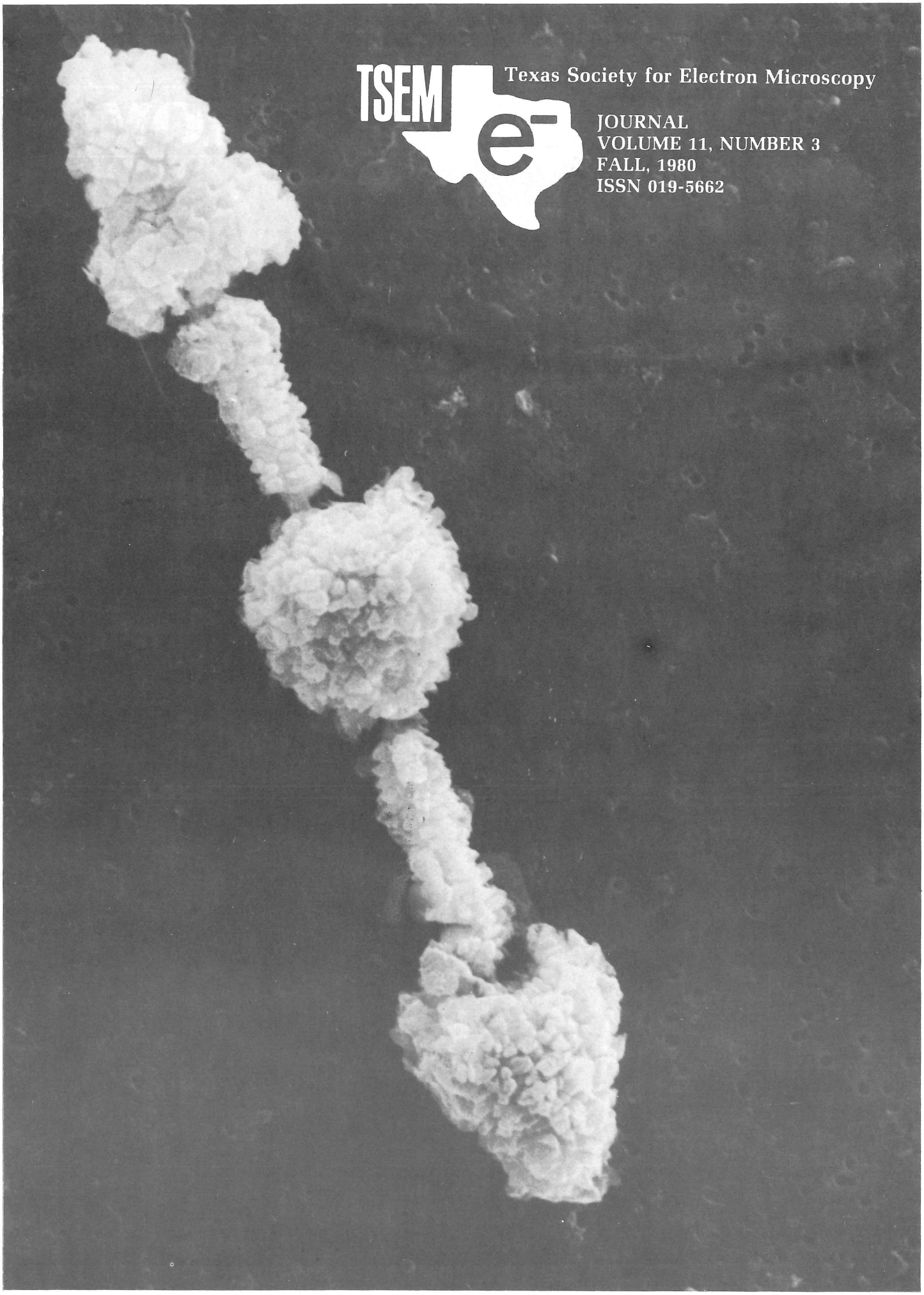

TSEM

Texas Society for Electron Microscopy

JOURNAL
VOLUME 11, NUMBER 3
FALL, 1980
ISSN 019-5662

# TWO NEW TEM'S FROM

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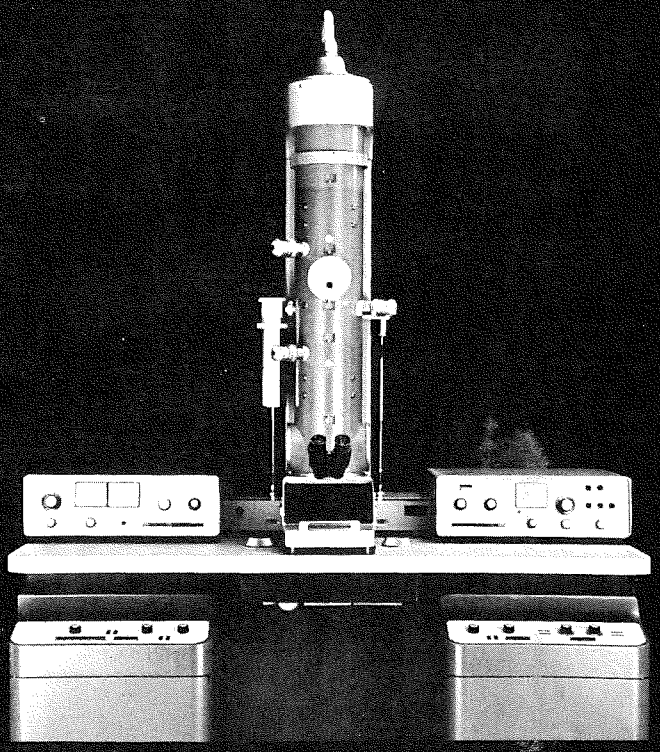

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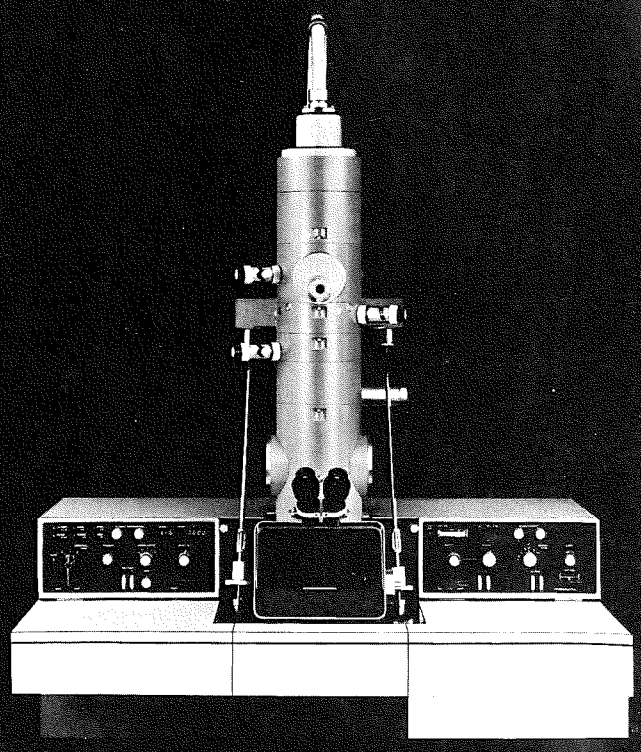

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# Contents

**Volume 11, Number 3**
**Fall, 1980**
**ISSN 019-5662**

## Texas Society for Electron Microscopy

*"For the purpose of dissemination of research with the electron microscope"*



## EDITORIAL POLICY

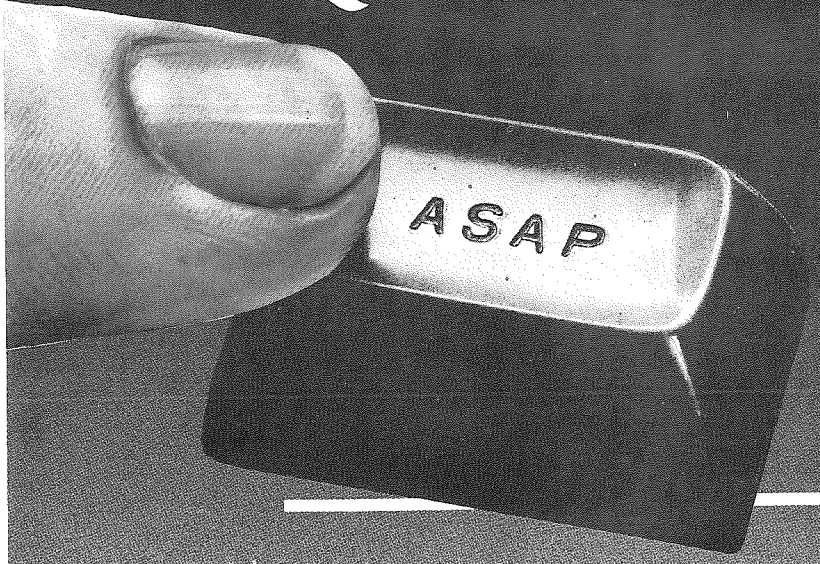
Feature articles, news, letters to the editor, and micrographs may be submitted. Feature articles should be 3-10 typewritten pages, double spaced, with figures, tables, and electron micrographs mounted for an 8-1/2 x 11 inch format. Three types of articles are solicited: 1) reviews 2) research reports 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

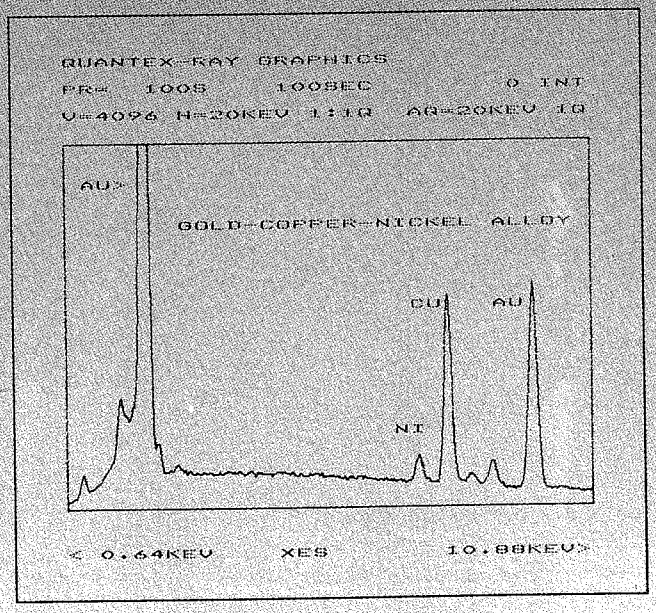
Scanning electron micrograph of a ferrugiuous body from a tissue digestion preparation. Submitted by Mr. Glenn Williams and Mrs. Deborah Piers, Department of Cell Biology and Environmental Sciences, University of Texas Health Science Center at Tyler. Magnification ×3,800.

# Quantitative microanalysis in 20 seconds or less with Kevex ASAP™




Many times standards aren't available for quantitative microanalysis by XES. Kevex ASAP is the first theoretically derived standardless analysis program for rapid quantification of a wide variety of materials. Just a single button activates ASAP—it's that easy. The analysis shown here was performed in 16 seconds—it's that fast.

ASAP and other Quantex-Ray™ programs such as Colby's MAGIC V and MATCH are revolutionizing microanalysis by eliminating the tedious work as well as the guess work. Chances are there's a Quantex-Ray program already in existence for your analytical requirements. Let us hear from you.

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SPECTRUM AUCU-1

STANDARDLESS EDS ANALYSIS
(ZAF CORRECTIONS VIA MAGIC V)

| ELEMENT & LINE | WEIGHT PERCENT | ATOMIC PERCENT | PRECISION 2 SIGMA | K-RATIO | ITER |
|---|---|---|---|---|---|
| NI KA | 2.52 | 5.83 | 0.20 | 0.0302 | |
| CU KA | 18.72 | 40.03 | 0.60 | 0.2266 | |
| AU MA | 78.45 | 54.13 | 1.09 | 0.6932 | 4 |
| TOTAL | 99.68 | | | | |

# President's Message

Recently I sent each of you a letter which is reprinted in this copy of the Newsletter. I'd like to reiterate our need to recruit new members to join our society and to encourage all of our members to attend our meetings more regularly. Use the membership form included in this TSEM journal. Copy it and distribute it to potential new members. The recruits will certainly flourish from their association with TSEM and the price is right.

The College Station and Fort Worth meetings are set and should be enjoyable and profitable both professionally and scientifically. Let us, the executive council, hear from you. We would like to hear your ideas, praises, disappointments, plans, and needs in order to know what the membership feels about their society.

Finally, I'd like to encourage every member to foster more lateral mixing both within the society and outside of it. Invite your friends in TSEM to give talks, seminars, visit your laboratory, answer your questions, help with your problems, help find, loan, or borrow equipment and/or positions. After all, we're all friends and we're in this good thing (TSEM) together. Everyone I've ever met in TSEM has always been eager to help one another and has taken pride in his membership. I'm confident of the outcome.

**Paul S. Baur, Jr.**
TSEM President

# Editor's Comments

I am grateful for the opportunity to serve as editor of TSEM Journal. This is sure to be a rewarding experience. Those who served in the past have done a fine job in building the status of this publication, and I hope to maintain the quality standards.

I thank the advertisers and contributors who have made the publication possible.

Each of you should feel compelled to contribute information from your institution or field to interest for upcoming issues. The broad base of this group is one of its strengths, and we should seek to magnify our diversity. Please make an effort to inform all of us of your ventures.

**Elaine McCoy**
TSEM Journal Editor

# Letters to the Editor

Dear Fellow TSEM Members:

It seems like only yesterday that I started to serve my last active year as an elected TSEM officer. In reality it has been more than two months and it's time you heard from me.

Our biggest news, is the council's decision to change from 3 to 2 meetings per year. This should stimulate better attendance, cut down on our society's and personal expenses, and allow us to coordinate better our meetings with the various universities' calendar year. Right now we plan to have a fall meeting in October and a spring meeting in March or April. The spring meeting has been regionalized; we will invite all our colleagues from neighboring states. We have our 1980 fall meeting scheduled for October 9-11 at Texas A&M, College Station and our spring meeting planned for April 23-25, 1981 at the Green Oaks Inn in Fort Worth. We recall the pleasant meetings held in College Station and, of course, the membership is anxiously looking forward to visiting Fort Worth. The Fort Worth Meeting follows the SEM, Inc. meeting to be held in Dallas, April 14-18, 1981. We hope to be able to profit from the fact that many commercial exhibitors will already be in the Dallas-Fort Worth Area and will only have to wait another week for our meeting. The following October meeting is in the formulative stage; Corpus Christi is being considered.

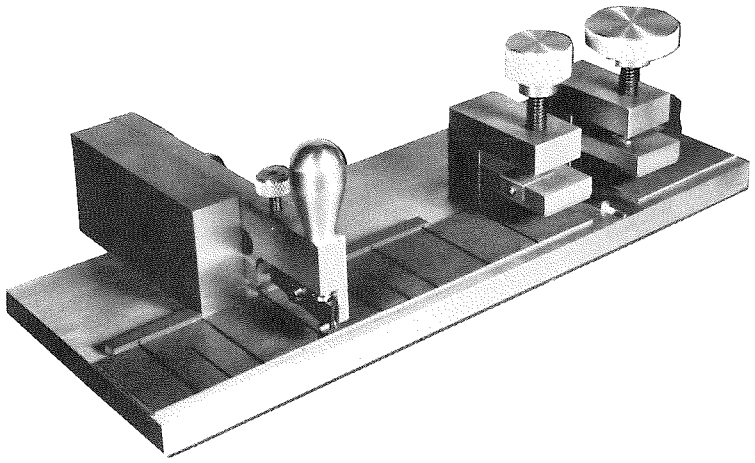
Obviously TSEM is not immune to the pressures of inflation so our net balance has fallen somewhat in the past year or two. We don't anticipate the need of raising dues nor registration fees, but we will have to control student funding and become more frugal with our planned social activities. We'll have to make every dollar count.

I think we ought to consider having one of our yearly meetings oriented more towards family participation. Spouses,

# LONGKNIFEMAKER and VIBRATOME

***aids for cytological sectioning***

***Medcast and Water Soluble Epoxy for EM & LM embedments***



## MI LONGKNIFE MAKER

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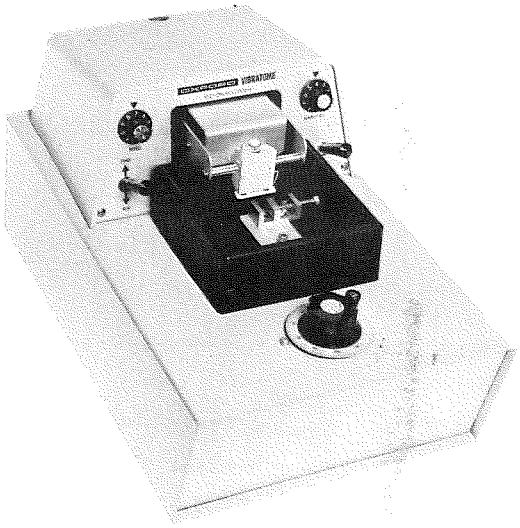
**8000** MI Longknife Maker



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| | | |
|---|---|---|
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children, and friends have enjoyed our gatherings in the past. Somehow over the years we've gotten into the habit of having closed meetings. The October 1981 assembly holds promise for a guest oriented meeting. We'll be in an exquisite area of the state, it will be at the end of the tourist season, the weather should be great. The meeting will adjourn at noon on Saturday so many of us could take the opportunity to "stay-over" one more day and enjoy the comradery and the beautiful setting. Let's think about even opening that meeting's sessions to our guests so they can hear our talks, listen to the speakers, and attend the socials.

Let me plea for your help in recruitment. We need to be revitalized by increasing our membership by at least 20%. Right now our roll stands at approximately 500. Use the enclosed TSEM application form, to enlist individuals who would profit from membership. Ask them outright. Help us grow.

I'd like to encourage our members to belong to other adjoining state societies, participate and recruit from their ranks. Cross "stateline" society mixing and the exchange of information, enthusiasm, and ideas will profit us all.

