal rudimentary cells and/or pinealocytes of reptiles, birds and mammals the usual compound present in the DCV is proteinaceous in nature.[4] Moreover, the coexistence of indoleamines and a proteinaceous compo-

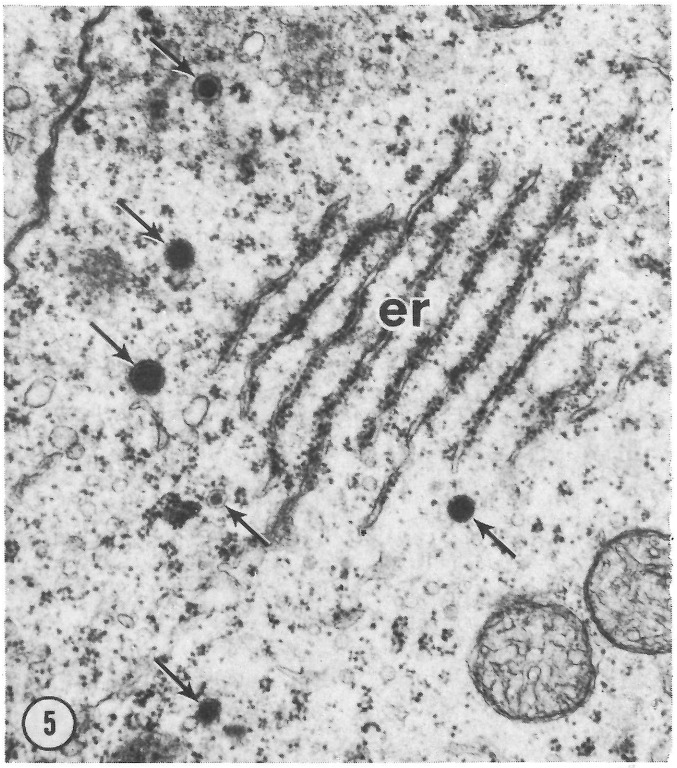

FIGURE 5. White-footed mouse. Numerous dense-core vesicles (arrows) in a pinealocyte perikaryon; er - granular endoplasmic reticulum. X36,000.

nent in the DVC of non-mammalian species has been shown.[4] The nature of this proteinaceous component may consists of (1) a carrier protein for indoleamine(s) product(s), or (2) a combination of peptidergic neurohormone(s) and indoleamine(s).[4]

Although it appears that the secretory process in the mammalian pinealocyte can be characterized morphologically, the nature of the secretory product(s) can not. Further investigations, including intracellular labeling studies (i.e., radioactive precursor studies or immunocytochemistry) as well as biochemical isolation (i.e., differential centrifugation) and identification of the contents of DCV are necessary before one can make more definitive statements concerning their role in pineal secretion.

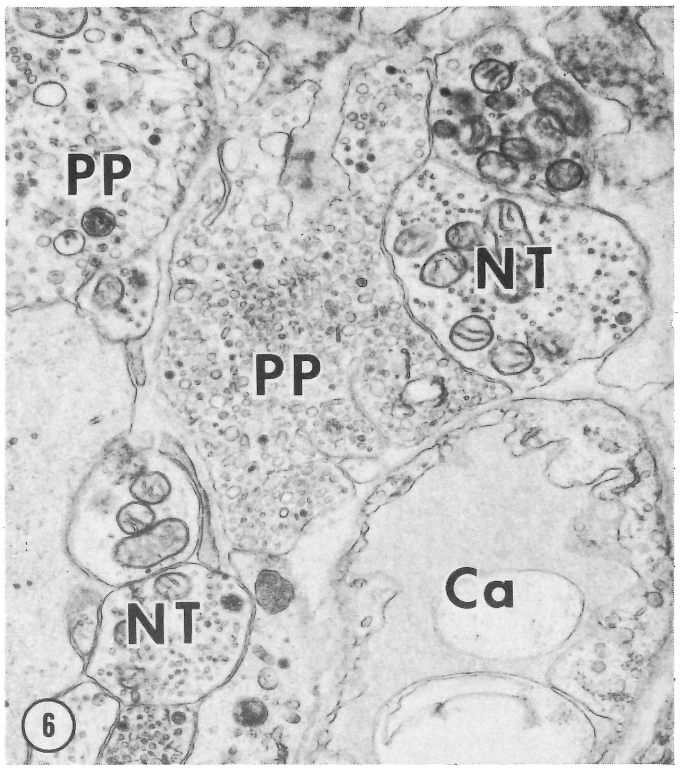

FIGURE 6. Laboratory mouse. Numerous pinealocyte processes (PP) in the perivascular space containing large numbers of both clear and dense-core vesicles; NT - nerve terminals, Ca - capillary. X22,000.

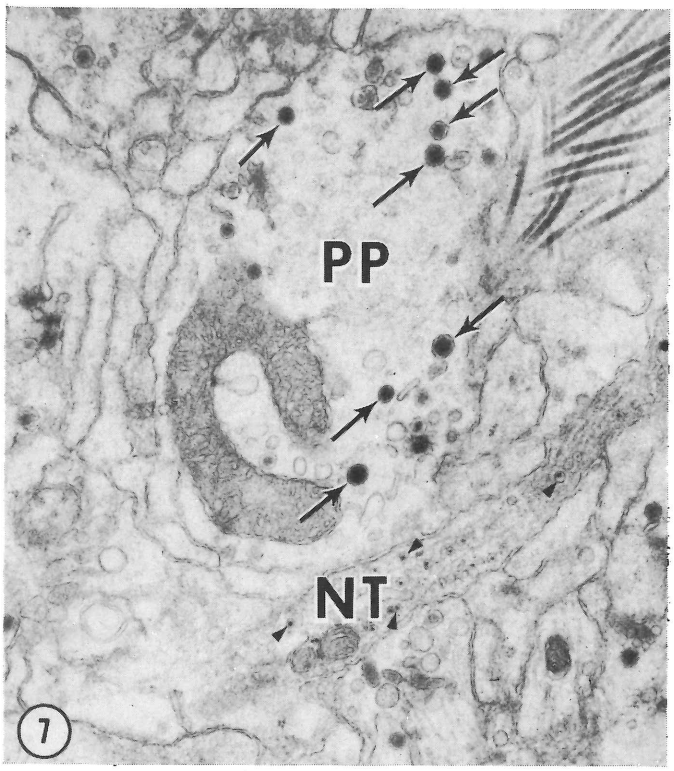

FIGURE 7. White-footed mouse. Numerous dense-core vesicles (arrows) within a pinealocyte process (PP); smaller, granular vesicles (arrow heads) in a presumptive adrenergic nerve fiber/terminal (NT). X18,000.

