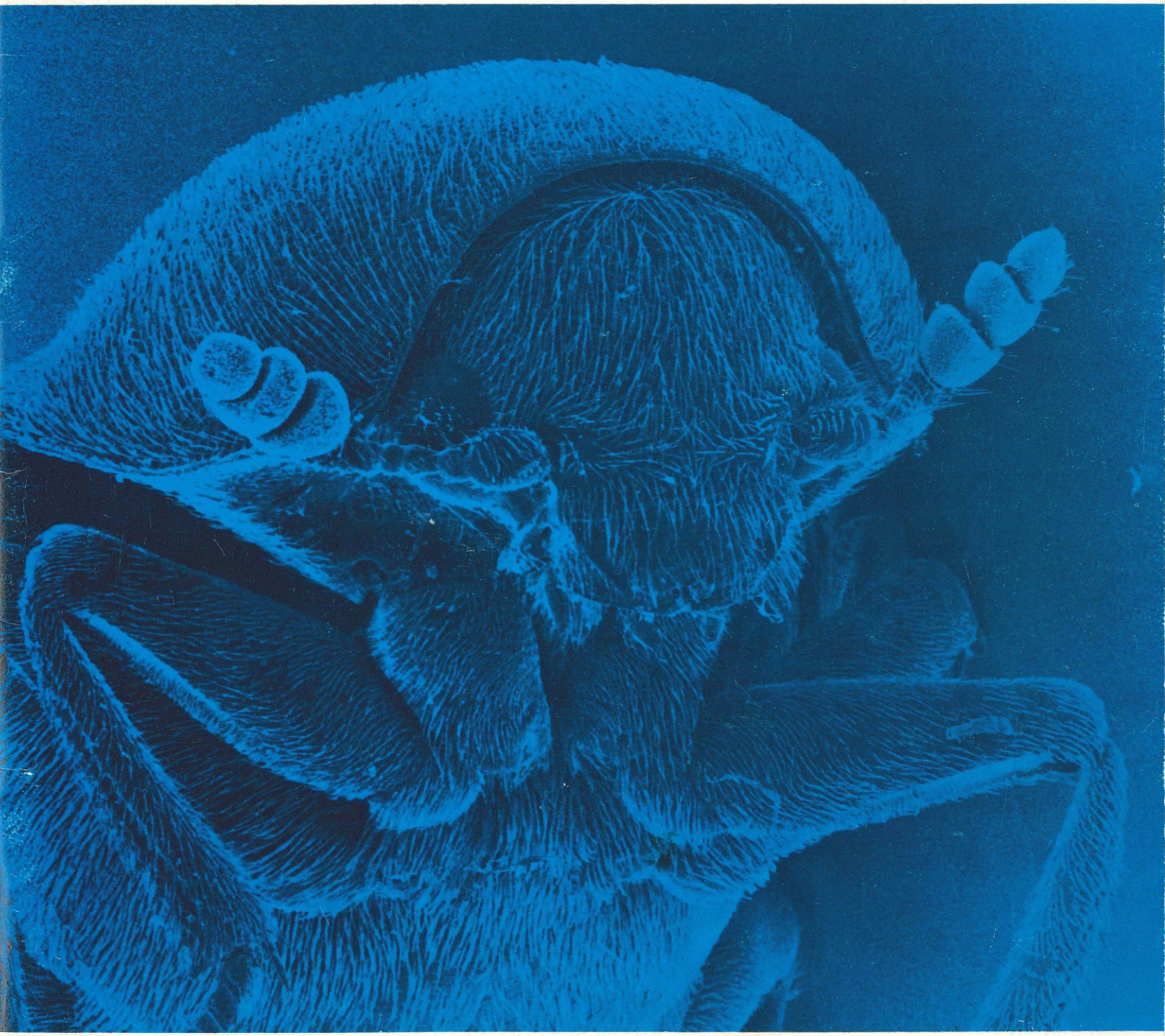
TSEM Texas Society for Electron Microscopy NEWSLETTER

Winter 1977



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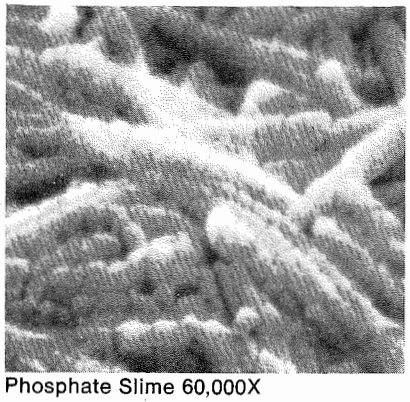