Finally, if you want or need to say something to our society officially or otherwise, drop me a note or give me a call. We need your input about plans, locales, meeting themes, etc. I promise your comments will be given every consideration. I know it's going to be a great year for us all.

**Paul S. Baur, Jr., Ph.D.**

# Financial Report

**TEXAS SOCIETY FOR ELECTRON MICROSCOPY**
**FINANCIAL REPORT**
Period Ending August 16, 1980

| | | | |
|---|---|---|---|
| **ASSETS ON APRIL 15, 1980** | | | |
| Certificate of Deposit No. 1099, Univ. Natl. Bank, Galveston | 2,000.00 | | |
| Certificate of Deposit No. 10-141345, Houston 1st Savings | 2,061.51 | | |
| Savings Account No. 10-502435, Fannin Bank | 1,201.67 | | |
| Checking Account, Fannin Bank | 1,299.85 | | |
| | 6,563.03 | | $6,563.03 |
| **RECEIPTS** | | | |
| Dues: | | | |
| Regular Membership | 440.00 | | |
| Student Membership | 34.00 | | |
| Interest on Cert. of Deposit No. 1099 | 37.50 | | |
| Interest on Cert. of Deposit No. 10-141345 | 51.97 | | |
| Interest on Savings Account No. 10-502435 | 65.34 | | |
| Refund from U.S. Postal Service | 134.48 | | |
| Waco Meeting: | | | |
| Registration | 572.00 | | |
| Refund, Holiday Inn | 15.66 | | |
| Corporate Donations: | | | |
| Amray | 100.00 | | |
| Edax | 200.00 | | |
| E.M. Services | 100.00 | | |
| Fullam | 125.00 | | |
| Kevex | 100.00 | | |
| I.S.I. | 100.00 | | |
| Pella | 100.00 | | |
| Polaron | 100.00 | | |
| | 2,275.95 | (+) | $2,275.95 |
| **DISBURSEMENTS** | | | |
| Waco Meeting: | 432.52 | | |
| Holiday Inn | 800.00 | | |
| Brazos Queen | 200.00 | | |
| Honoraria | 200.00 | | |
| Student Travel | 250.00 | | |
| Presidential Travel (EMSA) | 250.00 | | |
| Secretarial Expenses | 400.00 | | |
| Treasurer's Expenses | 14.00 | | |
| U.S. Postal Service | 70.00 | | |
| | 2,416.52 | (−) | $2,416.52 |
| **ASSETS ON AUGUST 16, 1980** | | | |
| Certificate of Deposit No. 1099, Univ. Natl. Bank, Galveston | 2,000.00 | | |
| Certificate of Deposit No. 10-141345, Houston 1st Savings | 2,113.48 | | |
| Savings Account No. 10-502435, Fannin Bank | 1,267.01 | | |
| Checking Account, Fannin Bank | 1,041.97 | | |
| | 6,422.46 | | $6,422.46 |

Respectfully submitted: Bruce Mackay, Treasurer.

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# Spectral Artefacts In Electron Eds X-ray Microanalysis Of Biological Specimens

By
**Nancy K.R. Smith**
Department of Anatomy
The University of Texas Health
Science Center at San Antonio
San Antonio, Texas 78284, USA

Key Words:
electron probe x-ray microanalysis
x-ray microanalysis
biological x-ray microanalysis
spectral artefacts

## INTRODUCTION

Elemental analysis of biological tissue at the cellular and subcellular levels by means of electron probe x-ray microanalysis is proving to have very broad applications. Evidence of these applications is given by many papers reporting specific applications as well as by several reviews of the applications in broad disciplines, such as physiology and pathology (1-3). X-ray microanalytical capability has become accessible to many biologists in recent years, especially by means of attachment of energy dispersive Si(Li) detectors to scanning and scanning transmission electron microscopes (SEMs and STEMs). Although many loboratories have been able to acquire the instrumentation required to perform x-ray analysis, it is in some cases considered as a magic black box which yields magic, unquestioned data. The biologist—microanalyst must be aware of the limitations of x-ray microanalysis and of the many possibilites for misinterpretation and error. Thus it behooves the analyst to try to understand the sources of extraneous and artefactual spectral information which might affect his results. The total understanding of spectral data involves even more than knowing about artefacts, especially if one is interested in accomplishing an absolutely quantitative elemental analysis. Discussion of basic x-ray theory is beyond the scope of this article. The reader is referred to books by Goldstein and Yakowitz (4) and by Woldseth (5). Discussion here will be limited to artefacts and extraneous peaks.

The reader is reminded that x-rays are of two types: 1) characteristic x-ray, which are characteristic of the elements from which they originate and 2) continuum x-rays, which represent a broad energy emission resulting from deceleration of electrons in the sample. The continuum constitutes a background above which the characteristic peaks of interest must be detected and, as such, is generally considered to be a nuisance, except where it is specifically utilized as in the Hall technique for quantitative analysis of thin sections (6). The continuum, however, is not an artefact but is related to such specimen parameters at density, average atomic number, and section thickness.

X-ray spectral artefacts and extraneous peaks can be broadly classified into 3 categories: 1) those resulting from the sample itself and/or its environment: 2) those resulting from the detection process; and 3) those resulting from the processing of the x-ray signal. The first category will be discussed in two parts: a) artefacts due to specimen preparation and b) artefacts due to the environment of the specimen.

## ARTEFACTS DUE TO SPECIMEN PREPARATION

The major problems remaining in biological x-ray microanalysis involve specimen preparative techniques. To date there has not been a consensus as to a practically

attainable preparative procedure which is considered to be ideal. Indeed, the ideal seems to be unattainable. The mere act of intervening into a biological system in any way represents a disturbance which raises doubt as to whether it is possible to make any measurement, especially at the microscopic level, which is representative of the natural, undisturbed state of the system. It is the goal of many x-ray microanalytical studies to make measurements on the unperturbed state, and preparative techniques for such studies almost invariably involve some variation of a cryotechnique, with tissue being frozen as rapidly as possible to minimize translocation of elements and to minimize crystal damage to the tissues. Tissue that has been frozen is further processed and is examined in either the frozen-dried or the frozen-hydrated state.

Some microanalysts microprobe tissue which has been processed by conventional, wet, ambient-temperature techniques, for example employing histochemical reactions and stains (7). Such studies can be meaningful if the investigator bears in mind the effects of the various steps of the preparative procedure on the tissue and makes his interpretation in such a context. Elements of interest may be lost, redistributed, or masked by other constituents. It is generaly acknowledged that every step of wet processing involves loss of elements and redistribution of elements in the tissue (8). It has been shown by Jones et. al. (9), using electron probe x-ray microanalysis, that conventionally accepted nonaqueous subcellular fractionation procedures lead to redistribution of elements.

Aside from loss and redistribution of elements, one should recognize that extraneous elements may be acquired by the specimen in any of a number of processing steps. Elements may be picked up from fixatives, buffers, or rinsing solutions. Examples are Os, P, and As. Embedding plastics may contain detectable elements, e.g., Cl. Stains used to increase electron contrast can lead to spectral peaks which overlap and obscure the peaks of interest. Examples are U and Pb. Table 1 gives examples of elements which could possible be detected following a total specimen prep and which could interfere with elements of biological interest when energy dispersive spectrometry (EDS) is employed.

Support films and mounting grids must be considered as sources of extraneous peaks. These sources are more properly categorized as artefacts due to the environment of the specimen but will be discussed as part of specimen preparation. Support films made from Formvar dissolved in ethylene dichloride will contain Cl. Our laboratory has bypassed this interference by using dioxane as the solvent for Formvar. We are also utilizing nylon support films, as suggested by Sauvermann and Echlin (10). These films contribute no characteristic peak to EDS spectra. If support films are coated to render them conductive, then the coating (e.g. Al) may produce a characteristic EDS peak. A mounting grid will contribute to the spectrum obtained from a sample (see later discussion of excitation of remote sources). Choosing a larger mesh grid will help to minimize this problem. A grid made of copper, for example, will give very large characteristic Cu peaks as well as a lot of extraneous continuum radiation. Depending on the application, it may be advisable to use a grid or support planchet made of a low atomic number element which yields minimal continuum and no detectable characteristic peak. Grids are commercially available made of Be, graphite polymer, and carbon-coated nylon. It should be noted that Be is toxic and should be handled with care. Nylon grids result in a detectable titanium peak.

Some investigators coat their specimens prior to x-ray analysis. In such cases, it should be remembered that not only will the elements comprising the coating (e.g., Au, Pd, Pt, Cr) result in significant characteristic peaks but also that the surface coating will absorb x-rays attempting to exit the specimen and hence will reduce the overall sensitivity of the analysis, particularly limiting the detection of elements present at the level of the margin of detectability.

Extraneous peaks may also result from gross contamination of the specimen during any step of the preparation. It is in this classification that we have finally been able to place a very serious Si contamination which plagued our laboratory for some time before its source(s) was tracked down. This problem will be discussed in detail in order to give the reader an example of how an artefact can interfere with an analysis and lead to invalid if

**Table I**

Extraneous x-ray peaks which overlap peaks from elements of biological interest (type emission included with elemental symbol)

| Extraneous element | Biological element |
|---|---|
| Cu L, Zn L | Na K |
| As L | Mg K |
| Os M, Pt M, Au M | P K |
| Pb M, Mo L | S K |
| Mo L, Ru L | Cl K |
| Ag L, U M, Pd L | K K |
| K K | Ca Kα |

not merely anomolous results.

Upon originally obtaining Si peaks in some of our spectra of biological tissue, we contemplated whether the Si could be of biological origin and soon rejected that notion. Levels of Si range from 2-20 ppm for parenchymal organs such as liver, lung, muscle, and brain and up to 100 ppm for connective tissue (11). Such levels would not be detectable by EDS. Sometimes the Si peaks in our spectra were minimal whereas at other times the Si peak would be larger than all the other peaks and then often increased in proportion to the other peaks in the spectrum as a function of time, i.e., with continued beaming of the specimen at one location. Pool et al. (12) carried out a time-series study in this loboratory under such a set of conditions and confirmed, as one would expect, that any attempt at quantitation under such conditions is totally invalid. It became imperative to find the source of such contamination, considering the tremendous investment of time, personnel, money, animals, and importance to a long-term project that is already involved in a preparation by the time a specimen reaches the stage of being microprobed.

Upon referring to the literature, it was found that the problem of an anomolous Si peak had been pointed out numerous times by biologists (13-18). In an early attempt at a biological application of x-ray microanalysis, Thurston and Russ (13) probed structured granules in Fischerella ambigua and reported the presence of a Si contamination artefact which increased in intensity as a function of time. The authors lamented that "perhaps all elements found to date represent artifacts of the experimental setup".

In 1973 Sutfin et al. (14) reported x-ray microanalysis of individual mitochondrial granules in chondrocytes and noted a difficult problem due to the buildup of a heavy contamination deposit on the specimen at the point of impact of the electron beam. They further noted that the deposit contained Si, that the Si increased with time, and that an anomalous Si x-ray peak was often the most prominent peak in the spectrum. The authors concluded that the sources of Si were Si fluids and greases used in the microscope vacuum system and also semiconductor devices studied in the SEM. By cleaning the vacuum system, changing to organic vacuum pump fluids and greases with very low vapor pressure, and playing a jet of inert argon gas on the tissue surface, it was possible to reduce the contamination to an acceptable, often negligible level. However, the authors noted that Si contamination occurs in microscopes in which Si vacuum fluids and greases have never been used, as in the case of Thurston and Russ (13) and as in the case of our laboratory. Silicon contamination was found to recur if integrated circuits were studied, even after the vacuum system was cleaned. Sutfin et al. (14) concluded that the Si peak is not of biological origin, that it is spurious, and that it depends on the nature of the material being analyzed and the history of the instrument. They also pointed out that different rates of contamination on different structures may lead to incorrect conclusions regarding Si content. They found that the spectra from the mitochondrial matrix. Similar effects have been observed in our laboratory, for example, detecting much more Si in the cytoplasm than in the nuclei of cardiac myocytes (19).

At a 1973 conference on microprobe analysis applied to cells and tissues, the problem of anomolous Si peaks was reported and discussed. In the discussion session, Russ (15) noted that surrounding a frozen specimen with a large baffle to improve the quality of the vacuum in the specimen region and to minimize ice formation on the specimen surface also reduced Si contamination considerably. Further points made regarding the problem were that no Si greases or vacuum pump fluids were used anywhere in the instrument, that the observed effect was above and beyond the internal fluorescence peak from the silicon dead layer of the detector, and that the observed effect actually built up on the surface of the sample as a function of time. In the same proceedings, Gullasch and Kaufmann (16) reported the presence of Si in spectra from red blood cells, and Hodges and Muir (17) reported Si from sections of bladder.

As recently as 1978, in a special 2-volume set of the *Journal of Microscopy* dedicated to a cryotechniques conference, authors (20) reported "a peak of unknown origin at 1.7 keV". And, in 1979, from an x-ray microanalysis study of macrophages (21), the Si peak in some spectra was off-scale with respect to the peaks from biological elements. It is the tendency of many biological microanalysts to deal with the Si peak just as they often treat other extraneous peaks, classifying them as biologically irrelevant and hence discarding them from further consideration.

After considering possible sources of Si in our laboratories, a task complicated by the fact that specimens were prepared in multiuser laboratories before being brought to the SEM-XRMA facility for analysis, and considering the fact that Si pump oils and greases had never been used in the SEM, it was concluded that the Si must be an artefact acquired during specimen preparation. Following an exhaustive effort, sources of Si contamination were identified.They will be discussed, and other potential sources of a Si peak will be identified as well.

1. Silicon contamination may originate from the vacuum system and conponents of the electron microscope itself. Si-containing molecules may be present in the vacuum system of the microscope, either as part of the residual gas composition or absorbed on the inner surfaces, due to backstreaming of diffusion or mechanical pump oils of the Si type (use of which is not recommended) and to volatilization of silicone vacuum greases used on components such as O-rings. Even when no Si oils or greases are used in the vacuum system, Si-containing molecules may be present on the inner surfaces of the microscope system due to the total past history of the vacuum ststem, such as irradiation of Si-contaminated specimens, semiconductors, etc.

2. Specimens may be contaminated by Si-containing oils and greases during the course of specimen preparation. Such specimen-borne contamination would explain increases in measured Si contamination with continued electron beaming. Fourie (22) attributed this type of effect

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An optical switch connects the fibre optics of two detecting elements to the photomultiplier. This allows rapid switching of detection modes, for example, simulating the change in illumination you would get if the specimen were rotated. An optional module allows selection, mixing, addition or subtraction of any two out of eight detector signals.

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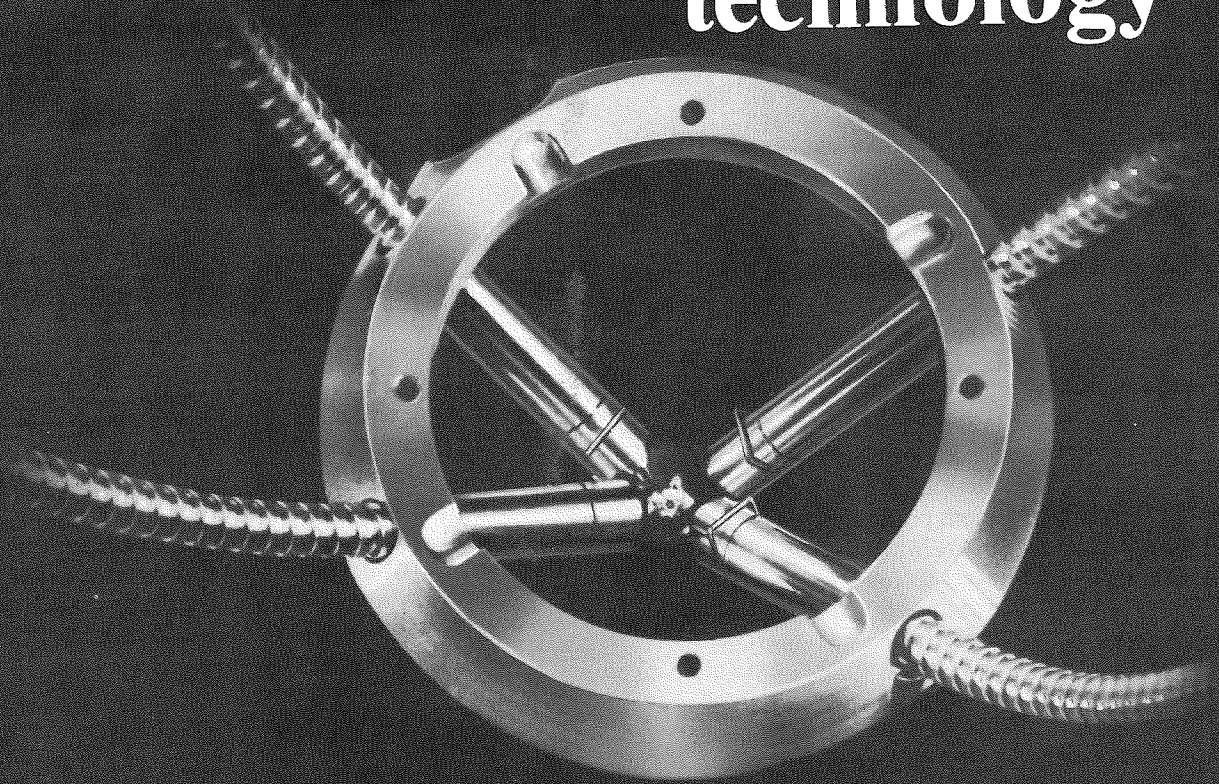
The MFD is described in our new SEM 505 brochure - along with many other special features. It will open your eyes to a new SEM technology. Send for it now.

# ...SEM 505 has the technology



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to random surface diffusion. In specimens with adsorbed contaminants, the contaminant molecules migrate along the specimen surface to the area where the primary beam is striking, interact with the beam to form nonvolatile and nonmobile cracking products, and hence surface contamination accumulates in the beamed area.

