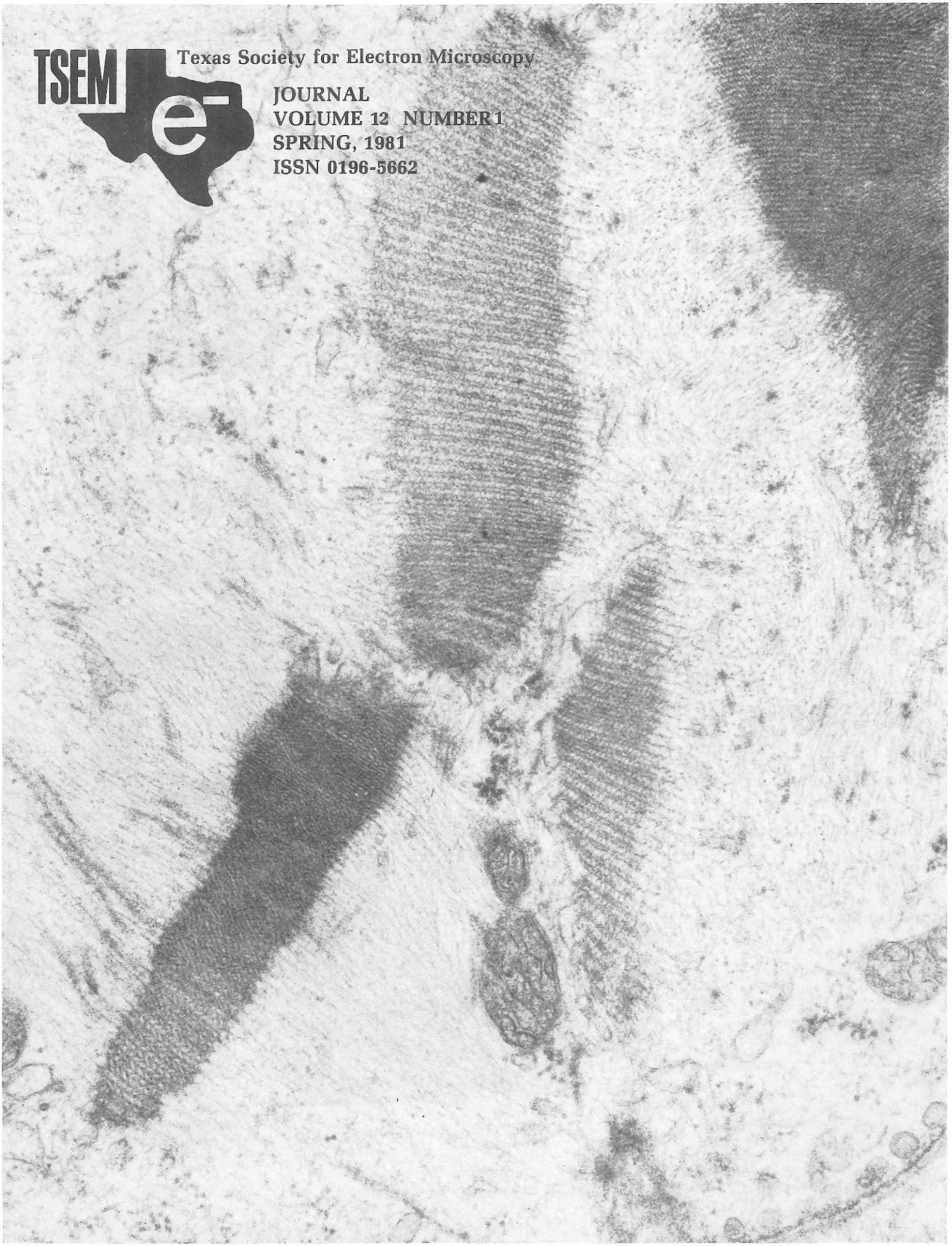

TSEM
Texas Society for Electron Microscopy
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VOLUME 12 NUMBER 1
SPRING, 1981
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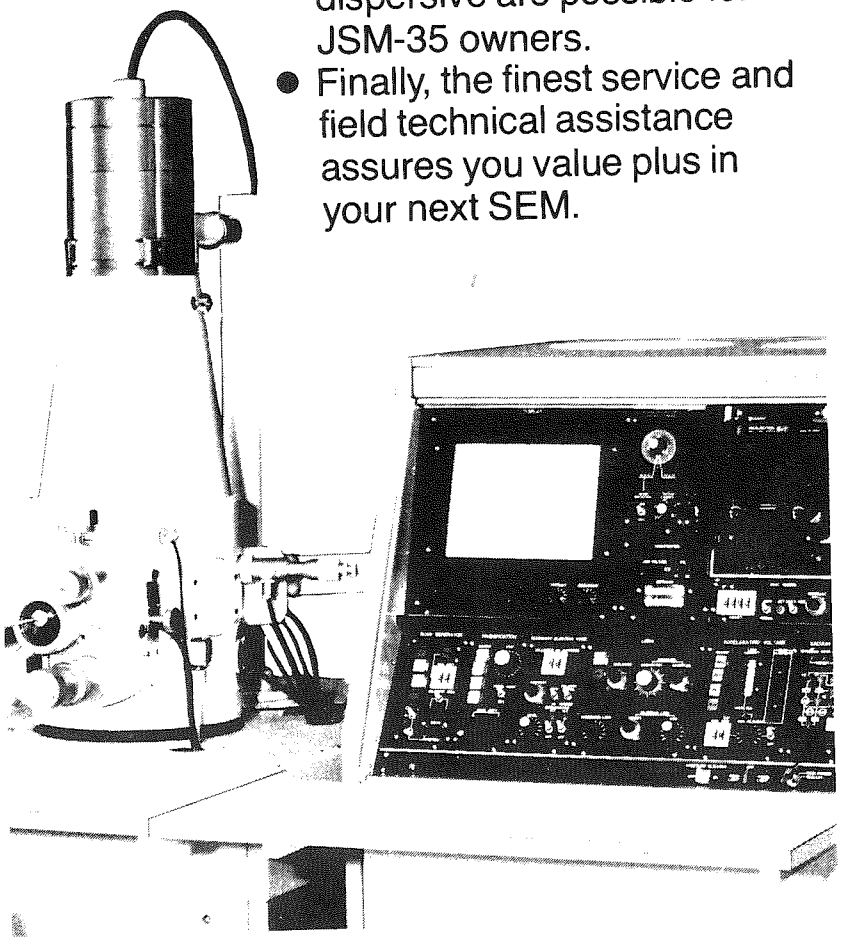

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EDITORIAL POLICY

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

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

ON THE COVER

Aberrant 2-band formation in human cardiac muscle fiber; x53,000. Submitted by Bruce Mackay, Department of Pathology, M.D. Anderson Hospital and Tumor Institute, Houston, Texas.

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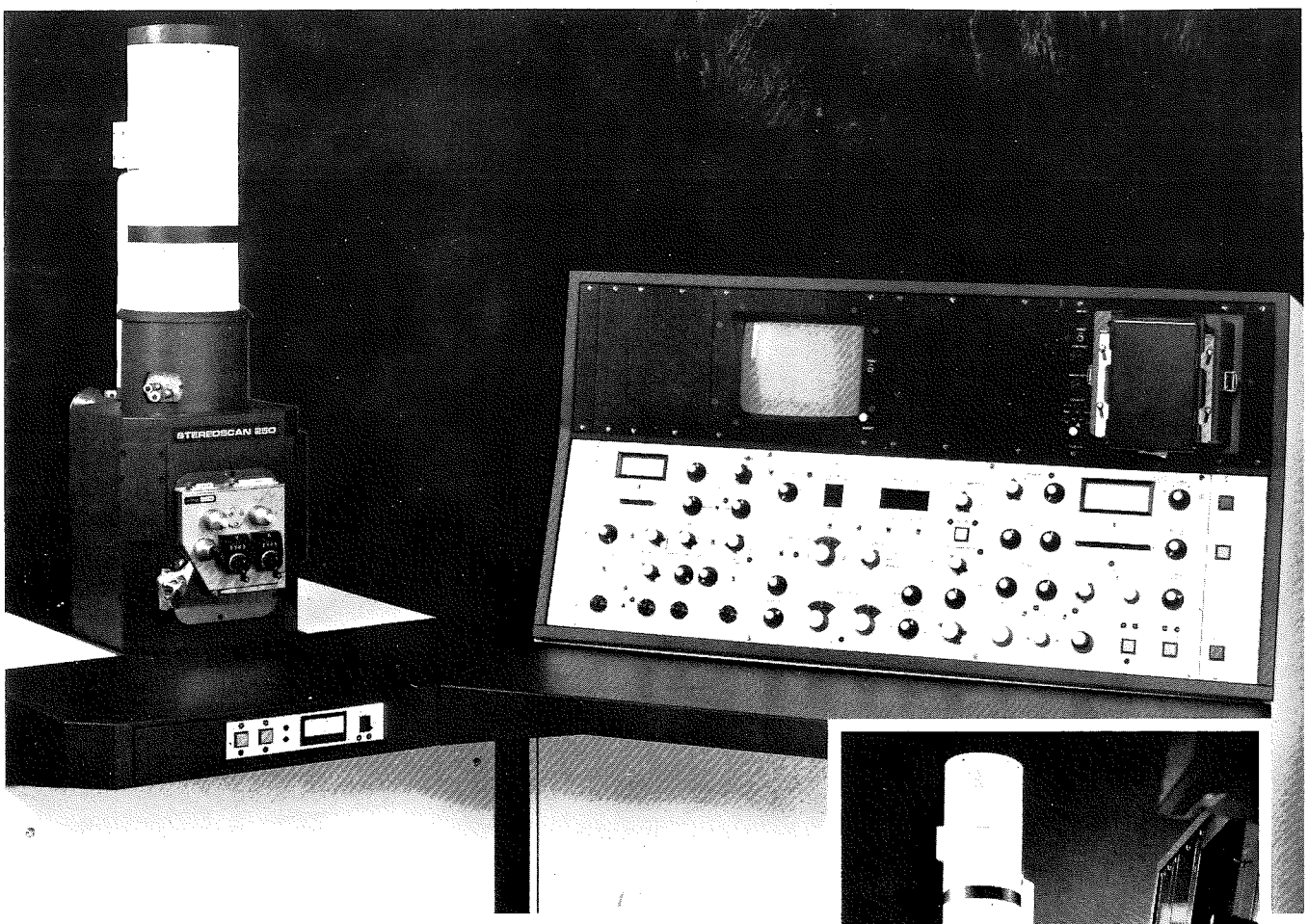

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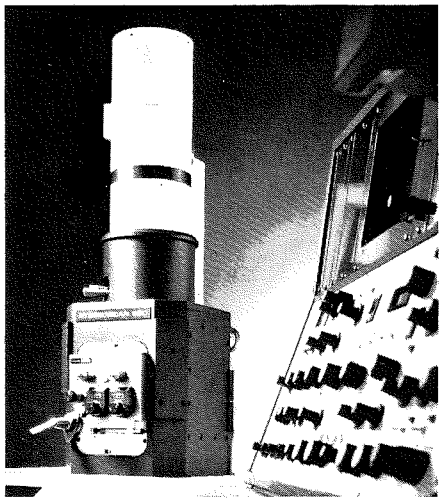

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President's Message

Dear TSEM Member:

Every year at this time the outgoing president writes "the farewell letter". The letters historically prognosticate about the future and thank the TSEM fellow officers. This year I'd like to take the liberties afforded the office and share with you some of my feelings and appreciations.

Although TSEM is in pretty good shape, I sense a softening of vitality and momentum in our society. I think I know at least some of the reasons for my perceptions.

First, our various EM facilities across this state are, for the most part, old, well established mini-institutions in themselves. Most are well equipped, thoroughly organized, and partially funded. As outside (federal) research funding hits a crunch, more and more of everyone's time is spent either directly or indirectly in the preparation of grants and in preliminary research useful in getting those grants with less time and energy available to devote to endeavors such as TSEM. Similarly, as the various research monies dry up the number of individuals involved in research either as technicians and/or graduate students gradually diminishes. It's survival and it's the absolute pits!

Secondly, Texas is becoming a very popular state for national meetings. Cell Biology, SEM, Inc., and EMSA have all been here in the past year or so and several will probably return in the next 4-6 years. That makes it and will make it difficult for us to "draw" to one of our local TSEM meetings.

Third, it seems to be the case that once service on the TSEM council has terminated, some afford themselves a period of pseudo-retirement from the society. We miss you all!

Fourth, I've noticed that other state EM societies have reached a level of participation and activity that we had several years ago. They have the advantage of newer laboratories, send monies, funding, and the vitality that always goes along with a new organization.

It sounds a bit glum but we have a great opportunity to dedicate ourselves to the revitalization of a noble enterprise — TSEM. We enjoy the best laboratories, finest instruments, highest levels of technical skills, and a grand assortment of lovely people. Let's just decide, to commit ourselves to pull together through these times, — unite our efforts in a common interest and keep TSEM the good and vital society it has always been. Let's develope a new membership drive, encourage the presentation of papers and attendance at our meetings, volunteering for hard jobs and face the future together. I know we can do it.

Paul S. Baur, Jr., Ph.D.
President, TSEM

P.S. My heartfelt thanks to all the officers who served with me this year — special thanks to Hilton Mollenhauer and Ernest Couch who both participated in miracles while serving as Local Arrangements Chairman. By the way, I'm volunteering for local arrangements — Galveston, 1982.

Editor's Comments

I am grateful to our advertisers for making this publication possible and to our contributors for making this issue interesting. Thanks to all of you for your participation.

Publication of a current list of TSEM members is projected for the July issue of this journal. Please be sure your dues are paid and make address changes with our secretary, Marilyn Smith, by the end of May to be included.

Your comments and suggestions regarding this publication are always appreciated.

Elaine McCoy
Editor

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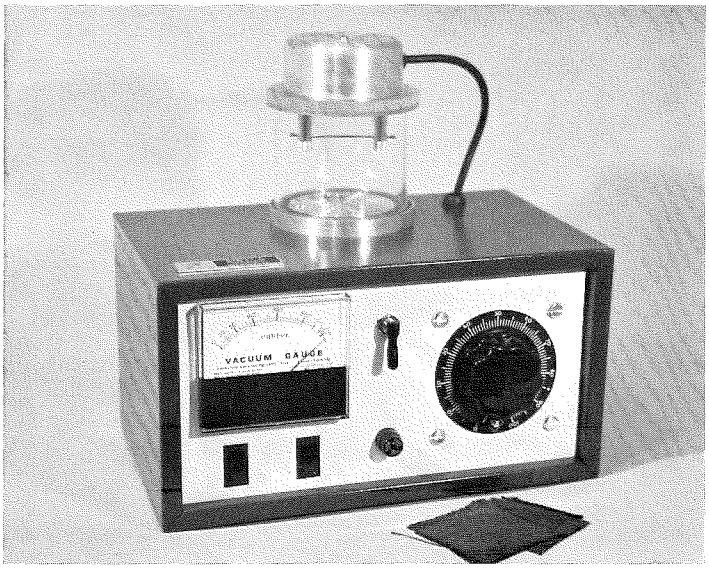

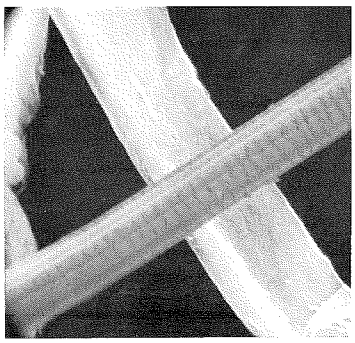

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CARDIAC BIOPSY

By
Bruce Mackay, Laura M. Keyes, Robert S. Benjamin
Michael S. Ewer, Sewa S. Legha, and Sidney Wallace

Departments of Pathology,
Developmental Therapeutics,
Medicine and Diagnostic Radiology

The University of Texas System Cancer Center
M.D. Anderson Hospital and Tumor Institute
Department of Pathology
Houston, Texas 77030

Supported in part by Grant NCI-CA-05831

Small pieces of myocardium can be obtained for histologic examination using a cardiac bioptome. The instrument was initially developed in Japan in the early 1960's[1], and technical improvements have produced the flexible bioptomes currently in use. The instrument shown in figure 1 is 50 cm. long. It is introduced into the internal jugular vein in the neck, and threaded under fluoroscopic control until its tips is in the right ventricle. The biting jaws (figure 2) can be manipulated with the forceps-like handle. The aim is to obtain the biopsies from the right ventricular septum close to the apex. The procedure is not uncomfortable for the patient, and meticulous attention to the details of the technique results in relatively few complications in the hands of an experienced operator.

Although some pathologic processes involving the myocardium can be recognized by light microscopy, the added dimension provided by the transmission electron microscope is necessary to detect more subtle alterations in the cardiac muscle fibers. The tissue obtained by heart biopsy is therefore routinely processed for E.M. Myocardial biopsies have been used to study changes in heart muscle in a wide range of disorders, and its contributions have on the whole been of limited significance. It has, for example, been suggested that the procedure is only of value in some 10% of patients with cardiomyopathy[2]. Cardiac biopsy has, however, been found extremely useful in defining the changes produced in the heart by certain anticancer drugs, notably Adriamycin, and in monitoring patients who are receiving this drug. Adriamycin is one of the most important agents currently available for the treatment of many solid tumors, but its administration has had to be restricted because of toxic effects on the heart, particularly a chronic cardiomyopathy that leads to biventricular failure. Attempts to prevent the cardiotoxicity by not exceeding an arbitrary cumulative dose level of 550 mgm. have met with limited success, since some patients develop myocardial damage at a considerably lower dose level[3]. Others can tolerate more of the drug and would therefore be denied its full benefit. An efficient method for monitoring the deleterious effects of Adriamycin on the heart is consequently desirable, and non-invasive techniques can be unpredictable. At the M.D. Anderson Hospital, over 250 cardiac biopsies have now been performed on patients receiving Adriamycin, and the results confirm that this method is of considerable value for detecting and quantitating myocardial damage.

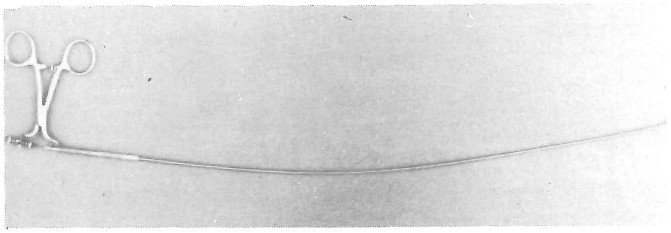

Figure 1. The cardiac bioptome.

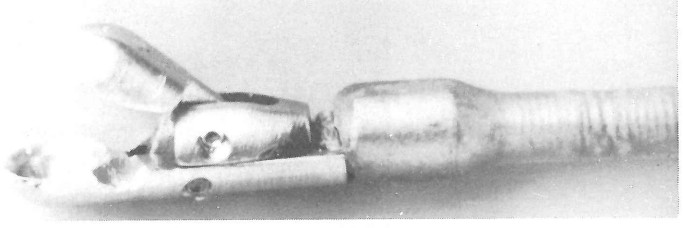

Figure 2. "Jaws" of the instrument.

PATHOLOGY:

Usually 3 pieces of tissue are obtained by the biopsy procedure, each roughly 2 mm. in size, and they are promptly placed in buffered 2% glutaraldehyde. The specimen bottle is taken immediately to the E.M. laboratory where the tissue is gently diced. From a single biopsy, 8 to 20 tissue blocks, each 1 mm. or less, are usually obtained.

Semi-thin sections are cut from every tissue block. Examination of those sections by light microscopy shows the degree of artefact present, either from squashing or more commonly as a result of contracture of the myofibrils. Some blocks may contain fat or blood clot. Light microscopy also reveals certain pathologic changes, such as an increase in the amount of subendocardial connective tissue, but does not allow a reliable evaluation of the early changes induced by Adriamycin in the myocardial muscle fibers.

We routinely thin-section 6 blocks, having found that at least 3 are necessary to provide a good indication of the extent of the pathology. Doubling this number gives a better opportunity to quantitate the findings. At least 3 sections are placed on each grid so that fibers partially obscured by grid bars can be examined. Changes in the fibers can be detected at a magnification of less than 2,000, and the 6 grids can therefore be screened in under one hour. The types of alterations present and number of involved fibers per grid are noted and a numerical grade is assigned (Table 1).