Table 1
Golgi profiles (GP) and dense-core vesicles (DCV) in various mammalian species

Species	Number of GP	Number of DCV	% of DCV in the perikaryon	% of DCV in the cell processes	Diameter DCV (in nm)
Vulpes vulpes domesticus (Fox)	1.6 ± 0.2	2.5 ± 0.3	48.0 ± 4.2	52.0 ± 4.2	65 - 150
Felix domesticus (Cat)	3.3 ± 0.1	4.0 ± 0.1	46.0 ± 2.3	54.0 ± 2.3	90 - 200
Spermophilus richardsonii (Richardson's ground squirrel)	5.3 ± 0.3	4.4 ± 0.4	53.3 ± 2.9	46.7 ± 2.9	80 - 150
Rattus rattus (Sprague-Dawley rat)	5.7 ± 0.5	4.8 ± 0.3	39.7 ± 0.6	60.3 ± 0.6	70 - 150
Tamias striatus (Eastern chipmunk)	5.4 ± 0.1	5.2 ± 0.2	48.9 ± 2.3	51.1 ± 2.3	55 - 140
Sigmodon hispidus (Cotton rat)	5.1 ± 0.1	8.3 ± 0.5	23.0 ± 2.3	77.0 ± 2.3	70 - 140
Phodopus sungorus (Djungarian hamster)	4.7 ± 0.3	11.8 ± 0.3	23.0 ± 1.0	77.0 ± 1.0	60 - 140
Peromyscus leucopus (White-footed mouse)	4.5 ± 0.2	25.2 ± 2.2	25.9 ± 1.1	74.1 ± 1.1	70 - 190
Mus musculus (Laboratory mouse)	11.8 ± 0.4	49.7 ± 2.6	26.7 ± 3.9	73.3 ± 3.9	70 - 180

For quantitative estimation GP and DCV were counted in 4 animals in the pineal tissue covering 1 randomly selected grid square (200 mesh). Data are expressed as mean ± SEM per 1000 μm^2 per animal. Animal species are ranked in order from lowest to highest involvement in neurosecretory-like secretory process based on the concentration of DCV.

ACKNOWLEDGEMENTS

The authors wish to thank Ms. Gwynne Duke for her excellent technical assistance and Mrs. Nancy Elms for typing the manuscript. This study was supported by a grant from the Polish Academy of Sciences, within project 10.4 to MK, by a NSF grant no. PCM 8003441 to RJR and by a NIH grant no. HD 12642 to AB. TSK is a postdoctoral fellow in the Center for Training in Reproductive Biology HD 07139. JTH is the recipient of a NIH RCDA K04 HL-00680.

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Regional News

AUSTIN
THE UNIVERSITY OF TEXAS AT AUSTIN, THE CELL RESEARCH INSTITUTE

Lectures

Dr. Dennis Brown spoke on "Development of a Virus Membrane" at Washington University, St. Louis Missouri, in September.

Publications

D.T. Brown: The assembly of alphaviruses. IN R.W. Schlesinger (ed.): The Togaviruses. Academic Press, New York (1981).

E.M. Education Possibilities

A course in special techniques for TEM applied to biological specimens, including negative staining, replication (metal) coating, freeze etching, radioautography and critical point drying, will be taught during the spring semester by Dr. Brown under the title Electron Microscopy II through the departments of Botany and Microbiology, using facilities of the Cell Research Institute.

DEPARTMENT OF BOTANY

LECTURES

Dr. Gary T. Cole spoke on "Morphogenesis, Ultrastructure and Biochemistry of the Fungal Propagule" at the University of Auburn, Auburn, Alabama in October.

In December at the University of Texas in San Antonio, Dr. Cole gave a seminar talk in the Dept. of Allied Health and Life Sciences on the subject, "Significance of the Fungal Propagule as an Infectious Agent in Plant and Animal Disease".

PUBLICATIONS

G.T. Cole: Application of scanning electron microscopy to studies of conidiomatal development in the fungi imperfecti. Scanning Electron Microscopy 1981 III: 305-312, 304. SEM, Inc. AMF O'Hare (Chicago), Illinois 60666 U.S.A.

L.M. Pope and G.T. Cole: SEM studies of adherence of **Candida albicans** to the gastrointestinal tract of infant mice. Scanning Electron Microscopy 1981 III: 73-80. SEM, Inc. AMF O'Hare (Chicago), Illinois 60666 U.S.A.

NEW EQUIPMENT

A Rebi cell fractionator originally made by DuPont Instruments has been refurbished for work on wall isolations of infectious fungi by Dr. Cole in collaboration with Dr. Milton Huppert, Director of the Mycology Research Lab, V.A. Hospital, San Antonio, Texas.

DEPARTMENT OF ZOOLOGY

LECTURES AND MEETINGS ATTENDED

Dr. Stephen Meier presented a paper at the Neurosciences Society meeting in Hong Kong, October, 1981. The paper was entitled, "Topographical association between somitomeres and primary brain parts of the embryonic neural plate".

PUBLICATIONS

S. Meier and C. Drake: Development of a latex-conjugated immunocytological marker for SEM analysis of quail-chick chimera. J. Cell Biol. 91:101a, 1981.

C.B. Anderson and S. Meier: The effect of hyaluronidase treatment on the migration of cranial neural crest cells in the chick embryo. J. Cell Biol. 91:162a, 1981.

C.B. Anderson and S. Meier: The influence of the metameric pattern in the mesoderm on migration of cranial neural crest cells in the chick embryo. Developmental Biology 85:385-402, 1981.

George D. Bittner: Trophic interactions of CNS giant axons in crayfish. Comp. Biochem. Physiol. 68A: 299-306, 1981.

T.A. Viancour, G.D. Bittner and M.L. Ballinger: Selective transfer of Lucifer yellow CH from axoplasm to adaxonal glia. Nature 293: 65-67, 1981.

S.J. Velez, G.D. Bittner, H.L. Atwood and C.K. Govind: Trophic reactions of crayfish muscle fibers and neuromuscular synapses after denervation, tenotomy, and immobilization. Experimental Neurology 71:307-325, 1981.

G.D. Bittner and M.L. Ballinger: Ultrastructural changes at gap junctions between lesioned crayfish axons. Cell Tissue Res. 207:143-153, 1980.

M.L. Ballinger and G.D. Bittner: Ultrastructural studies of severed medial giant and other CNS axons in crayfish. Cell Tissue Res. 208:123-133, 1980.

C.E. Hulsebosch and G.D. Bittner: Morphology and number of neurons in two species of polychaetes. J. Comparative Neurology 198: 65-75, 1981.

C.E. Hulsebosch and G.D. Bittner: Regeneration of axons and nerve cell bodies in the CNS of annelids. J. Comparative Neurol. 198:77-88, 1981.

NEW EQUIPMENT

Dr. John Ellison expects by January 1982 to have SEM equipped for cathode-illuminescence for the purpose of obtaining information from suitably treated fluorescent specimens at magnifications greater than those possible by light microscopy.