Any specimen preparative step involving a vacuum system with Si pump oil or vacuum grease could lead to specimen contamination by Si. Examples would be freeze-drying of tissue or coating by sputtering or by vacuum evaporation. One of the two sources of spurious vacuum grease to the stopcock of the freeze-drying apparatus by one of the users in a multiple-user facility.

The most malignant source of Si contamination of tissue in our laboratory was finally found to be direct contact with the oil (american Optical, cryocut microtome lubricant) used to lubricate the cryoultramicrotome. If the fine-tipped brush which is used to handle sections is laid down in the cryostat or brushed against any well-oiled surface, it acquires Si contamination which is subsequently transferred to the sections. The solution to this source of contamination is to carefully clean excess oil from the microtome and its cryochamber and to exercise extreme care in all aspects of tissue handling.

It has been suggested that the Si problem be circumvented by avoiding the use of Si oils and greases in preparative steps. In general, this is recommended. However, it would only be covering up the problem to simply switch, for example, to non-silicone microtome oil. If specimens were contaminated by direct contact with another oil and no characteristic peak due to the contaminant was observed in spectra, then the operator would be oblivious to the fact that his analysis was totally invalid. One simply connot overstress the necessity for absolutely meticulous care in the preparation of samples for x-ray analysis, especially if one's goal is to achieve a degree of consistency and reproducibility that is prerequisite to the performance of any type of quantitative analysis. In a recent paper, Isaacson et al. (23) discuss contamination as a "psychological problem" as opposed to its being just a technical problem. There is much to be said for the development of a mindset which would lead one to exercise extreme care in all steps of a preparative procedure for x-ray analysis and hence to hopefully avoid serving as a transfer agent of contamination.

3. A final, irreducible source of a Si peak in EDS spectra must be considered. That is the internal fluorescence peak from the Si dead layer of the Si (Li) detector (24). Such a peak appears in spectra when a Si K x-ray is generated in the Si dead layer at the front of the detector, enters the active region, and is detected. Such a peak imposes limitations on the minimum detectable limit for Si analysis by EDS. Fiori and Newbury (25) note that the internal fluorescence peak corresponds to an apparent concentration of 0.2 wt% or less Si in specimens. Figure 1, an x-ray spectrum obtained from a spectrographically pure carbon planchet, shows such a Si peak as well as an Al peak. The Al peak is inherent in our microscope system, being generated from the Al detector snout by x-rays and back-scattered electrons and is a prime example of an extraneous peak derived from the sample-detector-microscope environment.

## ARTEFACTS DUE TO THE ENVIRONMENT OF THE SPECIMEN

1. A source of spectral artefact due to the environment, which could alternatively be classified as an artefact due to the detection process, is that of microphony. The detector and associated electronics can act as an antenna and amplifier of miscellaneous noise such as voices, the ringing of a telephone, or the turbulence of small ice crystals in the dewar for the detector. It is important to route cables to minimize interference and to mechanically isolate the detection system as much as possible. Noise manifests itself spectrally as a very large peak at the low-energy end of the spectral distribution. One should strive to become aware of idiosyncrasies of the local environment which could lead to interferences. It has been found in our laboratory that the use of a small ustrasonic cleaner in the preparative laboratory adjacent to the SEM room causes interference with the x-ray analysis system; the reading on the deadtime meter becomes very high, and the system ceases to register counts for all practical purposes.

2. A second category of artefacts due to the sample's environment is comprised of extraneous peaks due to stray radiation. The stray radiation effects may be either electron or x-ray-induced.

Considering electron-induced effects, the first is that of direct entry of backscattered electrons into the x-ray detector. No problem should be observed when one is operating at low excitation voltages. However, backscattered electrons with energies of 25 keV and greater have sufficient energy to penetrate the Be window, gold contact layer, and Si dead layer of the Si(Li) detector and deposit their energy in the active volume. This problem shows up as an enhancement of the overall background level of the spectrum, not as an extraneous peak.

Backscattered electrons may strike any of many surfaces in the specimen enviroment and generate remote-source extraneous x-ray peaks. Such surfaces include the walls of the microscope chamber, the polepiece, the specimen holder and stage, and the collimator of the x-ray detector. Examples of such peaks, which may likewise be generated by fluorescence by stray x-radiation, are Cu, Zn, Fe, and Al in our system. The peaks obtained depend upon the elements composing the particular system as well as the exact setup (working distance, tilt, specimen-detector distance, etc.).

Another effect due to electrons is that of electron-beam tailing. The electron spot constituting the probe is not contained entirely within a point but has an areal distribution. A further spread may be contributed to the normal distribution of the electrons in the beam by electrons which are scattered by the final aperture. Such electrons, striking away from the central point of impact on the specimen, will lead to broader area excitation of the sample than expected and often even to remote electron excita-

tion of x-rays.

As mentioned, remote-source extraneous x-rays may also be x-ray-induced (i.e., fluoresced). X-rays generated by primary or secondary excitation may generate other x-rays upon interaction with the same surfaces mentioned above. Any x-ray which gets into the line-of-sight of the detector and which has sufficient energy to penetrate the detector can be registered. It is impossible to unambiguously identify the source of such x-rays by simple inspection of the spectrum.

A rather serious x-ray-induced effect results from thin metal foils used for the final aperture by some SEM manufacturers. In our SEM (JEOL JSM-35), the electron beam generates molybdenum x-rays when it strikes the Mo foil aperture strip. Mo x-rays have sufficient energy to penetrate the foil. They emanate from the foil and lead to wide-area excitation of remote sources, most noticeably resulting in Cu and Zn peaks from the brass specimen holder. This problem can be minimized by th use of a thick tantalum aperture beneath the foil to absorb the Mo x-rays. One can measure the extent to which electron beam tailing and fluorescence by x-rays from the aperture cause remote-source excitation by using a technique described by Bolon and McConnell (26).

3. A third category of extraneous peaks due to sample-environment effects is that of extraneous peaks due to contamination. Contamination may be in or on the specimen and includes the Si problem and other artefacts already discussed as part of specimen preparation. Contamination may also be deposited out on the sample in the microscope as residual gases in the vacuum system interact with the electron beam and form reaction products on the sample. Surface contamination absorbs x-rays emitted from the sample, thus interfering with the validity of quantitative analysis.

## ARTEFACTS DUE TO SIGNAL DETECTION AND PROCESSING

Before discussing artefacts in this category, a very brief review of the sequence of events occurring during EDS detection of x-radiation and processing of the resulting signal will be presented. In order to be detected, an x-ray photon must penetrate 1) a Be window, (typically 7.5$\mu$m thick) which isolates the cryogenic environment of the detector from the vacuum of the microscope; 2) a 200A thick gold layer, which provides electrical contact; and 3) the inactive silicon dead layer (200-2000A thick) of the semiconductor crystal. Within the active layer of the crystal, the photon interacts with a Si atom through the photoelectric process, leading to ionization of a Si atom, ejection of a photoelectron, and emission of a Si x-ray of Auger electron. Ultimately, the total energy deposit of the photon results in a number of electron-hole pairs which is proportional to the original energy of the photon. For example, a 3.8 keV x-ray photon should produce 1000± electron-hole pairs since 3.8 eV is the average energy required to produce an electron-hole pair. A bias voltage applied across the detector crystal causes the electrons to be gathered up, resulting in a charge pulse. A charge sensitive preamplifier converts the signal to a voltage pulse. The signal is transmitted through a cable to the main amplifier, which shapes and further amplifies the signal and sends it to the analog-to-digital converter (ADC). The ADC converts the analog voltage signal to digital information which is stored in the multichannel analyzer (MCA) and appropriately displayed as a spectral distribution. Proportionality between the original energy of the photon and the strength of the signal is conserved throughout the processing. For further details regarding the detection process and signal processing, the reader is referred to appropriate references (4,5). Artefacts from this source, which are important but are not of great interest to most biologists, will be listed but not described in detail. For further information regarding these sources of artefact, the reader is referred to works by Fiori and Newbury (25) and by Wolfseth (5).

1. Peak broadening, peak overlap. the typical full width at half maximum (FWHM) of an EDS peak is 140-170 eV whereas the natural spectral width of an x-ray emission is 1-2 eV. Peak broadening results from the detection process. The average amount of energy required to produce an electron-hole pair in the detector crystal is 3.8 eV. Since the production and collection of electron-hole pairs is a statistical process, a distribution of the number of electron-hole pairs produced results for photons of a particular energy. Due to the width of EDS praks, x-ray emissions from consecutive atomic number elements tend to not be totally resolved, i.e., they overlap. Computer-based spectral deconvolution or stripping routines hence become very important as part of the EDS analysis system. One must be ever aware of potential overlaps when interpreting spectra (see Table 1).

2. Peak distortion. Instead of having a normal distribution, EDS peaks may be skewed. Trapping, recombination of electron-hole pairs, or incomplete charge collection near the edges of the detector crystal can cause the number of electron-hole pairs collected to be too small for an energy deposit and thus result in a distortion of a peak's spectral distribution which is known as low-energy tailing.

3. Silicon escape peaks. Silicon x-rays are produced in the detector crystal. If such a Si x-ray escapes from the detector, the apparent energy dump will be too small by exactly 1.74 keV (the energy of the Si K x-ray) and will result in a peak known as a silicon escape peak, which will be located 1.74 keV below the parent peak. These peaks are usually not obvious by mere visual inspection of spectra obtained from biological samples. The magnitude of Si escape peaks varies with energy of the parent peak, being about 1+ for P and only .01+ for Zn.

4. Absorption edges. The Si dead layer and Au contact, through which all photons must pass to enter the active volume of the dectector, absorb x-rays and hence the absorption edges of these elements will appear in the EDS spectrum. The magnitude of these edges is such that they are not visibly noticeable in most spectra of biological samples. However, some computer programs take into consideration their existence as well as Si escape peaks for accurate spectral modeling.

5. Silicon internal fluorescence peak. This artefact

has already been described in the listing of sources of Si peaks. Itis properly classified as an artefact due to the detection process since it results from the Si dead layer of the detector.

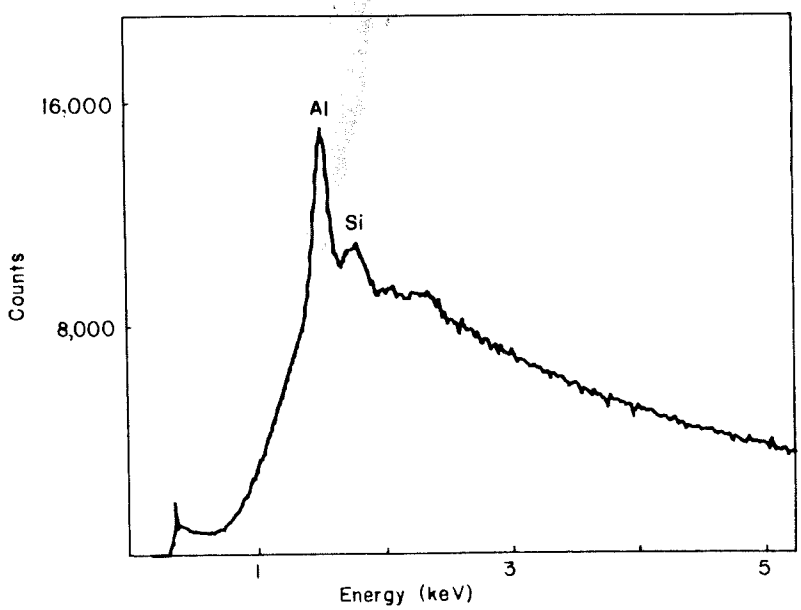
6. Deadtime effects. Photons are processed sequentially by the EDS system, making it necessary to have circuitry to gate off the system until the processing of one event is complete. Thus, not every photon entering the detector is registered in the final spectrum (concidence loss). In order to accomplish a certain length of livetime analysis, the internal clock of the MCA extends the real time of acquisition to compensate for the deadtime. In order to handle higher count rates, the time constant of the amplifier must be shortened. One should be aware that peak width, i.e., detector resolution, varies with time constant. One should collect under the same conditions all spectra which will be compared with each other. This is especially important when using reference spectra for deconvolution of unknown spectra. Count-rate dependent effects can affect the goodness of the fit (reflected by "chisquared" values in the Tracor Northern least squares fitting routine which we employ). Optimum operation of the detector system is at count rates of 1000-2000 counts per second (cps). It is recommended that the input count rate not exceed 5000 cps (25), although manufacturers rate their equipment at 20,000 cps. It is important to note that the input count rate actually refers to the entire energy range, up to the energy of the excitation source, and not just to the spectral range one might be displaying for inspection, which typically covers 0-5 or 0-10 keV for biological samples.

7. Pulse pileup, summation peaks. If two pulses arrive in the detector simultaneously, the pulses will be detected as one energy deposit equal to the sum of the two energies. The resultant artefactual peaks in the displayed spectrum are known as pulse pileup peaks or summation peaks. An example is that of two Cu K$\alpha$ peaks (8.04 keV) piling to give a peak at 16.08 keV. This problem is minimized by the use of pulse pileup rejection circuitry.

8. Peak shift and drift. The displayed peaks may shift or drift due to electronic instability in the system. Two parameters of particular importance are the amplifier gain and the zero shift of the MCA. Large electrical surges as well as slow drifts can cause problems. In our laboratory we have noticed that the amplifier is very sensitive to temperature and especially to drafts. Only through very careful stabilization of the environment has it been possible to stabilize the electronic drift. Shift and drift lead to errors in comparing spectra which were unknowingly collected under different conditions, with even fraction-of-a-channel shifts being very important when comparing reference to unknown spectra and being reflected in the chisquared value for goodness of fit.

## SUMMARY

The reader should be aware from the preceding discussion, which is by no means comprehensive, that there are a number of pitfalls involved in the interpretation of EDS spectra. By being aware of sources of spectral artefacts, the analyst can plan preparative procedures and set up analytical conditions in such a manner as to minimize these effects. If one is interested in performing quantita-




## FIGURE LEGEND

Fig. 1. X-ray spectrum from carbon planchet, showing Si internal fluorescence peak from Si dead layer in detector and A1 peak from detector housing.

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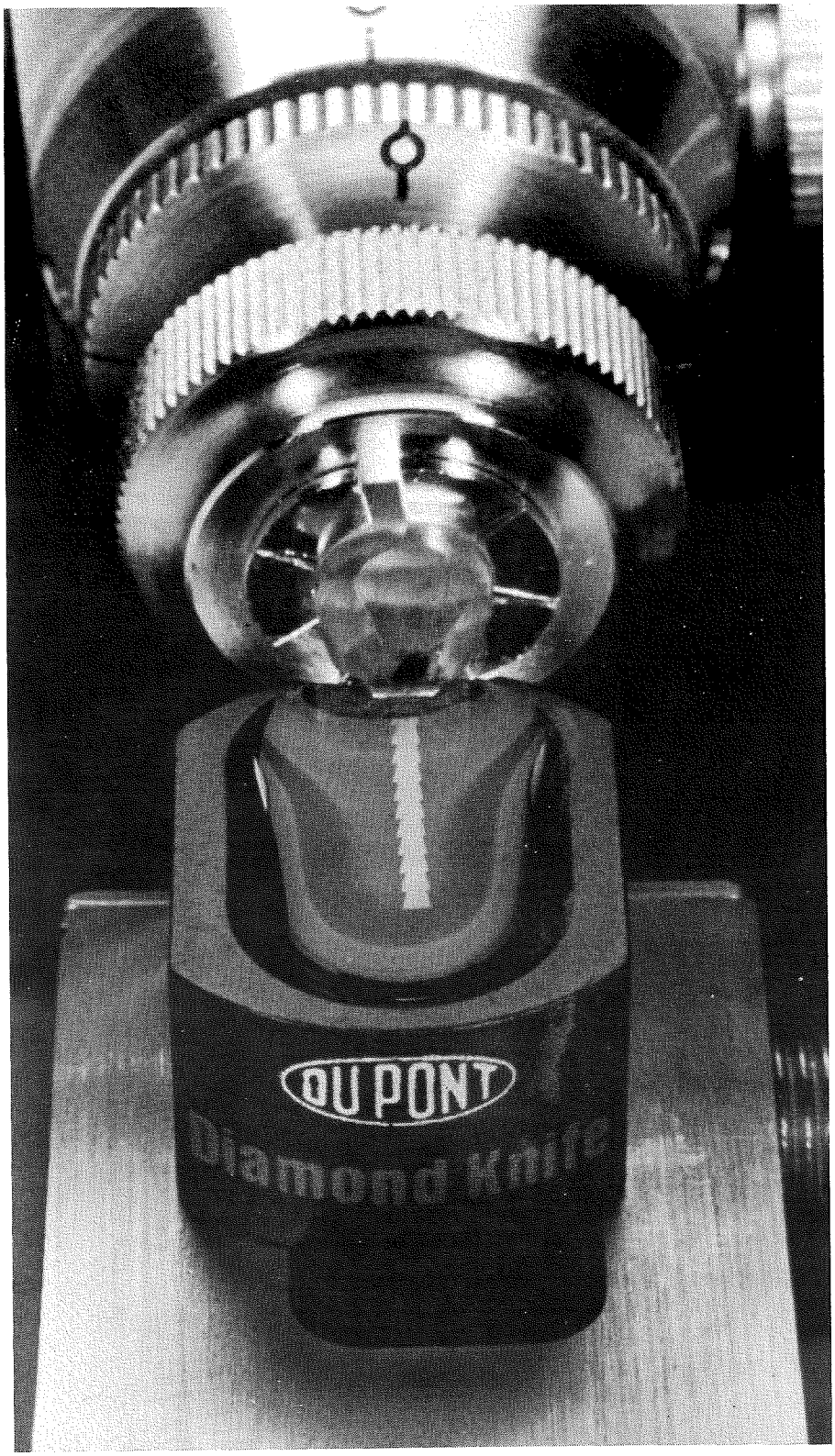


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tive analysis, there is a whole set of further effects which one needs to understand. Included are the effects of such parameters as excitation conditions of the electron probe, sample thickness, sample density and composition, sample roughness, beam damage, and mass loss. For bulk specimens, one must consider matrix corrections for absorption, fluorescence, and atomic number effects. For interpretation of EDS spectra, one should always bear in mind the total history of the sample and all the analytical conditions.