The pathologic changes produced in myocardial muscle fibers by anthracycline drugs including Adriamycin have been documented by the Stanford group [3,4], and we have been indebted to Dr. Margaret Billingham and her colleagues as our own program has developed. Essentially, 3 types of damage are seen, and they can be appreciated by comparing the appearance of a normal cardiac muscle cell (figure 4) which happens to be from one of our biopsies in which many of the cells did show pronounced alterations,

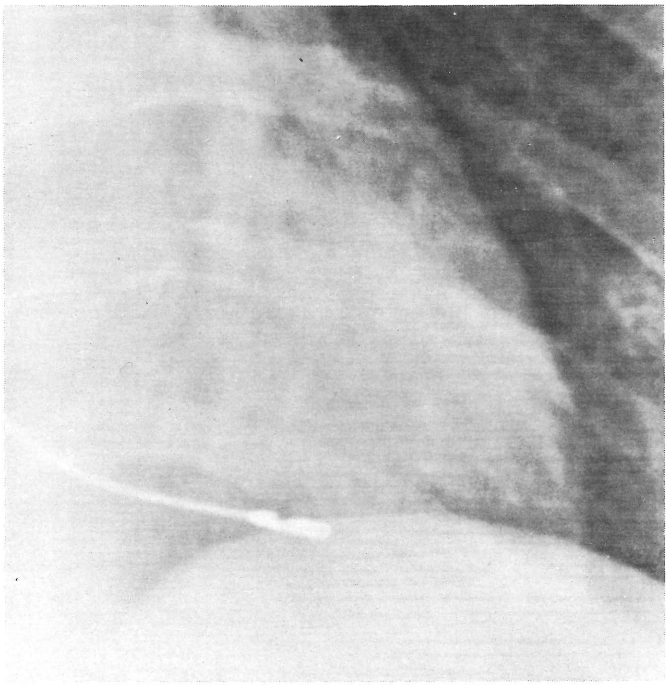

Figure 3. Radiograph showing the bioptome tip within the right ventricle.

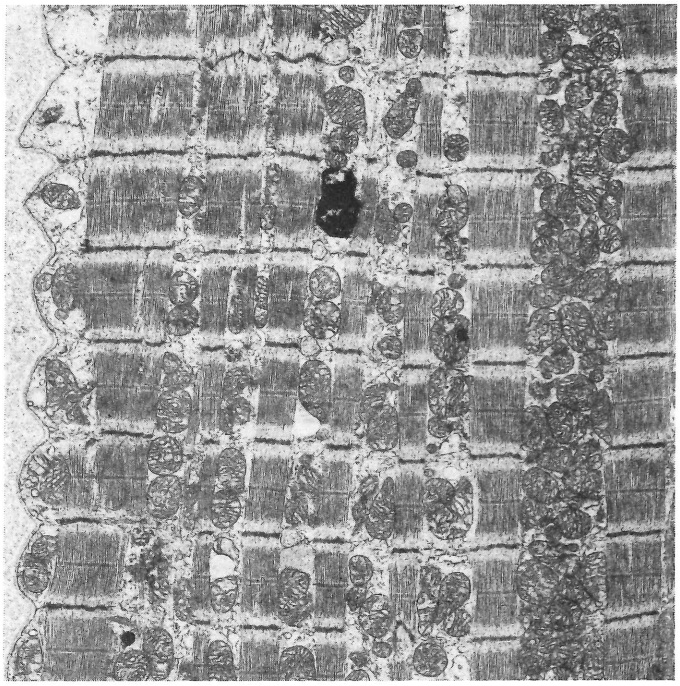

Figure 4. Normal cardiac muscle fiber from one of our biopsies. X5,500.

Table I

Grade	Vacuoles	Number of Muscle Fibers Showing Changes* Myofibrillar Dropout	Necrosis
0.5	<4	0	0
1	4-10	<3	0
1.5	<10	3-5	<2
2	-	6-8	2-5
3	-	>8	>5

*Reflects average number of abnormal muscle fibers per grid based on an examination of a minimum of 6 grids obtained from 6 blocks. A pathologic grade of 2 is an indication to discontinue administration of Adriamycin.

with figures 6 through 11. The changes must be distinguished from contracture artefact (figure 5). The first is a dilatation of tubules of the sarcoplasmic reticulum which may be focal within a fiber (figure 6) or more extensive with coalescence of the distended tubules (figure 7). Often associated with tubule dilatation, but in some instances seen alone, is damage to the contractile elements of the muscle fiber, preceded by disarray with distortion of the banding pattern (figure 8), and leading to a loss of myofibrils that ultimately results in an empty fiber (figure 9). Caution is necessary in evaluating apparent drop-out of myofibrils in the presence of severe contracture artefact, as the latter can produce empty areas within a fiber. The third type of change seen, and the most severe, is an accumulation of lysosomes (figure 10), leading to complete degeneration of the myocardial cell (figure 11). Small numbers of lysosomes are customarily seen in association with lipofuschin deposits, and are disregarded.

In addition to these established changes, we have encountered several other departures from normal morphology in some of our biopsies, including mitochondrial hyperplasia, mitochondrial bleb formation (possibly artefact), and giant mitochondria (figure 12). An intriguing observation has been the occurrence of abnormalties in myofibril formation in which aberrant Z-band material is produced (figures 12-14). The associated filaments appear to be only of the actin type. A similar appearance has been noted in heart muscle cells of patients with valvular disease[5,6], and the dense bodies resemble the nemaline rods of skeletal muscle fibers[7]. It is not known

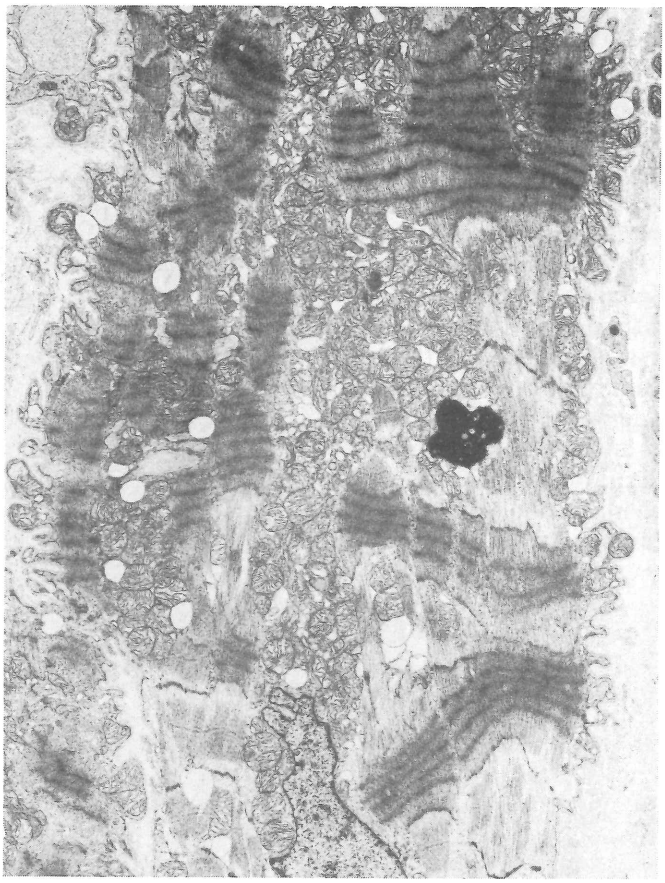

Figure 5. Contracture artefact. X4,300.

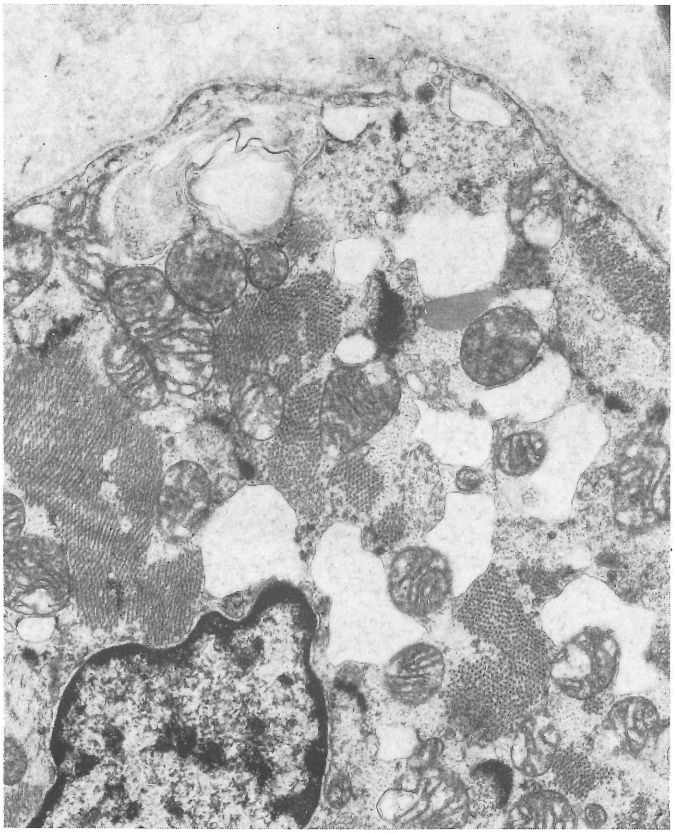

Figure 6. Focal dilatation of tubules of sarcoplasmic reticulum. X4,600.

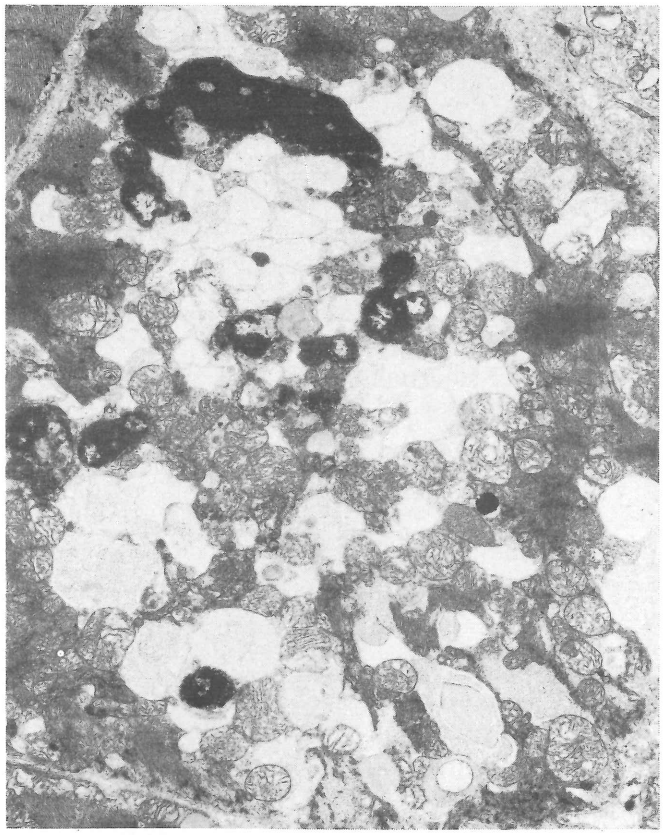

Figure 7. A more severe degree of vacuolation. X5,800.

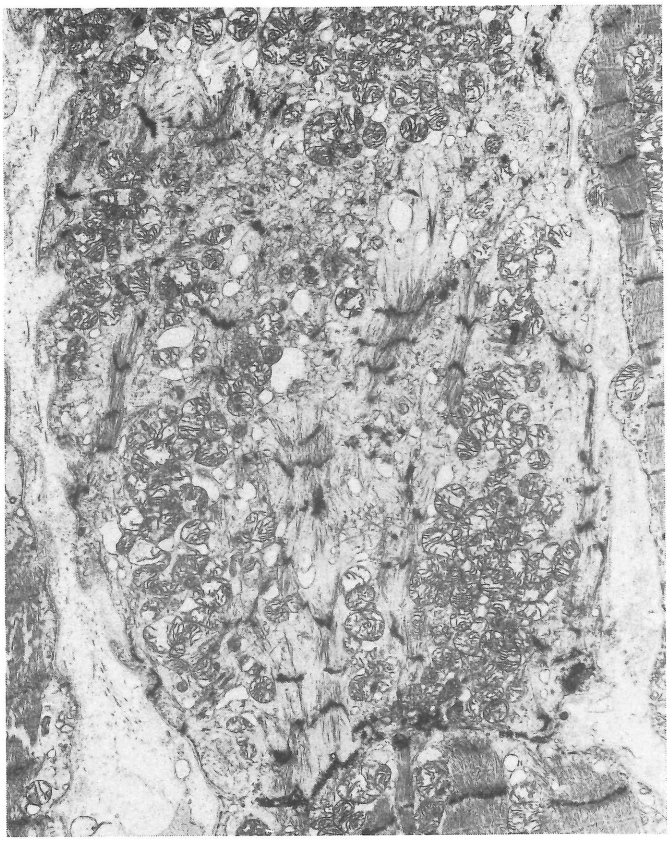

Figure 8. Disarray and fragmentation of myofibrils. X4,100.

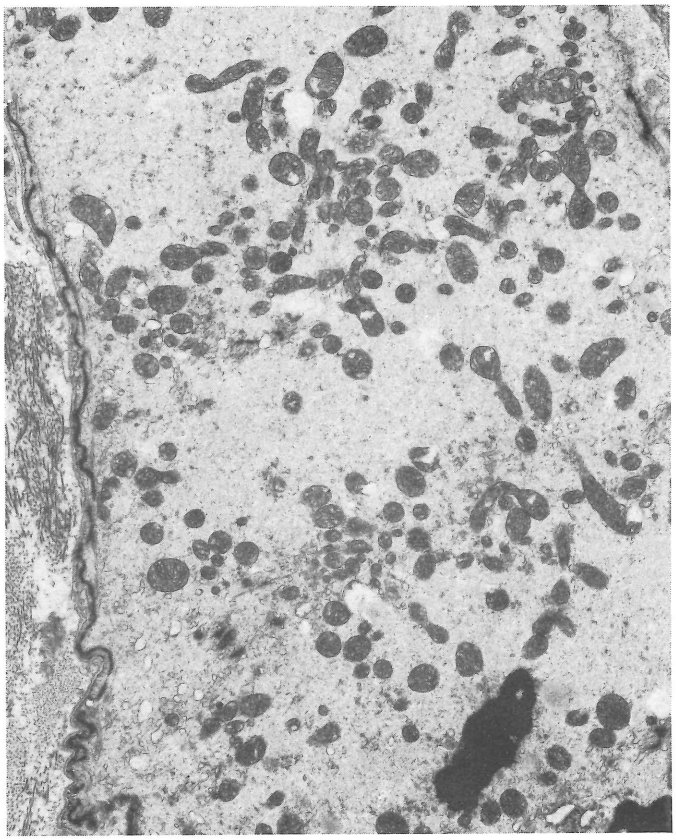

Figure 9. "Drop-out" of myofibrils. X5,900.

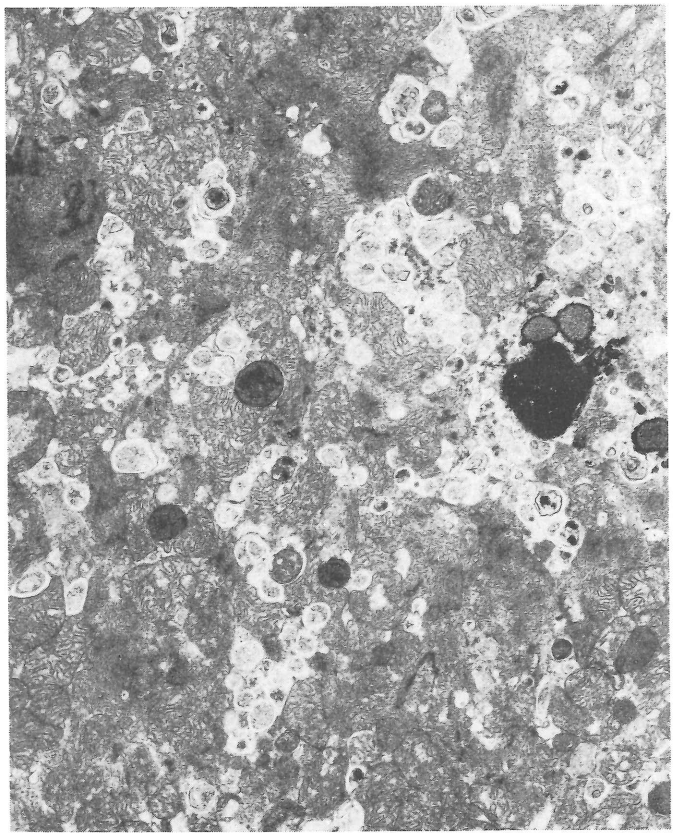

Figure 10. An early stage in the accumulation of lysosomes in a damaged fiber. X8,900.

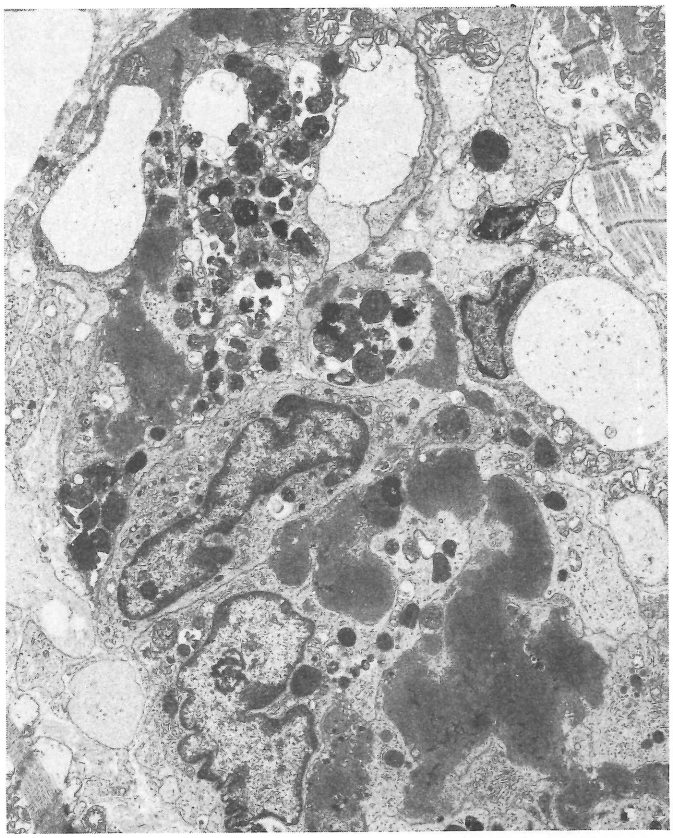

Figure 11. Severe degenerative changes. X4,000.

