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Texas Society for Electron Microscopy

JOURNAL

VOLUME 14, NUMBER 2

SPRING, 1980

ISSN 0196-5662

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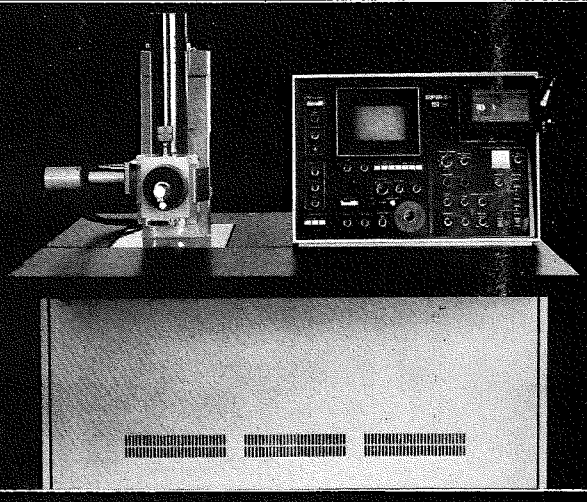

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Volume 11, Number 2
Spring, 1980
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Texas Society for Electron Microscopy

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

EDITORIAL POLICY

Feature articles, news, letters to the editor, and micrographs may be submitted. Feature articles should be 3-10 typewritten pages, double spaced, with figures, tables, and electron micrographs mounted for an 8-1/2 x 11 inch format. Three types of articles are solicited: 1) reviews 2) research reports 3) techniques papers. Reviews provide background material on a given research problem and often are condensed versions of review sections from current grant proposals. Research reports are short summaries of work published in part or in full in other journals but presented for a diverse audience with an interest in electron microscopy and allied technical approaches. Techniques papers describe new or rediscovered methods for improving or adding to existing techniques and give examples of the results obtained with these methods.

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

ON THE COVER

Crystal prisms in the hinge ligament of *Spisula solidissima*. Electron Micrograph courtesy of Dr. Mary Marsh, Biology Department, Rice University, Houston, Texas. Magnification ×12,000.

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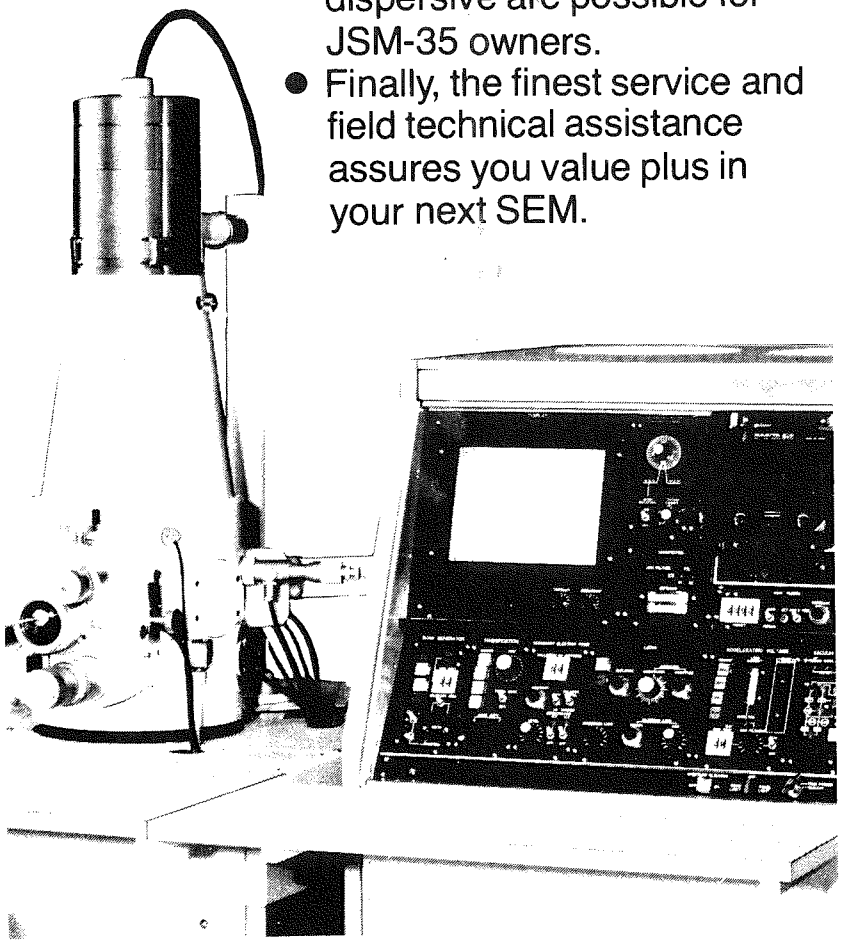

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President's Message

This has been a very trying but good year for the TSEM Society. Some very hard decisions were made, but I feel these were correct decisions and the society as a whole will benefit.

Two of these decisions will effect long term traditions of the society. First, the TSEM/LSEM Joint Symposium was abandoned in favor of having a centrally located meeting once a year in Texas. Second, our meeting format was changed from three meetings a year to two meetings a year. Both of these decisions were made with the membership in mind. We felt the cost of meetings and the difficulty of scheduling the spring meeting around spring final examinations warranted these changes. I feel the number of papers submitted for this Spring Meeting substantiate our fears that three meetings a year is too many.

Ann Goldstein made giant steps forward with the TSEM Newsletter. The name was officially changed to Journal and a library of Congress number was obtained. I would like to thank Ann for all of her hard work the past two years and wish our new editor all the luck in the world.

John Hansen ends his "long" term as secretary. This is probably the most difficult job in the society. Unfortunately, John did not have time to devote to a future office, but I hope he will continue as a strong member. Thank you John, for all the hard work.

Bruce MacKay still has one year to serve as treasurer of the society. I have enjoyed working with Bruce and know he will continue as a strong council member.

Charles Mims and Leon McGraw have again provided excellent scientific programs for each of our meetings. The task was especially difficult since the symposium was held in Texas this year. Charles will also be temporarily retiring from the council but Leon will take up where he leaves off providing excellent programs for next year.

We often forget that a student representative serves on the executive council. Thomas Drier did an excellent job keeping us informed on student needs and handled student travel each meeting. Thank you Thomas, for the year you committed during your busy graduate studies.

Ivan Cameron has provided experience and guidance to the council as past president. This is a very important link in the chain of events which take place each year as the officers change. Hopefully, I will be able to provide this same leadership next year.

I leave the society's affairs in the capable hands of Paul Baur. Paul's great interest and enthusiasm will make next year an even better one for TSEM.

Thank you for the opportunity to serve. I hope to see you all at future meetings.

Bill McCombs
President, TSEM

Editor's Comments

It has been my pleasure to serve as editor of the TSEM publication. I have learned a lot in our transition from newsletter to journal. This has been a cooperative venture and is the result of a successful combination of willing and enthusiastic advertisers who underwrite the cost; a good printer who works with us to maintain quality at a reasonable price; and a talented, verbal group including those who accept our invitation to publish articles in the journal, and our regional editors who send us the news from around the state. An editorial policy has evolved over the last two years and a formal statement of this is now included. An acceptable format has emerged and several other state societies are asking for information to use the TSEM Journal as a prototype. A number of free copies have been distributed to libraries, and we hope now to get letters from their acquisition departments requesting a subscription to the TSEM Journal.

Welcome to our new members in TSEM. I hope you will become contributors to the TSEM Journal. For those of you who are interested in a little past history I recommend past issues of the TSEM Newsletter, particularly Vol. 10 No. 3 which gives a short history of the society.

Our meeting in Houston was an interesting one and included presentations from people in materials science. We are grateful to our corporate members who bought exhibit space. Special thanks go to Jeol, Kevex and Amray who provided additional money for our social functions. We ate a lot of good Gulf shrimp and drank a lot of California wine. Some of you folks out there really missed a good time!

Our new editor is Ms. Elaine McCoy, Supervisor of Tissue Culture and Virology at Scott and White Clinic in the Microbiology Department. I know Mr. Jody Donaldson, our printer, will be glad to have the editor in the same town again. Ms. McCoy comes highly recommended from a group at Scott and White who have helped to shape the history of TSEM. I will continue as a consulting editor. You did not think I could just walk away without one last word, did you?

Ann Goldstein
TSEM Journal Editor

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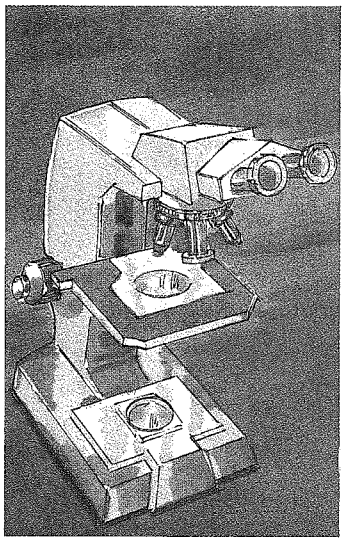

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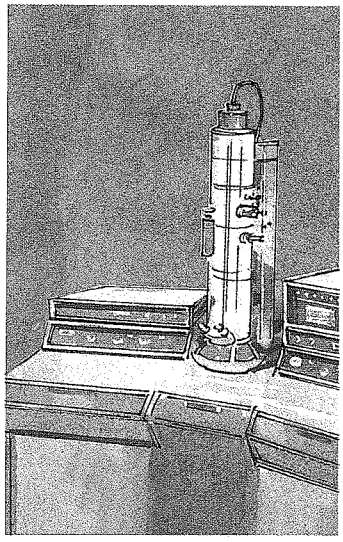

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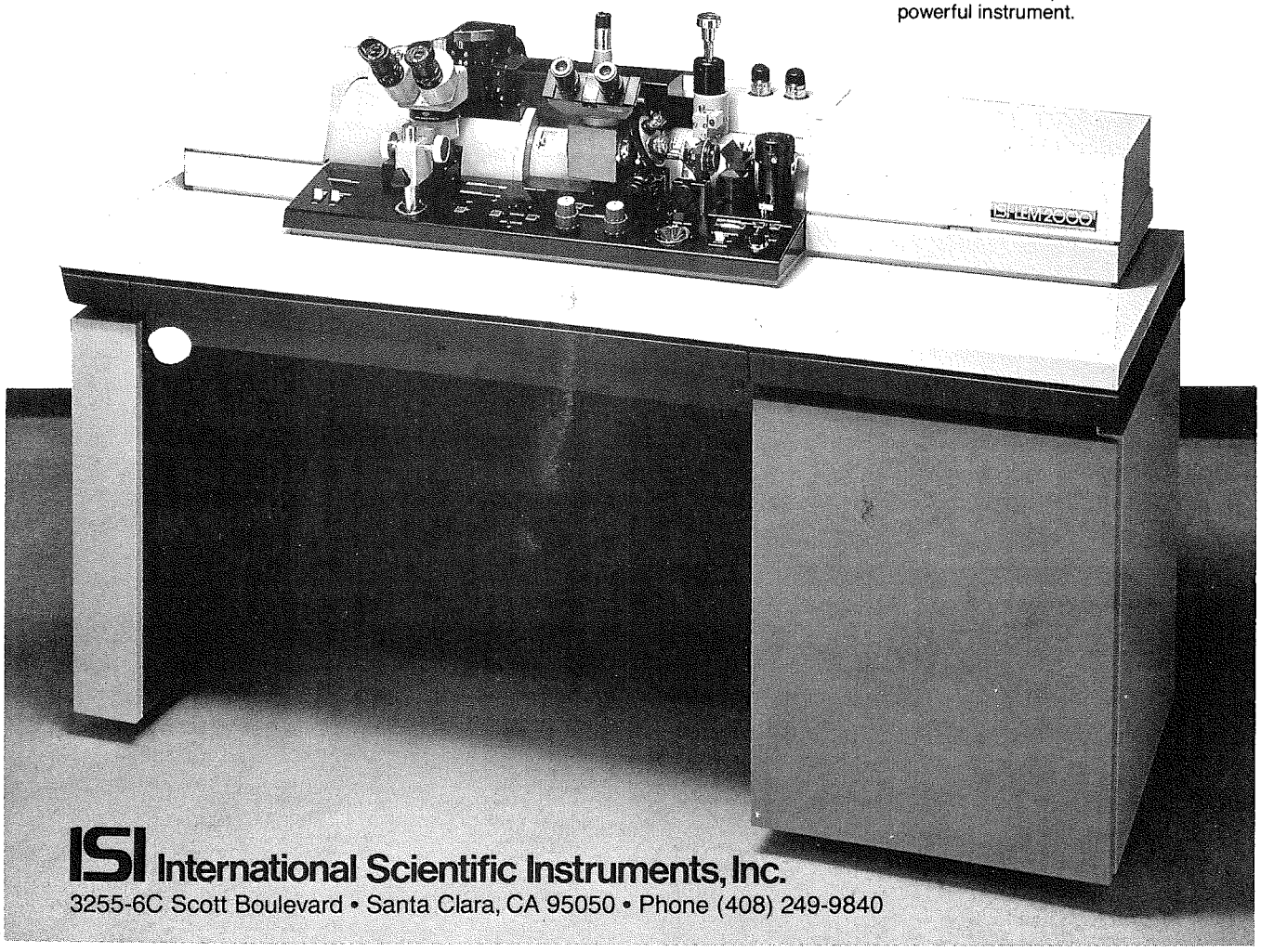

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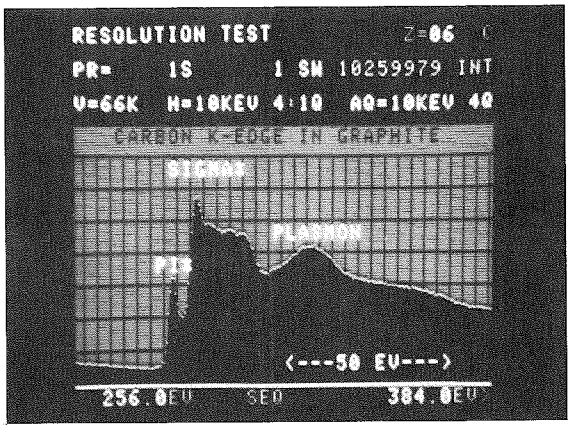

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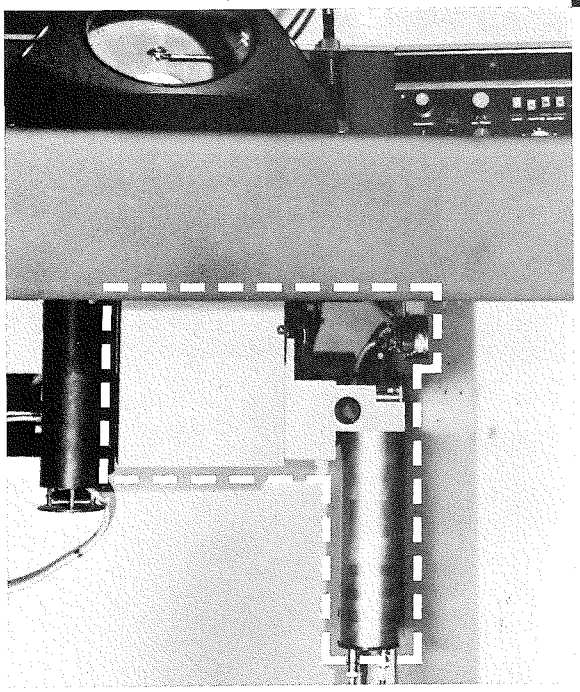

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Electron Microscopy For Rapid Viral Diagnosis

By
William B. McCombs, III,
Cameron E. McCoy, and **O. Dile Holton, III**

Not too long ago the identification of bacterial pathogens was assumed to be faster than the identification of viral pathogens. The dogma was that by the time a virus diagnosis was made, the patient had either recovered or died. Viral diagnosis was dismissed as being either of academic interest or to confirm the presence of an epidemic.

Thus, the possibility of presenting results of a virus identification to a clinician in a matter of hours has been the aim of many virologists over the past 10 years. Quick diagnosis might shorten the patient's hospital stay and prevent the patient from being given potentially toxic and often expensive antibiotics.

Knowing the agent causing the infection gives the clinician the academic satisfaction that he is dealing with a precise entity. This now can be of some benefit for certain viral diseases which can be treated with selected chemotherapeutic agents.

In light of the advances made in viral chemotherapy, and the generation of precise morphological criteria for certain groups of viruses, several techniques have been developed which give the virology laboratory rapid diagnostic capabilities. Techniques used for the rapid diagnosis of viral infections are listed in Table I. For the purpose of this article, we will limit our topic to the use of the electron microscope.