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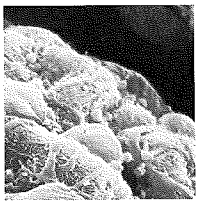

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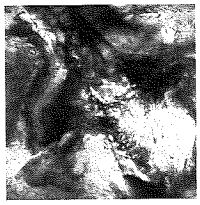

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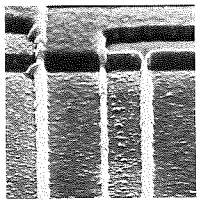

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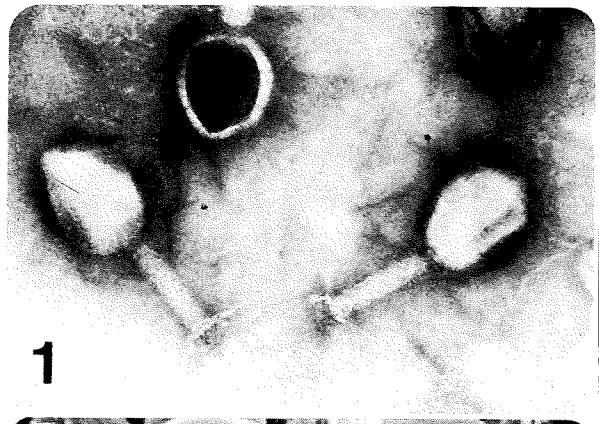


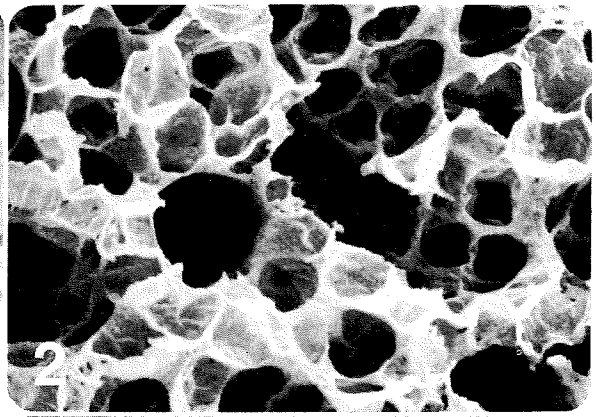


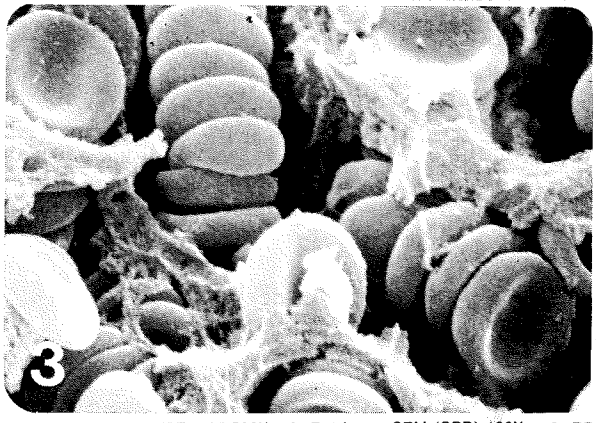


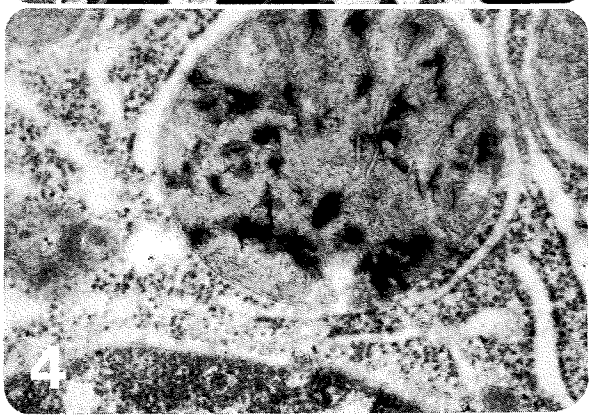


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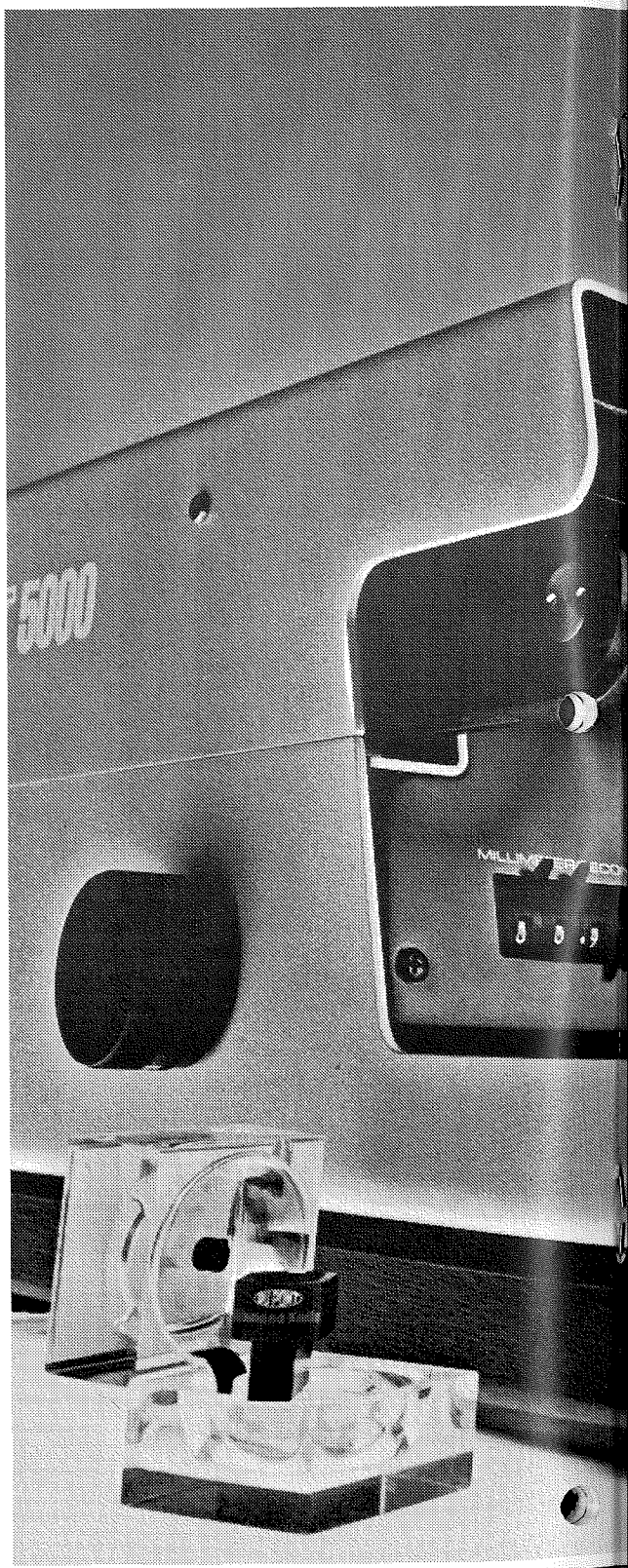

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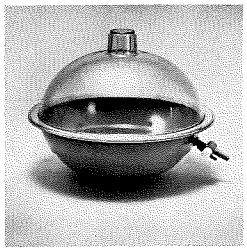

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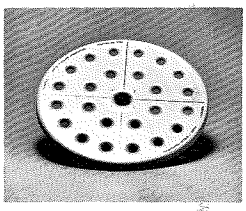

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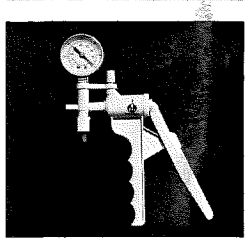

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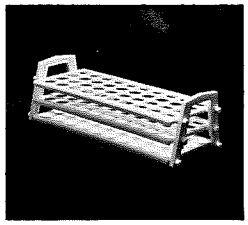

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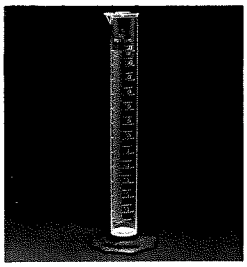

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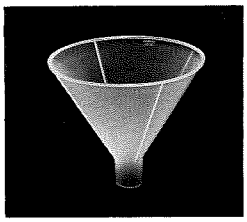

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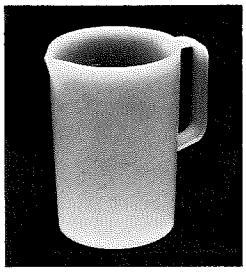

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# Heterogeneity Of Phosphatase-Containing Granules In Rabbit Neutrophils

By
**Daniel M. Zellmer** and **W. Allen Shannon, Jr.**
Veterans Administration Medical Center
and
The University of Texas Health Science Center at Dallas

Acid B-glycerophosphatase activity has been cytochemically demonstrated in small, pleomorphic granules (1,2,3) and in larger granules referred to as "azurophils" in neutrophils (heterophils) obtained from rabbit peripheral blood (1,2). The presence of this activity in both components has also been shown by biochemical assays of separated granules of fractionated cells (4). The activity has also been cytochemically demonstrated in immature cells (2,5), but, in the latter case (5), the authors did not detect any activity in mature cells and suggested that the activity detected in homogenates was due to contamination by mononuclear cells.

Alkaline B-glycerophosphatase activity has been cytochemically demonstrated by Bainton and Farquhar (5,6), Horn et al. (1), and Wetzel et al. (2). However, Bainton and Farquhar (6) suggested that the activity could only be demonstrated in the immature cell.

It has been biochemically determined that a separate acid phosphatase specific for 4-nitrophenylphosphate exists in the rabbit neutrophil (7). This has not yet been cytochemically demonstrated.

Recently, Oliver (8) was able to demonstrate acid phosphatase activity in the exocrine acinar cells of mice and rats which was specific for trimetaphosphate. The activity exhibited a different localization from that of acid phosphatases using B-glycerophosphatate or cytidine 5'-monophosphate as a substrate. She indicated that another type of lysosome had been discovered on the basis of its TMPase activity.

It has been our purpose to study the relationship and subcellular localization of these phosphatases in order to establish the relationships of each to prospective roles in the function of the cell.

Peritoneal exudate was obtained by a method described by Hirsch (9). The exudate was centrifuged 10 min. at 300 xg to obtain pellets of neutrophils. The pellet was resuspended in 0.15% NaCl for 30 s. Then, an equal volume of 1.5% NaCl was added, and the suspension was centrifuged at 300 xg for 5 min. This procedure was repeated until the red blood cells were lysed.

The neutrophils were fixed for transmission electron microscopy by resuspension in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.3, for 1 hr. at 4 C. The cells were pelleted at 300 xg for 30 s and rinsed 3 times in cold cacodylate buffer.

Fixed cells were reacted for alkaline B-glycerophosphatase by the method of Cutler et al. (10). The medium contained a final concentration of 20 mM Tris-maleate, pH 8.6, 0.24% sodium glycerophosphate, and 0.12% lead nitrate. The cells were incubated 30 min. at 37 C.

Acid B-glycerophosphatase activity was demonstrated by a modified Gomori procedure (11). The medium contained a final concentration of 50 mM sodium acetate, pH 5.0, 0.12% lead nitrate and 0.3% sodium B-glycerophosphate. The cells were incubated 30 min. at 37 C.

Acid trimetaphosphatase activity was determined by

the method of Oliver (8).. The medium contained 1.8 mg sodium trimetaphosphate, 0.45 ml of 0.1 M acetic acid, 5.0 ml distilled water and 1.0 ml of 1.5% freshly-prepared lead acetate. The medium was adjusted to pH 3.9 and filtered. The cells were incubated 30 min. at 37 C.

Acid nitrophenylphosphatase activity was determined by the method of Ryder and Bowen (12). The medium contained 0.1 M acetate buffer, pH 5.0, 3.8 mM p-nitrophenylphosphate and 2.64 mM lead acetate. The cells were incubated 45 min. at 37 C.

After incubation, the cells were rinsed three times in 0.1M cacodylate buffer, pH 7.3, and post-fixed 1 hr. in osmium tetroxide. They were counterstained 10 min. in uranyl acetate. The cells were dehydrated in an ethanol series with a final rinse in propylene oxide. They were embedded in Epon 812. Thin sections were prepared on a LKB Ultrotome, and unstained sections were examined on a Philips 301 electron microscope.

Alkaline B-glycerophosphatase activity (Fig. 1) is found primarily in granules of approximately 400 nm in diameter which appear slightly ovoid. The reaction product is peripherally distributed in the granule. There are usually ten or more granules in any cell profile which contains a nucleus. Some smaller granules of 150-200 nm in diameter are observed which contain somewhat denser deposits, but these may be sections through an end of a larger granule.

Acid B-glycerophosphatase activity (Fig. 2) is found in a granule of approximately 400 nm in diameter. Few of these granules are observed. Not all cellular profiles exhibit these granules, and those which do have only one or two. The reaction product forms a dense peripheral deposit just inside the rim and occupies about one-third of the granule. These granules appear to be almost spherical.

No activity is present in any granule when 4-nitrophenylphosphate is used as the substrate. The deposit is observed on about one-fourth of the plasma membranes and one-half of the nuclei. The product forms a fine deposit which uniformly covers the nuclei of some cells (Fig. 3).

Acid trimetaphosphatase activity (Fig.4) is observed in slightly ovoid granules of 420 nm diameter. These granules are more numerous than those containing acid B-glycerophosphatase activity, and the deposit is uniformly distributed throughout the granule. The granule is only about one-tenth as frequent as the granule which contains alkaline B-glycerophosphatase activity. Some fine deposit adheres to the plasma membrane and the nuclear membrane.

Alkaline B-glycerophosphatase activity is found in a granule which is much more frequent than either granule having acid phosphatase activity. Although Bainton and Farquhar (6) were unable to detect any such activity in the mature neutrophil, the presence of alkaline B-glycerophosphatase activity in small peripheral granules in human neutrophils has been shown (13, 14). Geddes et al. (15) indicated that the demonstration of this activity is inhibited by fixation in glutaraldehyde, indicating that it could have eluded Bainton and Farquhar due to fixation in 1.5% glutaraldehyde.

Acid B-glycerophosphatase activity has been observed in smaller granules as indicated previously, but we have never seen this small, plemorphic granule. The reactive granule we observed is similar in size to the alkaline B-glycerophosphatase-containing granule, but is not nearly so numerous. Segal et al. (16) have recently described an acid B-glycerophosphatase-containing granule in human neutrophils which is similar in size, shape and frequency to the granule described here.

Acid trimetaphosphatase activity was the basis for the identification of another type of lysosome (8), and, in our work, it was manifested in a granule which did not have the characteristics of the other described granules. It is much more frequent than the acid B-glycerophosphatase-containing granule and has a more uniform deposit of reaction product. It is much less frequent than the alkaline B-glycerophosphatase-containing granule. It is not nearly so frequent or as large as myeloperoxidase-or catalase-containing granules (unpublished results).

Nitrophenylphosphatase activity at acid pH could not be cytochemically localized to any granule under our cytochemical conditions. However, it might be noted that we were unable to detect acid B-glycerophosphatase activity in smaller granules as has been reported by several authors. It is possible that the reaction deposit diffuses from the cell or granule or that the fixation, although shortened, destroys the activity.

Baggiolini et al. (7) found acid 4-nitrophenylphosphatase activity in their "D-particle" fraction which consisted of small vesicles and membrane fragments. Since the only activity which we observed was on the membrane, it is possible that this enzyme is confined to the membrane.

There appear to be at least three different granules which exhibit phosphatase activity, each of which responds specifically to a different substrate. In addition, the membrane may contain yet another phosphatase. The functions and interrelationships of each of these granules is now of mounting interest.


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6. Bainton, D.F., and M.G. Farquhar, 1968,*Differences in enzyme content of azurophil and specific granules of polymorphonuclear leukocytes. II. Cytochemistry and electron microscopy of bone marrow cells*, J. Cell Biol. 39, 299-317.

Neutrophils from rabbit peritoneal exudate phosphatase activity. N=multilobed nucleus. (×14,300).

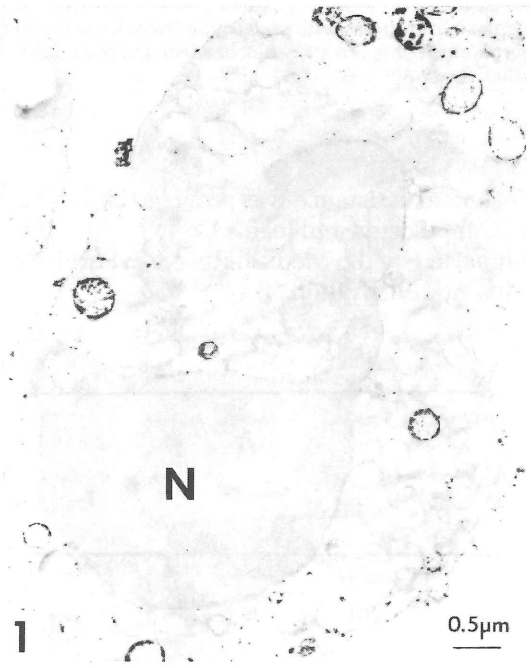


Figure1. Alkaline B-glycerophosphatase. Reaction product is a fine deposit located just inside the rim of the granule and occupies 1/4 of the granule. Granules tend to be perpherally located.