STAFF CHANGES

Joan Hunter has left The University of Texas to take a position in the Nancy Pritzker Laboratory, Stanford Medical School, Palo Alto, California.

THE TEXAS STATE DEPARTMENT OF HEALTH

NEW EQUIPMENT AND FACILITIES

Electron microscope facilities are being developed under direction of David L. Maserang. A Hitachi 8600 is in operation together with an LKB ultratome 5.

COLLEGE STATION
DEPARTMENT VETERINARY ANATOMY

PRESENTATIONS:

(1) H.H. Mollenhauer (U.S.D.A.): 1981 Cell Biology Meeting, Anaheim, California: Formation of Protein Bodies in Pea Cotyledon. (2) K.G. Thompson (Vet. Pathology): Nov., 1981,

American College of Veterinary Pathologists Meeting, Monterey, California: Hereditary Primary Hyperparathyroidism in German Shepherd Dogs.

PUBLICATIONS

G.K. Rieke, D.E. Bowers and P.E. Penn (all med. anatomy). 1981. Vascular supply pattern to rat caudoputamen and globus pallidus: Scanning electron microscopic study of vascular endocasts of stroke-prone vessels. Stroke, 12(216).

NEW EQUIPMENT

The Electron Microscopy Center announces the installation of an Energy Dispersive System with beam control (Tracor Northern) on the JEOL JSM-35 SEM. A Wavelength Dispersive Spectrometer (JEOL) is expected to be installed in the next few months. When complete, the system will be state-of-the-art in microprobe technology.

NEW STAFF MEMBERS

Miles Frey has joined the Veterinary Pathology Staff as an Electron Microscopy Technician II. Miles came to A&M after 16 years as a Research Technician in microbiology at the College of Veterinary Medicine, Cornell University.

*SPECIAL NOTE

Dr. E. Larry Thurston (Director of E.M. Center) who was injured in a car accident September 10, 1981 continues to make improvement in Hermann Hospital in Houston. Please remember him in your thoughts.

HOUSTON

BAYLOR COLLEGE OF MEDICINE SECTION OF CARDIOVASCULAR SCIENCES

GRANTS AWARDED

Dr. W. Barry Van Winkle has received a three year grant from the NIH to support his freeze-fracture studies of membranes in cardiac and skeletal muscle.

LECTURES

Dr. Margaret Ann Goldstein, postdoctoral trainees - Laurel Trager, Ph.D., Joiner Cartwright, Jr., Ph.D., and Senior Research Assistant, David Murphy attended and senior research assistant David Murphy attended the American Society for Cell Biology meeting in Anaheim. Dr. Goldstein chaired a muscle structure section on Nov. 12 at which Dr. Trager presented a paper on "Thin Filament Arrangement in Skeleton Muscle". Graduate Student - Danna Zimmer took a course in gel electrophoresis techniques in Woods Hole, Mass. in November.

PUBLICATIONS

Joiner Cartwright, Jr. and Margaret Ann Goldstein. Microtubules in Soleus Muscles in the Postnatal and Adult Rat. J. Ultrastructure Research, in press.

BAYLOR COLLEGE OF MEDICINE, DEPARTMENT OF CELL BIOLOGY

LECTURES

At the National Cancer Institute/National Institutes of Health, June 14-19, 1981, Dr. Robert L. Pardue conducted a workshop on **In Vitro** Immunization of Procedures for Monoclonal Antibody Production.

At the American Society for Cell Biology meeting in Anaheim, California, November 9-13, 1981, the department of Cell Biology, Baylor College of Medicine was represented by B.R. Brinkley, Ph.D., W.J. Deery, Ph.D., Sari Brenner, Graduate Student, Leo Simone, Ph.D., L. Wible, Research Associate, George Perry, Ph.D. Joe Tash, Ph.D., W. Wray, Ph.D., Susanne Gollin, Ph.D., and Eric Eastman, Ph.D. W. Deery presented a paper entitled "Microtubule Assembly from Endogenous Tubulin in a Lysed Cell System", Sari Brenner presented a paper entitled "Tubulin Assembly Sites in Mammalian Cells Characterized by Staining with Centrosome, Kinetochore, and Tubulin Antisera", L. Simone and L. Wible presented a poster entitled "Taxol-Induced Microtubule Initiation and Assembly in Mammalian Cells:, G. Perry and L. Wible presented a poster entitled "Microtubules in Dystrophic Chicken Cells **In Vitro**", J. Tash presented a paper entitled "Regulation of Protein Phosphorylation and Motility of Sperm Flagella by cAMP and Calcium", W. Wray presented a paper entitled "Chromosomal Localization of the Skeletal Muscle a-actin Gene in Chicken", S. Gollin presented a poster entitled "Isolation and Scanning EM of Prematurely Condensed Chromosomes", and E. Eastman presented a poster entitled "Two Dimensional Polyacrylamide Gel Analysis of Mononucleosonies from CHO Nuclei and Chromosomes".

PUBLICATIONS

Brinkley, B.R., S.M. Cox, D.A. Pepper, L. Wible, S.L. Brenner, and R.L. Pardue, 1981. Tubulin Assembly Sites and the Organization of Cytoplasmic Microtubules in Cultured Mammalian Cells. J. Cell Biol. 90:554-562.

Pardue, R.L., R Brady, J. Dedman, and C. Reading, 1981. Monoclonal Antibodies to Calmodulin Produced by **In Vitro** Immunization of Mouse Spleen Cells. J. Cell Biol. 91.

Snabes, M.C., A.E. Boyd, R.L. Pardue, and J. Bryan, 1981. A DNase I Binding Immunoprecipitation Assay for Actin. J. Biol. Chemistry 245:6295, No. 12.

Brenner, S., Differentiation and Growth Potential of Ciliated Cells. Lecture to Human Studies Collaborative Group Meeting, NIH, Bethesda, Maryland, May, 1981.

Brenner, S.L., and B.R. Brinkley, 1982. Tubulin Assembly Sites and the Organization of Microtubule Arrays in Mammalian Cells. **In** The Organization of the Cytoplasm, Cold Spring Harbor Symposia on Quantitative Biology, Vol. XLVI, Chp. 26 (in press).

NEW FACULTY AND/OR STAFF MEMBERS

W.J. Deery, Ph.D., has joined Baylor College of Medicine in Dr. B.R. Brinkley's laboratory. Dr. Deery will do his post-doctoral training with Dr. Brinkley.

George W. Smith, Ph.D., Associate Professor of Biology, Union College, is investigating the protein composition of the isolated mitotic apparatus utilizing immunofluorescence to study kinetochore and calmodulin localization as a visiting faculty member.

Nick Mace, Ph.D., is now an Adjunct Professor with the Department of Cell Biology. Dr. Mace's full-time position is with the Department of Pathology as Director of the EM Core Facilities.