The increasing availability of the electron microscope in the clinical laboratory has led to the development of its diagnostic use. Major advances have been made in the development of E.M. techniques for the routine identification of human viruses.

The simplest technique for E.M. identification of viral isolates is negative staining. Negative contrast electron microscopy enables virus particles to be visualized in clinical material in less than three hours under favorable circumstances, and sometimes in a matter of minutes. The technique is based on the addition of heavy metal salts to the specimen preparation to absorb electrons which would normally pass through organic matter. An extract of a clinical specimen in distilled water is mixed with a stain (either potassium phosphotungstate or ammonium molybdate in distilled water), a drop is touched on the surface of a formvar coated copper grid, the excess is removed with absorbent paper and then the specimen is allowed to dry. The virus particles appear as regular bright structures against a dark background. The stain penetrates into irregularities in the surface of the virion allowing its ultrastructure also to be observed.

As a routine method of morphological identification, negative staining is extremely practical. However, the procedure is actually destructive to many enveloped RNA viruses. These and other viruses can readily be seen in thin sections of fixed and embedded virus cultures, but because of the length of time needed for most routine E.M. embedding procedures (1 to 2 days), this approach is not useful for rapid identification.

There have been several reports of rapid embedding methods for electron microscopy (2, 6, 7). The shortest of these to date has been described by Doane et al (6), with the total processing time being reduced to approximately 2.0 hours without loss of ultrastructural detail. In our laboratory, we have found the sequential method of Doane, et al to be superior. Briefly, specimens are fixed for 15 minutes at 4°C in 2.5% glutaraldehyde in Millonigs phosphate buffer, rinsed three times in the phosphate buffer, 1 minute each, and post fixed for 15 minutes at room temperature in 1% osmium tetroxide in phosphate buffer.

The fixed specimens are then dehydrated through acetone as follows: 70% acetone, two changes in 3 minutes, absolute acetone, three changes in 5 minutes; absolute acetone, three changes in 5 minutes. After 10 minutes in a 1:1 mixture of absolute acetone and Spurr embedding medium (12) and two changes of 100% Spurr medium, 5 minutes each, the specimens are placed in fresh Spurr medium in a BEEM capsule and heated to 95°C for 60 minutes to achieve polymerizations of the embedding medium. It is important to keep the polymerization temperature at 95°C. Above that temperature the BEEM capsules show a tendency to soften, resulting in misshapen blocks. There is no obvious difference in cutting properties of specimens processed by this method, however, sections do not stain as sharply as those prepared by the more time consuming standard procedure.

The **in situ** technique (10) was developed in our laboratory to help determine rapidly what group of virus had grown out in tissue culture from a positive patient sample. Specific cell monolayers are inoculated with suspected viral specimens in 30 ml Falcon flasks. When cytopathic effect is first noted, the areas of interest are circled. The growth medium is decanted, and the cells fixed **in situ** for 1 hour in 3% phosphate buffered glutaraldehyde, washed for 15 minutes in the phosphate buffer, and postfixed for 30 minutes in 1% osmium tetroxide in phosphate buffer. The flask side opposite the monolayer is removed. The monolayer is then dehydrated with ethanol and hydroxypropal methacrylate according to Brinkley et al (4) and the cell layer is embedded in Epon (9) to a depth of 5 to 8 mm. Polyethylene BEEM capsules were filled with embedding medium and placed, open end down, over previously circled areas. The flasks are left open overnight in a 37°C incubator and then transferred to a 60°C oven for final polymerization. After 24 hours at 60°C, the capsules are snapped off with pliers, leaving the cells on the flat block surface ready for sectioning.

Another useful rapid technique, the pseudoreplica technique (Fig. 1), utilizes the negative stain approach to viral identification. This technique has advantages over other rapid techniques. First, as the fluid is absorbed on the agar surface, any virus present is actually concentrated on the agar surface. Second, the agar diffusion also reduces the salt content of the specimen, preventing salt from crystallizing and confusing interpretation. Third, the method requires very small quantities of specimen (0.025 ml). Most important, the technique takes less than an hour to perform.

Advances in electron microscopy pioneered by Almeida and Waterson (1) in their application of immuno-electron microscopy have also been the basis for another method for rapid viral identification. Here, immune sera are used to aggregate virus particles. The aggregates can then be negatively stained. This technique has been especially useful in the investigation of hepatitis A and B virus since virus from stool can be aggregated making them easier to locate. The availability of monospecific antisera is the main limitation of this technique. Also, one must screen for a single known-immuno-reactive group of viruses and this limits the testing for an unknown causative agent.

The main limitation of the rapid viral diagnostic techniques is the inability to completely identify the virus. These E.M. techniques merely show the shape and general morphology of the viruses and in many cases, viruses responsible for infections of diverse etiology have similar E.M. morphology.

However, general morphology will usually allow the investigator to identify at least the genus of the virus. Since this can often be done the same day, rapid techniques have found their place as routine tests procedures in the virology laboratory. Rapid techniques will never replace good diagnostic virology practices, but we feel that certain diseases can best be studied using rapid techniques in addition to routine culture methods (Table II).

Many diseases caused by viral infections are often life threatening and urgency is often required. Others, such as the rotavirus are not as serious but techniques are not currently available for successfully growing these viruses in tissue culture. Electron microscopy examination is now the accepted technique for the diagnosis of these hard to culture viruses.

INFECTIONS OF THE FETUS AND NEWBORN

There is a group of microbial and viral agents affecting the fetus and newborn which, because of their similar manifestations and the need for special laboratory tests in diagnosis have been grouped into the TORCH complex — Toxoplasma, Other, Rubella, Cytomegalovirus, and Herpes Simplex virus. Urgency is required in the diagnosis of these life threatening diseases. Two of these agents, Cytomegalovirus and Herpes Simplex virus lend themselves to study by electron microscopy.

Cytomegalovirus (CMV) is widespread in nature and few people escape infection during their lifetime. While primary infection in adults is usually benign, if infection occurs during pregnancy, this virus is capable of causing irrepairable damage to the fetus. Cytomegalic inclusion disease (CID) is now the most common cause of congenital infection, occurring at a frequency of approximately one per 100 live births.

Of the organs affected, the kidney, liver, and lung, in that order are the most common sites of obvious involvement. Isolation of the virus from a fresh urine specimen, a throat swab, a liver or lung biopsy from the fetus or neonate will establish that CMV infection is present.

Although these viruses grow readily in tissue culture, CMV often takes up to six weeks before viral cytopathic effect (CPE) is observed. With the availability of anti-herpetic drugs, i.e., idoxuridine, cytosine arobinoside, and adenosine arabinoside, the early diagnosis of these diseases are even more important since the earlier the treatment the better the chance for success.

If CMV infection is present, our laboratory requests on a routine basis a urine sample and processes it by the pseudoreplica technique. Lee et al (8) using the pseudoreplica technique have been able to identify CMV in the urine of 90% of infants known to have CMV infection. As stated previously, CMV might also infect the lungs or liver. Often the specimen of choice will be biopsy material. In these cases, the specimen is processed by the rapid embedding technique, giving the physician diagnostic information on the same day the specimen is taken.

Present information indicates that congenital herpes simplex (HSV) infection is very rare. The more common neonatal infection is acquired during parturition and does not usually present as an acute disease until 5-10 days of age. For this reason neonatal HSV infection is often difficult to diagnose on the basis of clinical manifestation alone since many clinical features are similar to those of many other infections and non-infectious conditions. When illness does occur, it is frequently severe, and is accompanied by seizures, encephalitis, respiratory distress and bleeding disorders. Vesicular skin lesions that tend to

cluster are usually present. If these skin lesions are present, they generally contain large amounts of virus. If fluid is present, it can carefully be taken with a syringe and processed by the pseudoreplica technique. Often, older lesions do not contain enough fluid. In these cases a punch biopsy is requested and processed by the rapid embedding method. The typical morphology of Herpes virus can easily be distinguished by electron microscopy (Fig. 2).

IMMUNOSUPPRESSED PATIENT

The wide use of immunosuppressive drugs in chemotherapy for neoplastic diseases, long-term treatment of organ transplant recepients, and even some patients with collagen-vascular disease — has created a large population of patients at increased risk of infection. Among sites for serious viral infection in the immunosuppressed patient, the lung is among the most common. Immunosuppressed patients can produce very little sputum, making the diangosis of pneumonia from sputum culture or smear very difficult. Since the usual clinical specimen is often negative and the clinician feels the urgency to start treatment with a specific drug, lung biopsies are often used.

With immunosuppressed patients, clinically evident interstitial pneumonia can be caused by CMV, Herpes, and **Pneumocytis carnii** infections. All of these can cause interstitial pneumonia and can not be readily cultured. Using the rapid embedding technique and electron microscopy, a lung biopsy can be studied and a diagnosis made the same day. Since the course of therapy is drastically different for each agent, the demonstration of the agent may preclude the need for additional laboratory testing or unwarranted antibacterial therapy.

Patients with Hodgkin's disease often develop disseminated herpes zoster infections. When this does occur, vesicles will usually be seen on the trunk of the body, although the lungs may also be involved. If vesicles are present, specimens may be processed by the pseudoreplica technique or punch biopsies processed by rapid embedding technique. Although this disease is usually not fatal, early diagnosis allows early treatment and a better prognosis.

HERPES ENCEPHALITIS

Herpes simplex, the familiar organism that causes fever blisters and other herpes simplex infections, can be deadly if it migrates from the nasopharyngeal mucosa along the olfactory nerve into the brain. The acute encephalitis that follows often brings coma within days and death in two or three weeks. It is now well established that early treatment with idoxuridine greatly enhances the patients chances for recovery.

Fever, coma, focal indications in neurologic tests, and a temporal or frontal lobe focus on a encephalogram make up a presumptive clinical picture of acute encephalitis. The most common specimen obtained when working up a suspected case of encephalitis is spinal fluid. In the case of herpes encephalitis, spinal fluid rarely contains enough virus to utilize the pseudoreplica technique. However, brain biopsies are now performed with far less reluctance and tissue taken from the proper site and processed by the rapid embedding technique readily demonstrates virus. It must be remembered that this encephalitis is a focal lesion and if the biopsy is taken from a non-infected site the virus will not be seen.

INFANT DIARRHEA

The cause of infant diarrhea has been a puzzle for many years, but recently the discovery of rotavirus by Bishop, et al (3) has given another diagnositc use for the electron microscope. It is now well established that rotavirus is the major cause of acute gastroenteritis in infants and young children. Rotavirus can easily be detected in as high as 73% (5) of patients having diarrhea during the peak winter season.

Since it still seems difficult to culture human rotavirus **in vitro**, the most widely used diagnostic method has been the pseudoreplica technique and electron microscopy. An appropriate stool sample is processed as outlined by Portnoy et al (11) for obtaining the suitable specimen for the pseudoreplica technique. The processed specimen, a homogenate supernatant, is then analyzed by the pseudoreplica technique for virions with typical rotavirus morphology (Fig. 3).

In conclusion, diagnostic electron microscopy especially the pseudoreplica technique and the rapid embedding technique, have found a practical use in the clinical virus laboratory. Rapid E.M. techniques enable a virology laboratory to obtain some day results on several different types of specimen. This rapid diagnostic capability in some viral diseases has a profound influence on the treatment chosen by the physician and ultimately the prognosis of the disease. Although the advantages of rapid E.M. for viruses are dramatic in some cases, caution should always be taken to use these techniques as only adjuncts to standard virology and electron microscopy techniques. Cultures should always be taken for standard CPE analysis and biopsies should always be processed by standard E.M. techniques.

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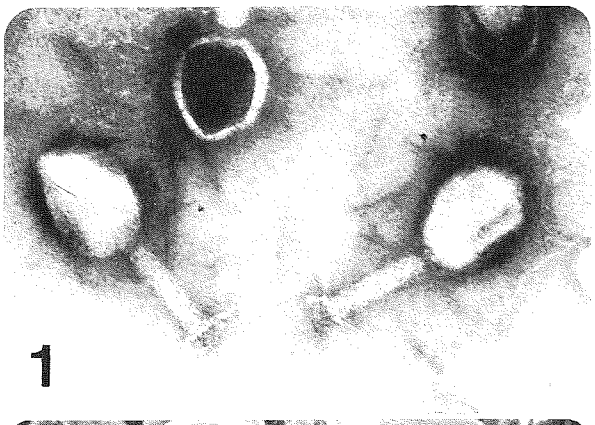

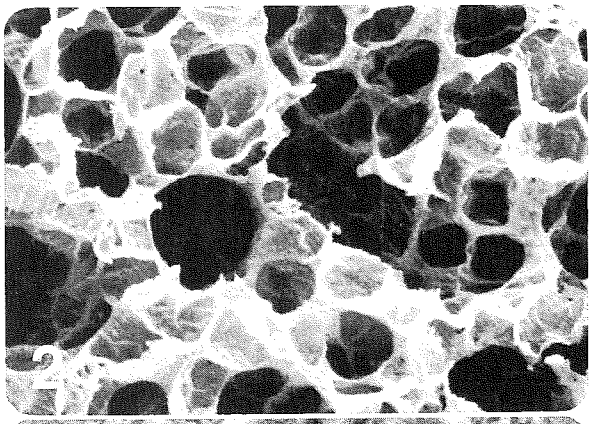

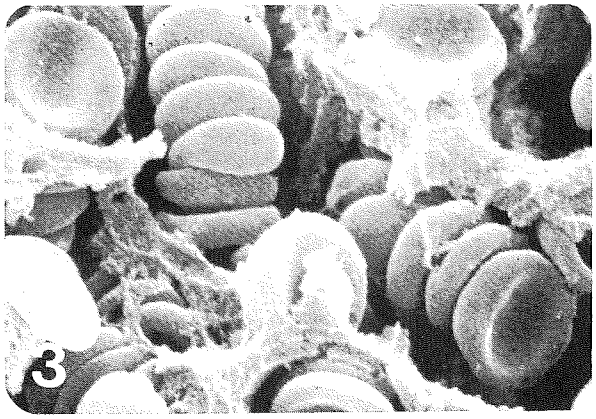

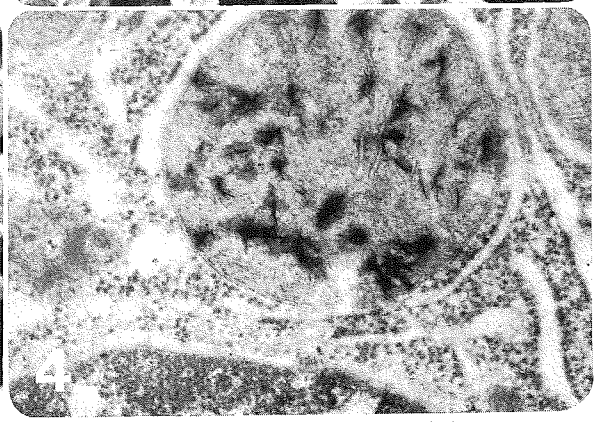

1. T-4 Phage, TEM (PTA) 97,500X 2. Rat Lung, SEM (CPD) 100X 3. RBC's In Situ , SEM (CPD) 3000X 4. Mitochondrial Inclusions Diseased Human Lung, TEM 19,000X