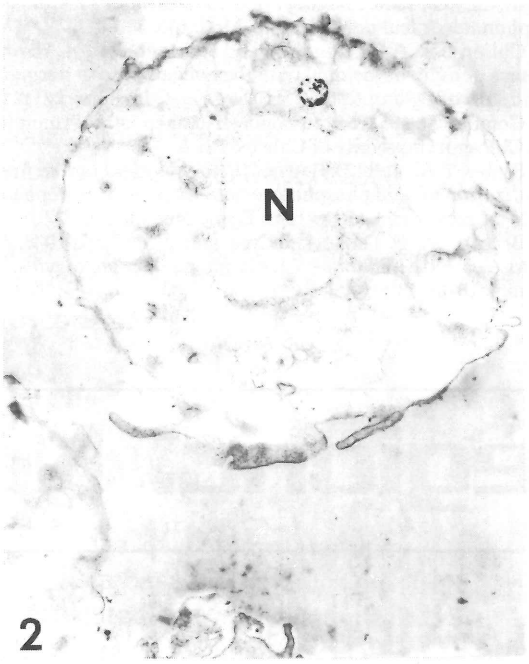


Figure 2. Acid B-glycerophosphatase. Granules are approximately spherical. Coarse reaction product is localized just inside the rim and occupies about 1/3 of the granule. Reactive granules tend to be perpherally located.

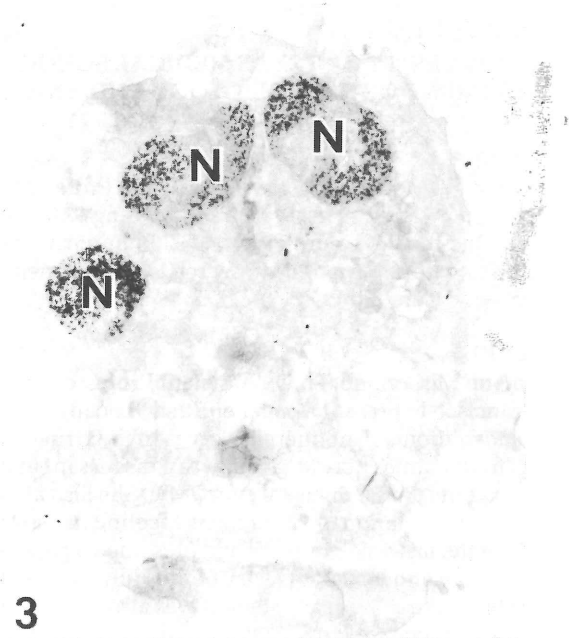


Figure 3. Acid 4-nitrophenylphosphatase. A reaction product forms a fine deposit which covers the nuclear heterochromatin of the cell. Sometimes, some product is also found on the plasma membrane (not shown). No activity has been observed in granules.

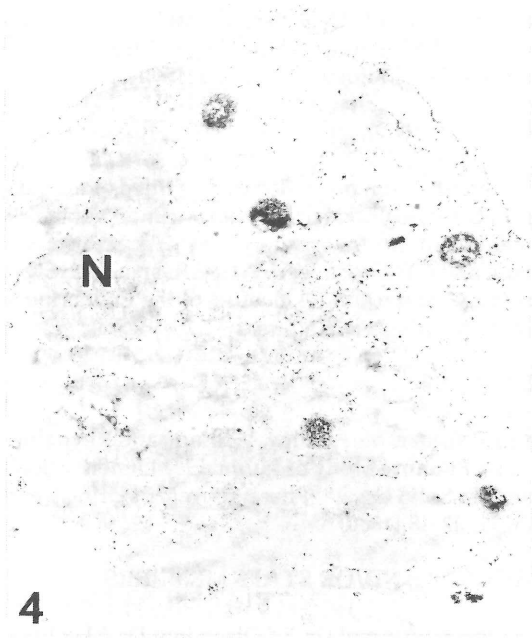


Figure 4. Trimetaphosphatase. Reaction product is a fine deposit distributed uniformly throughout the granule. Positive granules tend to be centrally located.

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## ACKNOWLEDGEMENTS

Technical assistance was provided by Jill Bast, Sally Bates, Kathy Porter, and Jeanie Collier.

Supported by the Medical Research Service of the Veterans Administration.

# Regional News

## DALLAS

### UNIVERSITY OF TEXAS HEALTH SCIENCE CENTER, DEPARTMENT OF PATHOLOGY

#### GRANTS AWARDED

Dr. Allen Shannon recently received a grant from the American Diabetes Association, North Texas Affiliate to continue studies on "F an D-Cells in Diabetes". Dr. Shannon directs the EM Unit at the Veterans Hospital in addition to being a member of the Cell Biology faculty, UTHSCD.

#### LECTURES

Dr. Allen Shannon presented papers titled "Pathology of Allozan Diabetic Dog Endocrine Stomach and Pancreas" at the annual meeting of the American Diabetes Association and "Pancreatic and Gastric Endocrine Cell Response to Hypophysectomy" at the annual meeting of the Endocrine Society both held in Washington DC, June 16-20.

#### PUBLICATIONS

Schulz, Werner, McAnalley, William, and Reynolds, Rolland: Freeze-Fracture Study of Pulmonary Lamellar Body Membranes in Solid Crystal Phase. Journal of Ultrastructure Research, 71, 37-48 (1980).

#### NEW FACULTY AND/OR STAFF MEMBERS

Dr. Kathy Muntz and Dr. Ed Olson join Dr. Max Buja's staff. Dr. Muntz is doing post doctrate work on localization of catecholamines and beta adrenergic receptors in the myocardium. Dr. Edwin Olson is a Cordiology Fellow. Drs. Allen Shannon, Daniel Zellmer and Dick Dey and technicians Jill Bast, Sally Bates and Joie Reavis recently moved into new E.M. facilities at the Dallas Veterans Administration Medical Center. Shirley Waggoner has transferred to the Veterans Hospital from UTHSCD. She is doing research EM with Dr. Shannon's group.

## HOUSTON

### THE UNIVERSITY OF TEXAS MEDICAL SCHOOL DEPARTMENT OF NEUROBIOLOGY AND ANATOMY

#### GRANTS AWARDED

David W. McCandless, Ph.D., Assistant Professor, has recently been awarded a research grant totalling $24,164 from the National Institute of Neurolgical and Communicative Disorders and Stroke. The subject of his research is "Experimental Seizures and Cerebral Energy Metabolism".

#### LECTURES

JoAnn McConnell, Ph.D., Assistant Professor, travelled to San Francisco to present a paper entitled "Localization and Characterization of Cholinergic Nerves in the Urinary Bladder" at the Urodynamics Soc. Mtg, and was a discussant at 2 research sessions at the Am. Urological Asso. Mtg. She also attended the American Association of Anatomists Meeting in Omaha, Nebraska the last week in April and presented a paper entitled "Morphology and Histochemistry of the Human Vas Deferens.

Robert Sikes, Graduate Student was also present at the American Association of Anatomists Meeting this year and gave a lecture on "The Cytoarchitecture of the Retrosplenial Cortex in the Rabbit: A Golgi Study".

Dr. Diana Redburn, Associate Professor, presented an invited seminar at the International Symposium on GABA and Glutamate in Sardinia, Italy as well as a talk in Rome at the Univeriseta Cottolica, May 16-18.

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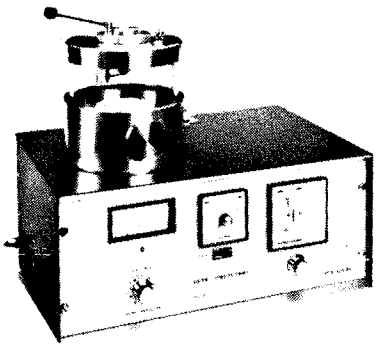

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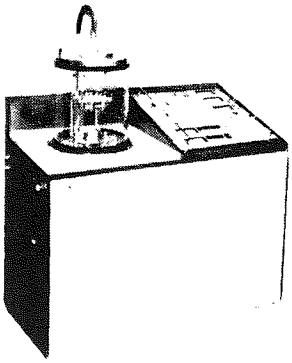

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# Abstracts

**INVOLVEMENT OF CYTOPLASMIC VESICLES IN TELIOSPORE GERMINATION AND BASIDIOSPORE FORMATION IN THE RUST FUNGUS GYMNOSPORANGIUM CLAVIPES.** Charles W. Mims, Department of Biology, Stephen F. Austin State University, Nacogdoches, Texas, 75962.

The involvement of cytoplasmic vesicles in hypal tip growth has been documented in numerous fungi. The results of this study indicate that similar vesicles are also involved in teliospore germination as well as the subsequent events leading to basidiospore formation in the rust fungus **Gymnosporangium clavipes**. These vesicles are numerous near the germ pore regions of germinating teliospores as well as in rapidly elongating germ tubes, developing sterigmata and basidiospore initials. Such vesicles migrate to the apex of elongating or enlarging structures and fuse with the plasma membrane. The membrane surrounding the vesicle appears to be contributed to the plasma membrane while the contents of the vesicle are added to the developing wall. The origin of these vesicles is unclear although in other fungi it has been suggested that they are derived from the Golgi apparatus. Well developed Golgi bodies are, however, not present in rust fungi.

**VEGETATIVE COMPATIBILITY/INCOMPATIBILITY IN HIGHER PLANTS. I. A STRUCTURAL STUDY OF A COMPATIBLE AUTOGRAFT IN SEDUM THLEPHOIDES.** Randy Moore, Biology Dept., Baylor Univ., Waco, TX

An ontogenetic study of a shoot autograft in **Sedum telephoides** was performed to document the cellular events that occur during a compatible tissue graft. Initial adherence of the cut surfaces occurs by 24 hours after grafting and is correlated both with a pronounced dictyosome activity along the graft interface and with callus proliferation in the stock and scion. A necrotic layer of 1-2 collapsed cells in thickness initially extends as a continuous barrier between the stock and scion, but the layer is fragmented by 2-3 days as the callus proliferation continues. Graft incision also induces a mild senescence in cells at the graft interface that is characterized by a reduced staining intensity in the cytoplasm, replacement of the large primary vacuole by numerous smaller vacuoles, and the occurrence of flocculent material throughout the cytoplasm. The cellular senescence never proceeds past and early, non-lethal stage, and cells along the graft interface completely recover by 3 weeks after grafting. Procambial differentiation occurs across the callus bridge by 10 days after grafting, and mature vascular continuity is established by 14 days.

**PULMONARY FREE CELL RESPONSE TO ASBESTOS AS DETERMINED FROM LAVAGE PREPARATIONS.** Deborah O. Piers, Marion G. Williams , Jr., and Ronald F. Dodson. Department of Cell Biology and Environmental Sciences, University of Texas Health Center at Tyler.

The acute response of the free phagocytic elements of the guinea pig respiratory system following intratracheal injection of "amosite" asbestos was studied by light microscopy, electron microscopy and x-ray energy dispersive analysis. The post injection intervals studied were two or four hours, or 1, 12, 14, 16, 18, or 30 days. The free cell population consisted of macrophages, neutrophils, eosinophils, lymphocytes and monocytes. The initial phogocytic response was carried out by the neutrophil population as was evident within two hours of injuction. The macrophagic components were predominantly involved in particle ingestion after the end of the first twenty-four hours. The asbestos was packaged within macrophages in either a membrane bound or intra-siderosomal form. Both inorganic and organic asbestiform-like entities were found within phagocytes and emphasized the need to document the composition of each particle by combined methodology such as used in this study.

**LUNG RESPONSE TO ASBESTOS.** Michael F. O'Sullivan, Marion G. Williams, Jr., and Ronald F. Dodson. Department of Cell Biology and Environmental Sciences, University of Texas Health Center at Tyler.

The acute response of guinea pig lung parenchyma to "amosite" asbestos was studied by light microscopy, transmission, scanning and scanning transmission electron microscopy. Particulate matter was classified by X-ray energy dispersive analysis. Animals were sacrificed at post injection intervals of 2, 4, 12 hours and 1 —7 days by intravascular perfusion which was found to produce better fixation than intratracheal fixation. The acute tissue response consisted of an intra-alveolar granulamatous reaction rather than interstitial alterations. Alveolar compression was commonly seen in areas adjacent to the localized accumulation of phagocytes. Phagocytic response was shared in the early periods between polymorphs and macrophages, while in the longer intervals macrophagic involvements predominated. Some edematous reaction was noted in type I pneumocytes but no indication of desquamative response was seen. Fibrotic involvement was limited to some intra-alveolar fibrin deposits. This study would suggest that the term "free asbestos fibers" refers to an extracellular state, while intracellular fibers are coated with either a membranous sheath, a siderosome or a classical ferruginous coating.

**LUNG MATURATION IN THE HYPOPHYSECTOMIZED EMBRYONIC CHICK USING DEXAMETHASONE** G. Russell Edwards and Robert V. Blystone, Trinity University, San Antonio, Texas 78284

Adrenal glucocorticoids have been shown to accelerate fetal lung maturation in mammals, including formation of surfactant. A number of maternal hormones cross the placenta and may affect fetal lung formation and this action makes interpretation of results derived from placental systems difficult. This placental factor does not exist in avians. The purpose of the study was to determine if exogenous glucocorticoid accelerates lung development in the hypophysectamized embryonic chick. Embryos hypophysectomized at 40-45 hours development were injected on day 14.5 incubation with 1$\mu$g of dexamethasone. Lung maturation was studied morphologically by light and electron microscopy as well as biochemically by analysis of whole lung phospholipid content. At 18.5 days incubation hypophysectomized embryos demonstrated a decrease in the number of lipid vacuoles when compared to controls. Hypophysectomized embryos which received dexamethasone showed an in-

crease in the number of lamellar inclusions and Golgi, but the quantity of lipid vacuoles remained roughly the same as compared to controls. At 18.5 days incubation lung phospholipid in hypophysectomized embryos was 397$\mu$g P/g in contrast to 690$\mu$g P/g in controls; whereas, dexamethasone treated specimens manifested a marked increase to 1140$\mu$g P/g. In this preliminary work the biochemistry agrees with the morphology in suggesting that lung maturation in hypophysectomized chicks was retarded but that concomitant treatment with exogenous glucocorticoid enhances surfactant synthesis.

**MYOFIBROBLAST ANCHORING SUBSTANCE (MAS)** P.S. Baur, Jr., Ph.D. and G.F. Barratt, M.S.

Contractile fibroblasts (myofibroblasts) are major cellular constituents of clinically active hypertrophic scars and scar contractures resulting from thermal insult. These cells appear to be implicated in the formation of these wound healing tissues. The premise that myofibroblast contractibility alone can account for the aberrant microarchitecture of these tissues is valid only if the cells are firmly attached to each other and the juxtoposed connective tissue (collagen fibers). A proteinecious fibrillar material has been routinely observed on the external surfaces of the myofibroblasts when scar tissues are examined by means of transmission electron microscopy. This material, tentatively called the myofibroblast anchoring substance (MAS), appears to firmly attach cells to cells or cells to adjacent collagen fibers. The MAS attachment sites appear to be relegated, for the most part, to those areas of the plasma membrane which overlie the terminations of the contractile bundles (actin filaments) found within the cytoplasm of the myofibroblasts. A more detailed elaboration of the fine structure of this substance and its role in scar contractibility will be similarly presented.

**EARLY ULTRASTRUCTURAL CHANGES IN THE MYOCARDIUM FOLLOWING THYROXINE-INDUCED HYPERTROPHY,** Cheryl Craft-Cormey and John T. Hansen, The University of Texas Health Science Center, San Antonio, Texas 78284.

The thyroxine-induced model of myocardial hypertrophy was studied with particular regard to the early ultrastructural changes in fractional volume of the mitochondria and myofibrils, and capillary distribution. Female Sprague-Dawley rats were divided into two groups: one group (T) received a single daily injection of L-thyroxine (25mg/kg IP) for 9 consecutive days while the other group served as vehicle injected controls (C), Twenty-four hours after the last injection, all animals were sacrificed by vascular perfusion with the appropriate aldehydes and the hearts excised, blotted dry and weighed. Tissue samples from the identical region of the anterior left ventricle were collected, routinely processed for electron microscopy and analyzed by accepted morphometric techniques. The heart weight/body weight ratios showed a significant increase ($P<0.001$) over C ratios. Likewise, the fractional volume of mitochondria (42%) was significantly increased ($P<0.001$) and there was no significant difference between the T and C hearts with respect to capillary luminal area/myocyte area. The mitochondria/myofibril ratio was increased in T hearts (.82) over that found in C hearts (.52). These results suggest that in the early stages of thyroxine-induced mycardial hypertrophy there is not an immediate increase in capillary area which may account for the ischemia and significant increase in mitochondrial volume which characterizes myocardial hypertrophy in this model.

**ANALYSIS OF VARIATION IN Z BAND WIDTH IN NORMAL, CONTROL AND ANOXIC MYOCARDIUM.** M.A. Goldstein, Department of Medicine, Cardiovascular Sciences, Baylor College of Medicine, Houston, Texas 77030.

Our studies of cardiac muscle using optical diffraction and reconstruction have revealed a similar Z lattice unit in Z bands of different widths. We have observed Z band widths 2 to 3 times the "normal Z width" in papillary muscle cells exposed to acute hypoxia (J. Molec. Cell. Cardiol. 9:285, 1977). At that time we had no data on how much variation in Z width occurs in normal papillary muscle, but used the value of 120 nm obtained from optical diffraction studies. Chase and co-workers (J. Molec. Cell. Cardiol. 10:1077, 1978) used a similar experimental model to determine statistically that the magnitude of Z band width changes in hypoxia was no greater than that in control muscle. We have now gone back to our study of hypoxic, control and normal **in situ** muscle to determine if variation at the level of individual Z bands can be obscured by a statistical pooling. We averaged Z widths ($n>25$) from a given myofibril ($n>7$), sampled myofibrils near intercalated discs and away from them (>10 sarcomeres away), and sampled several cells from a given block. Thus, a three level analysis was set up (group to group, cell to cell, myofibril to myofibril). Although sarcomere length is remarkably uniform in all three groups, the Z band width varies considerably within a myofibril and from myofibril to myofibril. Yet when individual Z width values are pooled according to animal, a histogram analysis shows the same distribution pattern observed by Salmons et al. (J. Anat. 127:17, 1978) for a control slow skeletal muscle. The pooled values for each group (control $n=591$, exp. $n=366$) are remarkably similar. There is considerable variation in Z band width in normal cardiac cells as well as in controls.