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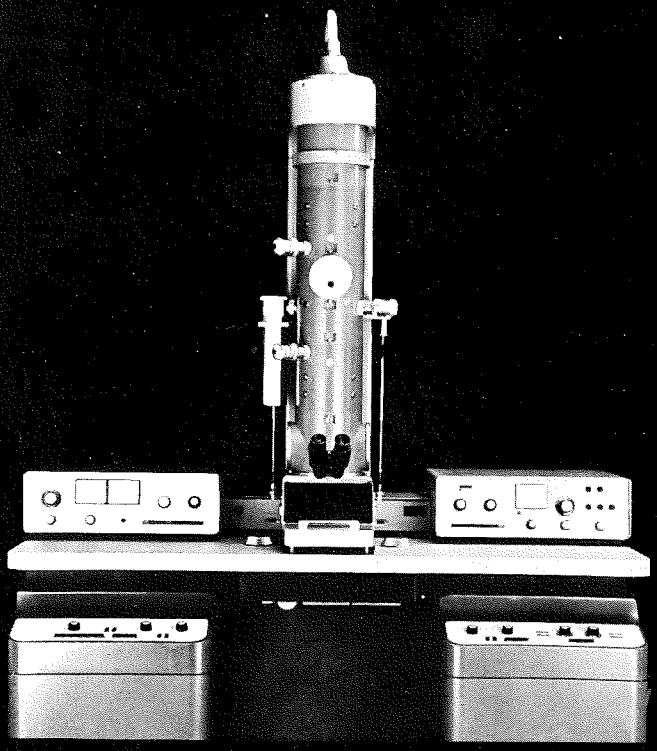

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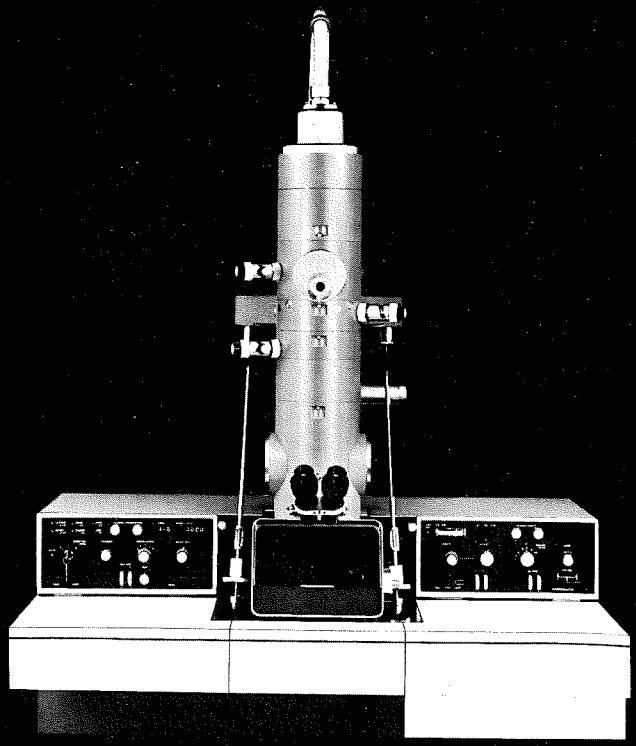

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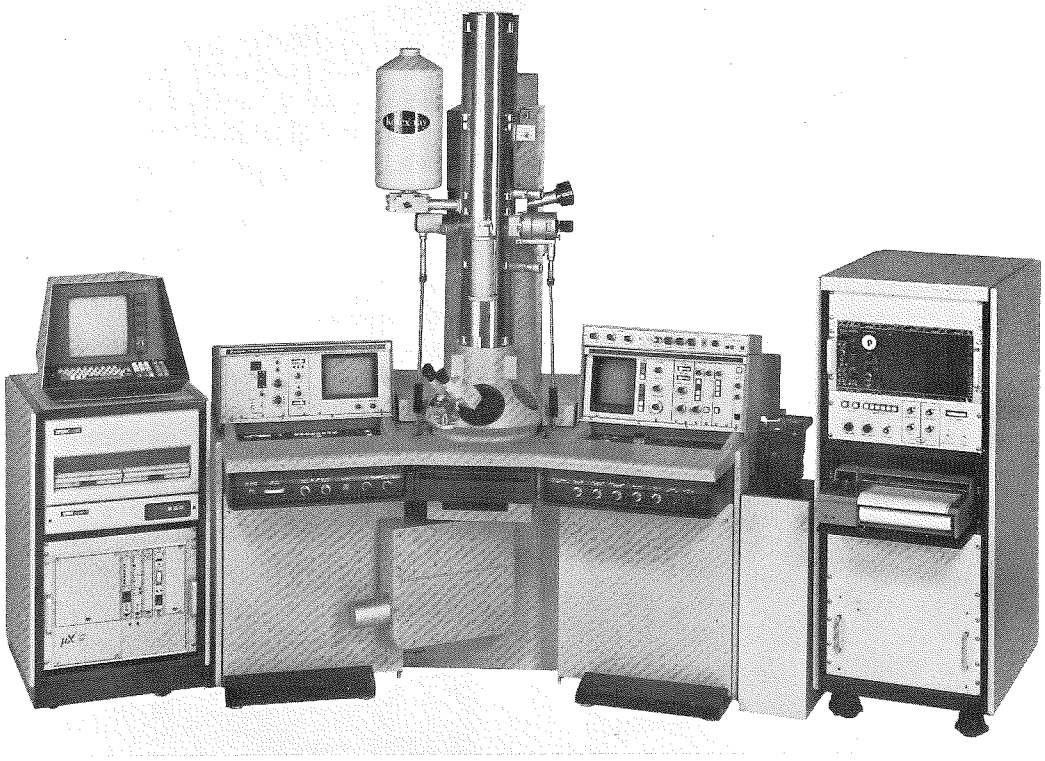

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whether they represent a response to the Adriamycin or are merely an incidental finding. Rarely the biopsy reveals the presence of metastatic tumor cells within the myocardium (figure 15).

The cytophysiologic mechanisms that lead to the various morphologic abnormalities are not well understood. A number of complex biochemical disturbances are produced by Adriamycin[8,9], including binding of the drug to nuclear and mitochondrial DNA, inhibition of enzymes in the mitochondrial electron transport system, and membrane disturbances that result in accumulation of cytoplasmic electrolytes (Na, Ca) and water. One could postulate that the nuclear damage alone might disrupt the orderly sequences of cytoplasmic synthetic functions to a degree manifested by visible derangement of organelle ultrastructure. It is known that radiation to the heart potentiates the cardiotoxicity induced by Adriamycin, but again the mechanisms responsible have not been elucidated.

The specificity of the various fine structural changes seen in anthracycline-induced cardiomyopathy might be questioned since similar alterations have been observed in other conditions. Vacuoles can be seen in electrolyte imbalance. Contractile elements may be lost in cardiac hypertrophy. Mitochondrial abnormalities have been reported in alcohol and cobalt-induced myocardial damage. However, there is a significant relationship between the degree of pathology and the cumulative dose level of the Adriamycin, and in most instances our patients have not been known to have an associated condition that might have produced changes in the heart muscle cells.

Cardiac biopsy does appear to provide a significant contribution as one method of monitoring patients who are receiving Adriamycin, and offers the potential to evaluate the effectiveness of drugs that might reduce the cardiotoxicity (such as alpha-tocopherol). Probably the single most valuable result from our own studies has been the demonstration that cardiotoxicity can be reduced by manipulating the dosage schedule. It was formerly the practice to give the drug in a single dose every three weeks, but its toxic effects on the heart can be significantly reduced when it is given using a prolonged continuous intravenous infusion[10]. Presumably this beneficial effect can be attributed to lower peak levels in the plasma. The modified schedule does not appear to compromise Adriamycin's antitumor activity.

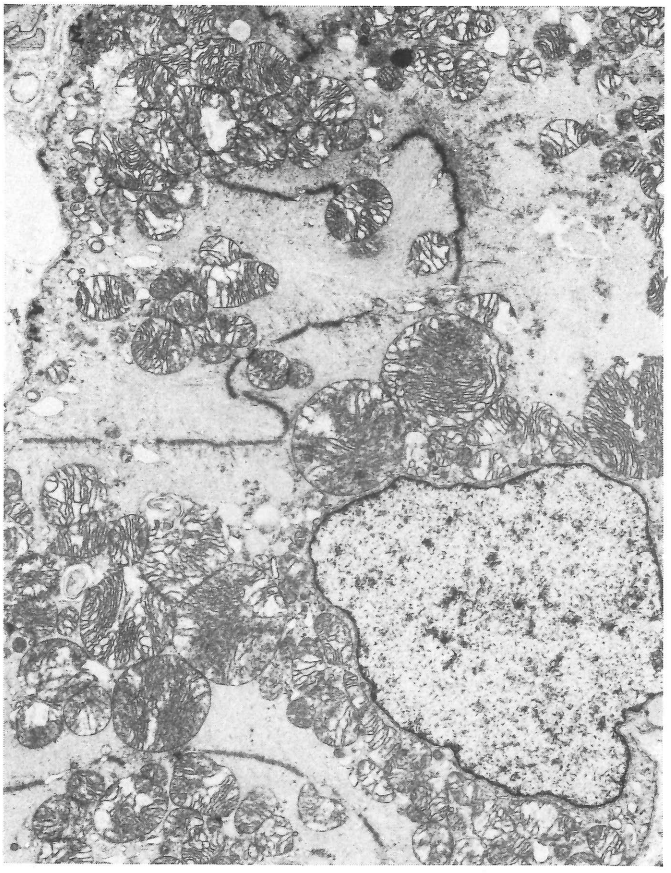

Figure 12. Abnormal myofibril formation with giant mitochondria. X5,500.

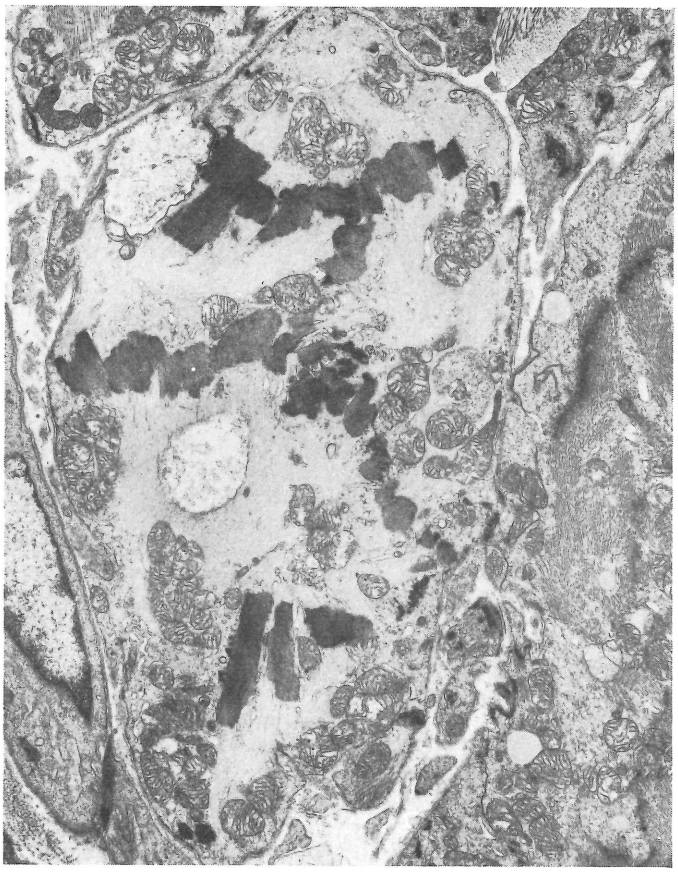

Figure 13. Rod-shaped bodies, probably aberrant Z-band formation. X5,500.

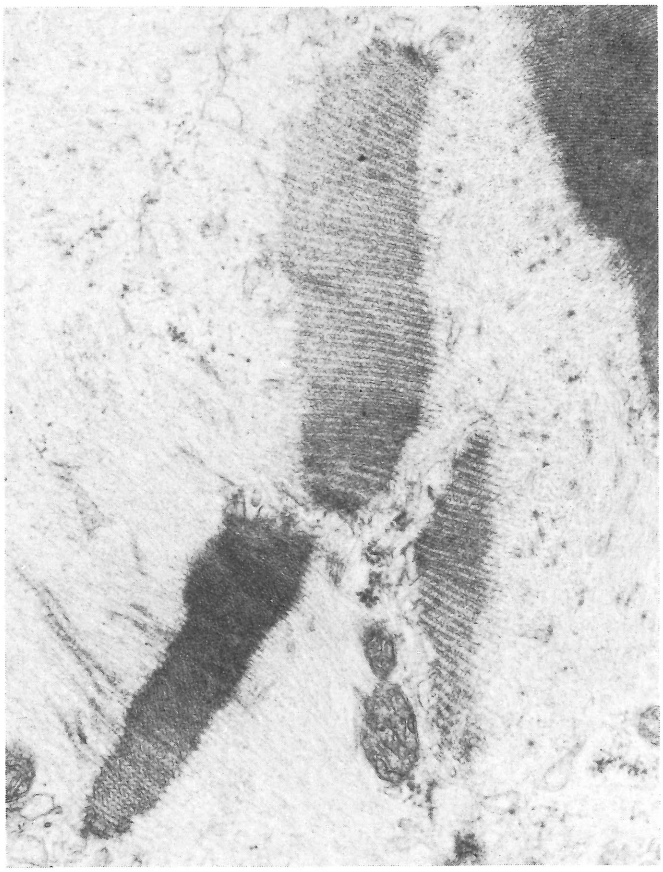

Figure 14. Detail of rod-shaped bodies. X25,300.

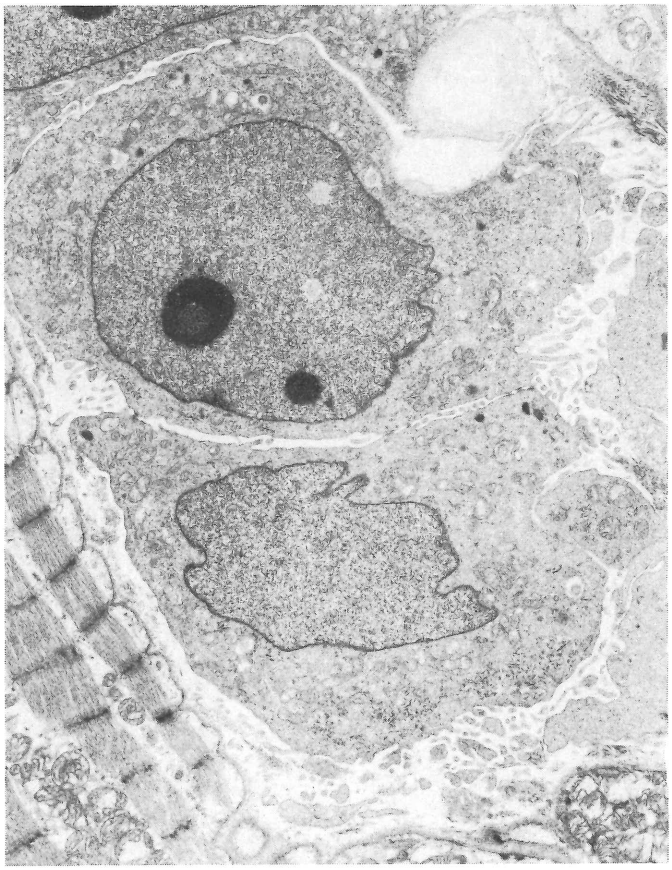

Figure 15. Metastatic breast carcinoma cells in myocardium. X6,000.

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Paracrystalline Inclusions in Blast Cells of Patients with Acute Leukemia

By
Gabriel Seman, M.D.
Department of Molecular Carcinogenesis
and Virology
The University of Texas System
Cancer Center M.D. Anderson Hospital
and Tumor Institute

Texas Medical Center
Houston, Texas 77030

During an ongoing electrom microscopy study of blood cells of patients with acute leukemia, we observed in three cases paracrystalline inclusions never before reported in the literature. Aside from its ultrastructural interest, this finding may lead to new insights into the cytology of this malignant disease.

Blood cells of 54 patients have been examined thus far 29 with acute myeloblastic leukemia, 16 with acute lymphoblastic leikemia, 2 with chronic myelogenous leukemia in blastic transformation, 3 with acute myelomonocytic leukemia, 1 with hairy cell leukemia, and 3 with undifferentiated leukemia). The patients nearly all had recently discovered, untreated disease, and all were in acute, highly cellular phase when their cells were collected and examined. Cells were collected by leukapheresis in the leukemia unit of this institution, headed by Dr. Kenneth McCredie. One of the three patients involved here (L.M.R.) was a 16-year-old boy with a history of Philadelphia chromosome-positive chronic myelogenous leukemia (CML) that had been treated for 4 years. He was in blastic transformation when admitted for treatment. The two other patients were women, one (E.K.) age 66, the other (D.H.) age 41, both with untreated acute myeloblastic leukemia (AML). All three patients had about 90% blasts when they underwent leukapheresis for the first time.

For electron microscopy, 1-2 ml cell suspension from leukapheresis bags was centrifuged for 5 min at 1000 rpm the supernatant was removed, and cell pellets were fixed overnight in the cold in 3% glutaraldehyde in Millonig's buffer, followed by 2% buffered osmium tetroxide for 2-3 hours. After dehydration in graded alcohols and propylene oxide, the blocks were embedded in epon-araldite. Thin sections were stained in uranyl acetate and lead citrate. Some sections were examined unstained.

The micrographs presented here show paracrystalline inclusions in leukemic cells of patients E.K. and D.H., who had about 10% and 5% inclusion-containing blasts in their blood, respectively. Patient L.M.R. had only occasional cells of this type. At low magnifications the inclusions could be seen as highly electrondense spots scattered in the cytoplasm, usually in front of a large, concave recess of the nucleus (figures 1-3). At higher magnifications (figures 4-6) the spots appeared as dark structures bristling with thin, hairy protrusions. In sufficiently thin sections (figure 5) it was possible to recognize that the structures were aggregates of densely packed, filamentous paracrystals. This was much more evident in unstained sections (figures 7 and 8). The lower contrast of paracrystals in unstained sections also proved that the paracrystals were picking up uranium and lead salts during staining.

Each of the aggregates was surrounded in part or entirely by a membrane (figures 4-6). This membrane was of endoplasmic origin, as demonstrated by its continuity, in some places, with dilated sacs of rough endoplasmic reticulum filled with a loose, reticulated component (figure 6). The presence of membranous bridges between aggregates (figure 5) also demonstrated that the aggregates were contained in a network of endoplasmic channels. This disposition was perhaps better in evidence in unstained sections (figure 7).

A feature of interest was the frequent association of abundant cytoplasmic fibrils with the paracrystalline inclusion systems. The fibrils formed either large parallel bundles (figures 2, 4 and 6) or a loose network between channels (figure 5).

The channel systems containing the paracrystalline aggregates did not belong to the Golgi apparatus. Dictyosomes or centrioles were never observed in their middle. In fact these organelles were nearly always located on the opposite side of the nucleus (figure 3).

By ultrastructural criteria, cells with aggregates were poorly differentiated myeloblasts in all three patients. This was of particular interest in the case of patient L.M.R., who was in blastic transformation of CML. The myeloblastic or lymphoblastic nature of transformation in CML has always been difficult to diagnose but important to determine in planning treatment. In the present case, by revealing in the cells of patient L.M.R. inclusions identical to those observed in cells of two patients with typical AML, electron microscopy allowed confirmation of the myeloblastic character of the transformation.

The nature and significance of the paracrystalline inclusions require further investigation. These inclusions bear a great similarity to hydroxyapatite of bone, which suggests that they may contain, or are principally made of, calcium phosphate. This point is currently being investig-

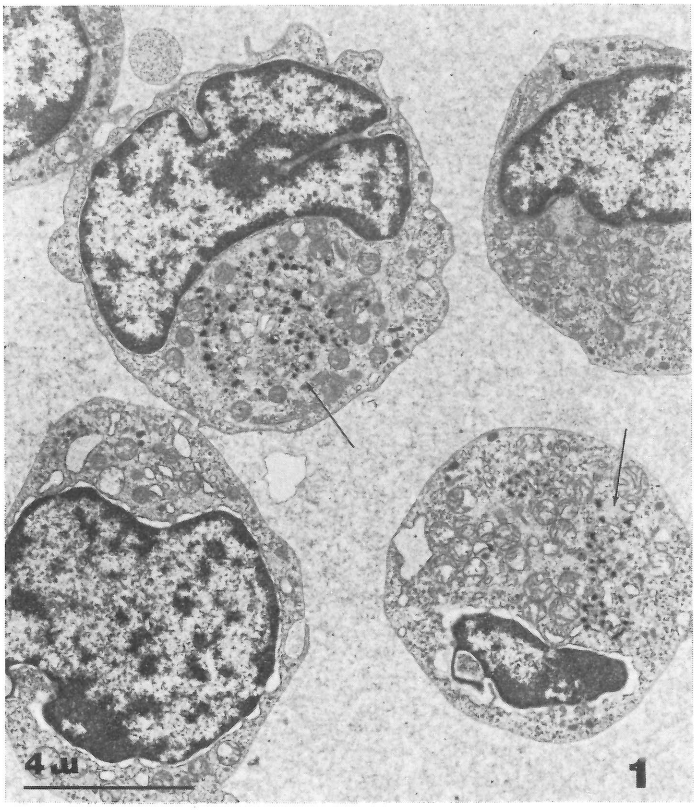

Figure 1. Patient E. K. with AML. Two blast cells contain inclusions (arrows).

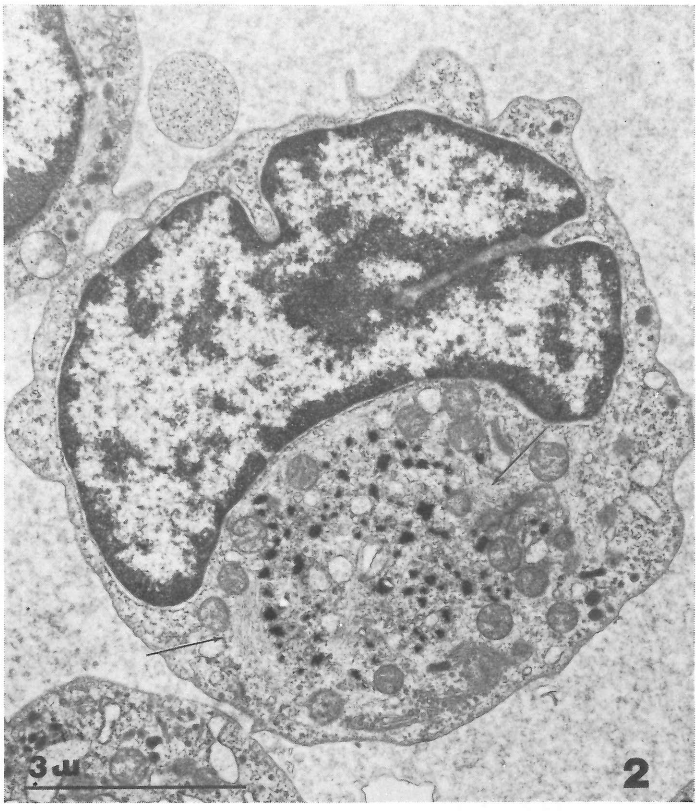

Figure 2. Patient E. K. Higher magnification of a cell shown in figure 1. Bundles of cytoplasmic fibrils surround the inclusions (arrow).