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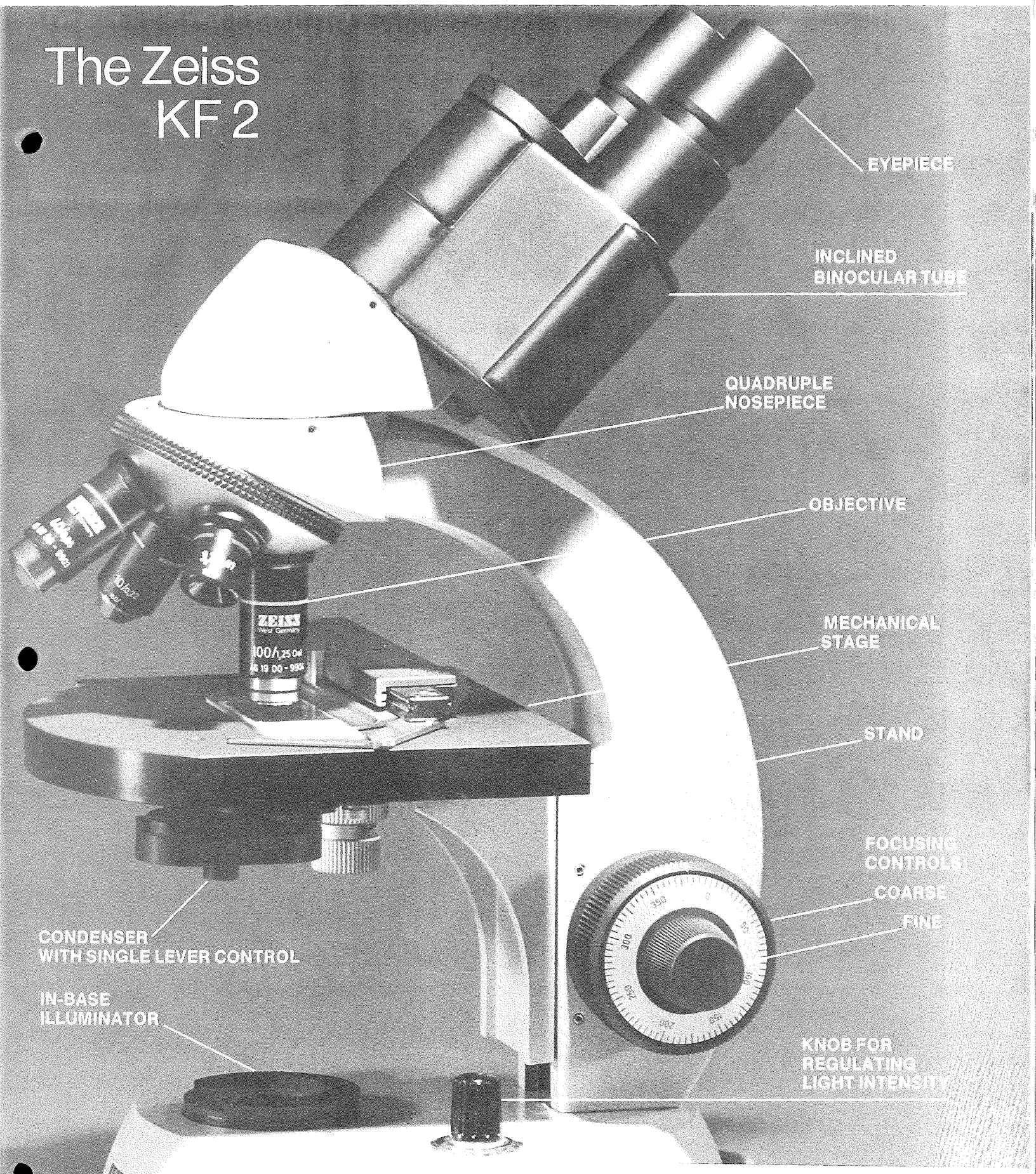

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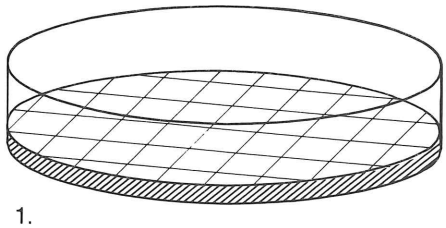
TABLE 1: Techniques for Rapid Detection of Human Viruses

TECHNIQUE	SPECIMEN	DETECTABLE VIRUSES
Fluorescent Antibody Techniques	Nasopharyngeal secretions	Influenza A and B Parainfluenza viruses 1, 2, 3, 4a and 4b Respiratory Syncytial virus Measles Adenovirus (group antigen) Rubella
	Brain biopsy	Rabies, Herpes simplex
	Tissue culture (occasional nasopharyngeal secretions)	Mumps
	Skin and Brain	Herpes simplex Varicella zoster
Immunoperoxidase	Urine and biopsy (occasional nasopharyngeal secretions) in cell culture	Cytomegalovirus Herpesvirus hominis
	Brain biopsy	Rabies
ELISA	Various	Hepatitis B
	Feces	Rotaviruses
Electron Microscopy	Various	Herpes simplex, Cytomegalovirus Rotavirus

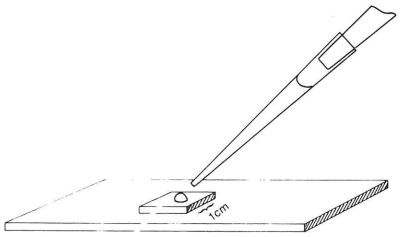
TABLE 2: Medically Important Human Viruses Which may be Studied by Electron Microscopy.

DISEASE	SPECIMEN	TECHNIQUE
Congenital Herpes	Biopsy	Rapid Embedding[1]
	Fluid	Negative Staining
Congenital CMV	Biopsy	Rapid Embedding[1]
	Urine	Pseudoreplica
Herpes Virus Encephalitis	Brain Biopsy	Rapid Embedding[1]
Infant Diarrhea (Rotavirus)	Stool	Pseudoreplica
Interstitial Pneumonia	Lung Biopsy	Rapid Embedding[1]

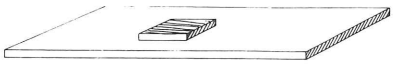
1. Routine processing of portion of specimen should always be done.



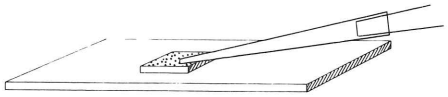
1.
Pour 2% Noble Agar to a depth of 2-3mm in petri dish. Cut into 1cm squares.



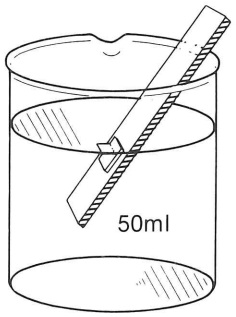
2.
0.025ml of specimen is placed on agar block (1cm²).



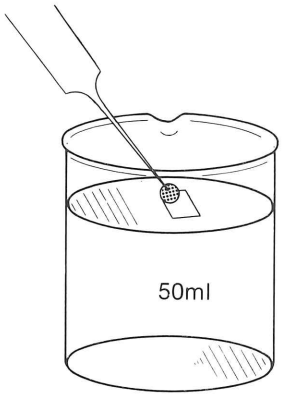
3.
Allow drop to diffuse and completely dry.



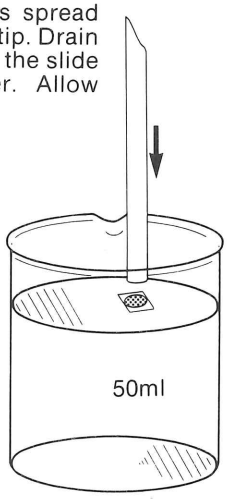
4.
0.025ml of 0.25% Formvar is spread over the surface with pipette tip. Drain excess immediately by tilting the slide against a piece of filter paper. Allow to dry 10 seconds.



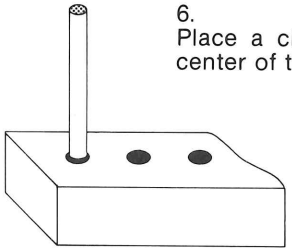
5.
Trim edges of agar block with scapel blade and float film onto surface of 3% phosphotungstic acid. Allow film to stain for 5 minutes.



6.
Place a clean 200 mesh grid in the center of the film.



7.
Retreive the grid and film by plunging the grid below the surface with a glass rod (3mm diameter). The film edges wrap around rod allowing grid to adhere to end of rod.



8.
Turn rod up and allow grid to dry.

FIGURE 1: Pseudoreplica technique.

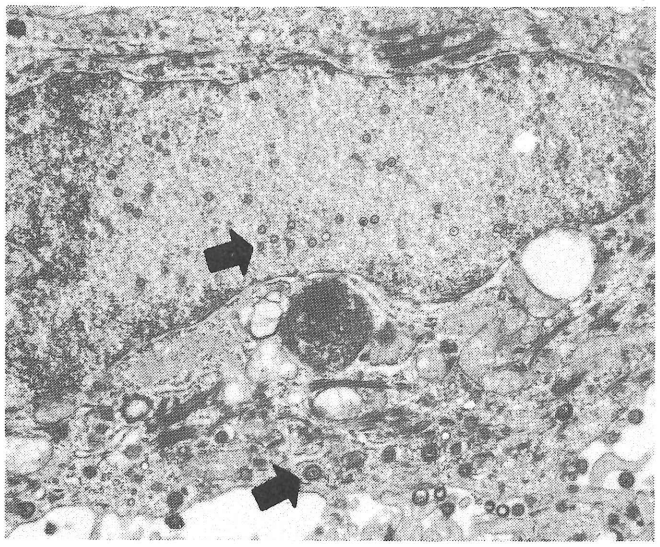


FIGURE 2: Electron micrograph of a skin biopsy taken from a Herpes simplex lesion. Both immature and mature virus particles are present (arrows). 21,000×

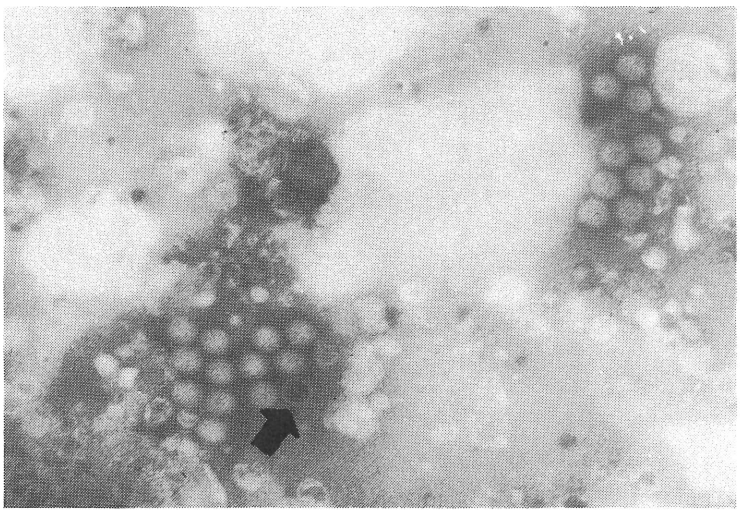


FIGURE 3: Electron micrograph of a diarrhea stool specimen processed by the pseudoreplica technique. Note the characteristic chart-wheel appearance of the rotavirus (arrow) 210,000×.

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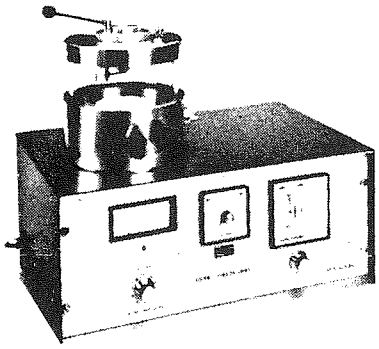

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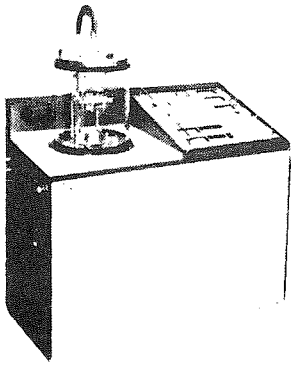

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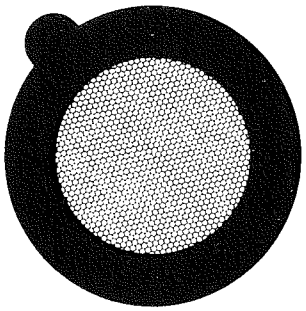

In the first pattern the grid bars are only 5 micron width resulting in a transmission of over 80%. The repeat distance is 460 lines/inch (18 lines/mm). Thus good specimen support is combined with very little obscurity of specimen.

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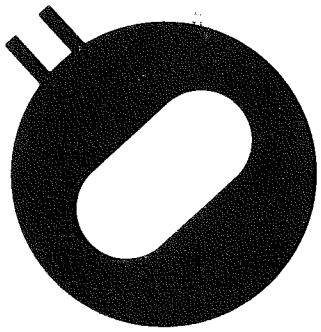

Long ribbons of ultramicrotomed sections can be supported on liquid drops suspended on a slot grid. The sections can then be dried onto a lower filmed grid. The orientation between the upper and lower grids is important if the ribbons are to be positioned correctly. This new slotted grid design has two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

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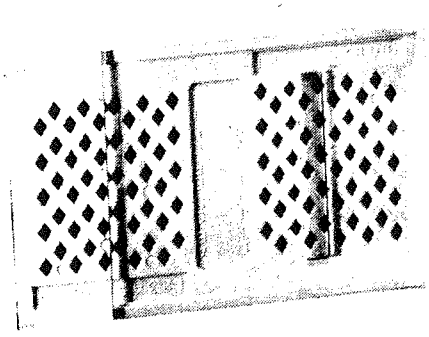

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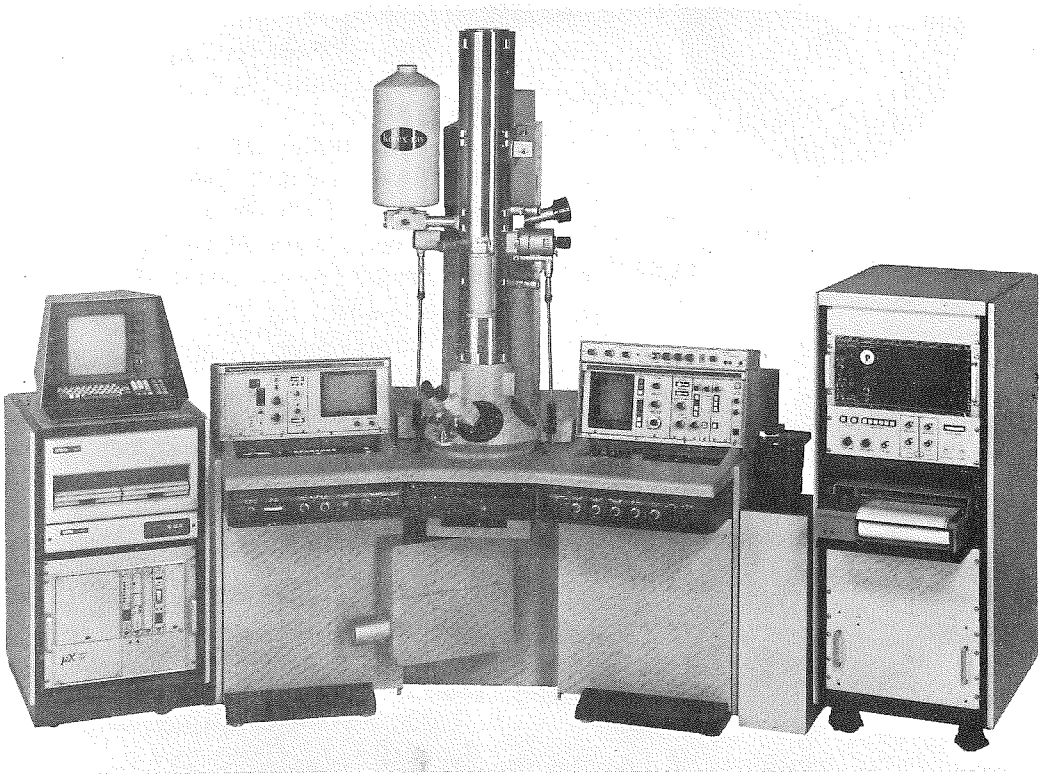

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Failure Analysis Applications Of The Scanning Auger Microprobe In The Semi-Conductor Industry

By
T. J. Shaffner
Central Research Laboratories
Texas Instruments, Incorporated
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INTRODUCTION

Similar to the conventional electron microprobe (EMP) equipped with x-ray detectors, the scanning Auger microprobe focuses a submicron spot of electrons onto the surface of a specimen. The spot can be scanned in a television raster in synchronism with the tracking beam on a viewing cathode ray tube, to form the scanning electron microscope (SEM) images that have become a familiar and valuable aid for a wide variety of production related applications. Recent development of sensitive electron energy spectrometers now makes it possible to sort from this large background of secondaries, those tiny contributions given by Auger production. Ejected Auger electrons have discrete energies dependent on the parent atoms, thereby availing one of a technique similar to EMP for detecting and identifying elemental species on a microscopic scale. The technique offers the additional capability for detecting single surface monolayers, and its value in the study of surface and interface chemistry has been well established during the last decade. Its success in failure analysis applications can also be attributed to high sensitivity for low atomic number elements, such as carbon, nitrogen, and oxygen, which give clues about contamination and cleanup procedures. The literature is beginning to stress importance of the scanning Auger as a failure analysis tool capable of qualitative routine application, much as the SEM is used today[1, 2, 3].

In our laboratory, we have for three years operated a JEOL JAMP-3 scanning Auger microprobe with 500 A spatial resolution capability, and observed its evolution from a novel research instrument into a practical cost-effective tool. This experience has brought clear definition of four characteristics which lead to solution of process related questions left unanswered by other techniques. This article gives examples of these which have had impact on practical aspects of our semiconductor manufacture and sales.

SUB-MICRON POINT ANALYSIS

Sub-micron point surface analysis is by design the most important capability of the microprobe. However, a single point by itself is seldom helpful unless compared with another from a control sample, background spot, or

cleaned surface. As routine procedure, we examine the specimen in situ, and then sputter clean the surface with argon for one or two minutes to remove airborne carbon and oxide contaminants absorbed prior to mounting. A follow-up spectrum is taken next at the initial point of analysis.