BAYLOR COLLEGE OF MEDICINE, DEPARTMENT OF MICROBIOLOGY

LECTURES

Texas A&M, Department of Medical Microbiology, College of Medicine, Dec. 3, 1981. "Adeno-associated viruses and their Interactions with Herpes and Adenoviruse".

TWO NEW TEM'S FROM

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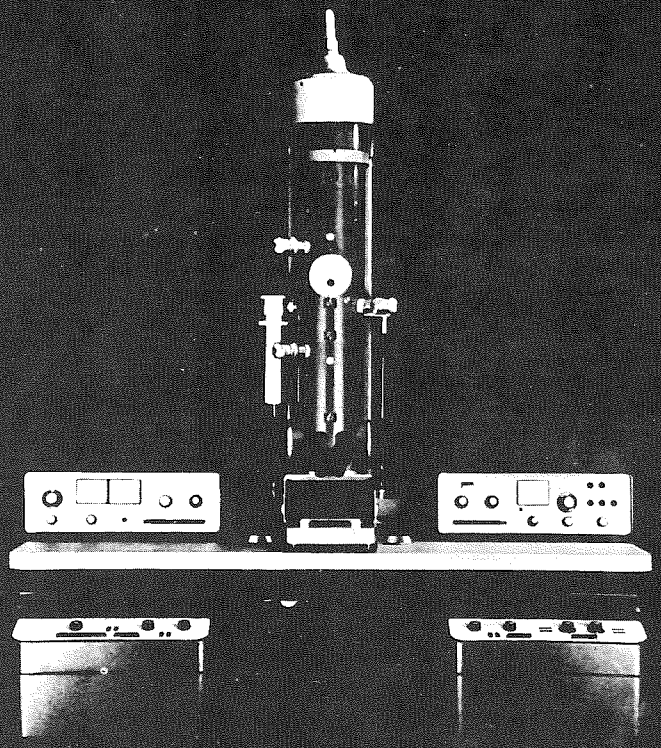

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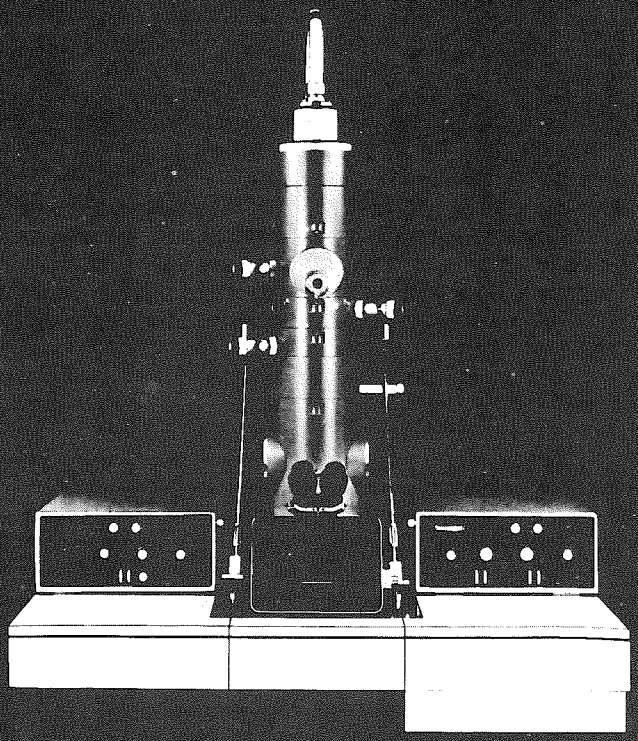

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THE UNIVERSITY OF TEXAS HEALTH SCIENCE CENTER, DEPARTMENT OF ANATOMY

GRANTS AWARDED

Dr. Ivan L. Cameron, "Glucose intake, gluconeogenesis, and cancer cochexia" from the National Cancer Institute, $91,625, from July 1981-June 1984.

PUBLICATIONS

A book, **The Transformed Cell,** ed. by I.L. Cameron and T.B. "Rusty" Pool, Academic Bress Inc., N.Y. May 1$981.

Herbert, D.C., F.J. Weaker, and P.J. Sheridan 1981 Autoradiographic demonstration of estrogen uptake by the armadillo pituitary gland. Experientia, 37: 1035-1036.

Herbert, D.C., and R.J. Reiter 1981. Influence of protein-calorie malnutrition on the circadian rhythm of pineal melatonin in the rat. Proc. Soc. Exp. Biol. Med., 166: 360-363.

Herbert, D.C., F.J. Weaker and P.J. Sheridan 1981. Localization of ^{3}H-dihydrotestosterone in the pituitary gland of the rhesus monkey. Cell Tissue Res., 215: 499-504.

Weaker, F.J. and D.C. Herbert 1981. Postnatal development of the ventral prostate gland in normal versus protein-calorie malnourished rats. Prostate, 2: 249-260.

NEW EQUIPMENT AND/OR FACILITIES

Quick-freeze device for preparation of rapidly frozen samples for freeze-fracture and TEM, John T. Hansen's laboratory.

POSITION AVAILABLE

Faculty level position: To work in all facets of Electron Microscopy, including TEM, SEM, STEM and EDX. Individual will participate in ongoing research programs as well as initiate additional investigations. Requirements: Doctorate degree with experience in Electron Microscopy research and a background in biological and/or physical sciences.

Send resume to: Dr. Ronald F. Dodson, Chief, Department of Cell Biology & Environmental Sciences, The University of Texas Health Center at Tyler, P.O. Box 2003, Tyler, Texas 75710, (214) 877-3451, Ext. 2504.

DEPARTMENT OF BIOLOGY, UNIVERSITY OF TEXAS AT TYLER

NEW EQUIPMENT AND/OR FACILITIES

The Dr. William Zuckerman Laboratory of Electron Microscopy at the University of Texas at Tyler was completed in August. It was made possible by an initial gift of $25,000 from Mrs. William Zuckerman in memory of her late husband, a Tyler Pathologist.

The laboratory consists of a Zeiss 109 transmission electron microscope, LKB microtome, dark room facilities, and other accessories. In addition to the general preparation room, there is a room to house a scanning electron microscope sometime in the future.

TYLER

THE UNIVERSITY OF TEXAS HEALTH CENTER, DEPARTMENT OF CELL BIOLOGY AND ENVIRONMENTAL SCIENCES

LECTURES

Dr. John R. Hoidal, Assistant Professor of Medicine at The University of Minnesota, was a Visiting Scientist in the Department of Cell Biology and Environmental Sciences and discussed the use of various animal models in the study of lung diseases.

Dr. Russell Martin, Professor of Medicine at Baylor College of Medicine, spoke October 22, 1981 on "The Effects of Smoking on Pulmonary Macrophages."