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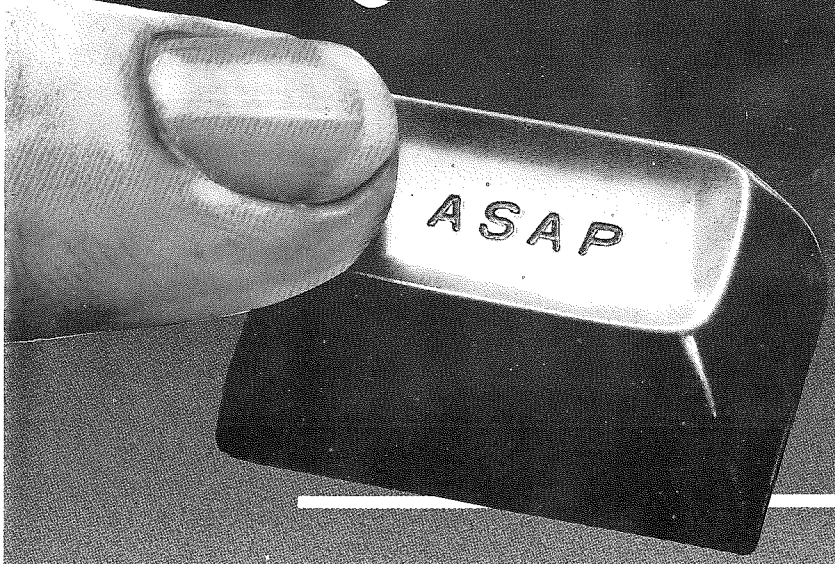

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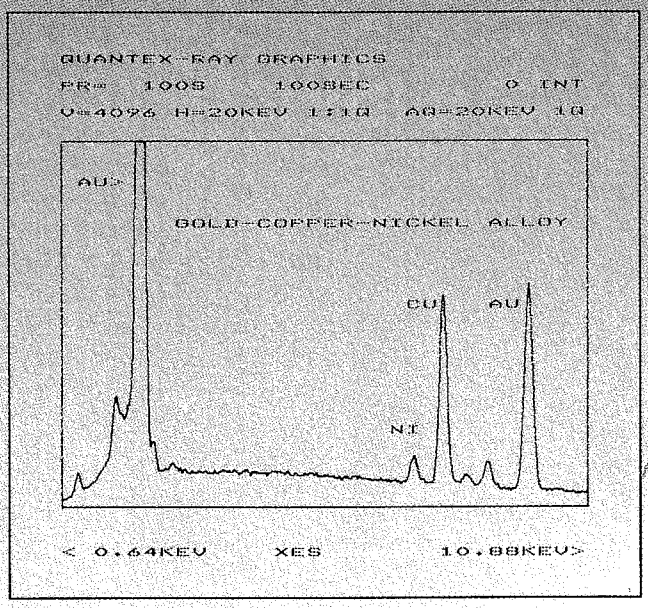

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AU MA	78.45	54.13	1.09	0.6932	
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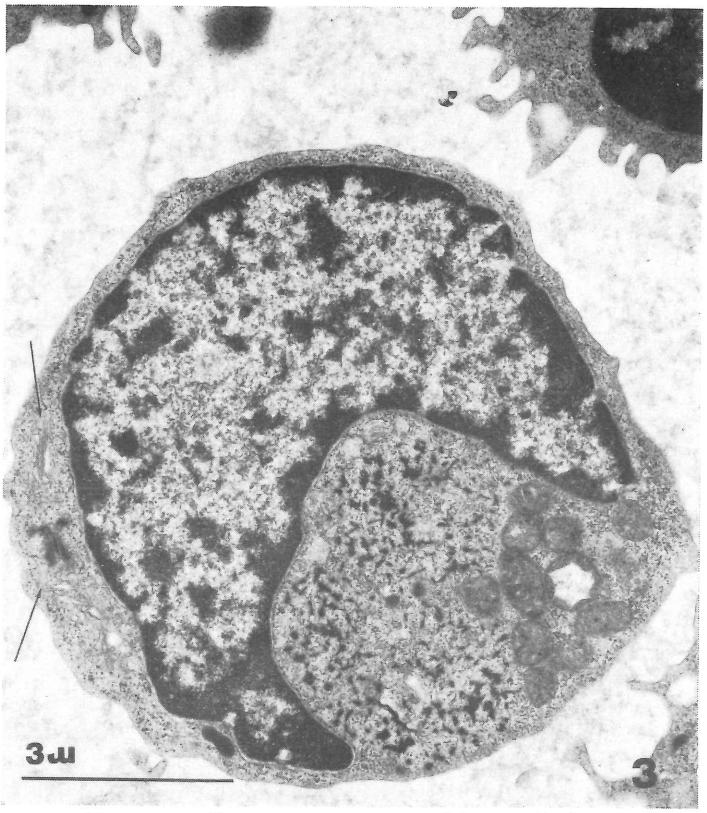

3. Paracrystalline aggregates in a blast cell of patient D. H. with AML. Centrosome and dictyosomes are on the opposite side of the nucleus (arrows).

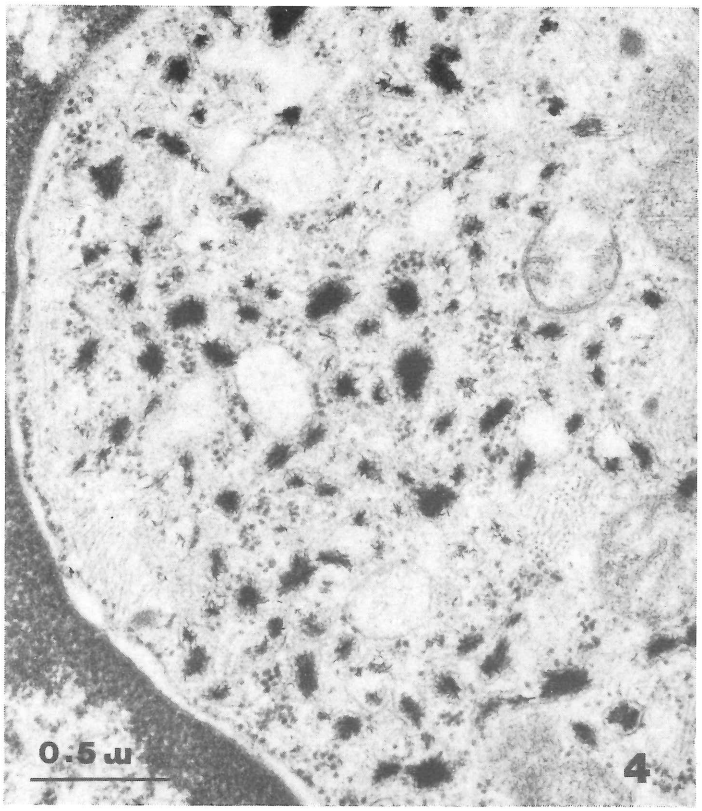

Figure 4. Patient E. K. Part of a blast cell with paracrystalline aggregates surrounded by a membrane. Bundles of fibrils abound in the cytoplasmic matrix.

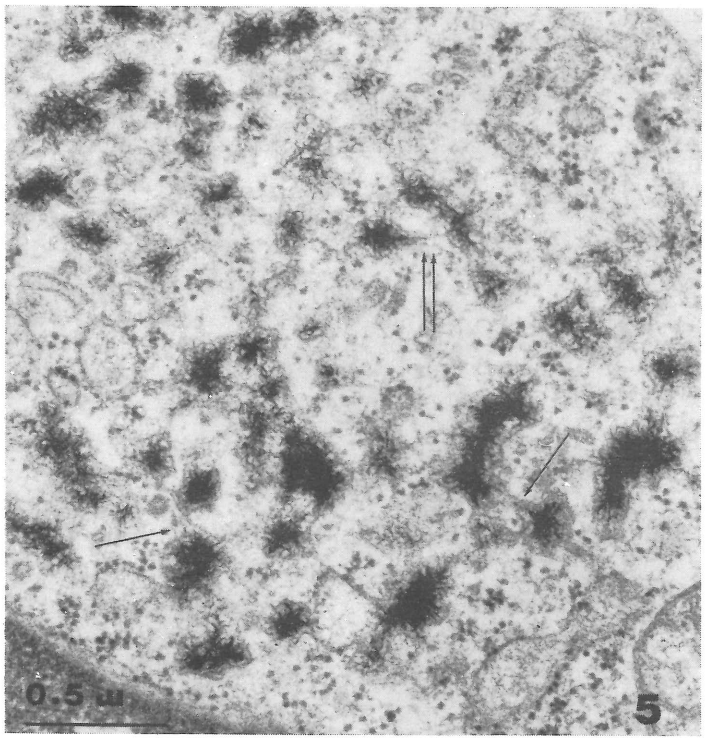

Figure 5. Patient E. K. The section is sufficiently thin to show the actual structure f paracrystalline aggregates. Endoplasmic membrane can be seen forming bridges between channels containing aggregates (arrows). A loose network of cytoplasmic fibrils (double arrows) connects some channels.

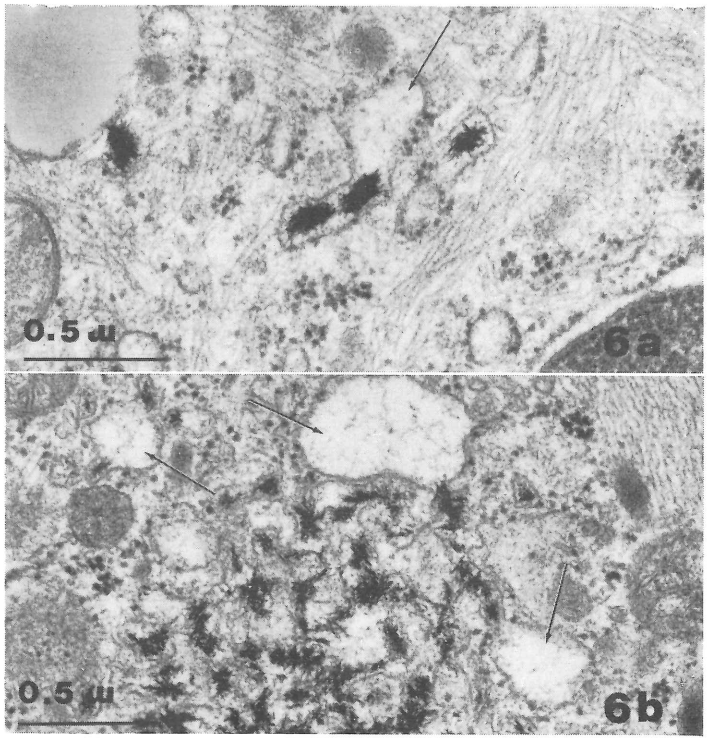

Figure 6. Patient E. K. Micrographs demonstrate the continuity of aggregate-containing channels with sacs of endoplasmic reticulum (arrows). Bundles of cytoplasmic fibrils run in all directions.

ated by x-ray probe analysis and other methods. A more difficult question is that of their origin. Did the inclusions preexist in live leukemic cells or were they the product of some enzymatic reaction during fixation? The latter is unlikely, since no reaction product similar to the paracrystalline aggregates has ever been observed in ultrastructural cytochemistry, especially hematology. Besides, cells of all the patients were processed in an identical fashion, yet only three patients showed aggregates. Thus, that paracrystalline aggregates, possibly of calcium phosphate are present in leukemic myeloblasts of some patients with acute leukemia appears a reasonable assumption. This assumption may lead to interesting questions concerning the metabolic activity of these myeloblasts. Finally, further research will be needed to determine whether the inclusions described here are distinct from or represent a particular form of the so-called B bodies observed in leukemic cells of 45% of the patients, mostly females, included in this electron microscopic study (1).

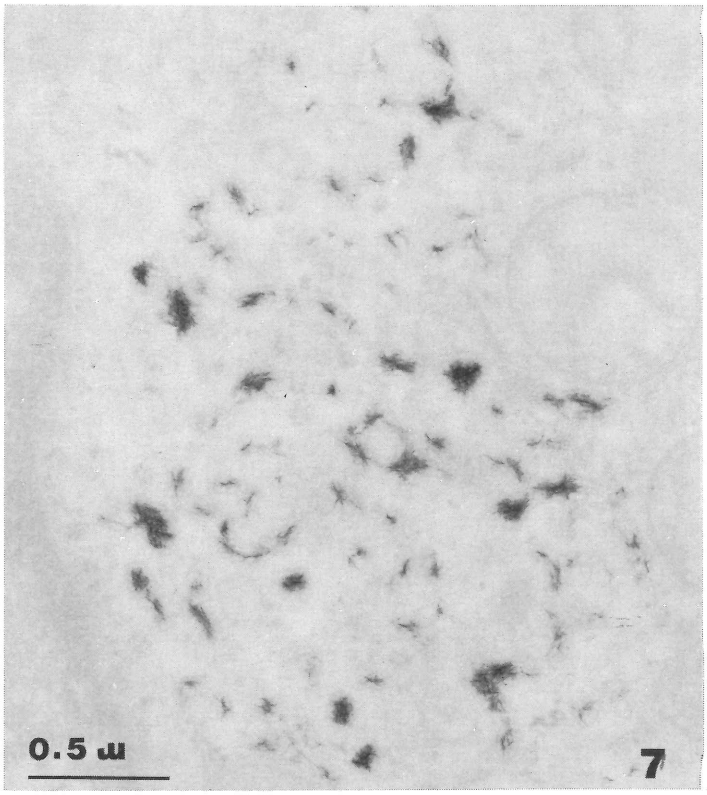

Figure 7. Patient E. K. Paracrystalline aggregates as seen is an unstained section of leukemic blast. The general disposition of the aggregates clearly reveals that they are contained in an endoplasmic channel system.

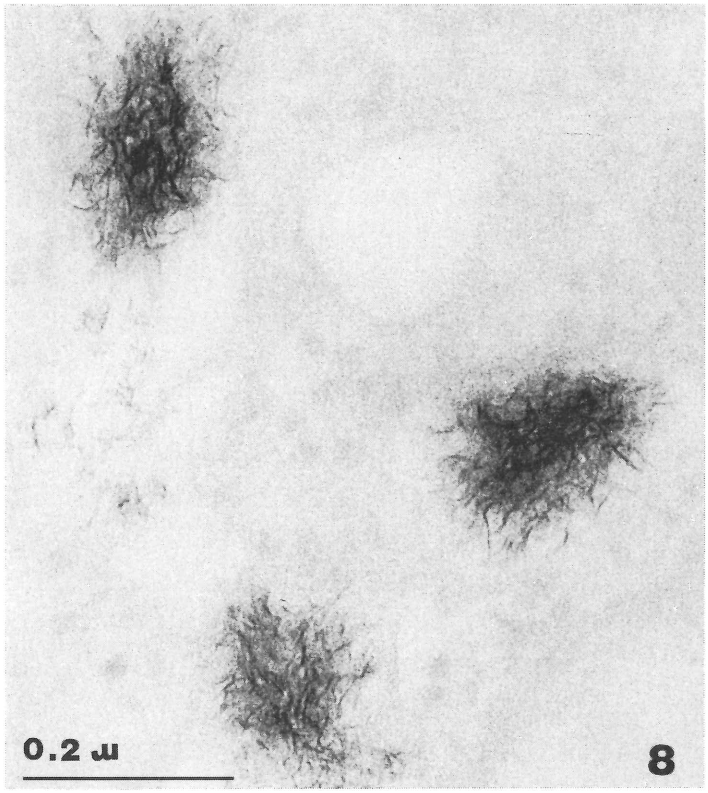

Figure j. Patient E. K. Very high magnification of paracrystalline aggregates in unstained section of a leukemic blast. The hairy paracrystals resemble hydroxyapatite of bone.

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ACKNOWLEDGEMENT

The skillful technical assistance of Cynthia Edwards and Tania Busch is gratefully acknowledged.

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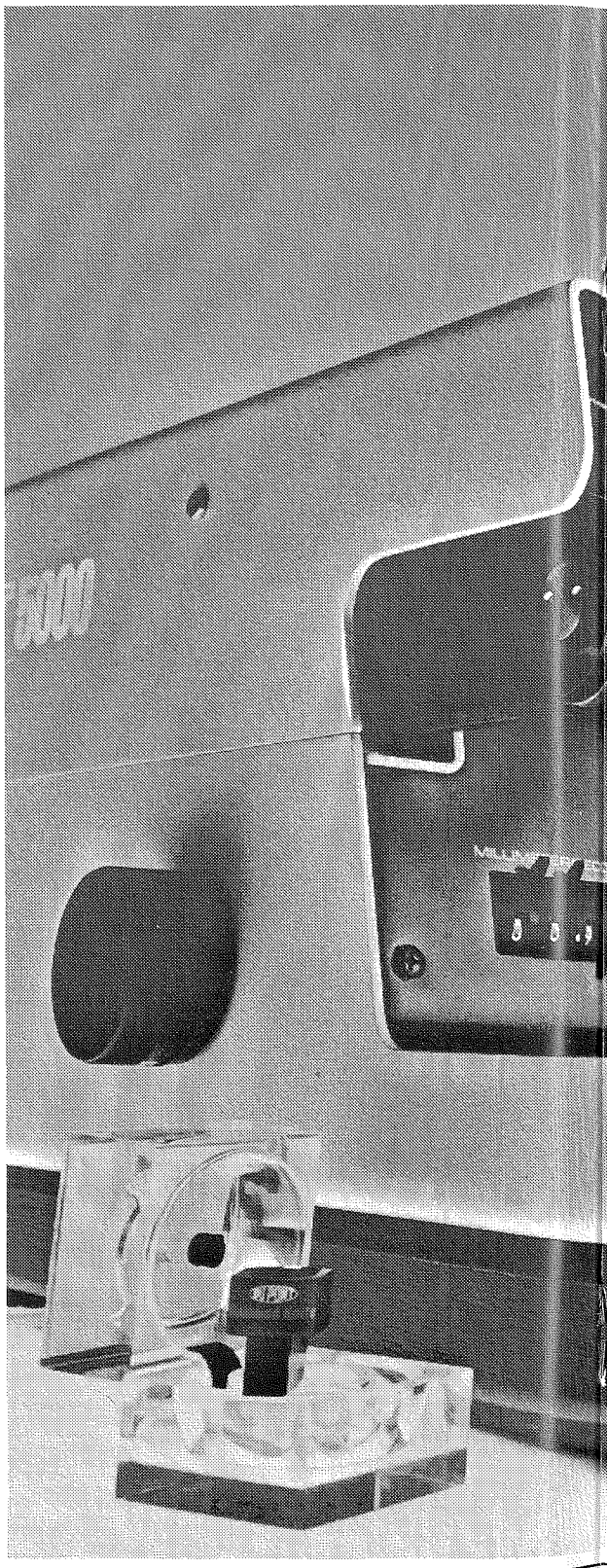

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Regional News

HOUSTON

THE UNIVERSITY OF TEXAS MEDICAL SCHOOL DEPARTMENT OF NEUROBIOLOGY AND ANATOMY

GRANTS AWARDED

Louise C. Moorhead, M.D., Research Scientist in Neurobiology and Anatomy, was recently awarded a research grant totaling $15,400 from the Retina Research Foundation. The title of the grant is "Medical Control of Vitreous Traction".

PUBLICATIONS

S. J. Enna, Ph.D., Professor of Pharmacology and Neurobiology and Anatomy, co-edited a book which was recently published by Chapman and Hall entitled "Neurotransmitter Receptors Part I (Amino Acids, Peptides and Benzodiazepines)".

NEW FACULTY AND/OR STAFF MEMBERS

The Department is pleased to welcome Dr. William Bennett who recently joined the staff as a Research Scientist. Dr. Bennett brought with him two research grants. He will be interacting with faculty members in ongoing research projects as well as continue his own research in the control of secretory mechanisms.