In Figure 1, a programmable read-only memory (PROM) circuit was analyzed. High logic is encoded on the chip by electrical continuity across thin aluminum strips which embody a Ti/W fusable link. Resistance heating is used to break select links and give open circuit, or low logic. This chip returned by a customer retained continuity across burned-open links leading to device failure. Carbon deposits were suspect, but Auger spectra revealed the presence of Ti and W. Following a three minute sputter cleaning, these vanish and open circuit is realized. To solve the problem, thickness and composition of the Ti/W were modified to insure complete vaporization of the link.

The best alternative technique for this analysis would be the EMP, which was also tried on the PROM circuit. Figure 2 illustrates why it was unable to resolve the problem. The monolayer of metal comprises about 1/10,000 of the micron sized volume excited by the probe, and detectable x-ray signal is produced throughout the entire volume. With a pure metal monolayer, the Ti signal borders on the 100 ppm detectability limit of the EMP, and even if detected, its surface character cannot be defined. On the other hand, the short mean free path of low energy Auger electrons, insures that only those within 10-30 A of the surface escape for detection, and this gives the technique its unique surface sensitivity.

MINIMAL SCATTERING EFFECTS

Calculations[4] and experiment[5] indicate that Auger spatial resolution is essentially determined by the primary beam diameter. Again, this is in contrast to the EMP, where scattered electrons excite detectable x-rays within the excited volume. The practical importance of the property cannot be overemphasized, since it leads to the possibility of performing analyses at points separated only by the diameter of the beam, without interference from nearby points on the surface. In Figure 1, notice that only Ti and W are detected; aluminum does not appear in the spectra. Spectra from the EMP have large aluminum peaks indicative of the metal positioned nearby.

Another example is shown in Figure 3, where a transistor bond repeatedly failing because of metal fracture was cross sectioned to expose a 3000 A thick contaminant layer within the gold metal itself. When the bond is under mechanical stress, fracture occurs across the film. Auger spectra with large carbon and nitrogen peaks suggest an air leak in the evaporator used for gold deposition. The leak was found and repaired. The absence of gold in the film's spectrum again demonstrates point-to-point independence on a sub-micron scale.

SIMULTANEOUS SEM IMAGES

Scanning Auger not only permits accurate probe positioning for analysis, but also encourages full diagnostic application of the SEM on every specimen. In the example of Figure 4, plant production of pocket scientific calculators was suspended pending solution of a battery contact problem. Dimples stamped in the metal for increased pressure against the battery were examined. Immediate identification of a stainless steel surface confirmed that the Ni plating required for resistance to corrosion and wear was absent. SEM images revealed a second unexpected problem with geometry where one smeared, flattened dimple resulted from inaccurate stamp alignment. Attention to these problems brought production back to normal.

To detect the Ni plating on the contact using EMP, one needs to compare plated versus non-plated samples, because this technique cannot distinguish a sub-micron thick Ni layer from bulk Ni in stainless. Such samples were not available; only "good" and "bad" were submitted to the laboratory. Auger confirmed that apparent performance differences could be resolved by correcting stamp alignment, and that both calculators, with only stainless surfaces, would ultimately fail.

PROFILING AND SPOT ANALYSES

Compositional depth profiling is possible when Auger spectra are measured at the same time sputter cleaning with argon is in progress. A 50 μm low power Schottky contact window in Figure 5 was profiled to determine causes for poor current/voltage characteristics, which were threatening to shut down production with unacceptably low yields. The high carbon content found throughout the layered structure testified to incomplete organic resist removal prior to metal deposition. Attention to chemical etch bath refresh schedules brought the problem under control.

Profiling can be accomplished with other instruments, such as the secondary ion mass spectrometer (SIMS) and Rutherford backscattering (RBS), but neither can achieve the combination of depth and spatial resolution required for this problem. Its solution resulted in a seven point yield increase. Loss of one yield point cost the industry between $50-100,000 per month, and we feel this application alone has justified purchase of the instrument.

DISCUSSION

The value of scanning Auger microscopy in routine failure analysis lies in its unique capabilities, and this article stresses four. Others will certainly be defined as further application accumulate. For example, high spot densities (near a microamp/μm^2) often lead to localized charge buildup and spectral degradation. Direct SEM imaging of charged surfaces offers flexibility to select spots free from the problem. This failing, a TEM grid of appropriate geometry can be used as a mask during thermal deposition of gold to apply grounded metal arbitrarily close to untouched spots preserved for analysis[6]. These spots can then be examined without spectral interference from gold. Other capabilities are now evolving to render elemental distribution maps useful in everyday situations. Currently, their utility is limited by

high background and low signal-to-noise of trace elements of interest, and the fact that spot analyses and SEM images often suffice. Computer enhancement of the images could subtract background and improve detectability, and such work has been reported [7]. It is perhaps another unique feature of the Auger microprobe that it lends itself readily to routine analysis, while remaining a topic of intense research and development.

ACKNOWLEDGEMENTS

The author is grateful to A. M. Williams for her help in acquiring and interpreting these data, and to the many coworkers who willingly supplied the samples and descriptions presented here.

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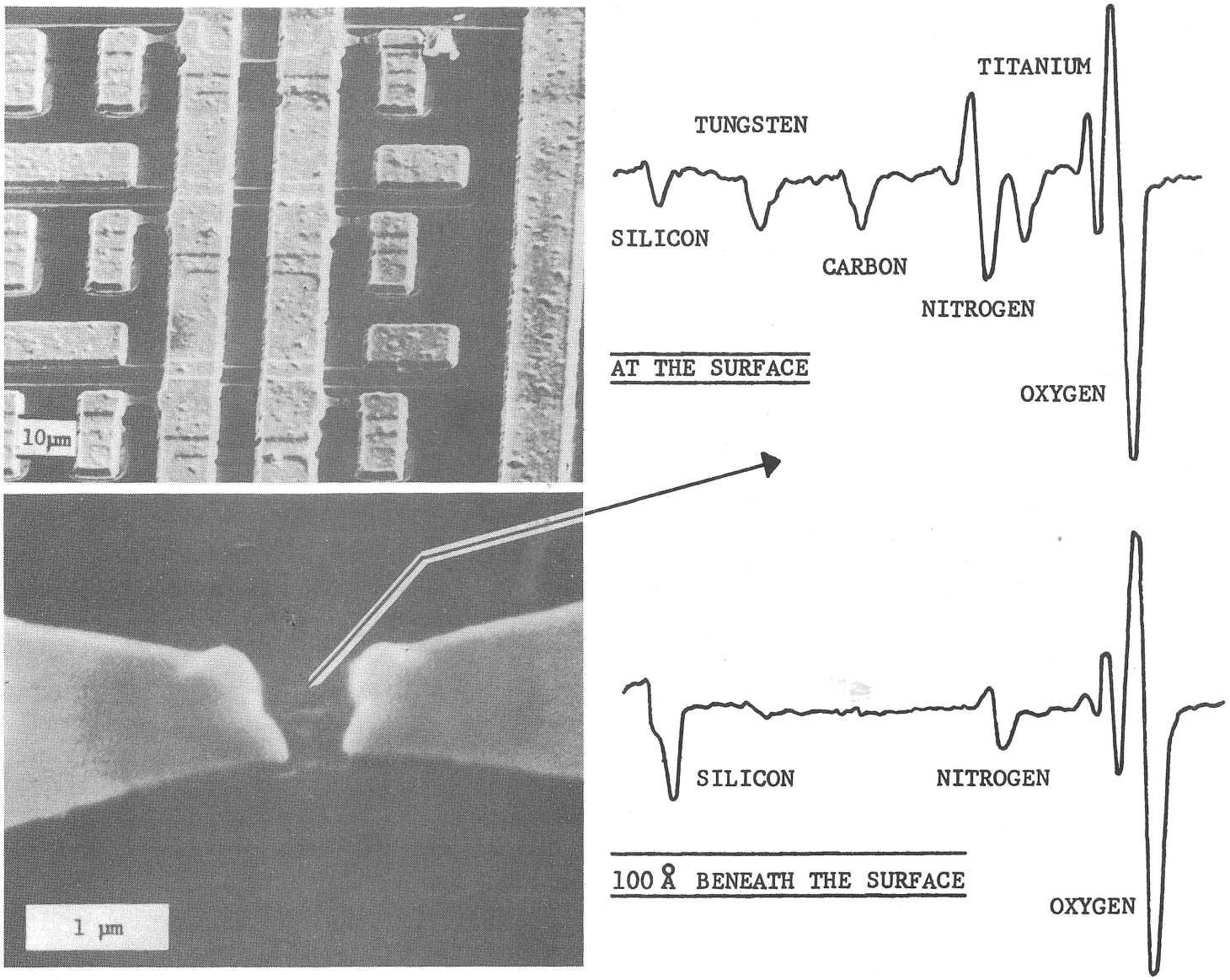

FIGURE 1: Short circuits in burned open PROM links are caused by a monolayer of Ti/W metal.

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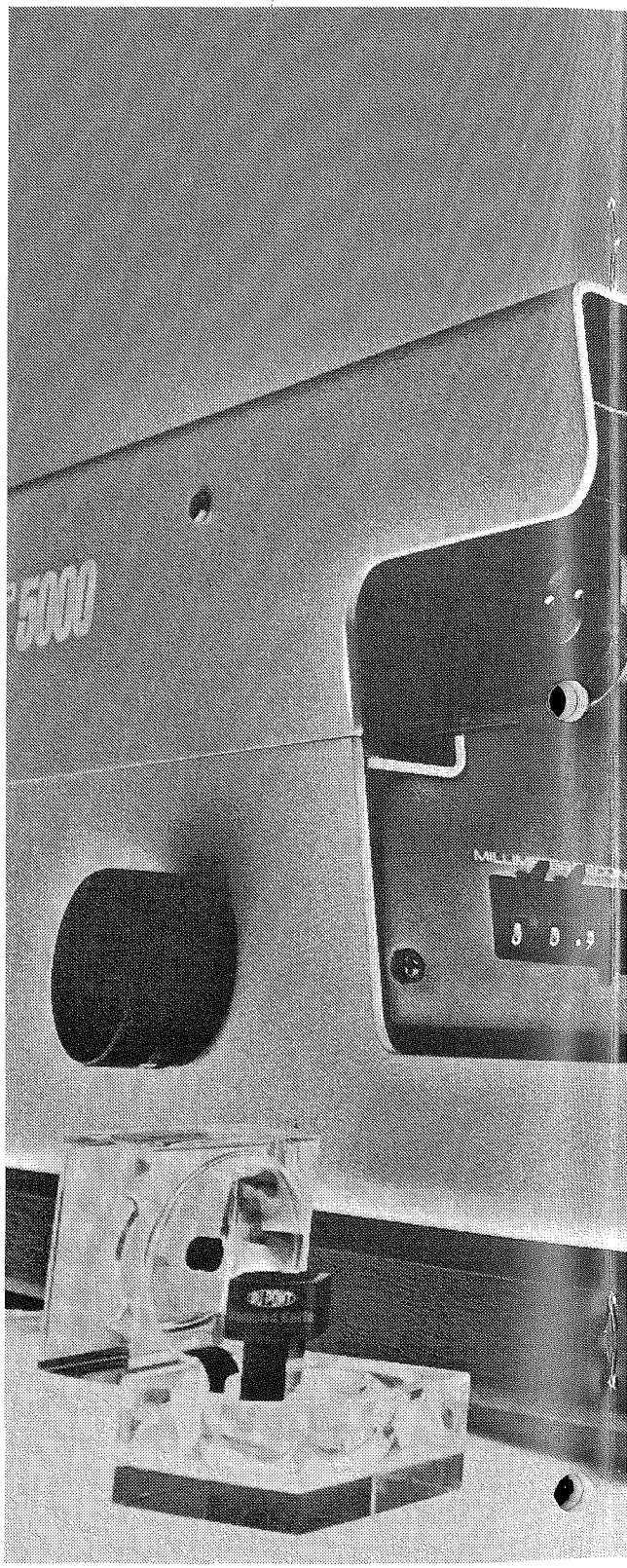

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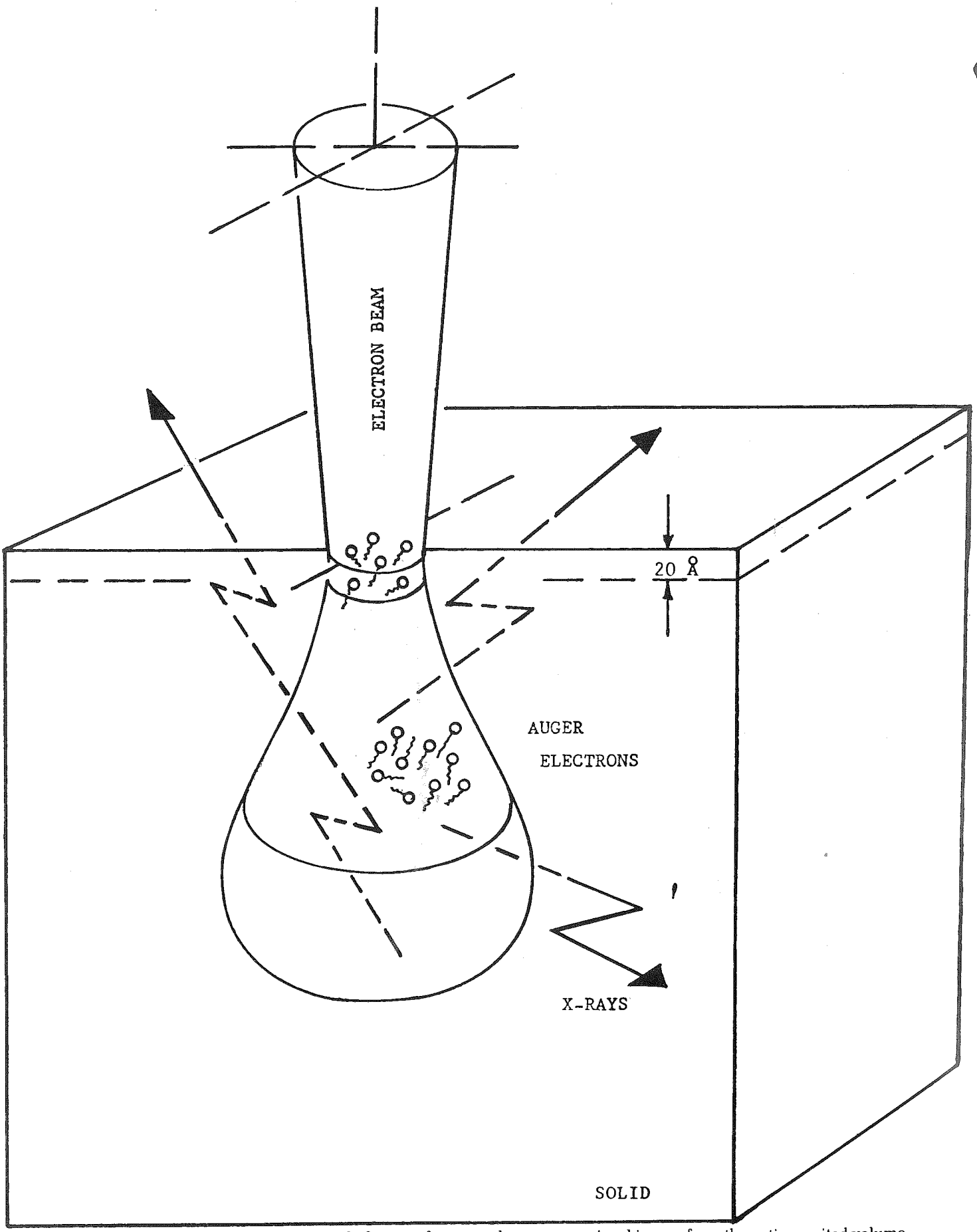

FIGURE 2: Detectable Auger electrons come only from surface monolayers; x-ray signal is seen from the entire excited volume.