PUBLICATIONS

Dodson, R.F., O'Sullivan, M.F., Williams, M.G., Jr., and Hurst, G.A.: Analysis of cores of ferruginous bodies from former asbestos workers. Eviron. Res.: In Press.

Dodson, R.F., Castillo, P., Hieger, L.R., and Williams, M.G., Jr. Ultrastructural and energy dispersive analysis of inorganic inclusions in a muscle biopsy. Ultrastruc. Path.: In Press.

Martin, R.R. and Dodson, R.F.: Human pulmonary alveolar macrophages phagocytize ash from Mt. St. Helens with release of chemotactic factors. Presented at the American Thoracic Society Meeting, Detroit.

Dodson, R.F., Williams, M.G., McLarty, J.W. and G.A. Hurst.: An ultrastructural study of particulate matter and ferruginous bodies in the sputum from former asbestos workers. Presented at Electron Microscopy Society of America, Atlanta, Georgia.

NEW EQUIPMENT

DuPont Sorvall MT 2-B Ultra-microtome, A.O. Dual Viewing Polarizing Microscope, Lanier Word Processor

WACO

BAYLOR UNIVERSITY

GRANTS AWARDED

$1,200 research grant awarded to Randy Moore from the American Orchid Society. Title of proposed study: "Hybridization of **Dendrobium** x **Brassavola** By Experimental Manipulation."

LECTURES

Invited research seminar presentations by Randy Moore at Stephen F. Austin State University and the University of Texas at Arlington. Title of seminars: "Graft Compatibility-Incompatibility in Higher Plants."

PUBLICATIONS

Moore, Randy. 1981. Graft compatibility and incompatibility in higher plants. Developmental and Comparative Immunology 5 377-389.

NEW FACULTY AND/OR STAFF MEMBERS

Dr. William D. Hillis-Departmental Chairman. Research Area: Immunology and Virology (Previously at Johns Hopkins).

E.M. EDUCATION POSSIBILITIES

"Biology 4402-Electron Microscopy." A formal course in electron microscopy, offered Fall and Spring Semesters in the Biology Department at Baylor University.

MEETINGS

Randy Moore attended the annual meeting of the National Association of Biology Teachers in Las Vegas, Nevada from October 22-25, 1981.

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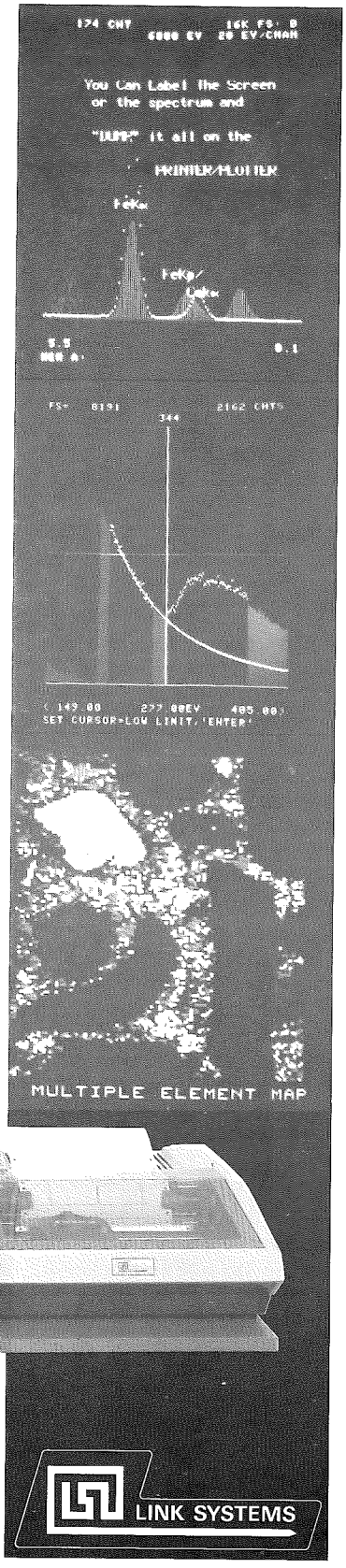

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Microphotography Workshop Summary

This summary of the workshop on microphotography at the Corpus Christi meeting is being published at the request of a number of our members.

OPTICAL THEORY AND CONTRASTING TECHNIQUES

By Butch Moomaw
Micro Specialist
Carl Zeiss, Inc.
Houston, Texas

Let's talk about light and physics and how they relate to microscopy. Each of us uses a microscope in his daily work, so everyday we take light apart, reorganize it, and put it back together again. What we create depends upon our application of the physics of light and our knowledge of our sample and microscope.

Light has some properties which belong to forms of energy that are characterized by straight line particle motion. These properties are called Geometric Optical Properties. There are, however, some properties which can not be due to particle motion but rather must be due to a waveform of motion and are called Waveform Optical Properties. In general, Geometric Optics are used to explain the optical paths of a microscope while Waveform Optics are used to explain the contrasting techniques and image formation in the microscope.

The properties which are of most concern to a microscopist are reflection, refraction, absorption, dispersion, diffraction and interference. Let's discuss each of these properties.

Reflection is the property of light which causes it to change direction by striking a surface but continue in a new direction at the angle of incidence but in the opposite direction. This can occur at any interfaces between two refractive indices.

Refraction is the property of light which causes it to change direction at the interface between a media of different refractive index which displaces the original ray path.

Absorption is the characteristic of light which occurs in all media and results in reduction or elimination of the light's original intensity. This generally occurs selectively by wavelength and is the reason we use colored stains in biological specimens. As the stains absorb different wavelengths of the "white" light being transmitted through the specimen, our eye is able to differentiate the various specimen details by the colors which are not absorbed.

Dispersion is related to refraction in that different wavelengths of white light are not equally refracted. This gives rise to the spectral fan effect of a prism in which white light is separated into its color components.

Diffraction is the physical effect of a solid substance on a light wave. A wave is bent by the edges of this substance according to the wavelength of the light and in the direction of the substance. As the waves are also bent more as the size of the spacing between edges becomes smaller, the angle of acceptance of light (the numerical aperature of the objective) becomes increasingly important as the size of the object is reduced. For example, look at a specimen with both course and fine structure and the diffraction image which it produces. To do this we can use an apparatus which Professor Abbe developed in the 1860's. This apparatus is called the "Abbe Diffraction Apparatus" and consists of a low magnification objective with an accessable rear focal plane, some specially designed metal shutters which may be inserted into the rear focal plane, a slide with regularly spaced geometric patterns, and several color filters.

By manipulating the orders of diffraction with the metal shutters in the objective rear focal plane we make fine lines appear course, course lines become fine ones, and a series of dots becomes vertical, horizontal or even diagonal lines; all due to modification of the original specimen's diffraction pattern. This diffraction pattern is composed of a bright central spot of light (The Zero Order) flanked by decreasingly intense spots called the +1 and -1 Orders, +2 and -2 Orders, etc.. The manipulation of these orders with the diffraction apparatus is analogous to the effect produced by the Aperture Diaphram, the Numerical Aperture of the objective or by some of the devices we use to produce contrasting techniques. It also serves to illustrate the principles of resolution and fidelity.