**ORGANIZATION OF CHROMOSOMES IN MITOTIC AND INTERPHASE CELLS.** A. Cole and R. Langley. Physics Department, University of Texas System Cancer Center, 6723 Bertner Avenue, Houston, TX 77030.

We have previously reported on the organization of mammalian mitotic chromosomes (Cole and Chen, 1980, TSEM Journal, 11(1):35; Cole and Langley, 1980, TSEM Journal, 11(2):31). These and other studies supported the view that the mammalian chromosome consists of a lateral organization of eight extended circular DNA molecules which are attached sequentially to a 120nm wide backbone ribbon at bands spaced every 100nm along the ribbon. The two duplex DNA strands from each circular DNA molecule are paired to form eight 7nm nucleoprotein fibers which pass through the 40nm thick attachment bands. Eight 6nm protein strands link successive attachment bands to generate the backbone ribbon. The DNA segments between attachment bands are wound on nucleosome cores and loop outward to form radiating fingers. In dehistonized chromosomes two groups of radiating loops were found, one which extends to a well defined border at about 0.5$\mu$m radius and the other which extends to variable distances to more than 4$\mu$m. We have recently discovered that the backbone structure is maintained in cell interphase and is organized at the nuclear periphery (membrane). The nucleohistone fibers radiate inward from the membrane as short and long loops to encompass the nuclear volume. These structures are pertinent to an understanding of chromosomal replication, function, evolution and variability, and to the induction and repair of chromosomal damage. The structures will be demonstrated using stereo electron micrographic projections. Supported in part by DOE contract DE-ASO5-76EV02832.

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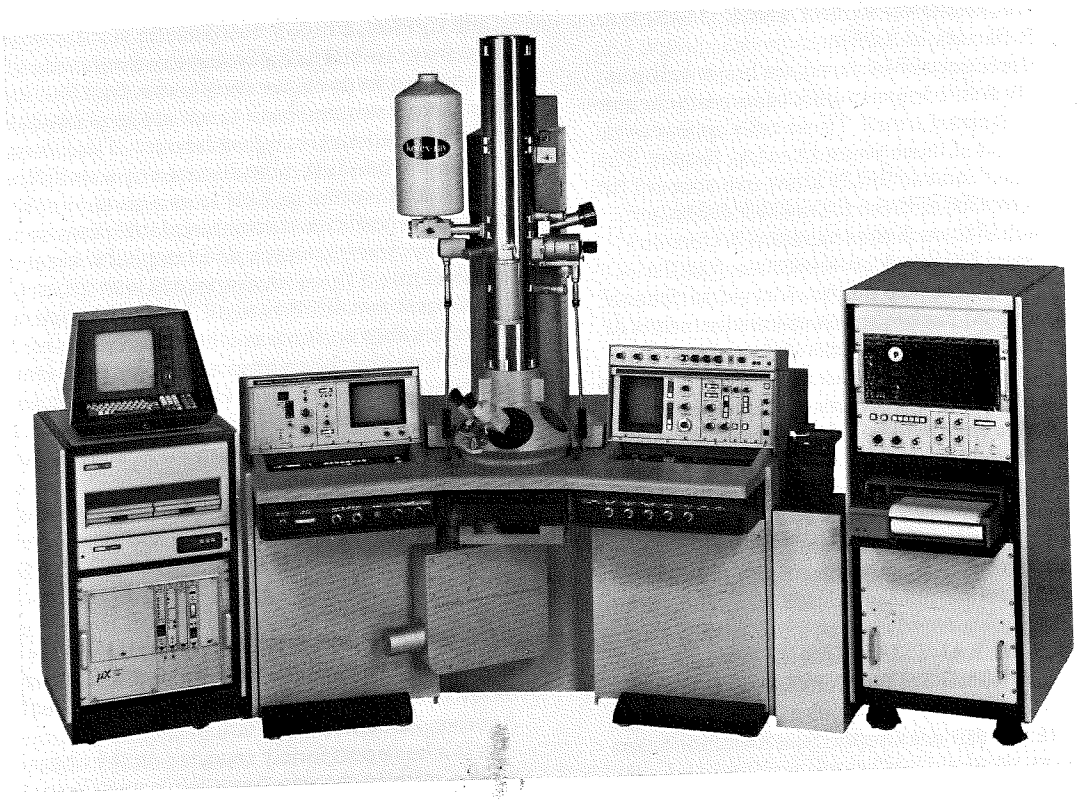

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**GAP JUNCTION AMPLIFICATION BY RAT OVARIAN GRANULOSA CELLS IN RESPONSE TO FOLLICLE STIMULATING HORMONE.** L. MacKenzie-Gragam, R.L. Matheson and R.C. Burghardt, Department of Biology, Texas A&M University, College Station, TX 77843.

Gap junction modulation in the granulosa cell compartment of the ovarian follicles of rats was examined following hypophysectomy and replacement therapy with estrogen and purified rat follicle stimulating hormone (rFSH).Multilaminar follicles of rats, hypophysectomized at 21 days of age, and examined 30 days later reveal small gap junctions within the granulosa layer. Administration of either exogenous estrogen or purified rFSH resulted in the amplification of granulosa cell gap junctions. While earlier studies (Merk, et al., **Endocrinology** 90: 992, 1972) suggested that junction growth in granulosa cells is a direct response to estrogen stimulation and not directly dependent on gonadotrophic hormones, the present studies indicate that rFSH is also capable of inducing gap junction amplification. Atrophy of the theca interna and interstitial cells, which normally follows hypophysectomy, was not prevented by rFSH administration indicating the absence of residual LH contamination in the purified gonadotrophin which would indirectly stimulate estrogen production in hypophysectomized animals. (Supported by a NIH Biomedical Support Grant; Purified rFSH was provided by Dr. A.F. Parlow, NIAMDD, Rat Pituitary Hormone Program).

**SCANNING ELECTRON MICROSCOPY OF THE RETINA OF THE COMMON MARMOSET, CALLITHRIX JACCHUS.** Robert S. St. Jules, Raymond Sis and Frank Stein, Department of Veterinary Anatomy, Texas A&M University, College Station, TX 77843

The structure of the retina of the common marmoset, **Callithrix jacchus**, was determined using scanning electron microscopy. The retina is thickest posteriorly and gradually thins peripherally, becoming about one third as thick near the ora serrata. A single row of capillaries is present in the outer portion of the inner nuclear layer while both capillaries and larger vessels occur in the ganglion cell later. Most striking is the great variation in shape of the photoreceptors in different areas of the retina. In the parafoveal region the photoreceptor inner segments are quite narrow and elongate, having about three times the length of those near the ora serrata. Cone inner segments in the peripheral retina, however, are about twice as wide as those in the parafoveal region. In contrast to the variation in form of the inner segments, the outer segments change little in length or width in different areas of the retina, and those of rods and cones cannot be distinguished. Finally, the close association of the outer segments with the very long, slender microvilli of the pigment epithelium can be readily visualized. This association is revealed best in areas where the retina has fractured, at the point of connection of the photoreceptor outer and inner segments, and has been removed.

**MORPHOLOGY OF THE INNER LIMITING MEMBRANE OF THE RABBIT RETINA AFTER VITRECTOMY.** Mannie C. Steglich, Louise C. Moorhead, Dianna A. Redburn, Department of Neurogiology and Anatomy, University of Texas Medical School at Houston, Houston, Texas 77025.

Pars plana vitrectomy was performed on pigminted rabbits using the Suction Infusion Tissue Extractor (Site by Keeler) in which the irrigation stream from the instrument tip is directed toward the retina, or the Peyman Bitrophage (by David Koff Systems) in which the irrigation stream is directed away from the retina. In one group of rabbits, surgery was performed in the right eye using the SITE. The left eye served for a control. In a second group, surgery was performed with the Vitrophage. In the right eye, the irrigation was directed through a separate site (Ocutome infusion tip) inserted through the pars plana. In all cases, the instrument cutting tip was held approximately 1mm above the retinal surface and 50 ml of irrigation fluid (BSS) were perfused through the posterior vitreous. Immediately following the vitrectomy, the globes were enucleated, and the posterior portion of the golbes were fixed, and then embedded in Epon 812. The tissue was then sectioned and examined at the light and electron microscopic levels. The vitreo-retinal junction was closely examined for any damage to the inner retinal surface. In the controls, the retinal surface was well preserved with only minor interruptions of the inner limiting membrane. In the other three groups, the technique of vitrectomy resulted in numerous microscopic cavities in the retinal surface. In these areas, the inner limiting membrane was ruptured, ganglion and Muller cells were avulsed, and debris was extruded into the vitreous. In some cases, these vitrectomy-induced disruptions extended through the ganglion cell layer to the inner plexiform layer.

**AN ULTRASTRUCTURAL EXAMINATION OF SPERMATOZOA IN THE COMMON MARMOSET, CALLITHRIX JACCHUS.** M. Lynn Davis and F.J. Stein, Department of Veterinary Anatomy Texas A&M University, College Station, TX 77843.

The common marmoset represents a relatively new prospect as a laboratory animal suitable for the study of human disease. For this reason, the raising of marmosets in captivity is imperative. The male reproductive system is one of the least studied of this organism, thus the lotrastructural organization of the spermatozoon is considered in this presentation.

Semen was collected by electroejaculation from marmosets housed at the Texas A&M Marmoset Facility (F.J. Stein, director) and prepared for electron microscopy, Both scanning and transmission electron microscopy were employed in the investigation. Basic sperm structure and that specific to **Callithrix jacchus** will be introduced.

**OPTIMUM DESIGN AND MECHANICAL FIXATION OF TITANIUM PROSTHETIC PYLONS WITH CANINE TIBIAL CORTICAL BONE.** R.R. Gleason and J.S. Caldwell, Dept. of Bioengineering, J.F. Junter and J.A. Allert, Dept. of Veterinary Physiology and Pharmacology, and C.E. Rothen, Dept. of Veterinary Anatomy, Texas A&M University, College Station, TX 77843.

Several methods and designs were used to mechanically fix titanium prosthetic pylons in the medullary cavity of the tibia in mongrel dogs. Assessment of fixation was made by utilizing Scanning Electron Microscopy. The prototype design exhibited incomplete interlocking of the bone and titanium which resulted in rotational destabilization. Early methods of mechanical fixation showed either gross loosening of the implant or poor mineralization in the bone. An optimum design has been reached that now enables the bone to mineralize fully, thereby providing axial and rotational stabilization.

**ULTRASTRUCTURAL OBSERVATIONS IN PONIES AFTER TREATMENT WITH MONENSIN.** Hilton H. Mollenhauer, Loyd D. Rowe, Sigmund J. Cysewski, and Donald A. Witzel, Veterinary Toxicology and Entomology Research

Laboratory, Science and Education Administration, Agricultural Research, U.S. Department of Agriculture, College Station, TX 77841.

Monensin, a biologically active compound derived from **Streptomyces cinnamonenis**, is used extensively as a feed additive for cattle to promote utilization of nutrient and weight gain. The equine species, however, is highly sensitive to poisoning by monensin. This greater sensitivity is of particular concern because horses may be accidentally poinsoned through feed mixing errors or by ingesting monensin-treated cattle feed. Several recent reports describe incidents of accidental poisoning of horses by monensin-containing feed. To help elucidate the mode of action of monensin, ultrastructural studies were made of myocardium, diaphragm, appendicular muscle, liver, and kidney of 3 ponies acutly poisoned with a single oral dose of monensin (4 mg/kg of body weight). These ponies developed severe signs of toxicosis and were killed 28-72 hours after treatment and as close as possible to their presumed time of death. Severe mitochondueal damage (swelling) and lipoidosis in myocardial tissues were observed in the 3rd pony. The hepatocytes of all of the ponies were characterized by increased amounts of smooth endoplasmic reticulum, large numbers of lipid droplets, vacuoles bounded by fibrous material, and a 2-fold increase in the number of peroxisomes per cell. Some hepatocytes also contained a membrane-bounded protein-like body. The observations indicate that heart mitochondria are primary targets of monensin poisoning in ponies.

**AN AUTOFADIOGRAPHIC TECHNIQUE TO DETERMINE INCORPORATION OF 3H-THYMICINE INTO THE EPIDERMIN OF THE STABLE FLY STOMOXYS CALCITRANS UTILIZING THICK RESIN SECTIONS AND TRANSMISSION ELECTRON MICROSCOPY.** Shirlee M. Meola, J. Mark Thompson, and John R. DeLoach, Veterinary Toxicology and Entomology Research Laboratory, Science and Education Administration, Agricultural Research, U.S. Department of Agriculture, College Station, TX 77841.

It has been reported that the insect growth regulator, dimilin, inhibits the histogenesis of adult epidermis in the stable fly. An autoradiographic study was done to determine if this effect was due to inhibition of DNA synthesis. This study required the development of both histological cna ultrasturctural techniques to detect the location of tritiated thymidine. The histological study involved the use of thick section resin embedded tissue post-stained with histological stains to determine the tissues in which the label was incorporated. These thick sections were correlated with adjacent TEM sections to determine the location and extent of incorporation of the 3H-thymidine within specific cells.

**ARSENIC LOCALIZATION IN LIVER CELLS BY ELECTRON PROBE X-RAY MICROANALYSIS.** N.K.R. Smith and E.M.B. Sorensen.* Dept. Anat. UTHSC, San Antomio, TX, and *Dept. Biol. MSU, Memphis, TN.

At present morphological, biochemical, and semiquantitative morphological data verify the existence of an intranuclear arsenic-induced inclusion in fish hepatocytes. These data indicate that the inclusion is a mon-delimited, electron-dense structure appearing in the nucleus of hepatocytes of green sunfish (**Lepomis cyanellus**) exposed to arsenic in the water. As exposure time increases, these inclusions enlarge and affect increasing numbers of liver cells. Subfractionation data show that the majority of the arsenic in a green sunfish liver homogenate is located within the nuclear pellet after only six days of exposure of these fish to arsenic. These data suggest but do not prove, that most arsenic is located within the dense inclusions.

Electron probe X-ray microanalysis was used to determine whether arsenic was localized in these inclusions. Liver tissue from fish exposed to arsenic was fixed in gluteraldehyde in phosphate buffer, degydrated in ethanol. and embedded in Epon. Sections 1/4 $\mu$m thick were cut for analysis using the JEOL JSM-35 scanning electron microscope with a United Scientific Si(Li) detector and Tracor Northern NS-880 X-ray analysis system. Inclusions analyzed for 40 to 300 seconds contain arsenic and sulfur in an approximate ratio to one another. Although sulfur was present in sufficient levels for dot mapping, arsenic was not. The possible significance of the association between arsenic and sulfur is discussed.

**INCLUSIONS RESEMBLING HYDROXYAPATITE IN ACUTE LEUKEMIA CELLS.** Gabriel Seman, M.D. Anderson Hospital and Tumor Institute, Houston, Texas, and Pierre Galle, Laboratory of Biophysics, Faculte' de Medecine de Creteil, France.

Blood cells were collected by leukapheresis from 54 patients with untreated acute leukemia and examined by electron microscopy. In 3 cases (two women with acute myelogenous leukemia and a boy with chronic myelogenous leukemia in blastic transformation) peculiar inclusions were observed in a few percent of the blast cells. These inclusions appeared as aggregates of electron dense, hairy paracrystals contained in membrane-bound cytoplasmic channels derived from rough endoplasmic reticulum. The channels formed an intricate network surrounded by mitochondria and often large bundles of cytoplasmic fibrils, and in places were opening into dilated endoplasmic sacs. Inclusions were always found in front of a large, concave recess of the nucleus. They did not belong to the Golgi apparatus, because dictyosomes and centrioles were nearly always located on the other side of the nucleus. The paracrystals were easily observed in unstained sections. Their morphology was similar to that of hydroxyapatite of bone. X-ray probe analysis revealed that they contained high above bockground levels of calcium, phosphorus and osmium, an indication that besides calcium phosphate they also include an osmiphilic protein or lipoprotein component. There are reasons to believe that the paracrystals are not artifacts but real structures preexisting in live cells. Treatment of sections with acids showed that the aggregates were centered on some kind of core. The presence of paracrystals rich in calcium phosphate in some leukemic myeloblasts raises interesting questions as to the metabolism of the cells.