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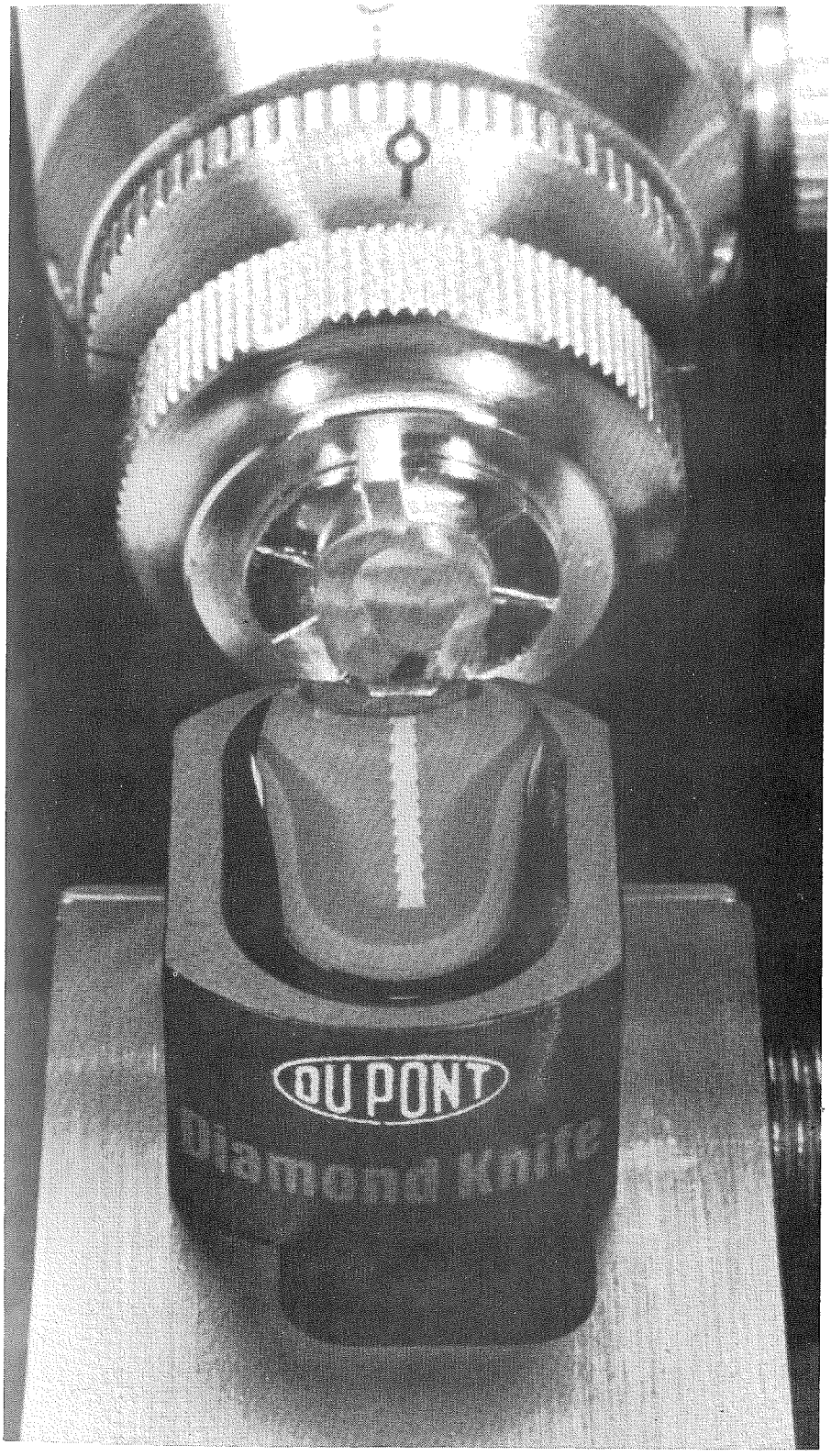

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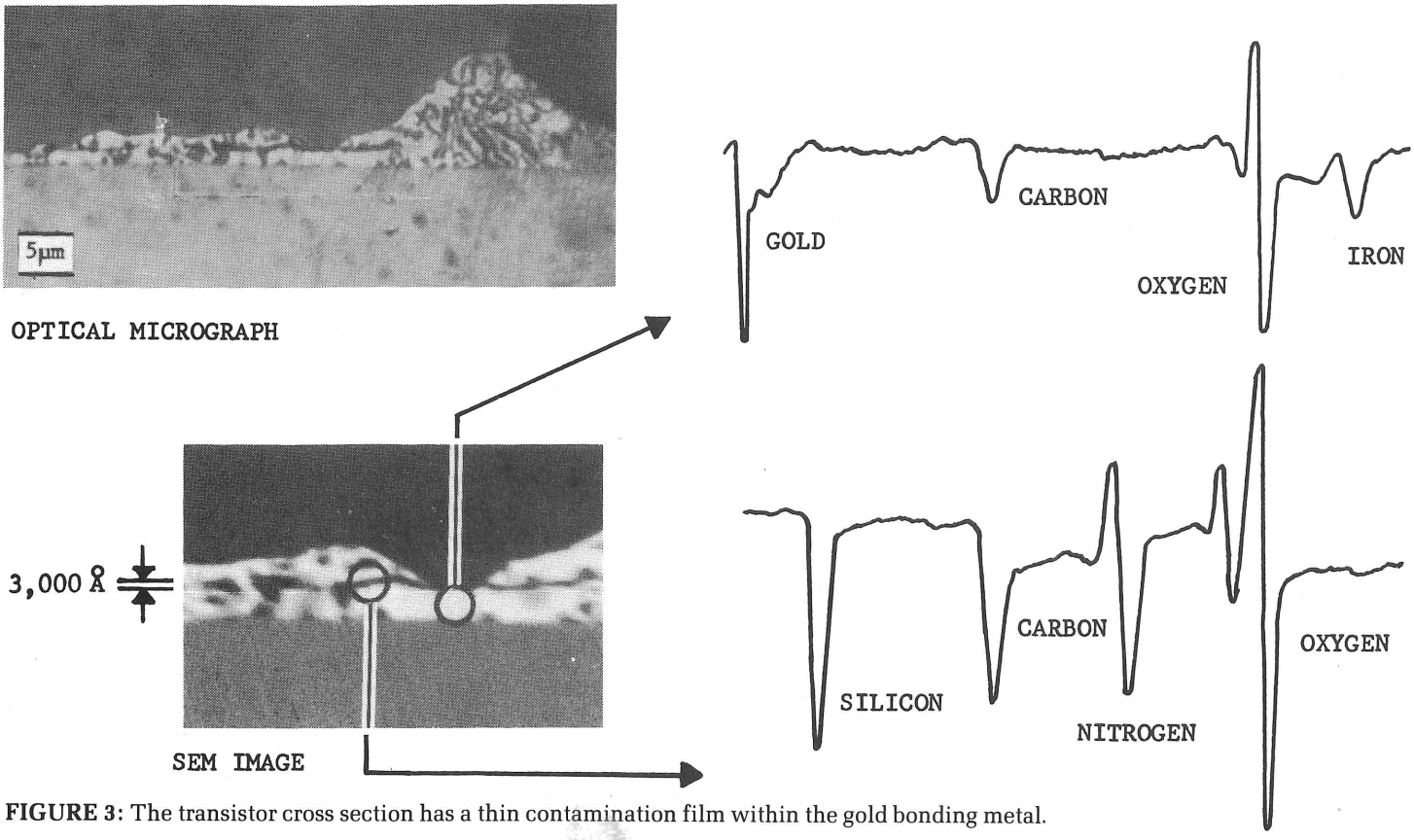

FIGURE 3: The transistor cross section has a thin contamination film within the gold bonding metal.

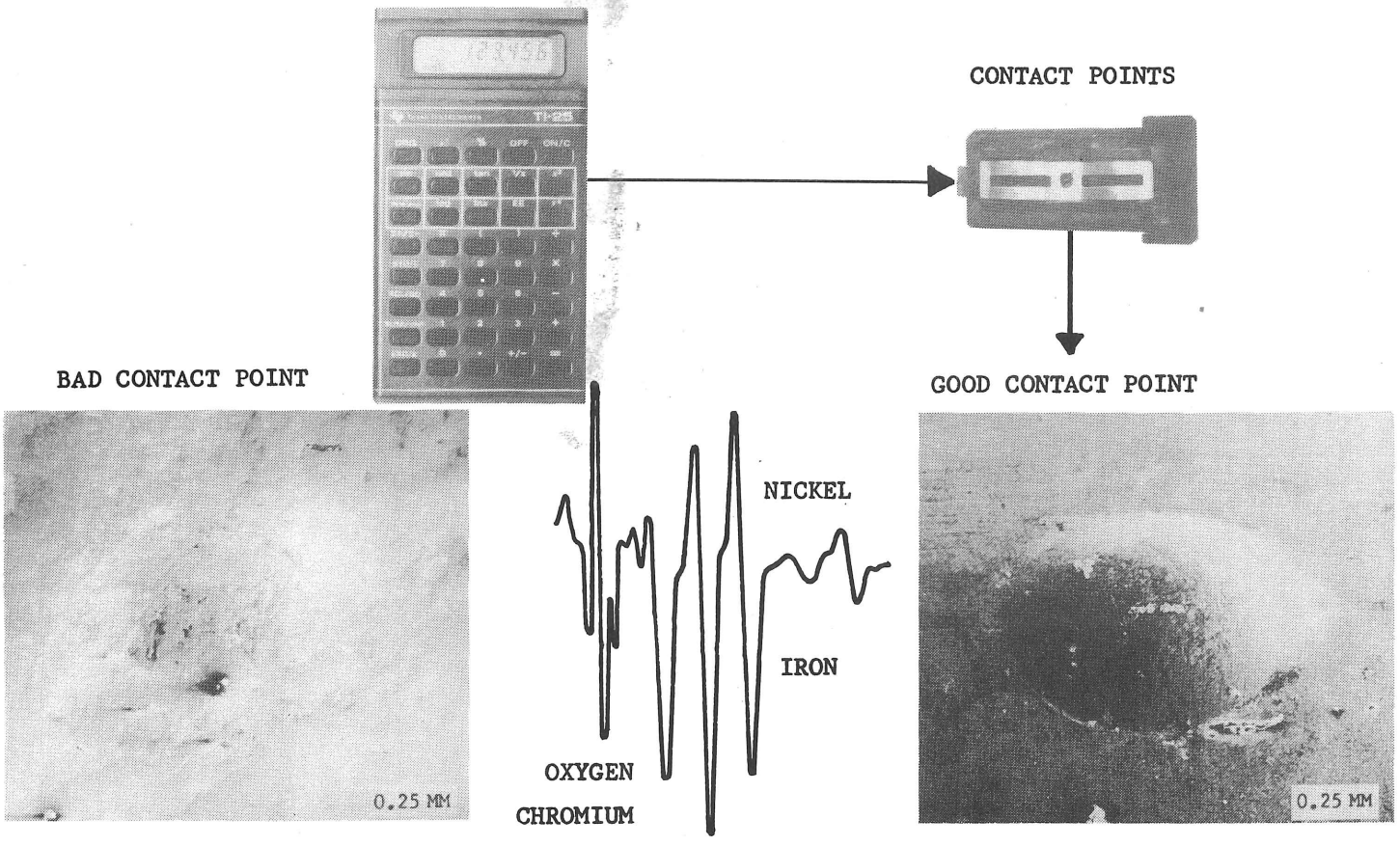

FIGURE 4: Poor battery contacts on pocket calculators expose two unrelated problems.

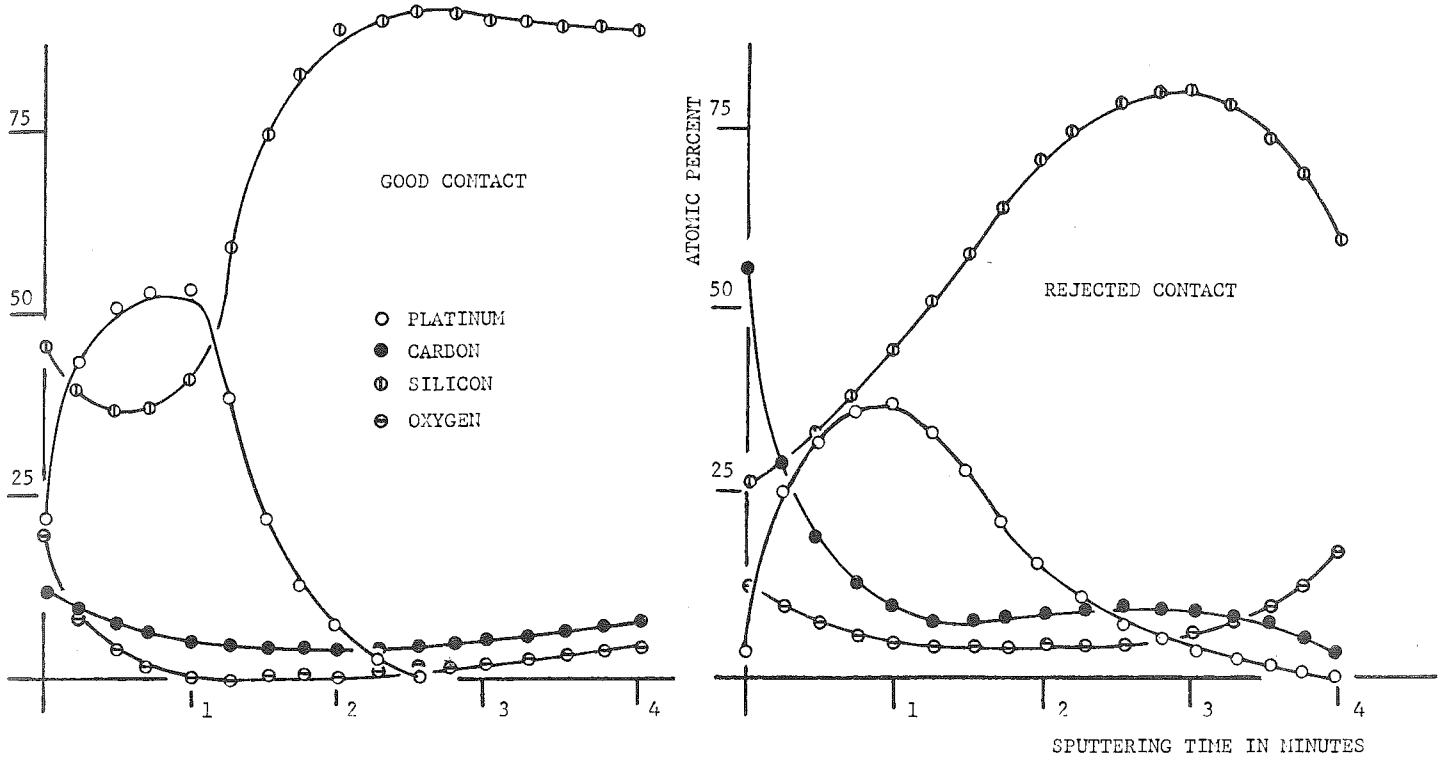

FIGURE 5: Carbon contamination throughout low power Schottky contacts testifies to incomplete predeposition cleanup.

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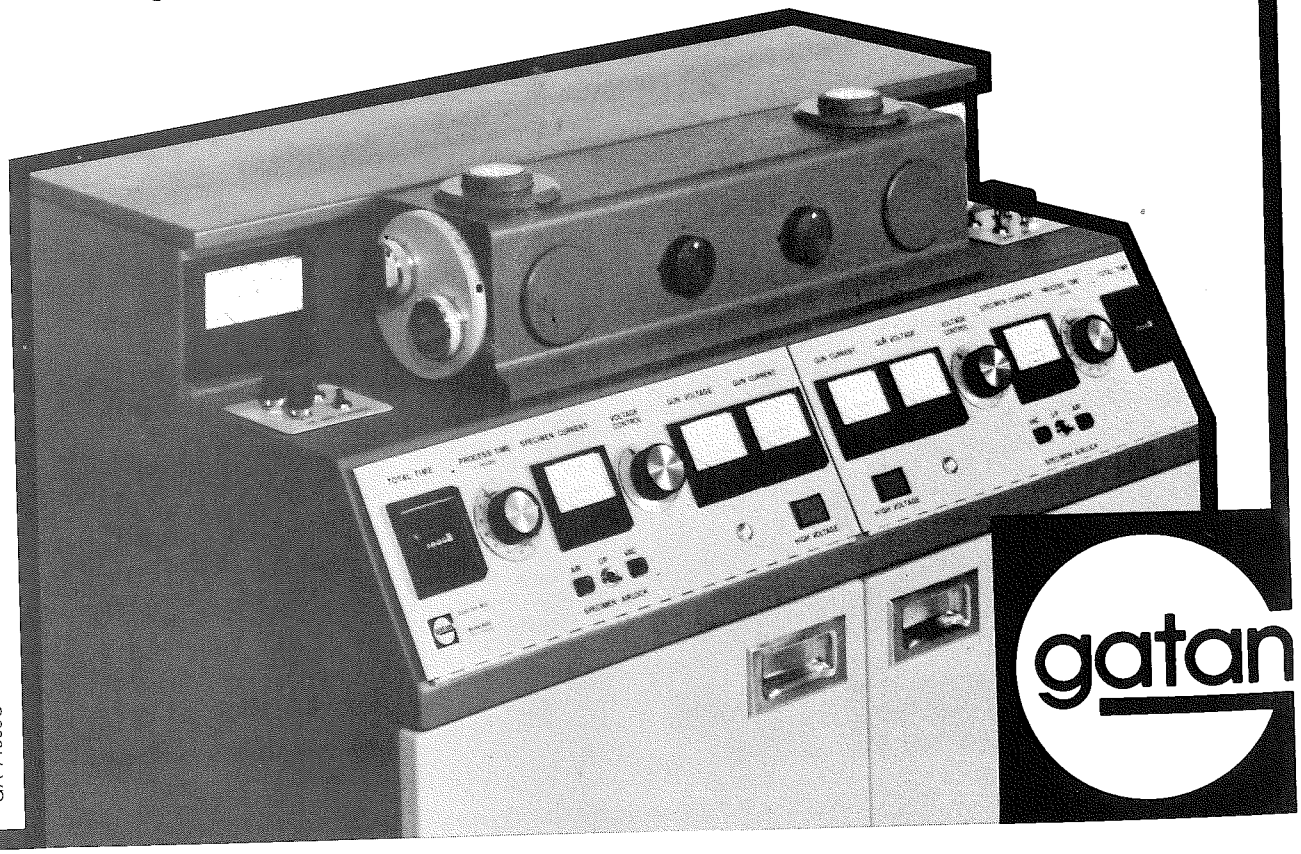

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Abstracts

RENAL MORPHOLOGY OF THE WHITE BASS, MORONE CHRYSOPS. Stephen C. Bennett Dept. of Biology, Stephen F. Austin State University, Nacogdoches, Texas.