PARTICIPATION IN SCIENTIFIC MEETINGS/SEMINARS GIVEN ETC.

S. J. Enna, Ph.D., Professor, chaired a symposium entitled "Recent Developments Relating to the Basic and Clinical Pharmacology of GABAergic Agonists" and made a presentation, "Neurochemical Actions of GABAergic Agonists" at the annual Americal Society for Pharmacology and Experimental Therapeutics Meeting in Rochester, Minnesota.

Jon F. DeFrance, Ph.D., Associate Professor, attended the International Symposium on Nucleus Accumbens in Sebago, Maine September 14-20 to present current research on nucleus accumbens and to help prepare a record of the proceedings.

Dr. Stephen Massey, Postdoctoral Fellow, presented a paper entitled "The Light Evoked Release of ACH from the Rabbit Retina **in vivo**" at the 4th International Congress for Eye Research in New York this fall.

Joe G. Wood, Ph.D., Professor and Chairman, participated in the Annual Meeting of the Association of American Medical Colleges in Washington D.C. October 25-29.

Robert McClung, Ph.D., Assistant Professor, visited the JEOL Applications Laboratory in Boston this fall to observe and discuss analytical electron microscopy techniques and to bring back new information to other Departmental members.

Louise C. Moorhead, M.D., Research Scientist, presented an exhibit of current research at the American Academy of Ophthalmology Meeting in Chicago October 30-November 6.

The Department was well represented at this year's Society for Neuroscience Meeting in Cincinnati, Ohio. Andrea Elberger, Ph.D., Assistant Professor, presented a paper entitled "Permanent deficit in visual acuity following neonatal surgical section of the posterior corpus callosum in cats". Jon F. DeFrance, Ph.D., Associate Professor, presented a paper by DeFrance, Sikes, Palmer, and Chronister entitled "Frequency specific effects of dopamine in the nucleus accumbens". "Biochemical pharmacology of kojic amine, a GABA receptor agonist" was the title of a slide presentation by Michelle Browner, Research Technician. Co-authors were Ferkany and Enna.

David Kendall, Teaching Associate, conducted a poster session entitled "Effects of γ-vinyl GABA on chemically induced seizures in mice: correlation with brain GABA content. Work was by Kendall, Fox and Enna. Elaina Mann, Research Technician, presented "Neurochemical and behavioral correlates of antidepressant drug action" by Mann and Enna. "Thalamic projections to the cingulate cortex of rabbits" was the topic of Robert Sikes', Graduate Assistant, presentation co-authored by DeFrance. John Haycock, Ph.D., Instructor, presented "Regulation of tyrosine hydroxylase in isolated chromaffin cells: Effects of 8Br-cAMP and ACh on DEAE-sephacel elution patterns" by Haycock. George and Waymire. Research Assistant Gregory Fuller presented a paper by Fuller and Wiggins entitled "Myelin synthesis following chronic postnatal methylxanthine administration". A paper by Patsalos and Wiggins was presented by Philip N. Patsalos, Ph.D., Postdoctoral Fellow, entitled "Postnatal synthesis of myelin and brain subcellular membrane fractions following postnatal phenytoin administration". John G. Linner, Ph.D., Assistant Professor, presented "Immunoelectron microscopic localization of substance-P in synapses of the substantia gelatinosa" and Zehava Gottesfeld, Ph.D., Assistant Professor, presented two papers: "Increased muscarinic cholinergic receptors in the deafferented habenula" and "Noradrenergic innervation in the habenula". Stephen Massey, Ph.D., Postdoctoral Fellow, presented a paper by Massey and Redburn entitled "Spectral sensitivity of light evoked ACH Release from the rabbit retina" "Pain and dorsal raphe inputs to parafascicularis nucleus of the rat" was the title of a paper by Andersen, Rigor and Dafny and presented by Graduate Assistant, Eve Andersen. Finally, S. J. Enna, Professor, presented two lectures at a Satellite Symposium of the Neuroscience Meeting entitled "Receptor Binding Methods" and "Radioreceptor Assays".

S. J. Enna, Ph.D., Professor, traveled to Puerto Rico December 13-19 to attend the American College of Neuropsychopharmacology Annual Meeting and to present two symposium lectures entitled "Interactions of antidepressants with brain neurotransmitter receptors" and "Neurochemical alterations in dying".

David W. McCandless, Ph.D., Assistant Professor, presented a lecture "Coma: Friend or Foe" at Tulane University in New Orleans in January. Dr. McCandless also visited The University of Miami and gave a seminar entitled "Energy metabolism in the reticular formation in the metabolic encephalopathies."

Dianna A. Redburn, Ph.D., Associate Professor, was an invited speaker at the Winter Brain Conference in Keystone, Colorado and presented two papers: "Synapto-synaptic interaction in retina" and "Receptors for neurotransmitters in the retina".

GENERAL NEWS

S. J. Enna, Ph.D., Professor, is the recipient of the 1981 Alumni Achievement Award from the University of Missouri-Kansas City. Dr. Enna is being recognized primarily for his contributions in the areas of neuropharmacology and neurochemistry.

On December 26th Dr. Joe G. Wood, Professor and Chairman, appeared on a panel discussion with members of the Living Bank organization on a local television show "Houston Live". The topic of the discussion was organ and body donation to medical science.

Chairman, Dr. Joe G. Wood and Assistant Professor, John G. Linner, Ph.D. are organizing a Chili Cookoff to be held May 16th

in a fund raising endeavor for the Houston Area Parkinsonism Society.

HOUSTON

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

GRANTS AWARDED

Renewal of Dr. Ann Goldstein's NIH grant HL 17376 entitled "Analysis of Z bands in Heart by Optical Diffraction" for 5 years. This grant includes partial funding for a JEM 200 CX electron microscope.

LECTURERS

Dr. Ann Goldstein gave a seminar on the "Z Band in Striated Muscle" at Rush Medical College, Department of Physiology and also at University of Illinois Medical School, Department of Anatomy in October, 1980.

PUBLICATIONS

Goldstein, M.A., Stromer, M.H., Schroeter, J.P. and Sass, R.L., "Optical Reconstruction of Nemaline Rods." Exp. Neurol **70:** 83-97, 1980.

EQUIPMENT AND/OR FACILITIES

Dr. B. Van Winkle has purchased an LKB ultramicrotome for use in his studies of sarcoplasmic reticulum in muscle.

NEW FACULTY AND/OR STAFF MEMBERS

Dr. Laurel Traeger is a postdoctoral fellow in Dr. Goldstein's lab and is working on Z bands. She is funded by an NIH training grant. Dr. Joiner Cartwright, Jr. is a postdoctoral fellow of the Muscular Dystrophy Association and is working on microtubules in muscle in Dr. Goldstein's lab. Miss Kerry Paape is working as a technician in Dr. Goldstein's lab until she goes to medical school in 1981.

BAYLOR COLLEGE OF MEDICINE, DEPARTMENT OF MICROBIOLOGY

LECTURES

Dr. Heather Mayor presented a lecture on the structure of viruses at the 5th Biennial Conference on Diseases of the Vulva and Vagina sponsored by the Office of Continuing Education, Baylor College of Medicine.

PUBLICATIONS

"A computer graphics approach to investigating the architecture of icosahedral viruses" by Philip Hendren, Philip Mayor, John H. Chalmers, Jr. and Heather D. Mayor. Computers and Biomedical Research **13,** 581-600, 1980.

BAYLOR COLLEGE OF MEDICINE DEPARTMENT OF CELL BIOLOGY

GRANTS AWARDED

B. R. Brinkley, Ph.D., has received a grant from the National Cancer Institute and the National Institutes of Health entitled "Cytoskeleton and Cell Transformation to Malignancy."

LECTURES

At the American Society for Cell Biology meeting in Cincinnati, Ohio, November 14-18, 1980, Baylor College of Medicine was represented by B. R. Brinkley, Ph.D., S. Brenner, graduate student, J. Bryan, Ph.D., A. R. Means, Ph.D., R. L. Pardue, Ph.D., D. A. Pepper, graduate student, G. Perry, graduate student, D. S. Turner, research assistant, Lei-Lei Wang, graduate student and D. B. Zimmer, graduate student. G. Perry gave a paper entitled "Microtubule-Calmodulin Interactions." S. Brenner presented "Autoantibodies in Human Serum Selectively Bind to the Centriole Region in Cultured Cells." D. A. Pepper addressed the topic "**In situ** Detection of Calmodulin Binding Sites in Cells **in vitro:** Association with Mitochondria and the Cytoskeleton." D. B. Zimmer discussed "Microtubules and Transformation: A Quantitative Electron Microscope Study." L.-L. Wang spoke about "Calcium Mediated Regulation of Actin Assembly in Human Platelet Extracts."

At the November meeting on Motility in Dallas, sponsored by Baylor and UTHSC at Dallas, Dr. J. Bryan presented a paper entitled "Calcium Regulation in Microtubules in the Cytoskeleton." Dr. R. L. Pardue addressed "Muscle and Non-muscle Cell Motility."

Dr. R. L. Pardue traveled to Washington D.C., in December 1980 where he delivered a lecture on "Microtubules and Aging."

Dr. B. R. Brinkley and Dr. R. L. Pardue presented a paper at the ICN-UCLA Meeting, February 22-March 1, 1981 in Keystone, Colorado, which dealt with Chemical Carcinogenesis. They gave presentations at the Endocrine Workshop in Houston, Texas March 2-6, 1981 as well. Dr. Brinkley spoke about "Techniques for Indirect Immunofluorescence" and Dr. Pardue discussed the "Preparation of Immunocytochemical Probes." Dr. Brinkley also attended a seminar at the University of Michigan, April 7-8, 1981, where he talked about "The Regulation of Microtubule Assembly in Eukaryotic Cells."

PUBLICATIONS

Brenner, S., D. A. Pepper, D. Turner, A. E. Boyd, and B. R. Brinkley, 1980. Immunofluorescent and immunoelectron microscopic characterization of a human antibody to centrioles and pericentriolar material. J. Cell Biol. 87(2): 240.

Brinkley, B. R., D. A. Pepper, S. M. Cox, S. Fistel, S. L. Brenner, L. Wible, and R. L. Pardue, 1980. Characteristics of centriole-and kinetochore-associated microtubule assembly in mammalian cells, **In** Microtubules and Microtubule Inhibitors (M. DeBrabander and J. DeMay, eds.).

Brinkley, B. R., S. M. Cox, and D. A. Pepper, 1980. Microtubule-plasma membrane interactions: Analysis by tubulin immunofluorescence and electron microscopy, **In** Testicular Development, Structure and Function. (A. Steinberger and E. Steinberger, eds.).

Pardue, R. L., M. A. Kaetzel, S. H. Hahn, B. R. Brinkley, and J. R. Dedman, 1981. The identification of calmodulin binding sites on mitochondria in cultured 3T3 cells. Cell 23:533-542.

Perry, G., B. R. Brinkley, and J. Bryan, 1981. Interaction of calcium-calmodulin in microtubule assembly **in vitro, In** Muscle and Cell Motility, Vol. 2, (R. M. Dowben and J. W. Shay, eds.).

NEW EQUIPMENT AND/OR FACILITIES

1. Dage Low-Level Light Camera model 650 SVX for Zeiss Photomicroscope
2. Dec Type LSI-11 computer
3. Hamatsu Model C1000 type 12 TV camera/photomicroscope with Panasonic Time-lapse VTR NV 8030 Video Recorder
4. Ortho Cytofluorograf model 50-H 164-05 Argon Laser

NEW FACULTY AND/OR STAFF MEMBERS

B. R. Brinkley, Ph.D., has been elected President of the International Federation for Cell Biology from 1980 to 1984. The next meeting of the Federation will be in Toyko in 1984.

Leo Simone, Ph.D., is investigating the effects of taxol on

cultured CHO cells as a visiting faculty member from the State University of New York, College at Potsdam.

R. L. Pardue, Ph.D., is a new instructor in the Department of Cell Biology. He will teach Cell Biology to graduate and medical students.

G. R. Edwards, M.S., is the new supervisor of the Electron Microscope Core Facility directed by Dr. B. R. Brinkley, Department of Cell Biology.

EM EDUCATION POSSIBILITIES

B. R. Brinkley, Ph.D., will teach a course entitled "Cell Biology and Histology" to graduate and medical students beginning in July 1981.

JOB OPPORTUNITIES

Position Sought: Asst. Prof. Cell Biologist with 3 years post-doctoral experience. Extensive electron microscopy, immunology, and good biochemical background. Interested in research, teaching or industry. Available 1981. Contact editor.

TYLER

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

LECTURES

Dr. S. Donald Greenberg, Professor of Pathology at Baylor College of Medicine, was an invited lecturer and visiting scientist in the Department of Cell Biology and Environmental Sciences on November 12-15, 1980. Dr. Greenberg's lecture was entitled "Sputum Cytopathology." Dr. Greenberg is involved as a coinvestigator with ongoing projects in the Department.

Dr. E. Clinton Lawrence, Assistant Professor of Medicine at Baylor College of Medicine, presented a seminar entitled "Bronchoalveolar Lavage" on December 5, 1980 and discussed collaborative efforts with the Department's staff.

Dr. Russell Martin, Professor of Medicine at Baylor College of Medicine, visited in the Department on February 10, 1981 to discuss the status of collaborative investigations involving his division and members of the Department concerning reactions of lavaged macrophages with particulate matter.

PUBLICATIONS

Dodson, R. F., Williams, M. G., and Hurst, G. A. 1980. A comparison of ultrastructural characteristics of bronchoalveolar macrophages in perfused parenchyma with those obtained by a fixative or physiological saline lavage. Cytobios 27:107-111.

Dodson, R. F., Williams, M. G., and Hurst, G. A. 1980. Early response of free airway cells to "Amosite": A correlated study using electron microscopy and energy dispersive x-ray analysis. Lung 157:143-154.

Spatz, M., Bembry, J., Dodson, R. R., Hervonen, H., and Murray, M. R. 1980. Endothelial cell cultures derived from isolated cerebral microvessels. Brain Research 191:577-582.

NEW EQUIPMENT

The following new equipment has been added to the laboratory:

JEM-100CX Temscan Analytical Electron Microscope
Zeiss Videoplan Image Analysis System
Tracor Northern TN-2000 Energy Dispersive System
IEC Cryostat

NEW FACULTY AND STAFF

Tomotoshi Akematsu, M.D., a visiting Clinical Pathologist, has joined the Department as a Postdoctoral Research Associate.

Joan Ford has joined the Department as an EM Researcher.

Carolyn Tuley has joined the Department as Administrative Assistant.

TEMPLE

Scott & White Clinic

LECTURES

Dr. William B. McCombs was an invited speaker at seminar in December given by the Department of Cell Biology, Mayo Clinic, Rochester, Minnesota. His topic was "Rapid Diagnosis of Viral Diseases".

GRANTS

O. D. Holton and Thomas Runyon, Tissue Culture Studies on Ciliary Body Epithelium, $27,000, funded by Scott & White Research Department.

O. D. Holton and W. B. McCombs, III, Isolation and Clinical Characterization of a Cell Line (SW 527) Derived Human Breast Tumor Associated Antigen, renewal for $20,000.

DALLAS

UNIVERSITY OF TEXAS HEALTH SCIENCE CENTER, DEPARTMENT OF PATHOLOGY

NEW FACULTY AND/OR STAFF MEMBERS

The Pathology Department welcomes the addition of Roger Gleason to Dr. Mary Lipscomb's lab. He was previously at Texas A&M.

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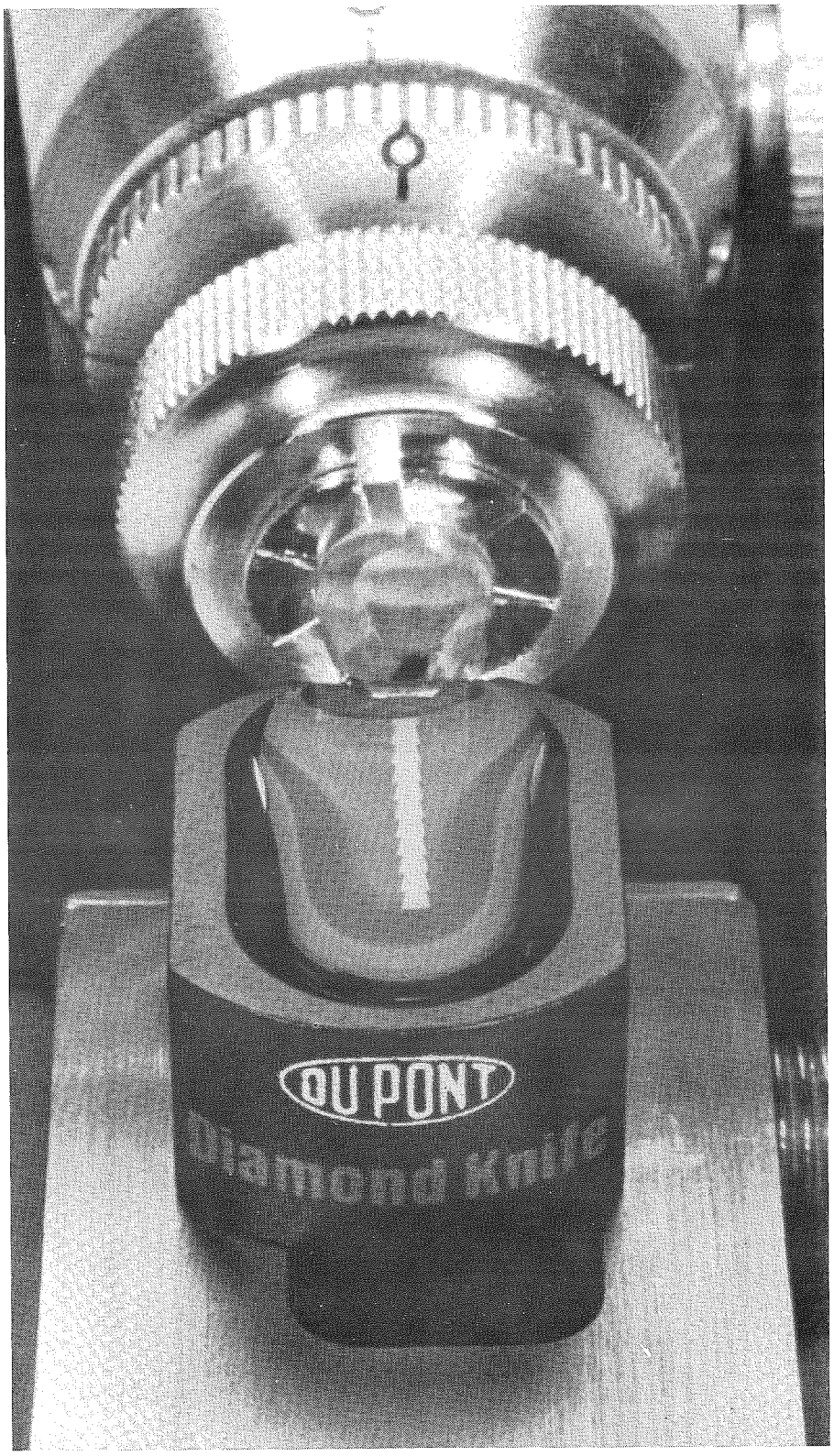

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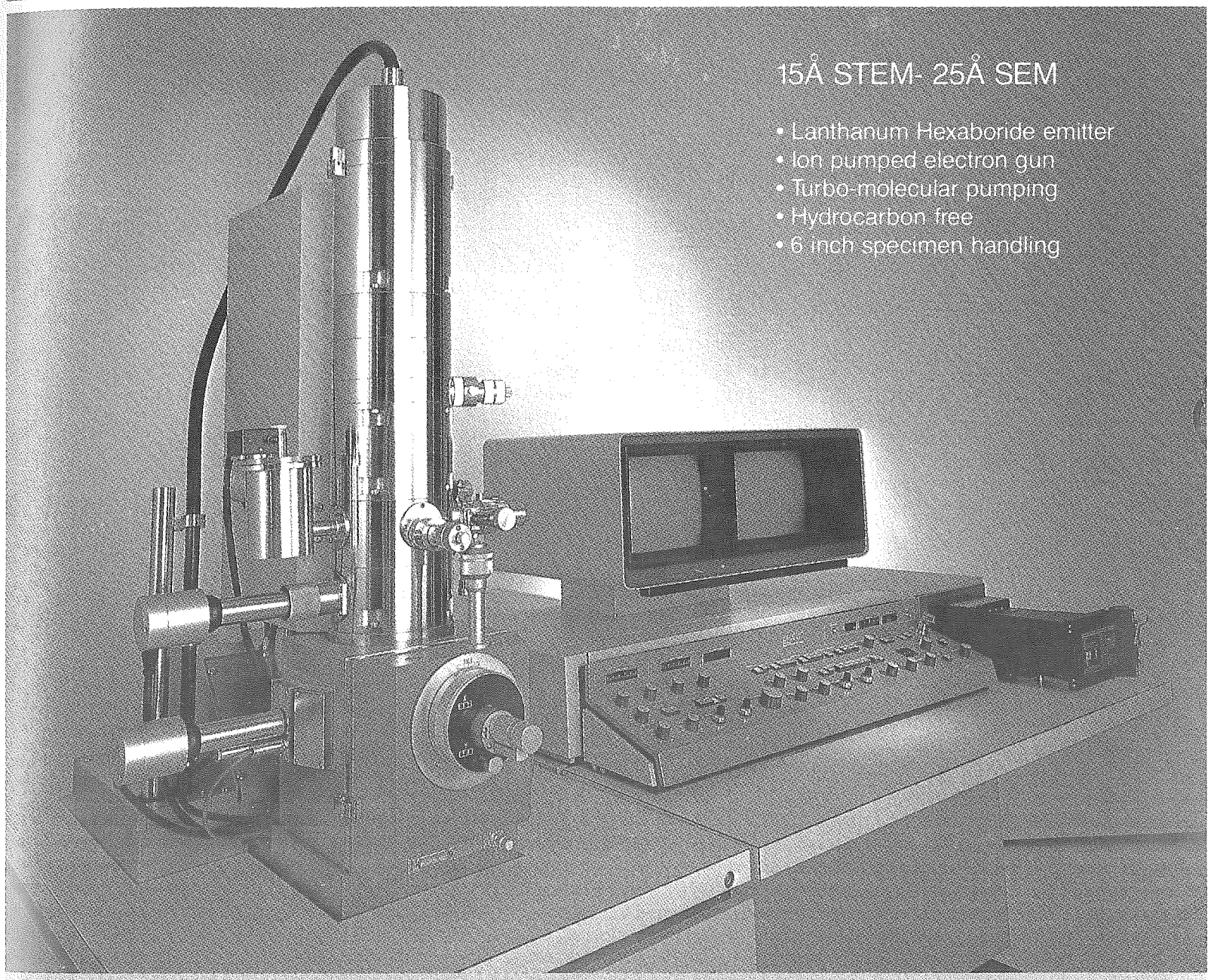

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PAST-PRESIDENTIAL SYMPOSIUM

The Use of the Electron Microscope in Medical Diagnosis

Chairman: William B. McCombs, III

HEPATIC DISEASE PROGRESSION ASSOCIATED WITH TRACE METAL OVERLOAD: ULTRASTRUCTURE AND X-RAY MICROANALYSIS STUDIES, S. S. Barham, R. H. Wiesner, and E. R. Dickson, Department of Cell Biology and Division of Gastroenterology, Mayo Clinic, Rochester, MN 55901.