**MICROTUBULES IN TUMOR CELLS**, Bruce MacKay, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030.

Rod-shaped bodies similar in appearance to the microtubules of the cytoskeleton are common in the cytoplasm of cells of some neural tumors including gliomas of the central nervous system, and Schwann cell tumors of the peripheral nervous system where they are longitudinally-aligned within cytoplasmic extensions. Groups of microtubules are also frequently found in the dendritic processes of neuroblastoma cells. Parallel arrays of intracisternal microtubules are present in more than 10% of human melanomas. The tubules are approximately 30 nm. in diameter and have a helical periodicity in cross section. The occurrence of microtubles in human soft tissue sarcomas is unusual. Two myxoid sarcomas will be shown in which aggregates of microtubules, lacking the geometric

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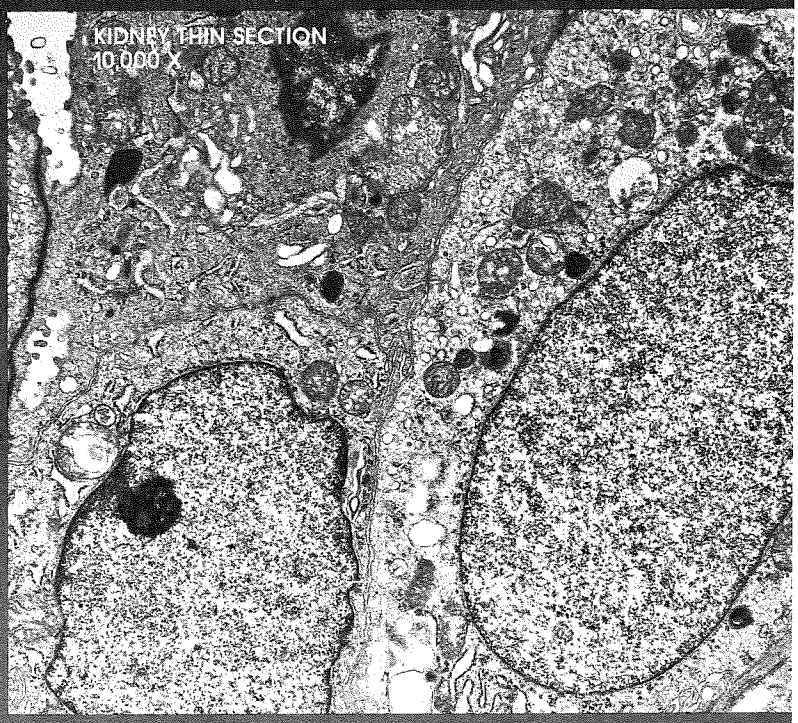


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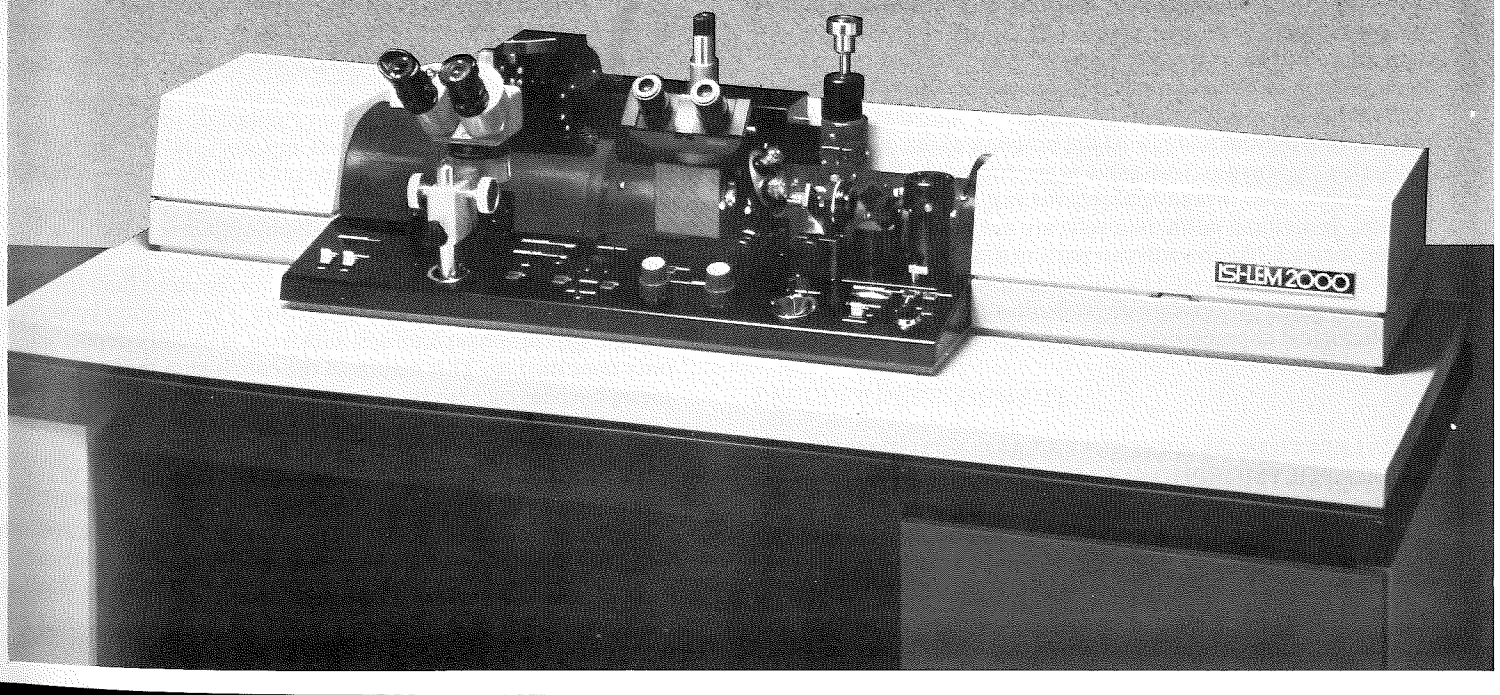


regularity seen in melanomas, were observed within distended cisternae in many of the cells.

**MEMBRANE-BOUND PARTICLES IN THE VENOM APPARATUS OF A PARASITOID WASP (HYMENOPTERA: BRACONIDAE).** K.M. Edson, M.R. Barlin, and S.B. Vinson, Department of Entomology, Texas A&M University, College Station, TX 77843.

Transmission electron microscopy revealed the presence of membrane-bound particles in the venom apparatus of the braconid endoparasitoid **meteorus leviventrils**. The venom apparatus consists of two gland filaments and a venom reservoir. Cytoplasmic stromata in secretory cells of the gland filaments contain hexagonal particles (approx. 50 nm in diameter) which have a dense core and capsid. These particles are also found in the cytoplasm and are often associated with vacuoles. The particles apparently bud from the cell at the bases of microvilli associated with a secretory apparatus. They appear to derive a membrane-bound particles are ovoid and measure approximatily 170 nm x 110 nm. Membrane-bound particles are found only in the secretory apparatus of gland filament cells and in the reservoir lumen. The secretory cells of the reservoir do not contain cytoplasmic stromata and membrane-bound particles are not found in the secretory apparatus of these cells.

**AN SEM STUDY OF THE ALIMENTARY CANAL OF THE GREATER WAX MOTH, GALLERIA MELLONELLA.** Cheryl A. Irons and Donald H. Whitmore. Biology Department, The University of Texas at Arlington, Arlington, Texas 76019.

Results of our SEM studies have yielded substantios gross morphological descriptions of the gut of the greater wax moth, **Galleria mellonella**, and have provided considerably more information concerning the arrangement of cells lining the lumen of the canal. In the foregut, the pharynx and esophagus are characterized by longitudinal fords of tissue, undoubtedly covered by a chitinous sheet or intima. A pronounced transition in cellular arrangement and morphology occurs in the crop and proventriculus regions where the cells are distinctly visable, but covered by a thick intima and many cuticular spines. The junction between foregut and midgut is highly constricted. A protion of the foregut intima appears to extend a short distance into the midgut cavity. The columnar cells of the midgut are covered with microvilli and appear to be more tightly packed in the anterior portion of the midgut. Numerous goblet cells can be seen, often in the process of secreting droplets. A delicate peritrophic membrane lines the midgut. At the junction of the midgut and hindgut, a highly muscular valve is encountered. The hindgut can be subdivided into several regions (e.g. ileum, colon, and rectum). Individual epithelian cells are not observed in this region; instead, like the anterior portion of the foregut, longitudinal folds covered by chitinous intima predoninate. An attempt is made to correlate physiological function with the morphological characteristics of each region of the alimentary canal.

**LOCALIZATION OF ARYLSULFATASE ACTIVITY IN THE RABBIT POLYMORPHONUCLEAR LEUKOCYTE.** Daniel M. Zellmer and W. Allen Shannon, Jr., Veterans Administration Medical Center and University of Texas Health Science Center at Dallas.

Polymorphonuclear leukocytes (PMN) were obtained from the peritoneal exudate of rabbits by a previously described method (brown et al., Proc. 37th Meet. Electron Micros. Soc. Amer., p. 78, 1979). The cells were fixed in 2.5% glutaraldehyde, 0.1 M sodium cacodylate, pH 7.3 and incubated in the arylsulfatase medium of Hugon and Borgers (J. Cell Biol. 33, 212-218, 1967).

Arylsulfatase is an acid hydrolase which has been reported in the azurophil of immature PMN, but not in mature PMN. Activity has been reported in small, peripheral granules of approximately 200 nm diameter. In our work, reaction deposit was also present in larger, centrally-located granules of approximately 400 nm in diameter. The reaction product is a fine deposit just inside the rim of these granules and occupies from 1/4 to 2/3 of the total volume. These granules correspond in size to the so-called "specific" granule.

Reaction product was frequently observed just inside the rim of larger granules, up to 800 nm in diameter. The deposit was dense, but seldom occupied more than 1/5 the volume of the granule. This granule would correspond in size to the "azurophil" granule.

Preliminary evidence suggests the pH may be very critical as to which type of granule is reactive. Since three different arylsulfatases with three different pH optima have been found in human PMNs, it is possible that different pHs result in different patterns of reactivity.

**LOCALIZATION OF TRIMETAPHOSPHATASE ACTIVITY IN RABBIT POLYMORPHONUCLEAR LEUKOCYTES.** W. Allen Shannon, Jr. and Daniel M. Zellmer, University of Texas Health Science Center at Dallas and Veterans Administration Medical Center.

Polymorphonuclear leukocytes were obtained from the peritoneal exudate of rabbits by a previously described method (Brown et al., 37th Ann. Proc. EMSA, p. 78). The cells were fixed in 2.5% glutaraldehyde, 0.1 M sodium cacodylate, pH 7.3 and incubated in the trimetaphosphatase medium described by Oliver (J. Histochem. Cytodhem. 28, 78-81, 1980).

Trimetaphosphatase activity has been demonstrated in exocrine acinar cells, and its presence led to the elucidation of a new and unique type of lysosome. Its presence has not previously been indicated in PMN.

Trimetaphosphatase activity was found in granules of approximately 400 nm in diameter. The reaction deposit is uniform throughout the granule. the granules tend to be more centrally located in the cell than are other phosphatase-containing granules.

Some activity is exhibited in cytoplasmic protrusions within the cell membrane. The deposit appears to be free in the cytoplasmic matrix which fills the protrusions. The significance of this is unknown.

The granule containing trimetaphosphatase activity is very similar in size to those containing alkaline B-glycerophosphatase. However, it is more frequent than the latter and much less frequent than the former. The reaction product, using B-glycerophosphate as a substrate, is usually deposited only near the rim. This results in the conclusion that trimetaphosphatase activity is in a different granule.

**THE PRESENCE OF CATALASE AND MYELOPEROXIDASE ACTIVITY IN SMALL GRANULES OF RABBIT POLYMORPHONUCLEAR LEUKOCYTES.** Daniel M. Zellmer and W. Allen Shannon, Jr., Veterans Administration Medical Center and University of Texas Health Science Center at Dallas.

Polymorphonuclear leukocytes were obtained from rabbit peritoneal exudate and were fractioned by a previously described method (Brown et al., 37th Ann. Proc. EMSA, p. 78). Cells and fractions were fixed in 2.5% glutaraldehyde, 0.1 M sodium cacodylate, pH 7.3.

Myeloperosicase (MPO), which is involved in the killing of bacteria by an MPO-mediated anto-microbial system, is usually located in the so-called "azurophil" granule. The presence of MPO activity has been shown in the miniscus region of fractionated PMN by several authors; however, it had been assumed that the activity was due to membrane contamination or free protein at the meniscus.

Catalase has been found in a granule similar to the azurophil and is involved in a catalase-dependent microbicidal activity, but its principal role may be to inhibit the MPO-mediated anti-microbial system.

Cytochemical reaction of PMN for catalase and MPO demonstrated numerous small granules of approximately 200 nm which contain reaction product. Incubation of the region just below the miniscus of gradients containing fractionated PMN confirmed the presence of these small granules which constitute 10-15% of the total fraction. Granules exhibiting many different forms show positive reaction.

**COATED PIT/VESICLE FORMATION IN OSTEOARTHRITIS SYNOVIAL FLUID MONOCYTES.** W. Allen Shannon, Jr., and Daniel M. Zellmer, University of Texas Health Science Center at Dallas and Veterans Administration Medical Center.

Synovial fluid aspirate was centrifuged to pellet cells and solid contents. The pellet was fixed in glutaraldehyde-osmium and routinely processed.

Thin section analysis revealed a heterogeneous cell population consisting primarily of monocytes, lymphocytes, macrophages and some neutrophils. All cells appeared coated, as with a glycocalyx. Dense fibrous material, possibly immune complex, was prevalent. This material appeared to be phagocytosed in small quantities by the monocytes. Indeed, there appeared to be a correlation between the presence of numerous membrane plaques, coated pits, and adjacent dense material. Microtubules were associated with many of the plaques and coated pits.

Fine structrual analysis indicates coated pit formation preceded by membrane plaque formation and internalization by microtubule activity. Coated pits appear to have little material within while larger internalized vesicles contain dense material.

## REGIONAL NEWS Continued...

Dr. Diana Redburn, Associate Professor, chaired a session and co-presented four presentations with Dr. Steve Massey, Sr. Research Assistant, Cindy Hampton, Graduate Student, and Mannie Steglich, Research Assistant at the Association for Research in Vision and Ophthalmology meetings in Orlando, May 2-5. At the same meeting, Dr. Michael Oberdorfer, Assistant Professor, presented "Distribution of Retinal Axons in Pigmented Embryo (Ferret) Eyes" with Nancy Miller, Research Assistant. Louise A. Moorhead, M.D., M.D. Research Scientist, also presented vision research.

Marilyn Munkres, Teaching Associate, took a course entitled "Scanning Electron Microscopy and X-ray Analysis" in Bethlehem, Penna. June 23-27, in order to learn new techniques and pass them on to students and associates.

Dr. Joe G. Wood, Professor and Chairman, chaired a session and presented a paper entitled "Chromium Tagged Norepinephrine Content Shown by X-ray Analysis" at the 38th Annual Meeting of the Electron Microscopy Society of America in Reno, Nevada August 4-8.

Dr. S.J. Enna, Professor of Neurobiology and Pharmacology, presented lectures on GABA receptors in May to the Canadian College of Neuropsychopharmacology in Edmonton, Alberta; to the Department of Pharmacology at Cornell U. School of Medicine in New York City; and at the International Symposium on GABA and Blutamate in Sardinia, Italy.

### FACULTY DEVELOPMENT

Dr. Nachum Dafny, Professor, left in June on a one year faculty development leave. Dr. Dafny is the recipient of a Fogarty International Center Fellowship and will be working with Dr. Joseph Terkel at Tel Aviv University in Tel Aviv, Israel.

### PUBLICATIONS

Dr. S.J. Enna, Professorof Neurobiology and Pharmacology co-edited a book entitled, *Receptors of Neurotransmitters and Peptide Hormones,* which was published in May by Raven Press.

### BAYLOR COLLEGE OF MEDICINE, DEPARTMENT OF MEDICINE, SECTION OF CARDIOVASCULAR SCIENCES

### LECTURES

Dr. Ann Goldstein gave a seminar on "The Z-lattice in Striated Muscle" at the UCLA Medical School Department of Physiology on Aug. 8 after attending the EMSA meeting in Reno, Nev.

### PUBLICATIONS

Golstein, Margaret A., Stromer, Marvin H., Schroeter, John P., and Sass, Ronald L.: Optical reconstruction of nemaline rods. *Experimental Neurology 69: (in press) 1980.*

### NEW STAFF MEMBERS

Mr. Brian Krdman has joined Dr. Ann Goldstein's lab as a research technician.

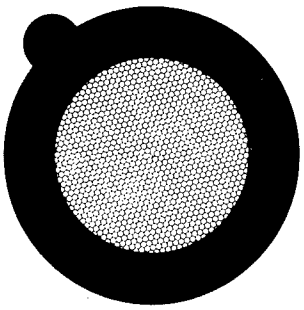
### FACULTY DEVELOPMENT

Dr. Ann Goldstein has been named to the Central Research Committee of the American Heart Association, Texas Affiliate.

Dr. Goldstein attended a one-week course in "Small Computers in Biomedical Research" taught by Drs. Larry Palmer and Lee Peachy at the Marine Biological Laboratory at Woods Hole, Mass. May 18-23, 1980.

# A New Approach to Bar Thickness And Definition

A new technology for producing high definition grids has led to the development of two new types of hexangonal grid pattern with a very thin straight grid bar.

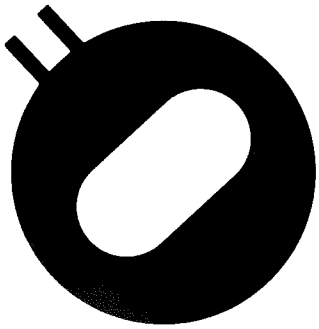


In the first pattern the grid bars are only 5 micron width resulting in a transmission of over 80%. The repeat distance is 460 lines/inch (18 lines/mm). Thus good specimen support is combined with very little obscurity of specimen.

Order details Type HEX 460 TB Grids
Tubes contain 100 grids. $21.00

Where the ultimate is really good specimen support is required, this grid mesh is a must. The repeat distance is approximately 700 lines/inch (28 lines/mm) with the same really thin bars as for the 460 pattern. Again very little specimen obscurity is encountered.

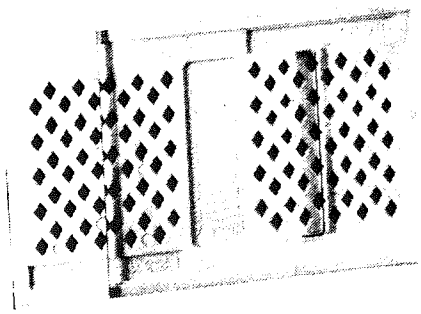
Order details Type HEX 700 TB Grids
Tubes contain 100 grids. $26.20



Long ribbons of ultramicrotomed sections can be supported on liquid drops suspended on a slot grid. The sections can then be dried onto a lower filmed grid. The orientation between the upper and lower grids is important if the ribbons are to be positioned correctly. This new slotted grid design has two wires attached, which, when bent, locate around the tweezers holding the lower grid.