The White Bass (Percicthyidae) is related to euryhaline species, and may have evolved from ancestors that lived in the sea and invaded fresh water in the early Cenozoic. The structure of the renal tubules is therefore of interest. The White Bass exhibits well vascularized glomeruli with a sparse mesangium. The visceral layer of the capsule is composed of podocytes that encircle the glomerular capillaries. The pedicels of the podocytes are of a more irregular nature than those of mammals. Fibrillar material is seen in groups around the nucleus, and often in the foot processes and pedicels. Preliminary measurements indicate that these fibers are larger than actin, and may be myosin, or tropomyosin. The structure of the renal tubules is similar to that of other primary division teleosts except that there is an extra segment of the proximal tubule. This segment is characterized by shorter, basophilic cells with centrally located nuclei, and vast amounts of rough endoplasmic reticulum in a perinuclear position.

A STEREOLOGICAL DESCRIPTION OF THE EFFECT OF DIET ON THE ULTRASTRUCTURE OF HEPATIC PARENCHYMAL CELLS. Jerry Berlin, Carmen Castro and Franklin Bailey, Texas Tech University, Lubbock, TX 79409.

In a previous study one of us (C.C.) reported diet-induced alterations in chromatin structure (J. Nutr. 110: 105-116, 1980). The present study was initiated to determine the effect of the same diets on the ultrastructure of the hepatic parenchymal cell.

Young male rats weighing approximately 150 g were caged individually and acclimated for a 4 day period with a stock laboratory diet. On the 5th day the rats were randomized by weight into 3 groups and fasted for 48 hrs. The following day the rats were offered ad libitum access to water and one of two semi-synthetic diets. The first diet was high carbohydrate and fat-free and the second diet was low carbohydrate and protein-free. Control animals were fed the stock laboratory diet. On the 5th day of feeding of the experimental or stock diet the rats were sacrificed by cervical dislocation and the livers were fixed in glutaraldehyde and embedded in Epon for morphological studies.

Cell volumes were determined by light microscopy from sections of known thicknesses. Additionally, the volume density (V_V) of nuclei and nucleoli were determined by planimetry from thick sections. Electron micrographs were printed with an overlaying grid for ultrastructural analysis. The V_V of cytoplasmic components (mitochondria, peroxisomes, lysosomes, lipid and glycogen) was determined by point counting. The surface density (S_V) of the outer mitochondrial membrane, mitochondrial cristae, RER and SER membranes per unit cytoplasmic volume was determined by linear analysis.

SEM STUDIES OF NAMA SEED COAT WITH POSSIBLE SYSTEMATIC IMPLICATIONS - Gail D. Chance and John D. Bacon. Biology Department, The University of Texas at Arlington, Arlington, Texas 76019.

Scanning electron microscopy studies of seed coat has shown a wide range of diversity in coat morphology among the species of **Nama** (Hydrophyllaceae). In general, **Nama** seeds fall into 3 major categories based on characteristic patterns on the coat: 1) a relatively smooth surface with little relief, save a few shallow depressions, 2) seed coat with numerous shallow depression in which adjacent cells are discernable, and 3) a reticulate surface which may be additionally ornamented with various wall thickenings and/or pitting patterns in walls of individual cells. Acetone extracted seed sections have demonstrated the multi-layered structure of the seed coat and, in general, all species possess a minimum of 2 layers. The cells of the external testal layer in seeds from categories 1 and 2 appear to be filled with secondary wall material and individual cells are rarely discernable. In some species the outer cell wall in this layer may be sunken, (but never absent), thus creating the shallow depressions. The reticulate nature of the outer testal layer in seeds from category 3 is the result of the outer cell walls being collapsed, torn, or absent. These various patterns, as well as the presence or absence of wall thickenings and pits, are of considerable taxonomic value and show trends among related species.

STRUCTURE OF THE MAMMALIAN CHROMOSOME: VISUALIZATION OF THE BACKBONE. Arthur Cole and Ruthann Langley, Physics Department, University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030.

Our studies have provided evidence that the mammalian chromosome contains eight circular DNA molecules arranged in a parallel array like eight stretched rubber bands laid side-by-side. At intervals of one to 20 μm a specific protein (Protein I) associates with outgoing and returning strands of each circular DNA molecule to form paired regions. Another protein (Protein III), found as 22 nm particles at the paired regions, associates laterally to form transverse organizational rows 22 x 176 nm in dimension. An association of 7 nm particles (Protein II) along specific DNA segments between organizational rows leads to a longitudinal condensation which, in the mitotic chromosome, produces a backbone ribbon consisting of a sequence of transverse organizational rows. DNA between organizational rows loops outward and condenses on 10 nm nucleohistone core particles to form fundamental chromatin fibers. These studies utilized the sedimentation of isolated mitotic chromosomes, through sucrose gradients containing selected treatment layers, directly onto electron microscope specimen supports. Salts, polyanions, and detergents were used to remove histone proteins and reveal backbone structures with radiating DNA loops. DNase digestion removed the radiating fibers to produce isolated backbone protein structures which retained the basic morphology of mitotic chromosomes. Results will be presented using stereo projections. Supported in part by Contract 76-EVO-2832 from the Department of Energy.

SQUAMOUS CELL CARCINOMA OF THE PROSTATE: REPORT OF A CASE Delcambre, Pamela S., Stinson, James C., Tormey, Albert R. Scott and White Memorial Hospital, Temple, Texas.

A case is presented of a 87 year old man who was experiencing symptoms of obstructive uropathy. Physical ex-

amination revealed an enlarged firm nodular prostate extending along the rectum. On general medical evaluation, there was no evidence of metastatic tumor. Tissue from a biopsy and transurethral resection showed the unusual occurence of a primary squamous cell carcinoma of the prostate. Ultrastructural as well as histochemical studies demonstrated mucous production as well as the predominant features of squamous differentiation.

EFFECT OF PILIATION OF KLEBSIELLA PNEUMONIAE ADHERENCE IN THE RAT BLADDER. R.C. Fader* and C.P. Davis, Dept. of Microbiology, UTMB, Galveston, TX.

Pili are believed to be mediators of **K. pneumoniae** adherence to bladder epithelium and thus may play an integral role in the pathogenesis of cystitis caused by the organism. By controlling the total time of growth in broth culture, piliated (P+) and nonpiliated (P-) populations of a single **K. pneumoniae** strain were obtained and injected into female rat bladders through a urinary catheter. The rats were prevented from urinating for 4 h and the animals were sacrificed at 4 h, 12 h, or 5 days post-infection. Bladders were processed and viewed by scanning electron microscopy for evidence of infection (ie., bacteria on bladder surface, areas of ulceration, swelling of bladder folds). No evidence of adherent bacteria, ulceration, or bladder swelling was observed in control animals injected with sterile buffered saline. 4 h after infection with P+, 5 of 6 bladders had bacteria on the bladder surface and in areas of ulceration. All 6 bladders showed evidence of swelling. In rats infected with P-, only 2 of 6 bladders had bacteria on the bladder surface; however, no ulceration was observed in the 6 bladders even though 2 of 6 had slight bladder fold swelling. At 12 h, all 6 rats infected with P+ demonstrated adherent bacteria, ulceration, and bladder fold swelling whereas in rats infected with P-, only one of six bladders were observed to be infected by day 5, four of six rats infected with P+ continued to show evidence of cystitis although only 2 bladders were observed with adherent bacteria. Only 1 bladder in the group infected with P- had evidence of cystitis and no bacteria were visible on the bladder surface. Thus it appears that piliations is an important parameter in the ability of **K. pneumoniae** to establish infection in the bladder.

CORRELATION AND LACK OF CORRELATION IN MUSCLE DISEASE AT THE LIGHT AND ELECTRON MICROSCOPY LEVEL. King, Tom, Turner, R.A. and Stinson, J.C. Scott and White Memorial Hospital, Temple, Texas.

Muscle disease is still a relatively new area of exploration, both by light and electron microscopy and the interrelation of muscle and the other systems of the body is still not well defined. The value of the electron microscope in the study of muscle is still not completely excepted by all.

In this presentation, examples are given in which there is good correlation between the light microscopic appearance of the abnormal muscle and electron microscopic appearance and other examples are discussed in which minimal changes were present by light microscopy but at the ultrastructural levels significant alterations are present. Some of these alterations described are poorly understood but will be useful for future comparision.

ULTRASTRUCTURE OF THE FUNGUS EXOBASIDIUM ON CAMELLIA SASANQUA. Charles W. Mims, Dept. of Biology, S.F. Austin State Univ., Nacogdoches, Texas.

Members of the fungus genus **Exobasidium** are all plant parasites attacking not only wild plants but also various ornamentals including varieties of **Azalea** and **Camellia.** This study concentrates on the ultrastructure of **Exobasidium camelliae** on its host **Camellia sasanqua.**Leaves of **C. sasanqua** infected with **E. camelliae** enlarge to many times their normal size. An extensive system of slender, septate hyphae develops beneath the epidermis of an infected leaf. Large, Club-shaped basidia develop from these hyphae and push through the epidermis and cuticle of the host. The hyphae within the leaf extend through the intercellular spaces of the leaf. Small finger-like haustoria develop from hyphae in close association with host cells. Haustoria penetrate the wall of the host cell and invaginate the cell membrane. Each haustorium contains a distinctive system of interconnected membranes. The structure of this membranous system is considered.

SOME COMMENTS ON THE CAUSE AND ELIMINATION OF CHATTER IN ULTRATHIN SECTIONS. Hilton H. Mollenhauer, Veterinary Toxicology and Entomology Research Laboratory, Science and Education Administration, Agricultural Research, U.S. Department of Agriculture, College Station, TX 77840.

Chatter is a relatively common problem associated with the ultrathin sections used in electron microscopy. Chatter is most often encountered during the testing of new knives but may also show up occasionally throughout the life of a knife. Many factors may contribute to chatter including knife sharpness, block hardness, microtome rigidity, and electrostatic charges that form on the knife surface and tissue block. Because careful adjustment of cutting angle sometimes eliminates chatter, cutting angle is often considered a primary cause of chatter. In this report the problem of chatter is discussed in light of the case histories of 7 new diamond knives all of which produced sections that were unusable because of chatter. In all of these examples the chatter problem was alleviated by various adjustments to the microtome or to the cutting angle of the knife. The conclusions are: 1) chatter is dependent on knife edge quality (i.e., sharpness). 2) Microtome rigidity may be important in reducing chatter. 3) Cutting angle is not very critical if the knife is sharp and if there is sufficient clearance angle between the knife and block face.

ENDOCRINE-LIKE CELLS IN THE MIDGUT EPITHELIUM OF THE HOUSE CRICKET (ACHETA DOMESTICUS), Timothy J. Molloy, and Ernest F. Couch, Department of Biology, Texas Christian University, Fort Worth, Texas, 76129.

Electron and light microscopic studies were made on the midgut epithelium of the house cricket **(Acheta domesticus).** Cross sections of the midgut region were fixed with glutaraldehyde, paraformaldehyde, and trinitroresorcinol in cacodylate buffer. After post fixation with 1% OsO_4, the samples were flat embedded in Araldite. Thin sections were stained with uranyl acetate and lead citrate and examined under a Philips 300 microscope.

The cricket midgut is principally involved in absorption and secretion, being roughly comparable to the small intestines of higher animals. Three general cell types were observed; columnar cells, regenerative cells, and basal granulated endocrine-like cells. We are much interested in the basal granulated cells which were found associated with the regenerative nidi, both of which are located beneath the columnar cells and along the basement membrane. These basal granulated cells resemble the endocrine cells found in vertebrate digestive tracts. Thus far, two distinct types of endocrine-like cells have been observed. The two types can be differentiated by granule size and granule electron density.

ELECTRON MICROSCOPICAL STUDY OF CAENORHABDITIS ELEGANS. Jay Pearce, James K. Butler, David Mitchell,

Biology Department, University of Texas at Arlington, Arlington, Texas, Boston Biomedical Research Institute, Boston, Massachusetts.

The free-living soil nematode, **Caenorhabditis elegans,** is of particular interest because of its genetical simplicity, its short life cycle, small size, ease of laboratory maintenance, and comparatively simple morphology. This preliminary electron microscopical survey of the morphology and internal organization of **C. elegans** is based mainly on scanning microscopy of intact and freeze fractured worms. Some thin sections were also examined.

INTERNALIZATION AND DISTRIBUTION OF CATIONIC FERRITIN AND SERUM-FERRITIN COAGULATE IN POLYMORPHONUCLEAR LEUKOCYTES (PMN). W.A. Shannon, Jr., VA Medical Center and Department of Cell Biology, University of Texas Health Science Center at Dallas, TX 75216.

The distribution and motility of anionic sites on the membrane surface of PMN were studied by cationic ferritin (CF) labeling and bulk presentation of serum-ferritin coagulate (FC).

PMN from rabbit peritoneal exudate were incubated at 37°C in the presence of CF. The cells were incubated in medium with and without serum. The cells were then fixed and prepared for thin section electron microscopy.

Within 5 min, serum-free CF was observed within the smallest class of vesicles which were seen throughout the cytoplasm and adjacent to the nucleus. CF-filled vesicles appeared to form by endocytosis. CF outlined the plasmalemma sporadically at some foci but en mass at others. Larger vacuoles were labeled immediately within their perimeters. The smaller vesicles often appeared adjacent to or connected to the larger vacuoles. Labeling of vacuoles within the Golgi area was also apparent. Outside the cells, clumps of FC were periodically apposed to the membrane. FC had basically the same pattern of distribution within the cells. Unlike with CF, large vacuoles were replete with FC and there was less obvious endocytosis with no localization in the Golgi regions.

It appears that, based on the mode of presentation to PMN, CF is either endocytosed or phagocytosed. Although both mechanisms result in internalization of the same marker, the primary mode of internalization and distribution differ.

SURFACE MORPHOLOGY OF AN ASPHALT-BEARING LIMESTONE FROM SOUTHERN OKLAHOMA. Lowell E. Waite, Department of Geology, University of Texas at Arlington, Arlington, Texas 76019.

The examination of external surface features of the Buckhorn asphalt, which is exposed in the Arbuckle Mountain region, southern Oklahoma, was performed using the SEM. The Buckhorn asphalt consists of asphalt-bearing limestones (grainstones) of Middle Pennsylvanian (Desmoinesian) age. The rock-unit has a residual bitumen content of greater than 8%, and is highly fossiliferous. Previous authors have noted the presence of unaltered aragonite in molluscan shells contained in the limestones, implying a lack of diagenesis due to the existence of the hydrocarbons.

Surface morphology was examined in both untreated samples (hydrocarbons present), and treated samples (hydrocarbons extracted with an organic solvent). Preliminary observations of both types of samples include the following: (1) a rock matrix consisting of large mollusk shell fragments, surrounded by fine-grained carbonate material; (2) numerous small holes occurring throughout the samples, thought to represent bioturbation structures; and (3) a lack of surficial diagenetic features.