Interference is the physical effect waves on one another. After diffraction has occurred in the specimen and the objective has gathered together as many of the resultant waves as its Numerical Aperture will permit, these diffracted waves will interfere. There can be Constructive Interference, Destructive Interference and Partical Interference. These gathered waves (the diffraction pattern) are brought together in the intermediate image plane of the microscope and run into each other. As the waves meet and recombine, bright areas will be seen where the waves interfere constructively and dark areas appear where the waves interfere destructively to produce the intermediate image in the microscope tube.

We take advantage of these concepts by organizing the microscope optically for best illumination, even background information and minimum stray light; ie., by conjugating the field planes, which control the observation ray path, and pupil planes, which control the illumination ray paths.

Field planes are planes or places in the microscope which are imaged simultaneously with the image of the specimen. That is, the field diaphragm, the specimen, the microscope eyepiece field stop, and the retina of the observer's eye.

Pupil Planes are planes or places in the optical system which are imaged in the exit pupil of the microscope. That is, the light source, the condenser front focal plane where the aperture diaphragm is located, the objective rear focal plane, and the

exit pupil of the microscope, and observer's pupil.

A clear understanding of diffraction and a clear understanding of interference lead to Abbe's classic definition of the Image Formation Process. "Image Formation is the result of interference of diffracted waves with undiffracted waves."

Koehler Illumination is the technique developed to maximize the Principles of Abbe's Theory of Image Formation. This maximization of diffraction in the specimen and the interference in the microscope occurs when the microscope is properly set up. A microscope is properly set up only when all optical elements are aligned, not only with regard to optical centration but also with regard to optical conjugation. When we learn to set up this optical conjugation we are able to see things properly, just as we conjugate our verbs so that we may say things properly. This conjugation occurs in two specific types of areas in the microscope. The first area is the Field Planes and the second area is the Pupil Planes.

How are Field Planes conjugated? Most modern microscopes have been designed so that many of these planes are already aligned or fixed in place but some adjustments are still required. Assuming that the microscope illumination is on, let's first look at the field planes.

First we must align the observer's retina to the optical system. Next, one should be sure the eye is focused to infinity since the top lens of the eyepiece produces parallel ray paths. The image of the eyepiece field stop (where the intermediate image is produced by the objective) will be imaged at infinity. In some eyepieces this can be checked by inserting a reticle at this point. In other eyepieces, one must just learn not to accommodate. Looking into the microscope eyepiece will automatically accomplish this but we will generally not be in focus.

Next, we move the specimen into the point in front of the objective which will produce a focused intermediate image in the plane of the eyepiece field stop. This is done either by moving the stage up and down, or the microscope limb up and down. We should now see a well-focused image of the specimen. We now have conjugated the retina, the eyepiece field stop and the specimen. There is one more field plane we must conjugate before we start on the pupil planes.

This is the imageof the field diaphragm of the microscope illuminator. This image is projected into the specimen plane by moving the condenser up to its stop and then slowly down. The field diaphragm should be closed far enough to fit within the field of view and centered with respect to the eyepiece field stop. A sharply defined image of the edges of the leaves is seen in the specimen image when the condenser has been adjusted to the correct height. The centration is done by moving the condenser in its mount, then open the field diaphragm until it just clears the field.

Now we should have well-conjugated field planes. It is sometimes the case with the field diaphragm that even with all this, the diaphragm may have red and blue fringes on opposite sides at the same time. This is generally due to the aperture diaphragm or the microscope illuminator being misaligned. This will be corrected as we conjugate the pupil planes.

In most modern microscopes, conjugation of the field planes automatically conjugates most of the pupil planes. The microscope operator still has a few to align and should be familiar with the rest so that he or she may check the microscope from time to time and isolate suspected problems.

The observer's pupil is automatically aligned to the microscope exit pupil whenever the entire field of view is visable. If there is a cut-off when looking into the microscope, it is generally due to the observer being misaligned to his microscope. This arises from being at the wrong angle, too close to the eyepieces, or most often, too far from the eyepieces. This is common when the observer uses his glasses with eyepieces which do not project the eyepoint high enough. A simple piece of white paper will illustrate the height of this exit pupil when placed above the eyepiece.

Once the observer's pupil and the microscope exit pupil are conjugated, the next step is to make sure the rear focal plane of theobjective is centered in the tube. This is almost always so unless the objective is mounted incorrectly, the nosepiece click stop is broken, or the objective is a centerable objective such as in polarizing microscopy. This, and all pupil planes are visable when looking into the microscope tube with an eyepiece removed.

As we look at the pupil planes, the next one we must conjugate is the front focal plane of the condenser. This is where the aperture diaphragm of the microscope is located. It should automatically be conjugated because we adjusted the condenser position when adjusting our field planes. In some condensers though, it is possible to make separate centering adjustments. If this is the case, the aperture diaphragm should be centered with respect to the rear focal plane of the microscope objective. Once aligned, it should be opened all the way to evaluate the next pupil plane, the image of the microscope illuminator (generally, a filament lamp). It may be necessary to swing out a diffuser in some microscopes at this point.

The filament lamp or most any microscope illuminator needs to be projected into the proper plane of the microscope. This is done with a collector lens of some type; either fixed or focusable. Ideally, an image of the actual filament itself is projected so that by viewing into the microscope tube one may see it. It should be projected at such a size that it covers the entire front focal plane of the condenser and is then projected again to cover the entire rear focal plane of the objective. Once this condition is met, it is perfectly acceptable to introduce a diffuser into the optical path near the microscope lamp to further even out the illumination. The advantage to doing so is that as you change objectives, the small differences in centering will not affect your illumination and the pupil will be evenly illuminated.

We now go back to the aperture diaphragm and close it slightly, about 1/3 to 1/4 of the total pupil plane diameter, to enchance the microscope contrast. This setting depends on the specimen. If we set it correctly we should have a good compromise between contrast and resolution. If we close it too far we get diffraction in the specimen. If we open it too far we get a lack of contrast.

With a properly set up microscope we can begin a discussion of the various contrast techniques. We should begin with Brightfield Microscopy as a basis for comparison.

As light passes through a specimen on the stage of a microscope set up for Brightfield Observation, several things occur simultaneously to the light.