Ref. Proc. Vol. 5 EM Society of Southern Africa
Dr. E.D.F. Williams, EM Unit, University of Cape Town

Order details Tube of 100 modified Slotted Grids
Type SL2X1W $6.00



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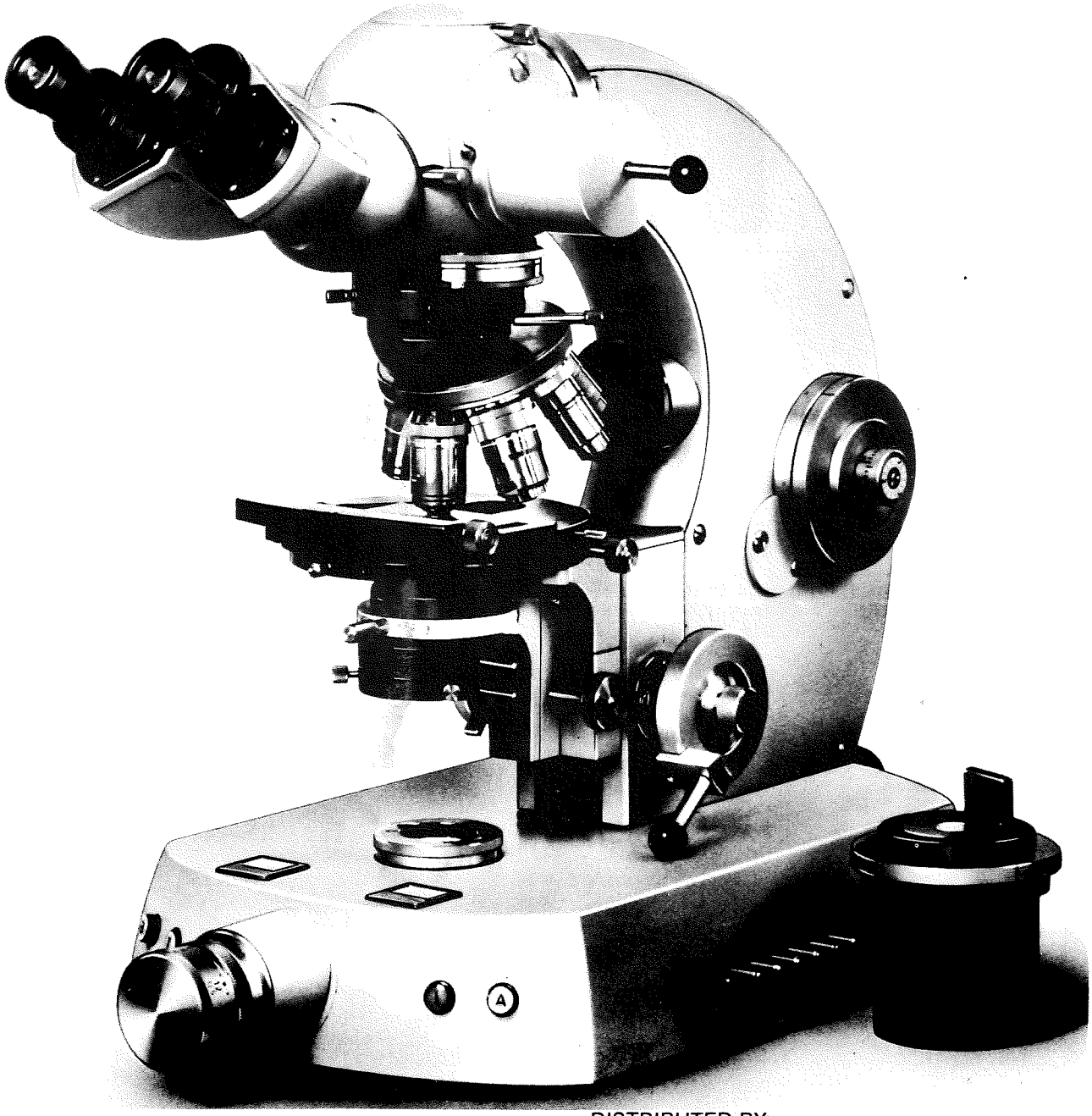

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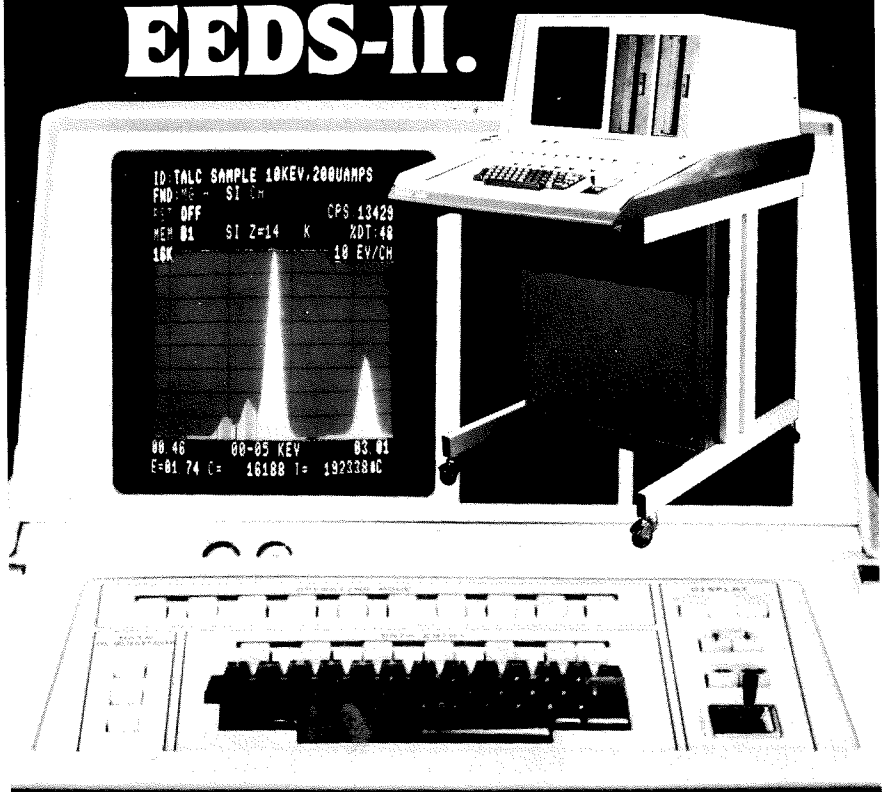


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# Application/Nomination For Membership

I hereby apply/nominate for Regular ☐ Student ☐ Corporate ☐ membership in the Texas Society for Electron Microscopy.

Name of institution applicant or corporation nominee person _____

P. O. Address _____

_____

Information as to position, degrees, and qualifications for Membership: _____

_____

_____

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This nomination is accompanied by a statement of interest in and contributions to Electron Microscopy and associated fields of science.

One year's dues in the form of a check or money order should be sent with the application for Membership form. (Regular $10.00, Student $2.00, Corporate $75.00)

Signature of one Member making the nomination:

_____

Dated _____ 19 _____

This application to Membership in the Society, or this application for transfer from the grade of Student to Regular Member, signed by one Member should be sent to the Secretary to be presented at the next meeting of the Council or approval by a majority vote of the Council. Notice of approval will be mailed by the Executive Secretary.

_____

Presented to the Council at _____ meeting. Date _____

Action _____

Remarks _____

Send Application to: Marilyn Smith
Texas Woman's University
Dept of Biology
Denton, TX 76204

# REGIONAL NEWS Continued...

### BAYLOR COLLEGE OF MEDICINE, DEPARTMENT OF MICROBIOLOGY

#### LECTURES

Dr. Heather D. Mayor — "Structure of Small DNA Viruses" — Instituto Superiore Di Sanita in Rome, Italy on June 27, 1980.

## LUBBOCK
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#### GRANTS AWARDED

"Characterization of Sertoli cells in Diabetic Animals" NIH April 1, 1980 — March 31, 1981. $96,831. James C. Hutson, Ph.D., Assistant Professor of Anatomy

#### PUBLICATIONS

Hutson, J.C., C.W. Garner, and D.M. Stocco. 1980. Effects of serum components on Sertoli cells in culture. Anatomical Record (In Press).

Childs, G.V., J.C. Hutson, and T.W. Bauer. 1980. Immunocytochemical defection of hormones at target cells. In: Monographs in Diagnowtic Pathology. Masson Publishing, U.S.A. (In Press).

Bolender, D.L., W.G. Seliger and R.R. Markwald 1979. A histochemical analysis of polyanionic compounds found in the extracellular matrix encountered by migrating cephalic neural crest cells. *Anat. Rec.* In press.

Fitzharris, T.P., R.R. Markwald and B. Dunn. 1980. Influence of Beta aminoproprionitrile on early cardiac cushion tissue morphogenesis. *J. Mol. Cell Cardiol.* In press.

Nathan, R.D., S.J. Fung, D.M. Stocco, E. Barron and R.R. Markwald. 1980. Sialic acid regulation of electrogensis in cultured heart cells. *Amer. J. Physiol.* (submitted).

Bolender, D.L., W.G. Seliger, and R.R. Markwald. 1980. A histochemical analysis of polyanionic compounds found in the extracellular matrix encountered by migrating cephalic neural crest cells. *Anat. Rec.* 196: 401-412.

Dalley, B.K. and W.G. Selinger. 1980. A new technique for the rapid screening and selection of large pieces of tissue for ultrastructural evaluation. Stain Technology, (In press).

Penelope W. Coates and Steven L. Davis. "Ependymal tanycytes on the floor of the third ventricle of ewes exhibit subtle alterations in surface features during the estrous cycle". (To be presented before the 10th Annual Meeting of the Society for Neuroscience, Nov. 9-14, 1980, Cincinnati, Ohio).

Penelope W. Coates. "A comparison of intraventricular nerve fibers and supraependymal cell clusters in the third ventricle of the monkey and sheep." (8th International Symposium on Neurosecretion, September 4-10, 1980, Friday Harbor, Washington).

Penelope W. Coates and Steven L. Davis. "Intraventricular nerve fibers in the cycling ewe". *Anat. Rec.* 196: 34A-35A, 1980.

Penelope W. Coates and Steven L. Davis. "Nerve fibers in the immature sheep third ventricle". Soc. Neuroscience Abs. 5: 426, 1979.

Dalley, B.K. andW.G. Seliger. 1980. A new technique for the rapid screening and selection of large

Dalley, B.K. and M.I. Bradley. 1980. Long term effects of smoooh muscle autotransplantation to the heart. *Anat. Rec.* 196: 40A-41A.

Bolender, D.L. W.G. Seliger, and R.R. Markwald. 1980. A histochemical analysis of polyanionic compounds found in the extracellular matrix encountered by migrating cephalic neural crest cells. *Anat. Rec.* 196: 401-412.

## TEMPLE
### SCOTT & WHITE CLINIC

#### NEW EQUIPMENT

In April 1980 a nem Hitachi H-600 was installed in R.A. Turner's EM Lab in the Department of Surgical Pathology.

#### GRANTS

W.B. McCombs, III and O.D. Holton, Clinical Evaluation of the Dorn Blood Culture Technique, $6,500.

W.B. McCombs, III and O.D. Holton, Detection of CEA and Tennagen in Human Colon Tumor Cell Lines, $10,000.

O.D. Holton and W.B. McCombs, III. Isolation and Clincal Characterization of a Cell Line (SW 527) Derived Human Breast Tumor Associated Antigen, $43,000.

# FOR SALE (Reasonable)

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Department of Botany
University of Texas at Austin
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# MISCELLANEOUS

Roger R. Markwald, Ph.D., Associate Chairperson and Professor of Anatomy elected chairman of the "Morphogenesis Club of the American Association of Anatomists" and received a Basic Science Teaching Award.

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**Cambridge-Imanco**, Steve Miller, 9551 Williams Street, Rosemont, Illinois 60018l (312) 671-4270.

**DuPont/Sorvall**, Jim Gordon, Instrument Products, Quillen Bldg., Concord Plaza, Wilmington, Deleware 19898. Phone: 800-441-7493 or (302) 772-5678.

**EG&G Ortec**, Dick Nieman, 21718 Rotherham, Spring, TX 77379. Phone: (713) 353-0078 or 1-800-251-9732.

**EDAX International Inc.**, Glen Gray, P.O. Box 135, Prairie View, Illinois 60069.

**Electron Microscopy Sciences**, M. J. Oulton, P.O. Box 251, Ft. Washington, Pennsylvania 17034.

**Ernest F. Fullam, Inc.**, I.T. Stoneback, Advertising Mgr., P.O. Box 444, Schenectady, New York 12301.

**Gatan Inc.**, Terry Donovan, 780 Commonwealth Dr., Warrendale, PA 15086.

**International Scientific Instruments**, Robert Ruscica, 3255 - 6C Scott Blvd., Santa Clara, Calif. 95050.

**JEOL, USA, Inc.**, Paul Enos, 200 Walnut Way, Euless, TX 76039. Phone: (817) 267-6011.

**Kevex**, Alan Weckerling, 2909 Sherwood, Colleyville, TX 76034.

**Ladd Research Industries, Inc.**,Dr. D.R. Cole, 1207 Todd Trail, College Station, TX 77840. Phone: (713) 693-8003.

**L.K.B. Instruments, Inc.**, Jack Kirten, 5300 Telephone Road, Houston, TX 77017.

**Montedison USA, Inc.**, Richard A. Steenrod, Jr., P.O. Box Drawer L, Bridgeton, Missouri 63044.

**Olympus Corporation**,Dan Fordan, 5201 Mitcheldale Suite B1, Houston, TX 77092.

**Perkin-Elmer ETEC, Inc.**,P.J. Breton, 3392 Investment Blvd., Hayward, CA 94545.

**Philips Electronic Instruments**, Bob Peterson, 7302 Harwin Drive, Suite 106, Houston, TX 77036. Phone: (713) 782-4845.

**Polaron Instruments, Inc.**, Dermot Dinan, 1202 Bethlehem Pike Line, Lexington, Pennsylvania 18732. Phone: (215) 822-3364/5.

**Polysciences, Inc.**,Paul Valley Industrial Park Warrington,PA 18976. Phone: (215) 343-8542.

**Princeton Gamma Tech.**, Rod Jensen, 8408 Bridgetown Dr., Austin, TX 78753.

**Schares Instrument Corporation**, Margot Martin, 2600 S. Gessner, Suite 315, Houston, TX 77063. Phone: (713) 468-4460.

**Rockwell International**,R.W. Max, Mail Station 406-146, Richardson, TX 75081.

**Spectrochemical Research Lab**, D. Raymond Slovinsky, 4800 West 34th Street, Suite A-12, Houston, TX 77092.

**Technics, Inc.**, Robert Barr, 5510 Vine Street, Alexandria, Virginia 22310. Phone: (703) 971-9200.

**Technical Instrument Co.**,John H. Meny, 4215 Beltwood Pkwy. Suite 106, Dallas, TX 75234.

**Ted Pella, Inc.**, Thomas P. Turnbull, P.O. Box 510, Tustin, California 92680. Phone: (714) 557-9434.

# ANNOUNCEMENTS

## SEM MEETING

This year the Scanning Electron Microscopy Inc. 1981 meetings will be held in Dallas at the Fairmont Hotel between April 14-18. These meetings provide an excellent opportunity for exposure to a wide diversity of subject areas and techniques in all fields of scanning electron microscopy. This year, as in the past, S.E.M. Inc. wishes to encourage student attendance at these meetings and is waiving registration fees ($20+) for interested students willing to work for 4 hours (one session) as a projectionist or monitor.

This opportunity is limited to students, both graduate and undergraduate, who wish to attend these meetings. Altogether, 40 students will be required to operate the projectors for the entire meeting. Experience with projection equipment is desirable but not essential.

Those of you who may be teaching EM or related classes this year or know of students who would be interested in helping out and enjoying the benefits of working at these meetings, please pass the information on. Interested students should contact:

Wayne R. Fagerberg
Department of Biology
Southern Methodist University
Dallas, Texas 75275

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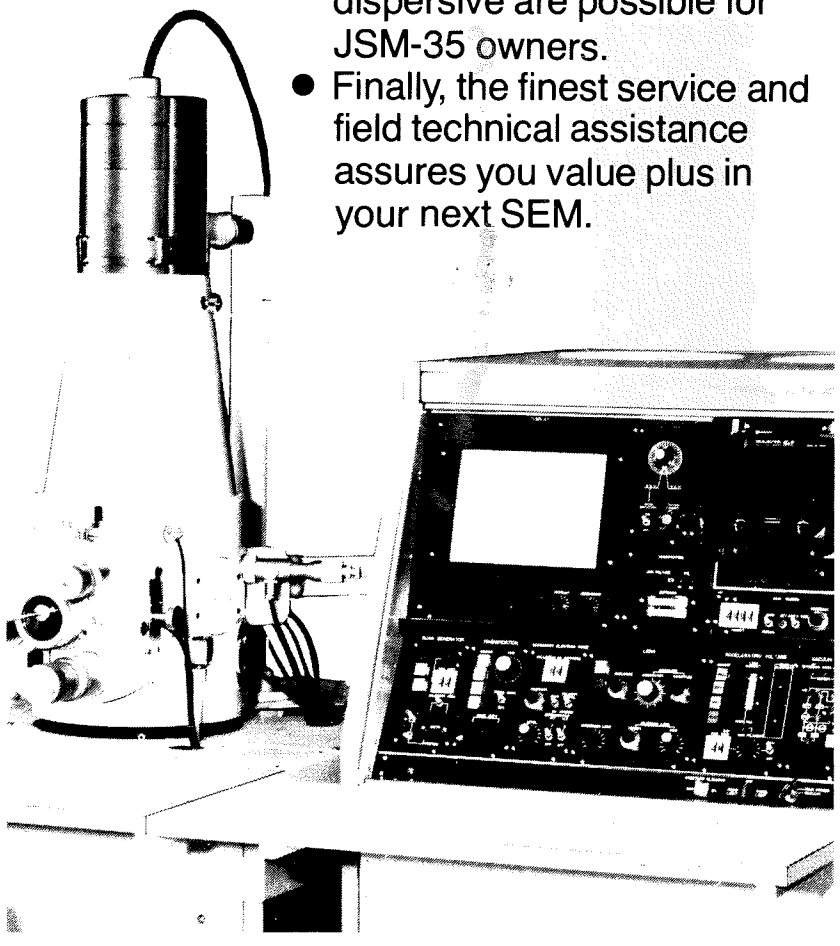

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