These initial findings agree with earlier reports that the impregnation of the limestones with hydrocarbons has prevented the occurrence of diagenesis. The SEM will be a valuable tool in further study of the Buckhorn asphalt, particularly in assessing the post-depositional history of the rock-unit.

STRUCTURAL FEATURES OF DORMANT SEEDS OBSERVED WITH SEM. Mary Alice Webb and Howard J. Arnott, Department of Biology, The University of Texas at Arlington, Arlington, Texas 76019.

Methods which were developed for using scanning electron microscopy (SEM) to study the mature dormant seed of zucchini **(Cucurbita pepo)** in its dehydrated state have now been applied to other dormant oil seeds, including **Yucca** and **Hibiscus** seeds. Methods used combine anhydrous preparations of seed tissue with a fracture technique which reveals intracellular features. Features such as undulating cell walls, polyhedral cytoplasmic units, and compact protein body structure seen in dormant zucchini seed with SEM have now been observed in these seeds. Arrangement of protein bodies in orderly arrays such as those seen in zucchini seed are not seen in **Yucca** and **Hibiscus** seeds. In order to sort out ultrastructural changes that occur with imbibition and aqueous fixation of the seeds, anhydrously fixed seeds are contrasted with aqueously fixed seeds which have also been fractured. Seeds which exhibit undulating cell walls when dehydrated have walls that are smooth and flat following imbibition. Protein body structure observed in anhydrously fixed **Yucca** differs from that observed following aqueous fixation, but lipid body structure in both anhydrous and aqueous preparations of **Yucca** appears similar. Cellular components of **Hibiscus** seed, also consisting of protein and lipid bodies, are readily observed using the methods described. Fracturing techniques also reveal large druse crystals in some cotyledon cells of **Hibiscus.**

FRACTIONATION OF POLYMORPHONUCLEAR LEUKOCYTES (PMN) FROM HUMAN SYNOVIAL FLUID. D.M. Zellmer and W.A. Shannon, Jr. VA Medical Center and Department of Cell Biology, University of Texas Health Science Center at Dallas, TX 75216.

PMN of peripheral blood from normal humans have been fractionated by isopycnic centrifugation. Fractions containing azurophilic and specific granules have been identified, as well as a granule which contains alkaline phosphatase activity.

We have fractionated PMN from the synovial fluid of a gout patient by velocity sedimentation. The fractions obtained were comparable to those obtained from PMN of normal peripheral blood.

The alkaline phosphatase-containing fraction consists of small granules exhibiting various shapes, from spherical to dumbbell (spherical, 150 nm; ovoid 150 X 196 nm; elliptical, 60 X 270 nm; dumbbell, 45 X 360 nm). The latter are thought to contain the bulk of the enzyme activity. The ovoid or elliptical specific granules are larger (ovoid, 196 X 226 nm; elliptical, 135 X 286 nm), and the contents are more electron dense, although they are not of uniform density. The azurophils are quite large (270 X 346 nm) and much more electron dense, although variable in density. The ovoid granules obtained by velocity sedimentation are more uniform in size than those obtained by isopycnic centrifugation. Enzyme assays indicated one difference in the two types of fractionation. Isopycnic sedimentation results in two peaks of lysozyme activity, whereas velocity sedimentation yields a single peak coincident with the specific granule fraction.

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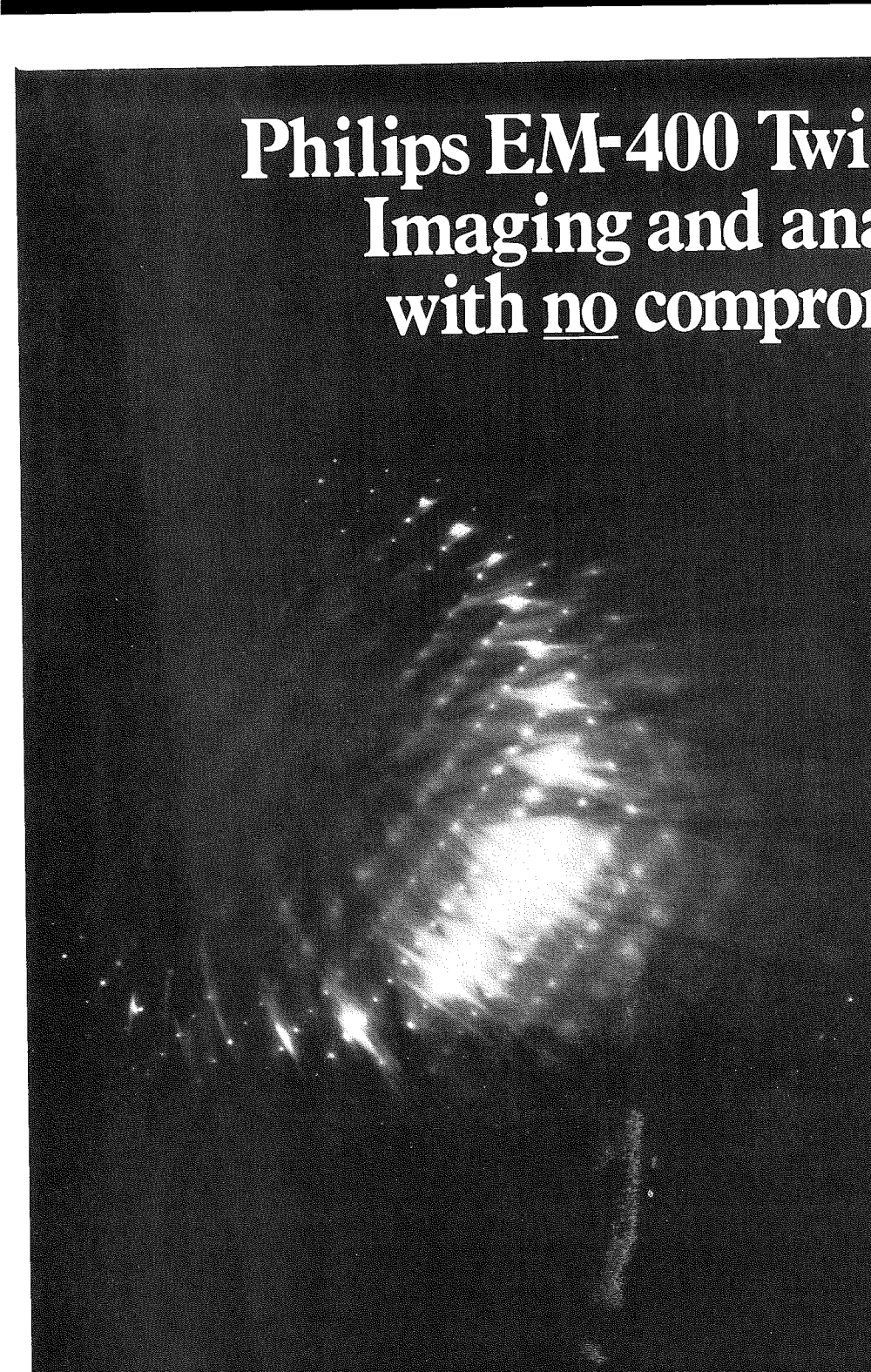

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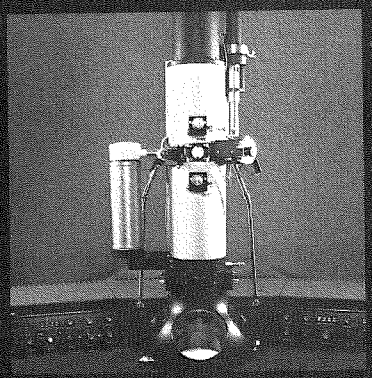

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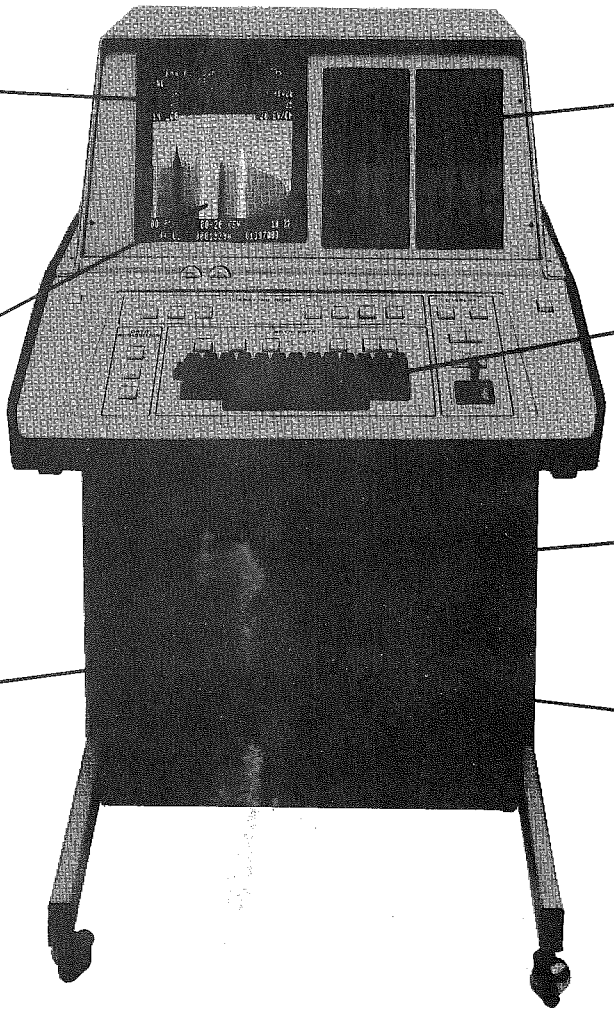

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Regional News

COLLEGE STATION
DEPARTMENT OF VETERINARY ANATOMY, COLLEGE OF VETERINARY MEDICINE

GRANTS AWARDED

A grant was awarded to Dr.'s D. H. Lewis (Vet. Microbiology), R. F. Sis (Vet. Anatomy) and R. R. Stickney (Wildlife and Fisheries Science) by the USDA. The project is entitled "Disease Management in Catfish Aquaculture" and seeks to evaluate the significance of ammonia stress on catfish at the microscopic and ultrastructural levels. (USDA Animal Health Research, Competitive Grant Program)

PRESENTATIONS

Kenneth L. White, asst. prof. Geography, "Paleo-subsurface galgai?: A preliminary report of SEM investigation of sand grain morphology." Soil Survey and Land Resource Workshop, Texas A&M University.

PUBLICATIONS

Greta A. Fryxell, assoc. res. scientist, Oceanography, co-author "The genus *Thalassiosira*: species with internal extensions of the strutted processes." Phycologia.

Greta A. Fryxell, assoc. res. scientist, Oceanography, "The genus *Thalassiosira: T. trifulta sp. nova* and other species with tricolumnar supports on strutted processes." Nova Hedwigia Beiheft.

Peter J. Rizzo and Robert C. Burghardt, asst. profs., Biology, "Chromatin structure in the unicellular algae *Olisthodiscus leteus. Cryphecodinium cohnii* and *Peridinium balticum.*" Chromosoma.

NEW EQUIPMENT

The Electron Microscopy Center has recently obtained a Phillips 400 transmission electron microscope. The microscope was purchased by means of an N. S. F. grant to several members of the Biology and Entomology Department faculties.

DALLAS
UNIVERSITY OF TEXAS HEALTH SCIENCE CENTER, DEPARTMENT OF PATHOLOGY

GRANTS AWARDED

Dr. Karen Burton, Pathology, has received a grant from the American Heart Association; Texas Affiliate.

PUBLICATIONS

Burton KP, Templeton GH, Hagler HK, Willerson JT and Buja LM: Effect of glucose availability on functional membrane integrity, ultrastructure and contractile performance following hypoxia and reoxygenation in isolated feline cardiac muscle. Journal of Molecular and Cellular Cardiology 12:109-133, 1980.

NEW EQUIPMENT AND/OR FACILITIES

Our JSM-35 has recently been equipped with a backscatter electron image system. The system will be used for particle detection in pulmonary projects and in forensic work. A Sorval JB-4 and MT-5000 will be joining our equipment roster soon.

NEW FACULTY AND/OR STAFF MEMBERS

Rebecca Swick, Research Assistant I, joined Dr. Karen Burton to work with her rabbit septal preparation.

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

AWARDS

S. J. Enna, Ph.D., Associate Professor of Neurobiology and Pharmacology received the John J. Abel Award in Pharmacology for 1980 on April 16 in ceremonies in Anaheim, Cal. This award is given each year by the American Society for Pharmacology and Experimental Therapeutics to an individual under 35 years of age for original and outstanding research in pharmacology. Dr. Enna received the award in recognition of his work on neurotransmitters, in particular, gamma-aminobutyric acid (GABA).

LECTURES AND MEETINGS ATTENDED

During the week of January 20th, S. J. Enna, Ph.D. attended the Winter Conference on Brain Research in Keystone, Colorado and made two presentations. They were entitled "Age-related alterations in neurotransmitter receptor binding and function" and "Neurochemical alterations induced by chronic administration of antidepressants."

Nachum Dafny, Ph.D. presented the lecture "Is the pineal also a neuromodulator?" at the University of Arizona in Tucson, at Cal-Tech in Pasadena, Cal., at the City of Hope in Durate, Cal. and at the Brain Research Institute at UCLA, February 5-8.

"The aging brain: A new theory" was the title of a talk given to the Houston Area Parkinson Society on February 8th by Dr. S. J. Enna. On February 26th Dr. Enna presented the seminar "Neurochemical alterations in aging and age-related disorders" to the Department of Anatomy and the Institute for Basic Research at the University of Rochester. On April 10th, Dr. Enna participated in the International Symposium on Psychopharmacology and Biochemistry of Neurotransmitter Receptors at the University of Arizona.

"Neuron secreting hormones of the CNS" was the title of Dr. Gerald Kozlowski's seminar presented at NIH on February 29th.

Sue Patterson, Neuroscience Librarian, attended the National Library of Medicine's initial online services training course for using Medline and related data bases at the University of Texas Health Science Center at Dallas March 3-7. Ms. Patterson is coordinating medline searches for the combined Neuroscience departments of the University of Texas Medical School at Houston.

Members of NB&A were active participants at the national meeting of the Society for Neurochemistry held in Houston the week of March 3. Zehava Gottesfeld, Ph.D. gave a presentation entitled "Noradrenergic sprouting in the partially deafferented habenula;" Dianna Redburn, Ph.D. presented work entitled "^{3}H-Muscimol binding in retina;" Richard Wiggins, Ph.D. presented the paper "Brain cyclic nucleotide responses to halothane" by Wiggins and Divakaran; and Philip Patsalos, Ph.D. presented

work entitled "Anticonvulsant drug teratogenecity" by Patsalos and Wiggins. As well, S.J. Enna, Ph.D. chaired a symposium on antidepressants where he presented a lecture entitled "Effect of antidepressants on neurotransmitter receptor binding."

On March 18th, Nachum Dafny, Ph.D. presented "Is the pineal the seat of the soul?" at the University of Iowa in Iowa City; on the 19th — "Are the basal ganglia involved in pain perception?" at the Mayo Clinic in Rochester, Minn.; on the 20th — "Are the basal ganglia involved in drug addiction?" at the University of Cincinnati; and on March 21st — "The search for morphine specific sites in CNS" was presented at the University of Michigan at Ann Arbor.

Joe G. Wood, Ph.D., Chairman, and Dianna Redburn, Ph.D. presented lectures for a course at the Women's Institute, March 19 through April 23, entitled "The normal and abnormal brain."

Jon DeFrance, Ph.D. visited the University of Mexico in Mexico City March 23-29 where he presented a series of lectures to the Department of Physiology.

Jo Ann McConnell, Ph.D. and John Linner, Ph.D. travelled to New Orleans in April to attend the annual meeting of the Histochemical Society. Dr. McConnell presented work entitled "Histochemical analysis of autonomic nerve fibers in the bladder of the human and the cat," and Dr. Linner participated in the Immunocytochemistry Workshop.

Richard Wiggins, Ph.D., attended and presented a lecture at the International Colloquium on Neurological Mutations Affecting Myelination in Seiliac, France, April 12-18.

At the recent FASEB meetings in Anaheim, Cal., April 14-17, David McCandless, Ph.D. Nachum Dafny, Ph.D., and members of Dr. Dafny's laboratory represented the department. Dr. Dafny's lab presented five papers: "Photic input reach the rat pineal via sympathetic and habenular complex," presented by Dr. Dafny; "Dose related effect of halothane on sensory evoked responses recorded from freely behaving rats," presented by Benjamin Rigor, M.D.; "Effect of substantia nigra, dorsal raphe, medial lemniscus and acoustic stimulation on single units in the caudate nucleus of the rat," presented by Avital Schurr, Ph.D.; "Horseradish peroxidase determination neuronal connection between the rat pineal and the habenular nuclei," presented by Mrs. Marjorie Brown; and "Modification of dose-dependent changes in sensory evoked potentials by chronic halothan administration," presented by Gregory Fuller.