Copper is thought to be hepatotoxic in Wilson's disease (WD) and in the Bedlington terrier dog (BT). The relationship of hepatic copper elevation to hepatic injury in primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) remains uncertain. The present study was initiated to determine hepatic copper levels and cellular and subcellular copper distribution during histologic progression of PBC and following treatment with D-penicillamine. Hepatic copper elevation and cellular and subcellular distribution were also sudied in PSC. Liver biopsies from 24 patients with PBC and 12 patients with PSC were analyzed by histologic copper staining, transmission electron microscopy, and x-ray energy spectroscopy. Total hepatic copper was measured by atomic absorbtion spectrometry. In liver sections from both PBC and PSC patients copper was elevated above normal levels and distributed throughout the hepatocyte but was further concentrated within lysosomes as has been observed in WD. Copper was observed to become progressively more concentrated within lysosomes as PBC progresses histologically, as noted previously in WD. Following D-penicillamine therapy in PBC, hepatic lysosomal copper was noted to remain relatively constant while extralysosomal cellular copper was noted to decrease. Hepatic copper elevation and cellular and subcellular distribution of copper were found to be similar in PBC, PSC and WD. This suggests that copper may be hepatotoxic in PBC and PSC.

THE USE OF THE ELECTRON MICROSCOPE IN THE RAPID DIAGNOSIS OF VIRAL DISEASES, William B. McCombs, III, Section of Microbiology, Scott and White Clinic, Temple, Texas 76501.

Until recently the identification of bacterial pathogens was assumed to be faster than the identification of viral pathogens. It was thought that a patient would either recover or die before a virus could be identified. Thus, a viral diagnosis was dismissed as being merely of academic interest and an unnecessary expense for the patient.

The aim of a rapid viral diagnosis should be to give an answer within a few hours following admission of the patient to the hospital. We have set our goals at Scott and White to have a diagnosis by the time the bacterial culture results are available, usually 24-48 hours after the specimen is obtained.

Several EM techniques have been developed and will be discussed. These include: the rapid embedding technique, the **in situ** embedding technique, the pseudoreplica technique, and immunoelectron microscopy. These techniques have been especially useful in the diagnosis of infections of the fetus and newborn, the immunosuppressed patient, herpes encephalitis cases, and infant diarrhea.

X-RAY ANALYSIS AND THE ELECTRON MICROSCOPE: AN IMPORTANT TOOL FOR MEDICAL RESEARCH AND DIAGNOSIS, Herbert K. Hagler, University of Texas Health Science Center at Dallas, Dallas, TX 75235.

Analytical electron microscopy is an important tool for use in medical research and diagnosis. A review of some of the basic principles of x-ray production, detection and analysis will be presented. The applications of the x-ray equipped transmission electron microscope and an automated scanning electron microscope will be reviewed. The analytical microscope's use in basic research to study electrolyte shifts with cell injury and the emergence of the automated scanning microscope for identification of particulate inclusions in biopsy material and its importance in diagnosis will be discussed. The field of analytical microscopy presents many new challenges for microscopists and increases the importance of microscopy in answering complex research and diagnostic questions.

ELECTRON MICROSCROPY IN TUMOR DIAGNOSIS, Bruce Mackay, M.D. Anderson Hospital and Tumor Institute, Houston, Texas.

Transmission electron microscropy has become accepted as an important aid to the pathologist in the identification of human tumors that cannot be classified by conventional light microscropy, and examples of its application from the following groups of neoplasms will be illustrated and discussed: Small round cell tumors, lymphoid neoplasms, melanoma, carcinomas, and sarcomas. Technical methods are particularly important, as the return from a diagnostic TEM study is directly related to the material that is provided; it must be representative, free from artifact, and properly processed. Pathologists must make full use of the available information from light microscropy on a particular case, including histochemistry where appropriate, and also the clinical data. Because of the small quantity of tissue needed for EM study, procedures such as fine needle aspiration biopsies are suitable provided the material obtained is representative. The pathologist/electron microscropist must work closely with the clinician to insure that appropriate tissue is procured and promptly and correctly processed. Interpretation of the EM findings require not only a familiarity with a range of the light microscropic appearances of human tumors, but in addition an understanding of the spectrum of the structure that these tumors may display at varying levels of differentiation, and the available information is at the present time woefully inadequate.

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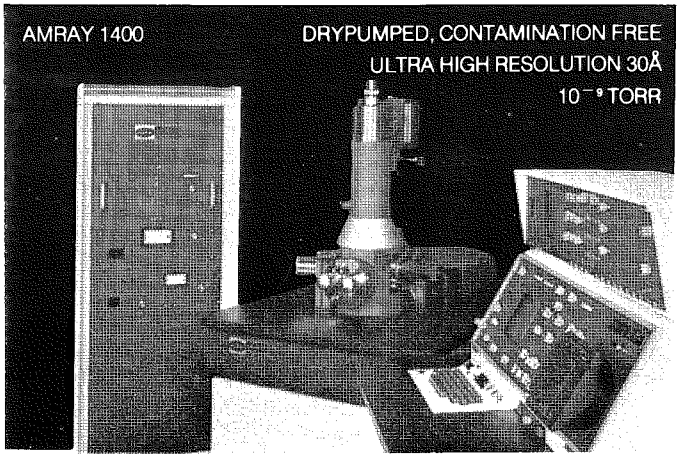

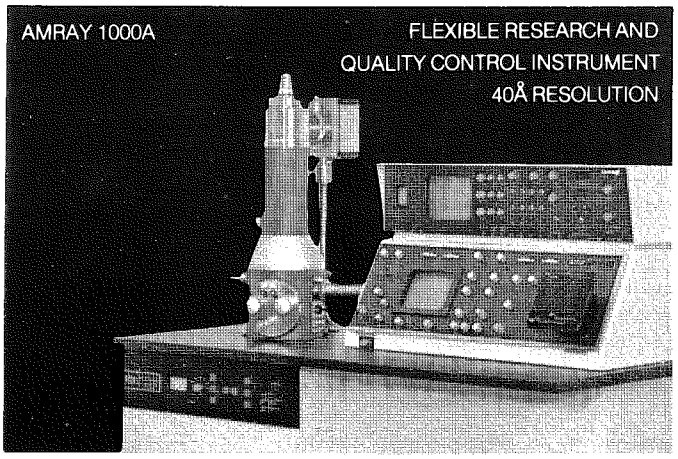

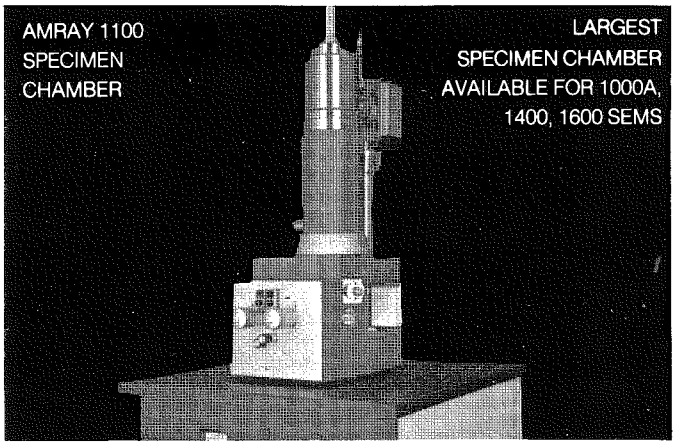

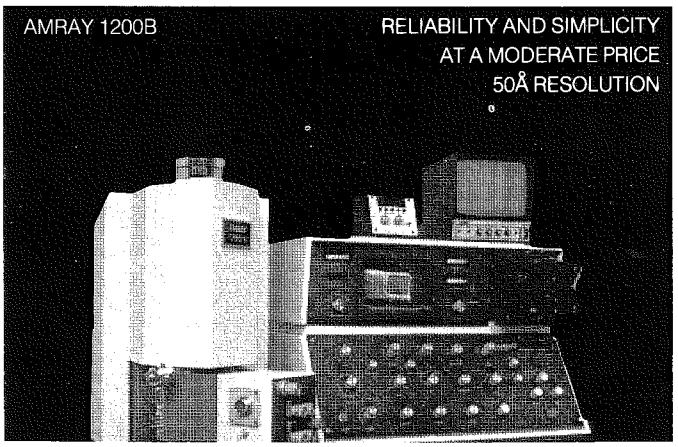

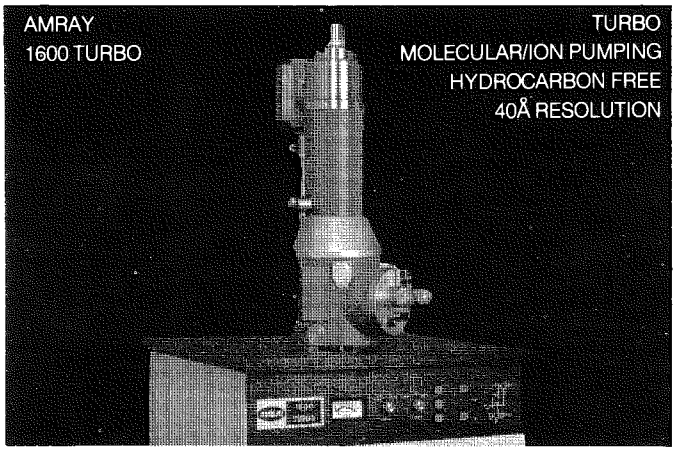

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Abstracts

MORPHOLOGY OF CLAYS. W. O. Milligan, The Robert A. Welch Foundation and Baylor University.

The morphology and structure of several natural clays will be delineated by electron microscopic and electron diffraction methods. Clays such as kaolinite, halloysite and others will be discussed, as well as a few soil samples. Shadow casting will be included in the electron microscopic studies and selected area diffraction will be included in some of the electron diffraction results. "Mountain Cork" will be demonstrated to show individual layers of about 10 A° in thickness. Rolled sheets of halloysite will be presented, illustrating hollow tubes comprising the halloysite.

AN ULTRASTRUCTURAL STUDY OF THE CEPHALIC REGION OF DROSOPHILA LARVAE. Ted D. Mullins and Randy Moore, Biology Department, Baylor University, Waco, Texas 76798.

Drosophila melanogaster is well known for its application in genetical studies. **Drosophila** larvae also provide an excellent system for studying tissue development and ultrastructure, since several different types of tissues are located in close proximity to one another. In this study, the cephalic region of the third instar larvae of **Drosophila** was examined in order to document the ultrastructure of some of the tissues present at this stage of development. The cephalic portion ofthe larvae consists primarily of muscle tissue. The bandings characteristic of muscle tissue were absent in longitudinal section. However, actin and myosin myofibrils were observed in cross section and were present at a ratio of 10 actin myofibrils to one myosin myofibril. Lesser amounts of nervous tissue were also present in the cephalic region of the larvae. This tissue exhibited typical cellular ultrastructure, and was characterized by the presence of oligodendrocytes, nerve cell processes, and axo-dendritic synapses. Microtubules were also abundant in the nerve cell processes and, to a lesser extent, in the oligodendrocytes.

VEGETATIVE COMPATIBILITY/INCOMPATIBILITY IN HIGHER PLANTS. II. THE INCOMPATIBLE HETEROGRAFT BETWEEN SEDUM TELEPHOIDES AND SOLANUM PENNELLII. Randy Moore, Biology Department, Baylor University, Waco, Texas 76798.

An ontogenetic study of a shoot heterograft between **Sedum telephoides** and **Solanum pennellii** was performed in order to characterize the cellular events that occur during an incompatible graft union. Adhesion of the stock and scion occurred by 21 hrs. after grafting and was correlated with an accumulation of dictyosomes in cells at the graft interface. The graft incision triggered a wound response in cells of both partners along the graft interface that was characterized by: 1) a reorientation of cytoplasm in cells adjacent to the cut surfaces, 2) deposition of cell wall, 3) starch accumulation, 4) a reduced staining intensity of the cytoplasm, and 5) callus proliferation. While **Solanum** cells recovered from the effects of wounding at an early and non-lethal stage, **Sedum** cells bordering the graft interface underwent a lethal cellular senescence characterized by: 1) cell wall suberization, 2) vesiculation of the cytoplasm, 3) degeneration of cellular organelles, 4) loss of membrane integrity, and finally 5) death and collapse of the cell. Thus, cellular necrosis in the **Sedum** partner characterized the incompatibility response between **Sedum** and **Solanum** and resulted in formation of a necrotic layer of collapsed cells and cytoplasmic remnants at the graft interface. This necrotic layer was never ruptured by callus proliferation and consisted of up to 8-10 collapsed cells by 10-14 days after grafting. Vascular redifferentiation did not occur in the callus masses at the graft interface. **Sedum** abscission occurred by 3-5 weeks after grafting, presumably due to desiccation of tissues. The possible roles of toxins that may elicit the incompatibility response and of a direct cellular interaction that may to procambial redifferentiation are discussed.

INTERCELLULAR BRIDGES AND CYTOPLASMIC CONTINUITY IN THE DEVELOPING SQUID EMBRYO, Joiner Cartwright, Jr., Baylor College of Medicine, Department of Medicine, Section of Cardiovascular Sciences, Houston, Texas 77030.

In the post-gastrulation embryo of the Atlantic squid, **Loligo pealei,** the cells of the developing blastoderm are joined together by intercellular bridges that very closely resemble those joining gonadal cells of many diverse species. In the squid embryo the cells are joined in groups that closely coincide with developing organ primordia. These bridges result from incomplete cytokinesis after mitosis followed by the breakdown of the spindle remnant and establishment of cytoplasmic continuity between cells. Cytokinesis ceases as the furrow base reaches a diameter slightly larger than the midbody. As furrowing stops, a dense material accumulates to form a cylindrical sheath 50 nm thick, lining the inner surface of the furrow base. Treatment of thin sections with proteolytic enzymes shows this material to have a significant protein component. As the midbody breaks down, vesicles line the inner surface of the bridge sheath. In this configuration, there is a channel 0.5 μm-0.6 μm in diameter through which cytoplasmic organelles can pass between cells.

When a cell within a group enters mitosis, the bridges already associated with that cell become temporarily closed. Vesicles enter the channel and fuse with those lining the inner surface of the sheath. The vesicles enlarge until the channel becomes occluded by a series of transverse cisternae attached to the sheath. After mitosis the transverse cisternae disassociate from the sheath and breakdown and the bridge reopens.

Introduced by M. A. Goldstein, Ph.D.

SCANNING ELECTRON MICROSCOPY (SEM) OF WOUND HEALING AND STOMATOGENESIS IN A REGENERATING CILIATE. John A. Garson. Dept. of Biology, Texas Christian University, Fort Worth, TX 76129, U.S.A.

Following postperistomal glass needle transection of the heterotrich ciliate, **Blepharisma japonicum,** SEM reveals complete loss of cortical ridges, kineties, and somatic cilia in the wound area. Wound healing is accomplished by constriction or involution of the wound perimeter followed by kinety fusion. Extension of kineties or ciliogenesis was not observed at the wound. Regenerative stomatogenesis of the astomatic cell fragments proceeds **extra situ** beginning ventrally at the primary ciliogenic kinety, the most central branched kinety. Evidence suggests somatic cilia are resorbed, accompanied by the ap-

pearance of groups of two to four closely aligned (1.30 μm apart), periodic, sprouting cilia. Often a decreasing height gradient (posterior to anterior) is observed in each group's cilia. This indicates the initial cilium in each group may act as a ciliogenic locus to direct assembly of each new successive cilium. Other kineties, beginning at the central branch, join in ciliogenesis. Later the inter-kinety ridge between adjacent ciliogenic kineties descends to form a rough-surfaced groove. Only then does ciliogenesis proceed in the inter-kinety zone. This indicates sub-pellicular structures are removed to permit unobstructed ciliogenesis. The adoral zone membranelles (AZM) are progressively assembled by the alignment of short cilia into equidistant transverse rows. Newly sprouted cilia are incorporated into the left row end, suggesting a predominance of ciliogenesis on the left side. Later the ciliogenesis of the undulating membrane and ciliary floor membrane begins, but confined to a kinety at the right side of the widening mouth. Complete ciliary rows of the developing AZM, previously equidistant, migrate together into groups of three to compose each membranelle. The cytopharynx develops from a triangular patch of short cilia as the cortical ridges to the left and right of the mouth curve and fuse. (Supported by NIH grant #GM-23878 to Dr. R. A. Jenkins, Dept. of Zoology and Physiology, University of Wyoming, Laramie, WY).

ULTRASTRUCTURAL EVIDENCE FOR A PURSE-STRING CONTRACTILE FORCE LOCATED IN THE EPIDERMIS SURROUNDING AN EXPERIMENTAL WOUND, Paul S. Baur, Jr., Ph.D.[1,2], George F. Barratt, M.S.[1], J. Darrell Hudson, M.S.[1], Donald H. Parks, M.D.[2], [1]Graduate School of Biomedical Sciences, University of Texas Medical Branch, Galveston, Texas 77550, [2]Shriners-Burns Institute, 610 Texas Avenue, Galveston, Texas 77550.

Wound edges are drawn together by a phenomenon known as wound contraction. Dermal myofibroblasts are thought to be the cells that mediate this contractile force. However, up to 25% of the surface area of a wound may close in the first few days, long before significant numbers of myofibroblasts can be found in the wound bed. This suggests that another contractile force may exist in the margins of the wound tissue. A contractile band comprised of fusiform shaped epidermal cells appears to mediate this other contractile force. The cells have been found to be circumferentially distributed with respect to and located within the wound margin. Microfilaments, similar in size to actin filaments and/or tonofilaments, can be observed in the cells comprising this band. The filaments appear to be aligned with the long axis of the "myoepithelial" initially involving cells found in the epidermis followed by the myofibroblasts of the dermis.