First some of the light is absorbed by the specimen and the stains which have been applied. This gives us the color differentiations we will ultimately see. Some of the light is diffracted by specimen details and these orders of diffraction exit the specimen at angles which are determined by the size of the structure which generated them. The microscope objective's job is to collect as many of these orders of diffraction as possible and refract them in such a manner that they are brought back together and allowed to interfere with one another. It is important to realize that this interference occurs only among diffraction orders which have the same origin. Also important is the fact that each order of diffraction carries a bit of information about the specimen and the more orders we can collect the more our image will resemble the specimen. All the light which does not pass near or through the specimen structures enters the microscope undiffracted and this is called "O" order light. This "O" order light appears to our eye as large bright areas surrounding the speci-

men or showing through areas of the specimen which have either no structure or structure which causes diffraction at angles beyond the acceptance angle of the objective. Since the "O" orders of diffraction carry only information about the background or field, this technique is called Brightfield. In this technique, we must depend upon natural contrast or staining to create enough contrast in the specimen for our eye to perceive the separate details.

We have some control over this contrast level even in Brightfield Microscopy. This control is the aperture diaphragm of the microscope. The aperture diaphragm is located in the condenser and its function is to change the maximum angle of illumination which strikes the specimen. By controlling the maximum angle of illumination we can control the number of orders of diffraction which are within the acceptance angle of the objective. As the number of these diffraction orders is reduced by closing the aperture diaphragm, the contrast is proportionately increased. This increase comes only at the expense of resolution. As we increase contrast by stopping or closing down the aperture diaphragm, we decrease the ability of the microscope to resolve small details.

The aperature diaphragm is only fully effective when the condenser is in its proper position, that is, at or near the top of its focusing travel. A common mistake is to lower the condenser rather than use the aperature diaphragm. This does create more contrast, but it also disturbs the entire optical conjugation of the microscope.

If we wish to observe a specimen which has little or no inherent contrast and we can not or do not want to stain it histochemically, we must resort to optical contrast or staining techniques. In order to do this we can manipulate and select from the various orders of diffraction which the specimen generates in order to create the contrast we need.

One of the first methods devised for this purpose was called Oblique Illumination. In this contrast technique the microscopist allows the "O" order to enter the microscope objective only at an oblique angle instead of the normal omnidirectional angles of Brightfield. This allows the microscope objective to collect more orders of diffraction from one side of the specimen and fewer from the other side. This creates a "Shadow" side of the image while the opposite side of the specimen appears in highlight since there are fewer diffracted orders to interfere with the "O" order and reduce its intensity. This technique is sadly almost forgotten today.

A more common technique used today is called Phase Contrast. This technique was developed in 1936 to study live cells. It is called Phase Contrast because we manipulate the brightness and the phase (Synchronization) of the "O" order to allow the lower amplitude orders of diffraction to more successfully interfere with it. We first use a special condenser to generate an omnidirectional but hollow cone of light. This cone of light is focused on the specimen. If we look into the pupil planes of the microscope when using a phase condenser, we can see this hollow ring which is properly called a Phase Annulus. When the hollow cone strikes the specimen most of it passes through the sample with very little change in amplitude or brightness but diffraction does take place. The rays which pass through undiffracted are once again called "O" Order and contain only background information. The diffracted orders are picked up along with the "O" Orders by the objective but would normally not have sufficient amplitude to effectively interfere with the "O" Order. In the phase microscope, we are able to manipulate the "O" Order and give the diffracted orders a better chance to interfere. This manipulation is done by putting a special ring in the back of the objective which precisely matches the image of the hollow cone with which we illuminated the specimen. This ring intercepts the image of the hollow cone with which we illuminated the specimen. This ring intercepts the image of the hollow cone which we saw as a ring of light, before it interferes with the diffracted orders. The ring is composed of special coatings which decrease the brightness of the "O" Order and at the same time put it 1/4 of wave further out of step with the diffracted orders. This additional 1/4 step phase difference is the real key since it, plus the small phase or step differences caused by the passage of the light through the specimen, enhance the ability of the weak diffraction orders to create a visible contrast difference in an otherwise low contrast situation.

Another technique which is commonly used for increasing the contrast of unstained specimens is called Darkfield. As one might suspect this technique differs from Brightfield in that the background is dark or black. The specimen however appears brightly illuminated. This bright specimen against a dark background gives us the highest contrast levels of any technique. Once again we achieve this by manipulation of the orders of diffraction. In this instance we simply illuminate the specimen with a hollow cone of light similar to the one we used for phase contrast but this time we make the angle greater than the acceptance angle of the microscope objective. This means that all the "O" order of diffraction, or the undiffracted light, does not enter the microscope and thus plays no part in the image formation process. Rather we simply allow the diffracted order to interfere with each other. It is noteworthy that in darkfield we have the best chance to gather even the furthest or highest angle orders of diffraction which means that we have outstanding resolving power in addition to tremendous contrast.

A more exotic contrasting technique has come into popular use since its development in 1954. The technique is called Differential Interference Contrast after Nomarski. This technique offers the possibility to continually adjust the contrast in unstained or stained specimen and do so either by different levels of grey or by color. The equipment needed for this technique can also be used perfectly well for Brightfield. Nomarski Interference as this technique is popularly called has its basis in polarized light. Polarized light has special properties which allow us to separate a single beam of light into two components of exactly equal amplitude and exactly equal phase called vectors. These two vectors can be considered as parallel beams of light, each vibrating at 90° to the other. As the beams pass through different portions of the sample, each is affected a little differently and when recombined will no longer be of exactly the same amplitude or may not be of exactly the same phase step. If either or both of these are changed then the beams will interfere destructively and cause a difference in brightness in the area through which they passed. This will cause contrast between this area and the surrounding area which we then perceive as an image detail.

To use Nomarski Inteference in a microscope, the microscope must have a relatively powerful illuminator. Next we must have a polarizer with its orientation direction east-west. In the optical path above the polarizer and in a special place called the Condenser Front Focal Plane we must insert at 45° a Wollaston Prism with Nomarski's modification. It is this prism which separates the polarized light into its two vector components or beams. The thickness of the prism determines the distance between the emerging beams and this distance must be less than the smallest resolving power of the microscope objective in use. This prism is generally built into a special condenser which has several other prisms in it for different magnifications. Once our two equal beams have been generated, we allow them to pass through the specimen. If the specimen has structures in that area which are not uniform in thickness, not uniform in refractive index, or not uniform in absorption characteristics, our two beams will emerge from the specimen either or unequal brightness, unequal phase steps, unequal col-

ors or a combination of the three. As these two unequal beams are collected by the microscope objective, they are brought to focus on the second Nomarski prism in the system which is located in another special place in the microscope called the Objective Rear Focal Plane. This prism is exactly like the one in the condenser but is put in exactly opposite in direction. When the two beams strike this prism, they are recombined to form a single beam again. This single beam still can not create an image in the microscope, the two components can not yet interfere because the vibration directions are still at 90° to each other. We insert another polarizer in a North-South direction, called an Analyzer, to rotate each of the components of this single beam 45° so that they will then vibrate in the same direction again. Now they can and do interfere with each other to create contrast and our image of the specimen.