Dianna Redburn, Ph.D., presented the seminar "Dopaminergic amacrine cells of the mammalian retina" at the Neuroscience Seminar Series, Baylor College of Medicine, on April 27.

Drs. S. J. Enna and Dianna Redburn have been named to the editorial board of the Journal of Neuroscience Research.

NEW FACULTY AND STAFF MEMBERS

We are pleased to welcome Mrs. Ingrid Goldie as our new Administrative Assistant. Mrs. Goldie joined us on March 17th. Another newcomer is Andrea Elberger, Ph.D. Dr. Elberger, Assistant Professor, completed her postdoctoral work in the Department of Anatomy of the University of Pennsylvania School of Medicine.

BAYLOR COLLEGE OF MEDICINE, DEPARTMENT OF CELL BIOLOGY

LECTURES

Frances L. Miller and debbi E. M. hodges will be giving a joint presentation entitled "Light and Electron Microscopy of Tissue Culture Monolayers" for the annual meeting of the Texas Society of Histo-technology, Inc., in San Antonio, May 14-17.

PUBLICATIONS

Micrographs from Dr. Myles L. Mace, Jr. and debbi E. M. hodges will be on display for the opening of the new Texas Commerce Bank - Med. Center in Houston, Texas.

E.M. EDUCATION POSSIBILITIES

There is talk of an informal meeting of E.M. technicians working at the TMC campus for the purpose of exchanging information and ideas. Is it just a pipe dream or will it come to pass?

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

LECTURES

Dr. Margaret Ann Goldstein Presented a talk on "The Z-lattice in Mammalian Striated Muscle" at the Department of Medicine Research Seminar on April 17, and at the Department of Cell Biology Seminar on March 19.

PUBLICATIONS

"Optical Reconstruction of Nemaline Rods" by Margaret A. Goldstein, Marvin H. Stromer, John P. Schroeter, and Ronald L. Sass. **Ex. Neurology**. In Press.

NEW FACULTY AND/OR STAFF MEMBERS

Cassandra J. Gross has joined Dr. Ann Goldstein's lab as a research technician. John Bucher is leaving to attend dental school.

BAYLOR COLLEGE OF MEDICINE, DEPARTMENT OF MICROBIOLOGY

LECTURES

Dr. H. D. Mayor, February 1980, University of California — "Defective Dora Viruses."

PUBLICATIONS

James F. Young and Heather D. Mayor, Adeno-associated virus-an extreme state of viral defectiveness, Prog. med. virol. **25**:113-132 (1979).

SAN ANTONIO

THE UNIVERSITY OF TEXAS HEALTH SCIENCE CENTER, DEPARTMENT OF ANATOMY

GRANTS AWARDED

Dr. Thomas B. Pool has received a new three year grant from the National Institute on Aging — N.I.H. entitled "Quantitative Changes in Human Fibroblasts During Aging." Dr. John T. Hansen has also received a new three year grant from N.H.L.B.I. - N.I.H. entitled "Arterial Chemoreceptor Function."

LECTURES

Dr. Ivan Cameron and Dr. Nancy Smith gave an invited review paper at the Biological Microanalysis Workshop at the Scanning Electron Microscopy meeting in Chicago, April 20-25, 1980. Their review deals with x-ray microanalysis of the intracellular concentration of elements in relation to cell reproduction.

PUBLICATIONS

Herbert, D. C. 1980 Growth patterns and hormonal profile of male rats with protein-calorie malnutrition. Anat. Rec., in press.

Job Opportunities

Position Available — Polaron Instruments Inc. is looking for the following personnel to market/ service its range of E.M. products and instrumentation:

(a) regional representatives
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Interested individuals should contact D. O. Dinan, Polaron Instruments Inc., 4099 Landisville Road, Doylestown, Pa. 18901, Phone: (215) 345-1782.

Position Available — For a senior electron microscopy technician, (Technician II) in the Department of Veterinary Pathology, Texas A&M University. Applicant should be capable of: 1) performing all preparatory functions required for routine electron microscopic examination of biologic specimens, 2) operating the electron microscope and performing routine maintenance required for operation and 3) performing photographic duties associated with ultrastructural evaluation of tissues. Applicant should have a minimum of two years full-time experience as technician in electron microscopy laboratory or equivalent formal course work in the field. A bachelor's degree in a scientific field is desired. For further information direct inquiries to: Dr. Ralph W. Storts, Department of Veterinary Pathology, Texas A&M University, College Station, Texas. Phone: (713) 845-2651.

Position Available Immediately — Electron Microscope Technologist II (Service Lab Technician), permanent full time. Must be proficient in preparation of biological tissues for TEM, ultramicrotomy with diamond knives, EM photo procedures, working knowledge of TEM, thin film prep and ability to instruct persons in routine procedures of EM. Experience with SEM preps for biological samples also preferred. Minimum requirements include 2 years University training in biological or physical sciences and 1 year transmission electron microscope work experience in an EM lab. MORE EXPERIENCE DESIRABLE.

Send vita and references to: Dr. Judith A. Murphy, Center for Electron Microscopy, Southern Illinois University, Carbondale, IL 62901, Phone: (618) 453-3730. SIU is an equal opportunity employer. Salary range $12,000 - $14,400/yr. JOB OPEN IMMEDIATELY.

Position Available — Electron Microscopy Technologist, permanent position for an individual with a minimum of one year of experience in electron microscopy. Must be familiar with specimen preparation for transmission and scanning electron microscopy. Emphasis on thin sectioning. Bachelor's degree in science preferred but not required. Salary up to $975/month depending on qualifications. Call (405) 624-6748 for further information or send resume to: Dr. Charlotte L. Ownby, Director, **or** Ms. Jane Ramberg, Manager, Department of Physiological Sciences, Oklahoma State University, Stillwater, OK 74078.

Oklahoma State University is an equal opportunity/affirmative action employer.

Position Desired — Chief Electron Microscopist in Houston-Galveston area. Certified EM technician with 15 years experience, publications, C.V. on file with Journal Editor.

Position Desired — Chief Electron Microscopist or Research Associate. Has bachelor's and master's degree with 18 years experience in EM, scanning EM, etc. and has trained numerous people in EM techniques. C.V. on file with Journal Editor.

Position Available — The Department of Materials Sciences at Southwest Research Institute has a specific opening for an Electron Microscopist with at least two years experience in the operation of TEM and SEM equipment. This position will include both maintenance and operation of the microscopes as well as preparation of specimens for examination. There will be considerable inter-action with senior staff members on a wide range of basic and applied projects dealing with the behavior of metals and alloys. Qualified candidates should submit resume in confidence, including salary requirements, to: Personnel Department, Southwest Research Institute, P.O. Drawer 28510, San Antonio, Texas 78284.

FUTURE MEETINGS

EMSA/MAS: Aug. 2-9, 1980. San Fransisco, California. Contact: Dr. G. T. McKinley, Xerox, Palo Alto, California 94304.

REGIONAL NEWS Continued...

Chappel, S. C., W. E. Ellinwood, C. Huckins, D. C. Herbert, and H. G. Spies 1980 Active immunization of male rhesus monkeys against luteinizing-hormone-releasing hormone. Biol. Reprod., 22, 333-342.

Sheridan, P. J., and D. C. Herbert 1980 Nuclear uptake and retention of androgen by the pituitary gland of the hamster and the rat. Cell Tissue Res., in press.

Herbert, D. C., and D. J. Tindall 1980 Epididymal androgen binding protein in protein-calorie malnourished rats. Endocr. Res. Commun., in press.

Herbert, D. C. 1980 Morphology of the mammotrophs and gonadotrophs in the anterior pituitary gland of protein-calorie malnourished rats. Am. J. Anat., in press.

Morgan, W. W., and D. C. Herbert 1980 Early responses of the dopaminergic tuberoinfundibular neurons to anterior pituitary homografts. Neuroendocrinology, in press.

Welsh, M. G., J. T. Hansen and R. J. Reiter, 1979 The pineal gland of the gerbil, **Meriones unguiculatus**. III. Morphometric analysis and fluorescence histochemistry in the intact and sympathetically denervated pineal gland. Cell Tiss. Res. **204**: 111-125.

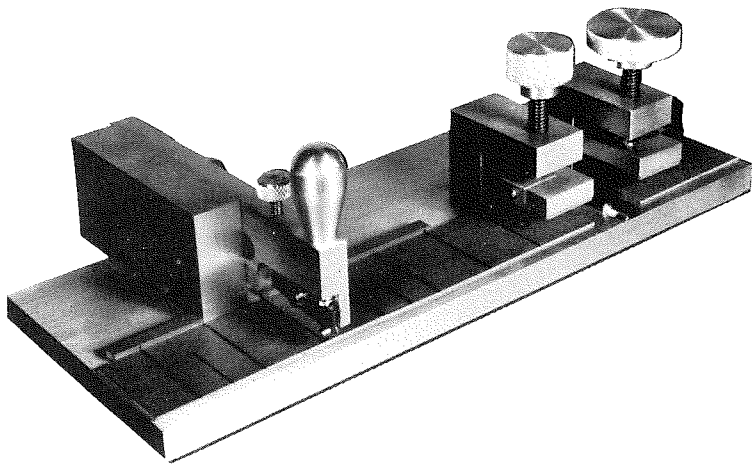
Hansen, J. T. 1980 The Carotid Body, **In**: McGraw-Hill Encyclopedia of Science and Technology, McGraw-Hill Book Co., New York, in press.

Cameron, I. L., N. K. R. Smith, T. B. Pool and R. L. Sparks 1980 Intracellular concentration of sodium and other elements as related to mitogenesis and oncogenesis **in vivo**. Cancer Research (May issue).

LONGKNIFEMAKER and VIBRATOME

aids for cytological sectioning

Medcast and Water Soluble Epoxy for EM & LM embedments



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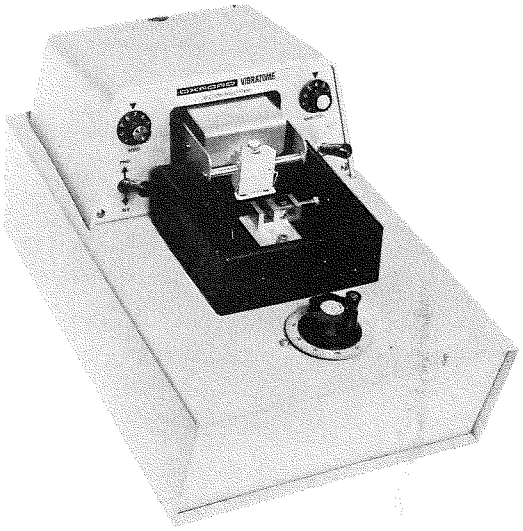
"Ralph" knives can replace metal knives

The Longknife Maker opens new horizons for sectioning large face histological size blocks in the semi-thin (1 - 4um) range using an ordinary rotary microtome. These knives cut glycol methacrylate plastic or paraffin embedded specimens. Knife edges are 25 - 38mm long.

8000 MI Longknife Maker



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cuts fresh material or fixed, in liquid bath
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100 Vibratome

New **MEDCAST**

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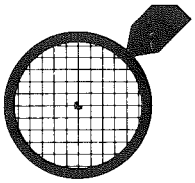
18001	Pelco Medcast Kit	$23.00
18009	Pelco Medcast Resin	12.00 (500g)

New **WATER SOLUBLE KIT**

light colored, flexible water soluble plastic for Light Microscopy embedments - Quetol 523

18086	Quetol 523 Kit	$29.50



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Financial Report

TSEM FINANCIAL REPORT
Period Ending April 15, 1980

ASSETS ON JANUARY 1, 1980:		
Certificate of Deposit No. 1099, Univ. Natl. Bank, Galveston	$ 2,000.00	
Certificate of Deposit No. 10-141345, Houston 1st Savings	2,000.00	
Savings Account No. 10-502435, Fannin Bank, Houston	4,201.67	
Checking Account, Fannin Bank, Houston	464.81	
Total Assets	$ 8.666.48	$8,666.48
RECEIPTS:		
Dues:		
Regular Membership	$ 1,300.00	
Student Membership	116.00	
Corporate Membership	900.00	
Interest on Cert. of Deposit No. 1099	37.50	
Interest on Cert. of Deposit No. 10-141345	61.51	
Houston Meeting:		
Registration	2,645.00	
Booth space	322.97	
Corporate contributions —		
Amray Inc.	100.00	
Jeol Inc.	200.00	
Kevex Inc.	100.00	
Subtotal	$ 5,782.98	(+) $ 5,782.98
DISBURSEMENTS:		
U. S. Postal Service Bulk Mailing Permit	$ 200.00	
Secretarial expenses	300.00	
Badges for Meetings	130.00	
Preparation of I.R.S. Return	90.00	
Houston Meeting Expenses	6,566.43	
Student Travel, Houston	600.00	
Subtotal	$ 7,886.43	(−) $ 7,886.43
		$ 6,563.03
ASSETS ON APRIL 15, 1980:		
Certificate of Deposit No. 1099, Univ. Natl. Bank, Galveston	$ 2,000.00	
Certificate of Deposit No. 10-141345, Houston 1st Savings	2,000.00	
Savings Account No. 10-502435, Fannin Bank	1,201.67	
Checking Account, Fannin Bank	1,299.85	
Total Assets	$ 6,563.03	$ 6,563.03

Regional Editors

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TWO NEW TEM'S FROM

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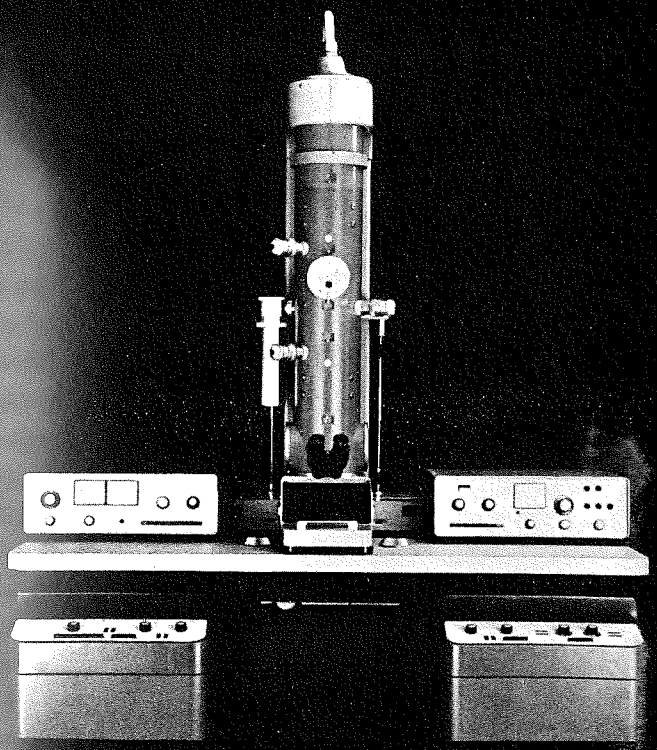

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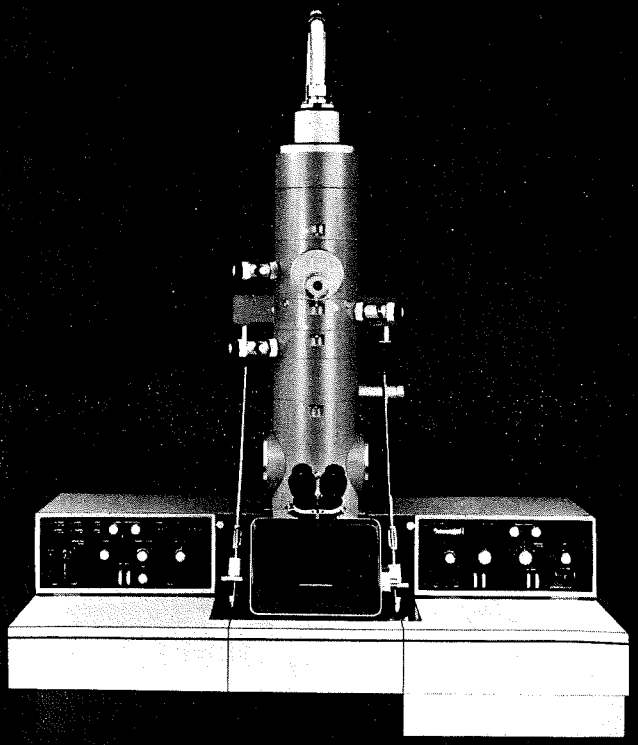

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