AN SEM STUDY OF AECIOSPORE FORMATION IN THE RUST FUNGUS PUCCINIA BOLLEYANA. Charles W. Mims, Department of Biology, Stephen F. Austin State University, Nacogdoches, Texas 75961.

Aecia of **P. bolleyana** are produced primarily on the under surface of infected leaves of **Sambucus canadensis.** The aecium is a cup-like depression bounded by a peridium consisting of a single layer of thick walled cells. The aecium is filled with chains of developing and mature aeciospores. Lining the base of the aecium are many cylindrical sporogenous cells that give use to a succession of aeciospore mother cells. Once formed the mother cell enlarges slightly and undergoes an unequal division to form a larger cell and a smaller disjunctor cell that remains attached to the larger cell. The disjunctor cell eventually dies while the larger cell develops into an aeciospore. The surface of the aeciospore is at first smooth but large surface granules eventually develop in a ring around either the middle or more typically the distal end of the spore. The primary wall of the spore disintegrates and is sloughed off the spore to reveal many small, cog-like surface ornamentations. Such ornamentations are, however, absent from the distal end of the spore in the region at which the spore contacted the disjunctor cell of the next spore in the chain.

AN ULTRASTRUCTURAL STUDY OF OVARIAN TISSUE IN PASPALUM DILATATUM GROWN IN VITRO. Denise Roper and Randy Moore, Biology Department, Baylor University, Waco, Texas 76798.

Cellular and tissue interactions are a basic aspect of eukaryotic growth and development. Plant tissue culture provides an excellent system for studying differentiation and organogenesis in vitro as they relate to various developmental processes in the intact organism. The purpose of this study was to document the ultrastructural changes that occur in ovarian cells of **Paspalum** throughout floral development. Excised carpels from immature, mature, and flowering stages were grown in vitro on a modified Murishige and Skoog medium and compared with control samples grown in vivo at similar developmental stages. Cells from cultured ovaries were distinguishable from those grown in vitro by 1) increased levels of cell division, 2) thin cell walls 3) increased vacuolation, and 4) fewer numbers of cellular organelles. In spite of these differences, however, the ovaries maintained a vitro appeared healthy and potentially functional. Ontogenesis of the ovarian tissue as well as experiments to determine any possible functional effects of an vitro culture will be discussed.

ULTRASTRUCTURAL ASPECTS OF GEOREACTION IN LATERAL ROOTS OF PHASEOLUS VULGARIS. Steve Ransom and Randy Moore, Biology Department, Baylor University, Waco, Texas 76798.

Special cells (statocytes) have been shown to be responsible for gravity perception in the primary roots of many plants. These statocytes, which are located in the rootcap columella, contain special starch storage organelles called amyloplasts. Amyloplasts sediment under the influence of gravity and have been strongly implicated as one of the primary organelles responsible for gravity perception in roots. In contrast to the large body of work on primary roots, georeaction in lateral roots has been little investigated and is poorly understood. In this study, lateral roots of **Phaseolus** were fixed **in situ** and examined with light and electron microscopy. The presence, distribution, and orientation of the amyloplasts were noted and correlated with the direction of the incident gravitational vector. The root caps of the lateral roots possess gravity sensing cells. These cells are characterized by the presence of many amyloplasts. The subcellular distribution of these "gravity detecting" organelles as related to root orientation and experimental manipulation will be discussed.

MORPHOMETRIC ANALYSIS OF PALISADE CELLS IN WATER STRESSED COTTON. J. D. Berlin, J. E. Quisenberry, B. L. McMichael, and F. Bailey, Department of Biological Sciences, Texas Tech University, Lubbock, Texas 79409; AR-SEA, USDA, Lubbock, Texas 79401; and AR-SEA, USDA, Plant and Soil Sciences Department, Texas Tech University, Lubbock, Texas 79409.

Sterological techniques were used to evaluate the effect of water stress on cotton leaves. Field-grown leaves of paymaster 266 were collected July 31, 1980 from irrigated and nonirrigated plants (leaf water potentials of the midday collections averaged

-13.5 and -26.5 bars, respectively). The samples were initially fixed in glutaraldehyde and routinely processed for light and electron microscopic examination. Quantitative analysis of subsequent micrographs of palisade cells revealed stress-induced changes in numerous subcellular components when reported as fractional volume (i.e., % cell volume), absolute volumes (μm^3 per cell), number per cell or volume of individual organelles. For example, the number of chloroplasts, mitochondria and peroxisomes per cell was increased by water stress. The mean size of individual chloroplasts and mitochondria was increased by stress whereas peroxisomes were smaller. The water stress imposed in this study resulted in larger palisade cells but there were fewer palisade cells per leaf. Thus, the decrease in cell number (presumably due to a mitotic sensitivity to stress) was partially compensated for by an increased cell volume.

SCANNING ELECTRON MICROSCOPY OF COTTON FIBERS. Jerry D. Berlin and J. F. Bailey, Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409.

Cotton fiber samples were examined with the scanning electron microscope to find treatment-induced surface changes which might alter the processing characteristics. The examinations were made on a "blind" basis to exclude biased interpretations. Samples were fixed to specimen stubs, coated with gold, and photographed at magnifications from 200 to 5,000 diameters. Several differences were apparent in the external areas of the fibers. Untreated and unwashed fiber had typical convolutions and surface features, including spiral ridges and grooves, reversals, and various types of folds. Washed fibers were clean, possessed the above features, and apparently lacked the outer cuticular or primary cell wall. Washed fibers treated to enhance their spinning properties had a surface layer that frequently peeled off in patches.

This work was supported by Cotton Incorporated.

A COMPARISON OF TANNIC ACID GLUTARALDEHYDE (TAG)-FIXED AND GLUTARALDEHYDE-FIXED POLYMORPHONUCLEAR LEUKOCYTES (PMN) AND GRANULES. W. Allen Shannon, Jr., Daniel M. Zellmer, William J. Brown, and William J. Snell, Veterans Administration Hospital and The University of Texas Health Science Center at Dallas.

Tannic acid in conjunction with glutaraldehyde (Saito et al., J. Cell Biol. 79: 601, 1978) has been used to reveal PMN membrane structure not apparent in conventional, glutaraldehyde and osmium-fixed materials.

TAG-fixation revealed a greater quantity of membrane-associated material, i.e., protein, and more refined detail than did other types of fixation. The trilaminar appearance of the plasma membrane exhibited enhanced density and finer resolution. The mitochondrial cristae were more distinct, and the membranes of the granules were more readily apparent. Granular matrix was elucidated that was not discernable by glutaraldehyde fixation alone.

TAG-fixed cell membranes averaged 70 A thick with a 30 A dense coating. At least 4 rather distinct azurophil (AG)-type granules and 5 specific (SG)-type granules were observed in granule fractions. Membrane coating measured 114 to 157 in AG vs 77 to 117 in SG. Glutaraldehyde-fixed cell membranes averaged 77 A thick with no apparent coating retained on the membrane. Only one type of AG was readily apparent. Their membranes had a coating averaging 89 A vs 57 A for the SG.

The asymmetry in TAG-treated sarcoplasmic reticulum vesicles (Saito et al.) is apparently referable mainly to the unidirectional orientation of the Ca^{2+} pump protein. Calcium binding is a requisite for phagocytosis.

This method strongly enhances the electron-density of membrane coating material, i.e., extracellular proteins. The demonstration of morphological heterogeneity in an attempt to further characterize the nature of the granules is greatly facilitated.

A COMPARISON OF TANNIC ACID-GLUTARALDEHYDE (TAG)-FIXED AND GLUTARALDEHYDE-FIXED POLYMORPHONUCLEAR LEUKOCYTES (PMN) TREATED WITH THE IONOPHORE, A23187. Daniel M. Zellmer and W. Allen Shannon, Jr., Veterans Administration Medical Center and The University of Texas Health Science Center at Dallas.

PMN were obtained from rabbit peritoneal exudates by a previously described method (Brown et al., Proc. 37th-EMSA, p. 78, 1979). The cells were suspended in HBSS with: 1) O.1% DMSO, 2) 10^6M A23187, 3) 10^6M A23187 and 10,000 PS latex beads/cell, or 4) no additives. Aliquots were removed at 0, 1, 3, 5, 10, and 30 min and fixed with tannic acid-glutaraldehyde (Saito et al., J. Cell Biol. 79: 601, 1978) or in glutaraldehyde. Both were osmicated after en bloc staining with uranyl acetate. A23187 induced complete degranulation. Other organelles, e.g., mitochondria, were also depleted. The membrane of one type of granule disappeared as the granule approached the cell membrane. The crystalline contents, presumably proteinaceous, apparently diffused through the cell membrane. Membranes of other types of granules remained intact until apparent fusion with the cell membrane. Tag-fixation indicated that the outer layer of the membrane of A23187-induced cells was considerably thicker and stained more intensely, indicating a significant increase in protein. Pentalaminar profiles involving granule membrane and cell membrane were observed at the site of fusion. The membranous layers at the site of fussion are of equivalent thickness and intensity. Since A23187 induces Ca++ binding, it might be assumed that the proteinaceous concentration could concern a calcium binding protein on the outer membrane, except at the site of fusion. The thickening was not discernable in glutaraldehyde-fixed cells. Lamellated membrane stacks were present in both preparations. These arise at the site of fusion and are cell-granule lamellae or lamellated granule membranes.

HISTOLOGICAL AND ULTRASTRUCTURAL CHANGES IN THE OVARY ACCOMPANYING THE AGING PROCESS IN THE FEMALE HAMSTER. Janice J. Sissom, Univ. of Texas Hlth Sci. Ctr at Dallas, Dallas, TX, and Eugene W. Hupp, Texas Woman's Univ., Denton, TX.

The present study was designed to describe ovarian histology and ultrastructure representative of the estrous cycle in sexually mature female hamsters and to correlate these structural variations with those described in senescent hamsters. Hamsters exhibiting at least 3 consecutive cycles were anesthetized. The ovaries were removed, processed, and subjected to scanning electron microscopy and light microscopy. Proestrous ovaries were characterized by smooth surfaces with blisterlike projections. As ovulation approached, granulosa cells enlarged, cell layers increased in thickness, lateral wall cells enlongated, apex cells began to slough off, and epithelial intercellular spaces widened. After ovulation, the cells within the area of rupture became smooth, devoid of microvilli, and hypertrophied to produce the cells of the corpus luteum. Ovaries from acyclic aged females contained very few nonatretic follicles, and the developing follicles present showed signs of early atresia that included shrunken oocytes with irregular contours,

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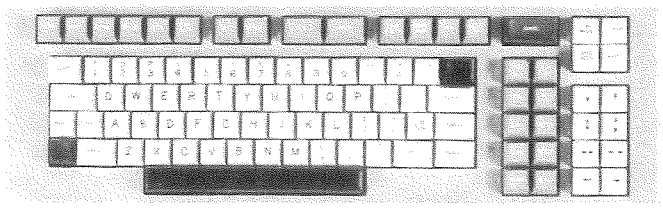

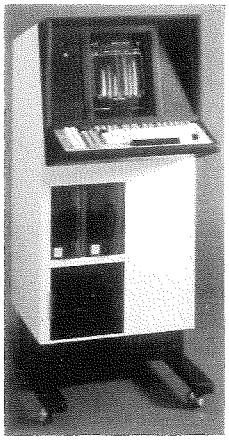

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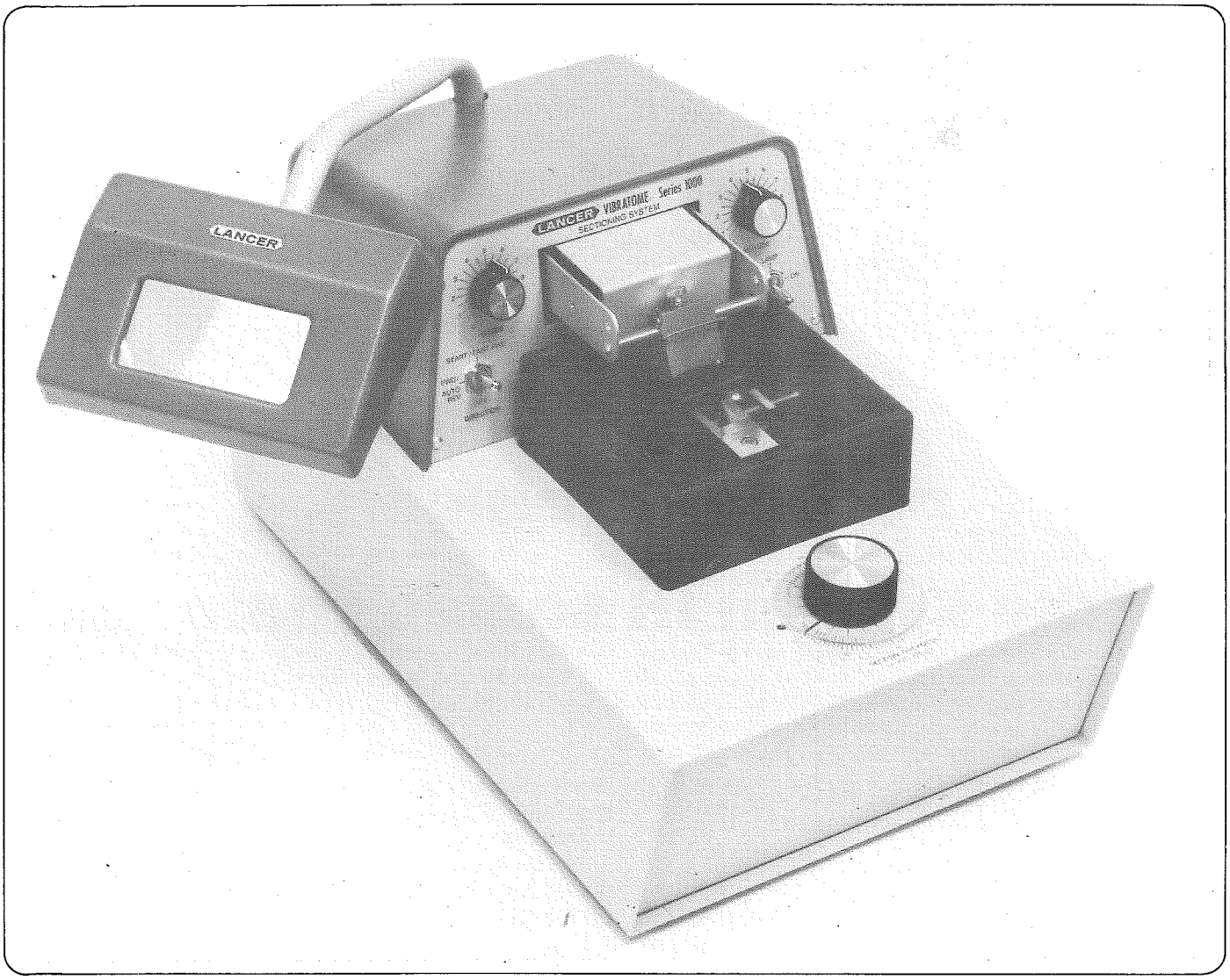

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flocculent or condensed cytoplasm, broken zona pellucida, and disrupted granulosa cell patterns. The surface was smooth and devoid of any large, distinctive bulges or corpora lutea. Blisterlike projections on the ovarian surface contained a deposition of erythrocyte-containing fibrin and were invaginated at the apex. Papillary-like outgrowths of the germinal epithelium were evident on these surfaces. This study demonstrated progressive degeneration of the epithelial covering of the follicle. Increased pressure within the follicle produced by edema ruptured the weakened areas. Evolution of the corpus luteum followed release of the ovum. Extensive changes accompanied the aging process within the senescent hamster ovary, and no signs of ovulation were found.

OVARIAN ULTRASTRUCTURE OF MICE EXPOSED IN UTERO TO DIETHLYSTILBESTROL (DES). Robert Wordinger and Ginger Miller, Department of Anatomy, North Texas State University/Texas College of Osteopathic Medicine, Ft. Worth, Tx. 76107.

Prenatal exposure to DES in mice is associated with genital tract abnormalities in female offspring. Some of these abnormalities may be ovarian-dependent since most offspring are in a persistant estrogenic state even at 12-18 months of age (McLachlan **et al**, 1980, Cancer Res. 40: 3988). This preliminary study was designed to examine the histology and ultrastructure of ovaries from offspring whose mothers had a single exposure to DES. Random bred Swiss mice were placed with individual males and examined daily for vaginal plugs. The presence of a vaginal was considered Day 0 of pregnancy. Pregnant mice were given a s.c. injection of DES (10 μg/kg in 0.1 cc corn oil) on Day 15 of gestation. All animals were allowed to give birth and offspring were weaned at 4 weeks of age. Female offspring were housed in groups of 6 until 5 months of age and then sacrificed. The ovaries were removed and processed for routine histological and ultrastructural examination. In comparison to control animals, the ovaries from DES exposed mice lacked corpora lutea and contained follicles in various stages of growth and atresia. Numerous enlarged and vacuolated interstitial cells were seen with light microcopy. Electron microscopy revealed these cells to be filled with lipid droplets in various stages of degradation. The cells were seen in close approximation to atretic follicles and also accumulated varying amounts of ceróid pigment. These results indicate that prenatal exposure of mice to DES creates a persistant estrogenic state in female offspring. Numerous follicles are in various stages of growth but become atretic, fail to ovulate and thus fail to produce corpora lutea. Transformed theca interna cells form the lipid-filled interstitial cells.

MORPHOLOGY AND ULTRASTRUCTURE OF THE SPERM AND SPERMATOPHORES OF FIVE SPECIES OF THE GENUS UCA. D. S. Mace, Jr. and J. Leon McGraw, Jr., Department of Biology, Lamar University, Beaumont, Tx. 77710.

The sperm and spermatophores of five species of **Uca** (Class Crustacea, Order Decapoda) were compared to provide data which may aid in the elucidation of the phylogenetic relationships between these species. The spermatophores were compared on the basis of size, number of sperm per spermatophore, staining characteristics, solubility and the ultrastructure of the spermatophore wall. Mature sperm were compared on the basis of size, type and quantity of cellular organelles.

The spermatophores were found to be morphologically and structurally similar; however, on the basis of size, similarities were noted which correspond with proposed phylogenetic relationships. Differences were observed in sperm ultrastructure which allow species identification.

DISTRIBUTION AND ORIGIN OF DLS IN GUINEA PIG TESTES. Hilton H. Mollenhauer and D. James Morre, Veterinary Toxicology and Entomology Research Laboratory, AR/SEA, USDA, P.O. Drawer GE, College Station, TX 77841 and Department of Medicinal Chemistry and Pharmacognosy and Department of Biological Sciences, Purdue University, West Lafayette, IN 47907.

Dictyosome-like-structures (DLS) that superficially resemble the stacked cisternae (dictyosomes) of Golgi apparatus, are present in spermatocytes of guinea pig testes. DLS are first visible in late spermatogonia as single saccules of irregular shape. DLS multiply during the first part of spermatocyte development until they constitute more than 25% of the total endomembrane of the cell. As the DLS multiply, the saccules become more uniform in shape and are more often stacked into the dictyosome-like configuration than they were previously. DLS decline in numbers and lose their stacked configuration during the latter part of spermatocyte development and only a few saccules remain through spermatid development. DLS reappear very late in germ cell development and are present in considerable numbers in both residual bodies and in the cytoplasmic droplets of the sperm.

The origin of DLS remains problematical though several observations implicate the Golgi apparatus as a potential source of DLS membrane. Our observations show: 1) DLS membranes closely resemble the thick cisternae of spermatid Golgi apparatus. 2) DLS saccules accumulate several cytochemical markers characteristic of mature Golgi apparatus cisternae. 3) DLS membranes appear structurally similar to a class of "thin-membrane-vesicles" (TMV) associated with the distal poles of Golgi apparatus. 4) DLS saccules and TMV are abundant in spermatocyte Golgi apparatus when DLS are forming. From these observations, we postulate that the membranes of the TMV are derived from Golgi apparatus and serve as precursor pools in DLS formation.

INTESTINAL ENDOCRINE CELLS IN THE LARVAL AND ADULT AFRICAN CLAWED TOAD, XENOPUS LEAVIS. Bridges, G. Baylor Univ. Med. Ctr., Dallas and Couch, E. F. Tex. Christian Univ.

Adult and larval stages of **Xenopus leavis** were examined by light and electron microscopy to determine the presence and types of neuroendocrine cells in the gut. Five distinct neuroendocrine cell types were found in the control specimens. Certain areas of neural crest tissues were removed from larval stages grown in culture. Upon examination, these specimens were found to have three distinct neuroendocrine cell types. In addition, portions other than neural crest tissues were removed from other cultured larvae. These specimens exhibited the same types of neuroendocrine cell types as the control samples.