I mentioned earlier that we could continuously vary the contrast with this technique. This is done by displacing the upper Nomarski prism laterally. As the two beams are being recombined, the prism will no longer be exactly equal to the first prism. This means that one wave will find more resistance to its passage than the other and will be slowed down more than the other. When the beams emerge from this second prism they will be out of step by the amount induced by the passage the sample plus the amount induced by the passage through the Nomarski prism. This second factor is continuously adjustable so that for any particular amount of difference induced by the specimen we can either eliminate that amount or amplify that amount to produce the amount of contrast best suited to that particular specimen or even a particular part of a specimen. Caution: Watch for orientation and interpretation errors.

If we shift the upper prism far enough, we will soon find that we start to generate the beautiful colors in the background and the specimen which one normally associates with Nomarski. These colors are produced when the amount of phase step difference is exactly 1/2 the wavelength of any wavelength of light. When for instance we have a 250 NM shift in our phase step, all the light with a 500 NM wavelength, blue light, will totally destructively interfere. In this situation no blue light will be visible and we will see a cyan color. As the prism is shifted we generate phase step differences from 0 up to over 500 NM so all colors are possible. Normally the best resolution occurs in the grey, colorless regions, but the color contrast is very useful for photographic presentations since it makes descriptions easier to follow.

A thorough understanding of the principles involved in microscopes, contrasting techniques, and image formation should eliminate some of the mystery from your microscope. With the mystery gone, confidence and creativity take over and who know where they will lead?

SIMPLE EQUIPMENT APPROACHES TO PHOTOMICROSCOPY

Donald L. Chaput
University of Texas Medical School
at Houston Media Center

Simple Equipment Approaches To Photomicroscopy

It is not necessary to have at your disposal an elaborate, sophisticated and expensive photomicroscope in order to take photographs through a microscope. Although photomicroscopes are capable of taking high quality photographs with relative ease, very good results can be achieved with systems far less sophisticated.

The most important piece of equipment in the system is, of course, the microscope. The quality of its optics is the greatest determining factor in the quality of the finished photograph. Actually, in order to take photographs through a microscope, no camera is needed at all. You merely need to hold a piece of film above the eyepiece and expose it to the light exiting from the eyepiece. The camera serves merely as a film-holding device.

It is not meant to be inferred that a simpler system will have the quality or the ease of operation of a more sophisticated system, but rather that it is an alternative.

Attaching The Camera To The Microscope

Some method must be devised to hold the camera in place over the eyepiece. Microscope adapters are available and can be purchased for many camera models to attach them directly to the microscope tube, but these will often translate vibrations from the camera's shutter through the microscope causing a blurred exposure.

If you are using a camera with its own internal shutter it would be best to fix the camera to its own separate stand, not fixed to the microscope itself. A copy stand is ideal, and many enlarger columns can be adapted for the same purpose. The microscope can be positioned underneath the camera for exposing and moved aside for viewing and focusing.

Virtually any camera is capable of taking photographs through a microscope, whether it be a view camera, an expensive single lens reflex, a rangefinder or even a simple box camera. There are advantages and disadvantages to each camera type and the methods for using each type differ slightly.

Cameras With Attached Lenses

While cameras with lenses that cannot be removed can be used to take photomicrographs, they are somewhat inconvenient to use and seem to offer more disadvantages than advantages. Consider their use only when a camera with removable lenses is not available.

If the camera has focusing capabilities, set it at the infinity setting. If f-stops are available, set the camera's aperture wide-open. Exposure control is achieved with shutter speeds and neutral density filters; f-stops do not control exposure on the microscope.

The camera must be positioned so that the eyepoint of the eyepiece is at the front surface of the lens, that is, the point where the light rays emerging from the eyepiece converge. The position of the eyepoint can be determined by moving a piece of white paper up and down 1-2 inches above the eyepiece until the circle of light formed is just a small dot. This is the eyepoint and the front surface of the lens should be positioned at this point. Having the camera attached to a copy or enlarger stand will

facilitate the positioning.

If a box camera with fixed focus and fixed apertures is to be employed, use a "high eyepoint" eyepiece and position the camera so that the eyepoint is at the camera's diaphragm leaves.

The disadvantages to these types of cameras in photomicroscopy are many. Since the image can not be seen through the camera, it will be necessary to first find the area to be photographed on the microslide by viewing directly into the microscope, and then positioning the camera and microscope together without disturbing the field. Since these cameras do not have internal light meters, exposure determination becomes difficult. Finally, almost the entire microscope field of view is photographed, including the out of focus edge areas.

CAMERAS WITH REMOVABLE LENSES

This type of camera will most commonly be a Single Lens Reflex (SLR) type. If you have a rangefinder type camera with a removable lens, you will probably find it easier to use with the lens in place as described in the previous section. Since critical focusing must be done at the film plane, rangefinders without lenses are difficult to use in this type of arrangement.

SLR cameras are used for photomicroscope work without their lenses. In essence, an SLR or a view camera becomes a mere film holder above the microscope.

An SLR has the distinct advantage that the microscope field can be viewed directly through the viewfinder of the camera, and since "what you see is what you get" with an SLR, focusing and image field size present no unique problems. Focusing can be done directly through the viewfinder and the camera's field will not include the out of focus edges from the eyepiece, but will be a rectangle within the field.

The camera itself can be positioned at almost any distance above the eyepiece. The further away from the eyepoint the camera is positioned, the greater will be the resultant magnification; the closer to the eyepoint, the greater the field of view.

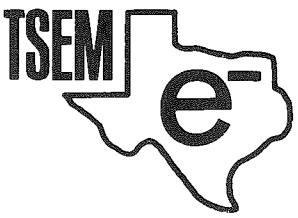
One disadvantage to using an SLR is that the ground glass focusing screens in these cameras are quite course and make fine detail difficult to see. A drop of mineral oil on the ground glass will produce a clear area to focus through, facilitating focusing on fine detail. The mineral oil, however, cannot be easily removed and may be objectionable if the camera is used for conventional photography. If interchangable focusing screens are available for the camera, it is highly recommended that the drop of mineral oil be placed on a screen which can then be used only for photomicroscopic purposes.

No matter what camera type is used, it is necessary to work in a darkened room or to manufacture some sort of light-tight collar to go between the camera and the microscope, to keep out extraneous light that might deteriorate the photograph.

CONCLUSION

These systems are not meant to compete with photomicroscopes. A "rigged" system will have neither the speed nor the ease of function of the more elaborate factory-designed systems, but good quality photographs are possible at a minimum investment.

There are many factors involved with capturing a quality image on film other than positioning a camera over the microscope. Perhaps the best "how-to" manual on the market is Kodak's publication #P-2 entitled: "Photography Through the Microscope." It covers fairly well the entire range of variables that must be controlled from proper illumination and exposure methods through specialized techniques and trouble-shooting.



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