ANALYSIS AND DETECTION OF SYRIAN HAMSTER PINEAL GLAND DERIVATIVES OF (^{3}H) SEROTONIN AND MELATONIN IN ORGAN CULTURES STIMULATED WITH NOREPINEPHRINE. Cheryl M. Craft and Russel J. Reiter, Department of Anatomy, The University of Texas Health Science Center at San Antonio, San Antonio, Texas.

The purpose of this study was to determine the viability of the hamster pineal in organ culture and to determine the effect of norepinephrine (NE) on (^{3}H) serotonin derivatives. The pineal glands were cultured with NE (5 x 10-^{5}M), and ^{3}H) serotonin derivatives were determined using thin layer chromatography and scintillation techniques. In this study, elevated levels of melatonin (7-fold, $p<.05$), 5-hydroxytrytophol (5-fold, $p<.001$), 5-methoxy-tryptophol (1.78-fold, $p<.05$), and

depressed levels of 5-hydroxyindoleacetic acid (3.8-fold, $p<.02$) and methoxyindoleacetic acid (1.78-fold, $p<.05$) were detected. Melatonin concentration in the media was also periodically measured by radioimmunoassay to determine the viability of the organ culture over a four-day period. The melatonin level on day 2 (2321±106 pg/gland, n=7) was significantly higher ($p<.01$) than on day 3 (1542±86 pg/gland n=7) or day 4 (805±39 pg/gland, n=9). The levels on day 2 were higher than those reported for **in vivo** studies of denervated hamsters for the same period (1440-2520 pg/gland/24 **hr** period). The time study of the media was without NE stimulation. All the indoles studied would have to be produced and secreted by the organ culture. Since new NAT must be produced for melatonin production to occur and inasmuch as the indole is not normally stored in the pineal gland (Rollag **et al,** Endocrinology 106:231, 1980), the organ culture must be producing NAT each day **in vitro.** The results of these experiments verify the viability of the hamster organ culture and also show that stimulation with NE is similar to stimulation of the pineal gland **in vivo.** (Supported by NSF Grant PCM 8003441.)

MORPHOLOGIC, IMMUNOCYTOCHEMICAL, AND MORPHOMETRIC COMPARISONS OF HYPOTHALAMICLESIONED WEANLING RATS AND J129 DIABETIC MICE IN ENDOCRINE PANCREAS. Richard D. Dey, W. Allen Shannon, and Richard E. Dobbs. Veterans Administration Medical Center and The University of Texas Health Science Center at Dallas.

Hypothalamic lesions in rats cause increased insulin secretion and hyperglycemia. However, the possibility of associated morphologic changes in pancreatic islet cells after hypothalamic lesions has not been investigated. The purpose of the present study is to evaluate possible morphological changes in islet cells of weanling rats after hypothalamic lesions (HL) and of the J129 diabetic mouse. Radio frequency generated lesions were produced bilaterally in the region of VM and DM hypothalamic nuclei. The location and extent of lesions were verified microscopically in cryostat sections. Pieces of pancreas from rats and J129 diabetic mice were processed for immunofluorescence and electron microscopic analysis. The distribution and size of islets and number of A, B, and D-cells per islet were determined by the immunofluorescence. TEM was used to count the number of granules in A and B cells of the islets. Some sections of pancreas were processed for immunoreactive insulin using the protein A-gold complex technique. Immunofluorescence studies of the HL rats showed marked islet confluency and apparent hyperplasia. There was an increase in endocrine/exocrine area and the average area per islet, and decreases in the number of islets/mm^2 and small islets present. Fewer A-and B-cell granules/cell were observed. In some B-sells there was an apparent fusion with adjacent exocrine cells, and some B-cell granules appeared in the exocrine cell cytoplasm. TEM of the J129 islets also revealed cell fusion and the occurrence of B-cell granules in exocrine cell cytoplasm. These studies indicate a marked pancreotrophic effect after hypothalamic lesions and in J129 diabetic mice.

USE OF ELECTRON DENSE TRACERS TO DETERMINE CHANNELING PARAMETERS TO PANCREATIC ENDOCRINE CELLS. Jill Bast and W. Allen Shannon, Jr., Veterans Administration Medical Center and The University of Texas Health Science Center at Dallas, TX 75216.

The possibility exists of treating B-cells of pancreatic islets with hormones or chemicals to revitalize their insulin-producing capability. We have studied sice limitations for such factors by perfusion of electron-dense tracers of different M.W. and dia into capillaries of the endocrine pancreas. Ferritin (MW 500K, 5-10 nm dia.), lanthanum (MW 210, <2 nm dia), or myoglobin (MW 16.8K, 3 nm dia.) visualized with diaminobenzidine were perfused into the pancreas cia the dorsal aorta for 2-3 seconds. The pancreas was immediately processed for thin section EM. Survey sections of 2 μm were examined for pancreatic islets containing capillaries. Areas with islets were thin-sectioned and studied in the transmission electron microscope for the degree of penetration of each tracer.

Ferritin was present in islet (n=5) capillaries but had not, to any significant degree, reached the interstitial space. In four out of seven islets, myoglobin was found to have penetrated into the intracellular spaces. All islets (n=6) observed for lanthanum channeling showed penetration into the intracellular spaces. Non of the tracers showed true intracellular involvement. Small tracers are being rested.

It was concluded that effective channeling of substances on the order of 3 nm dia. but not as large as 5 nm dia. can rapidly reach the endocrine cells. However, substances as small as <2 nm were not internalized by the cells.

A COMPARISON OF THE ULTRASTRUCTURAL MORPHOLOGY OF HEPATIC NECROSIS IN BDF1 MICE. Becky Jackson, Biology Department, Baylor University, Waco, Texas 76798.

Preliminary investigations have indicated a similarity in ultrastructural morphology of hepatic cells in response to starvation and cyanide-induced necrosis. To further investigate the mechanism(s) of cellular necrosis in BDF1 mice, controls were compared with a group of senescent animals, a group subjected to starvation, and a group of animals with acetaminophen or cyanide induced toxemia. Normal (ie. healthy) hepatic tissue is characterized by densely granular rough endoplasmic reticulum (RER), abundant stores of glycogen, and ultrastructurally normal mitochondria. Cells with acetaminophen induced toxemia exhibit reduced RER with increased amounts of dialated RER, which is apparently converting to the smooth endoplasmic reticulum (SER) form, loss of mitochondrial integrity, and a reduction in glycogen storage. Senescent cells exhibit an increase in SER and a loss of glycogen stores. Investigation of the senescent SER suggests that the SER arises from degranulated, vesiculated RER. Cells from starved animals exhibit RER that is reduced, disrupted, and dialated, a reduction in glycogen stores, and an increase in the SER. The cyanide treated cells are characterized by disrupted RER and mitochondrial membranes, and a depletion of glycogen stores. These results indicate that similar ultrastructural events during necrosis are characterized by a reduction in RER, an increase in SER, decreased mitochondrial integrity, and decreased amounts of glycogen. That is, there is a general sequence of ultrastructural events for cellular necrosis. The cellular mechanism(s) controlling this necrosis remain under investigation.

IN VITRO MORPHOLOGY OF HUMAN CILIARY EPITHELIUM. O. Dile Holton, Thomas E. Runyon, Cameron E. McCoy, and Marie P. Morgan. Department of Microbiology, Scott & White Clinic, Temple, Texas 76508.

Ciliary body epithelium from human eyes obtained through the Scott & White Eye Bank was dissected away from the major structures and processed as explants in L-15 medium supplemented with 10% FCS. The pigmented and unpigmented layers of the attached tissue fragments could be differentiated by light microscopy in a manner correlating with fixed section morphology. Initial cell outgrowth appeared to be of the unpig-

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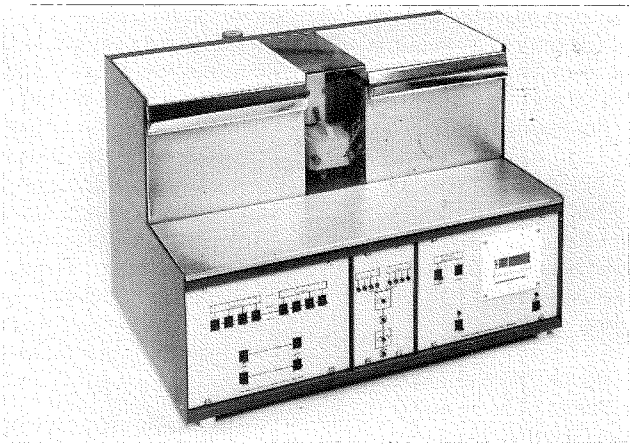

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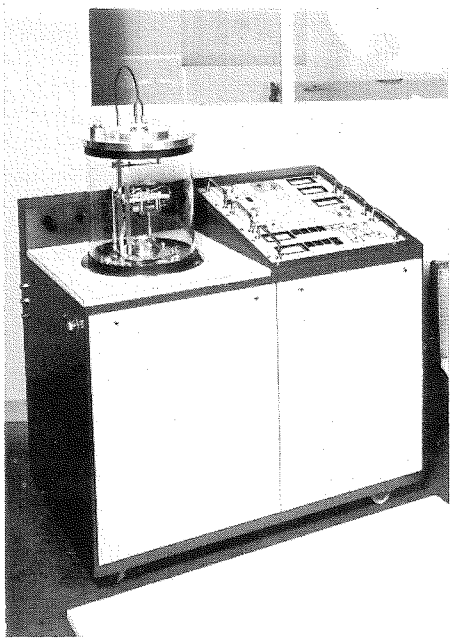

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mented variety, and first confluency varied from 2-18 weeks. The average doubling time was 4 weeks.

Pigment granules could be demonstrated ultrastructurally in cells of the first two passages of each cell culture. Loss of the granules may be explained by failure of the pigmented cells to divide or by **in vitro** loss of the cellular capacity to form pigment granules. Morphometric analysis of the mitochondria and of the cytoplasmic ratio reveal degenerative changes in cells of increasing passage levels which indicate possible nutritional deficiencies of the growth medium.

Maintenance of these cells in continuous culture gained additional importance with the discovery that they produce Endothelial and Nerve Growth Factors **in vitro.**

The clinical relevance of these cells makes basic research on the growth and morphology **in vitro** essential prior to using them as models for the same cells **in vivo.**

NEUROSARCOMATOUS TRANSFORMATION OF MALIGNANT MELANOMA. Bruce Mackay, Sheila DeMaio and J. Leslie Smith, Pathology, M.D. Anderson Hospital, Houston.

The cells of human malignant melanomas can assume a wide range of morphology both at the light and the ultrastructural levels. This can be particularly evident in metastases. Spindle cell transformation can be seen in primary and metastatic melanomas, and the spindle cells may retain the fine structural features of melanoma cells, notably the presence of melanin and premelanosomes, or they may be amelanotic. We have examined tissue from six patients in whom there was a well documented history of a cutaneous primary configuration malignant melanoma, who subsequently developed a spindle cell neoplasm at the site of excision of the primary skin neoplasm, or in one instance in regional lymph nodes. The cells have long cytoplasmic extensions containing microfilaments and microtubules, but no melanosomes or premelanosomes. The tumors closely resemble neurosarcomas, which are considered to be of schwann cell derivation, and it may be that the melanoma cells are expressing a normally latent aspect of their neural crest origin.

TUMORS OF SYNOVIUM AND TENDON SHEATH. Dietmar Schmidt and Bruce Mackay, University of Kiel, and U.T. System Cancer Center.

There are controversies concerning the malignant tumors that have been ascribed to synovium and tendon sheath. Synovial sarcoma is, as its name implies, believed to originate in synovium, but histologically acceptable examples occur some distance from the nearest joint and this assumption has been questioned. The so-called clear cell sarcoma of tendon sheath appears from ultrastructural studies to be a tumor of melanocytes. Epithelioid sarcoma is an enigmatic soft tissue neoplasm that has been said to resemble synovial sarcoma by electron microscopy, but we consider this an erroneous interpretation. In an attempt to clarify the nature and histogenesis of these tumors, we have studied normal human synovial membrane and tendon sheath by T.E.M. and S.E.M. The two tissues look remarkably similar, and it would seem reasonable to suggest that tumors arising from them might have similar histologic features. Among the three tumors that have been linked to synovium and tendon sheath, only the biphasic form of synovial sarcoma shows any resemblance to the cells of these tissues. Clear cell sarcoma is probably a soft tissue melanoma, and epithelioid sarcoma may fall within the broad group of malgnant fibrous histiocytomas.

SARCOMATOUS TYPE OF MALIGNANT MESOTHELIOMA. Marcella Klima, M.D., V.A. Hospital, and Mattie J. Bossart, Ph.D. St. Luke's Episcopal Hospital, Houston, Texas.

Mesotheliomas are currently classified as a benign solitary fibrous mesothelioma and as diffuse malignant mesothelioma. The latter one is divided into 3 groups based on the histological pattern: epithelial, sarcomatous and mixed type. Criteria for diagnosing a mesothelioma as sarcomatous are not as yet commonly delineated.

In this series of 37 diffuse malignant mesotheliomas, only 2 are of a mixed type and 11 are designated as sarcomatous in light microscopy. Nine of these 11 cases were examined by electron microscopy; one in multiple samples because of repeated surgeries. The mesothelioma tissues were compared with normal pleura, hypertrophic mesothelium, benign solitry mesothelioma, and the mixed type of mesothelioma.

By light microscopy, the sarcomatous type is composed of sheets of spindle cells and showsan undifferentiated pattern, resembling mesenchymal tissue. By electron microscopy, the tumor cells appear to be primitive cells with some mesenchymal features. In addition, attempts of epithelial or glandular differentiation were seen in 4 cases under the electron microscope.

While in the epithelial type of mesothelioma the cells have expressed epithelial characteristics, in the sarcomatous type the cells maintain a biphasic differentiation potential.

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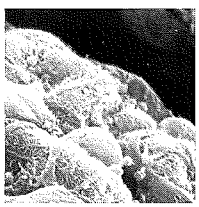

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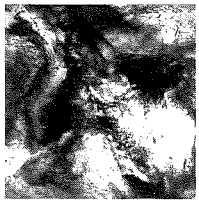

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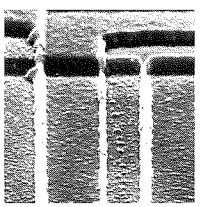

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Consider TECHNICS for support equipment such as vacuum systems or water chillers and recirculators...or a TECHNICS specimen supply kit to complete the needs of your Electron Microscopy Laboratory. **So give your Electron Microscope quality specimens ... with TECHNICS specimen preparation equipment.**

TECHNICS
Electron Microscopy System, Inc.

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Financial Report

TSEM FINANCIAL REPORT
Period Ending December 31, 1980

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	
Total Assets	$ 6,422.46	$ 6,422.46
RECEIPTS:		
Dues:		
Regular Membership	$ 270.00	
jstudent Membership	58.00	
Corporate Membership	75.00	
Interest on Certificate of Deposit No. 1099	50.00	
Interest on Certificate of Deposit No. 10-141345	109.12	
Interest on Savings Account No. 10-502435	33.98	
Refund from Secretary (Hansen)	117.71	
Registration, College Station Meeting	1,696.00	
Subtotal	$ 2,409.81	$ 2,409.81 (+)
DISBURSEMENTS		
College Station Meeting:		
Texas A&M University		
Rooms	145.50	
Food Services	904.30	
Holiday Inn	296.00	
Texas Hall of Fame	300.00	
Presidential Travel	57.80	
Secretarial Expenses	74.07	
Treasurer's Expenses	38.90	
U.T.H.S.C. San Antonio (Cwmsing Hansen Account)	11.19	
U.S. Postal Service	100.00	
Subtotal	$ 1,927.76	$ 1,927.76 (-)
ASSETS ON DECEMBER 31, 1980:		
Certificate of Deposit No. 1899, Univ. Natl. Bank, Galveston	$ 2,000.00	
Certificate of Deposit No. 10-141345, Houston 1st Savings	2,222.60	
Savings Account No. 10-502435, Fannin Bank	1,300.99	
Checking Account, Fannin Bank	1,380.92	
Total Assets	$ 6,904.51	$ 6,904.51

CORPORATE MEMBERS

AMRay, Inc. Thomas Levesque, 5209 Kisor Drive, Box 83416, Lewisville, TX 75056, (214) 247-3542.

Cambridge Scientific Instruments, Mike Webber, 3945 Farrington Dr., Marietta, Georgia 30066, (404) 926-9636.

Carl Zeiss, Inc., Dietrich Voss, 3233 Weslayan, Suite 191, Houston, TX (713) 629-0730.

EBTEC Corp., Margrit Barry, 120 Shoemaker Lane, Agawam, Mass. 01001, (413) 786-0393.

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E.I. DuPont de Nemours & Co. Inc., Biomedical Products Division, Harry Vacek, Concord Plaza-Quillen Building, Wilmington, Delaware 19898, (800) 441-7493 or (302) 772-6024.

EG&G Ortec, Dick Nieman, 21718 Rotherham, Spring, TX 77379, (713) 353-0078.

Electron Microscopy Sciences, Richard Rebert, Box 251, Ft. Washington, PA 19034, (215 646-1566.

Ernest Fullam, Inc., Richard Kemmer, 900 Albany Shaker Rd., Latham, NY 12110, (518) 785-5533.

Gatan, Inc., Terry Donavan, 780 Commonwealth Dr., Warrendal, PA 15086, (412) 776-5260.

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International Scientific Instruments, Robert Ruscica, 3255-6C Scott Blvd., Santa Clara, Calif. 95050, (408) 727-9840.

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

KEVEX Corp., Dick Cushing, 1101 Chess Dr., Foster City, CA 94404, (415) 573-5866.

Ladd Research Industries, Margaret Ladd, P.O. Box 901, Burlington, Vermont 05402, (802) 658-4961.

LKB Instruments, Inc., Jonni Fischer, 2407 W. Settlers Way, Woodlands, TX 77380, (713) 228-4082.

Olympus Corp., Precision Instruments Division, Susie Miles, 5201 Mitcheldale, Suite B-1, Houston, TX (215) 965-9761.

Polaron, Dermot O. Dinan, 4099 Landisville Rd., Doylestown, PA 18901, (215) 345-1782.

Polyscience, B. David Halpern, Paul Valley Industrial Park, Warrington, PA 18976, (215) 343-6484.

Princeton Gamma Tech, Dave Kane, 4005 Highland Dr., Austin, TX 78734, (512) 836-0188.

Rockwell International, R. W. Max, Mail Station 406-146, Richardson, TX 75081, (214) 996-6973.

Technical Instruments Co., John J. Meny, 4215 Beltwood Parkway, Suite 106, Dallas, TX 75234, □ 14) 387-0606.

Technics EM Systems, Inc., Diane A. Hurd, 7653 Fullerton Road, Springfield, VA 22153, (703) 569-7200.

Ted Pella, Inc., T. P. Turnbull, Box 510, Tustin, Calif. 92680, (714) 557-9434.

April 10, 1981

To: Texas Society for Electron Microscropy

Dear Fellow TSEM Members:

It is with great personal and sorrow that I report to you of the death of our friend, colleague, and fellow TSEM member, Paul Enos, who died on the evening of April 8, 1981. Paul had been a member for TSEM since 1973, and was corporate representative of JEOL, Inc. Paul was professional and successful in his business dealings. He was a past member of the TSEM Executive Council, having served as program chairman in 1976. His service to the council during that year was outstanding.

Paul Enos was one of TSEM's most avid supporters and benefactors. Time after time when we needed extra financial support, personal involvement, or just plain "leg work" Paul was always there; no questions, no expectations, just a friendly smile and "you bet". He will be a difficult resource to replace; we will all miss this good man.

In view of his contributions and involvement in our society, I will propose to TSEM Executive Council on April 23, 1981, that Paul be awarded an honorary TSEM membership, to be awarded post-humously, and that his name be carried on our rolls in perpetuity. Furthermore, I will introduce a motion that a special account be created by our treasurer to receive memorials made in Paul's behalf. I further propose that the revenues from this benefit be used as an annual TSEM award or awards for outstanding research.

In view of these proposals, I would like to encourage all of his friends and associates to participate in this worthy memorial.

Paul would not have liked us to be morose over his death. He would, I imagine, prefer that we pause, reflect, smile, and go on with our work. However, upon occasion when I find myself in close proximity to an electron microscope, I will think of Paul Enos, my friend . . .

Sincerely,

Paul S. Bauer, Jr., Ph.D.
President, TSEM 1980-81

Secretary
Texas Society for Electron Microscopy
Box 23971
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Denton, Texas 76204

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