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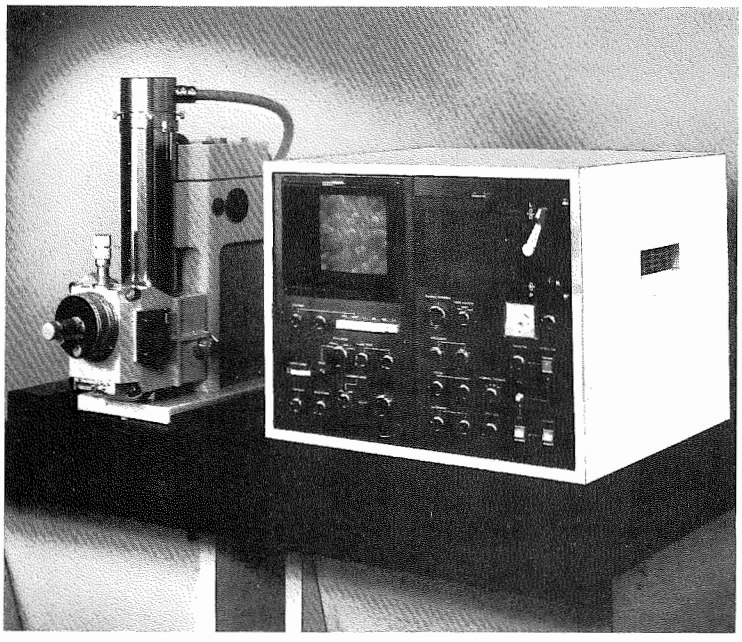

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Winter 1977

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ON THE COVER

A demestid beetle is pictured on the front cover at 100X, made from a negative (SEM) furnished by Dr. Margaret Barlin, Department of Entomology, Texas A&M University, College Station, Texas.

TSEM — LSEM — SEEMS
SIXTH ANNUAL JOINT SESSION
FEBRUARY 3-5, 1977
MONTELONE HOTEL — NEW ORLEANS, LOUISIANA

THURSDAY, FEBRUARY 3, 1977

AM & PM	Registration in Place de Iberville Foyer (2nd floor)
NOON	Commercial exhibits open in La Nouevelle Orleans East and West Rooms
PM	Scientific Sessions in Queen Anne Ballroom
EVENING	Beer & Pretzel Welcome at the Part II Establishment

FRIDAY, FEBRUARY 4, 1977

AM	Scientific Sessions in Queen Anne Ballroom
PM	Concurrent Scientific Sessions in Queen Anne Ballroom and Iberville North & South Rooms
EVENING	Joint Symposium Socializer in Queen Anne Ballroom

SATURDAY, FEBRUARY 5, 1977

AM	Joint Symposium Breakfast in Queen Anne Ballroom
AM & PM	Scientific Sessions

Commercial Exhibits will remain opening during meeting times for the duration of the Joint Symposium

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President's Message

The spring meeting of TSEM will be held at the Villa Capri Motel, Austin, Texas, in conjunction with the University of Texas. Mr. Rubin Mitchell, Cell Research Institute, University of Texas, is the local arrangements chairman for this meeting and if you have any questions, call him at (512) 471-3965.

This meeting will replace the traditional spring "student" meeting. However, students are encouraged to present their work. Every contributed paper session will be chaired by a student and travel monies will be allocated for student travel providing the individual is presenting their original work from the platform. Complete details regarding the application for these funds will be forth coming. A symposium and/or panel discussion is planned on a specific topic; however, the exact nature of this discussion has not been fully established.

There is increasing effort on the part of TSEM to recruit and incorporate physical scientists into our organization. The University of Texas at Austin campus was specifically chosen to host this meeting in that they have a large faculty of physical scientists employing electron beam instrumentation. It is hoped that their cooperation and the input from other members of the physical science community will make this the most ambitious physical science program yet undertaken by TSEM.

I hope to see you in Austin to enjoy science and the pleasures of Texas' Capitol City.

E. LAURENCE THURSTON
President

Letters

By the time I has read half way through Wood's Letter to the Editor (Fall, 1976) I felt the whole world was performing daily rituals of the burning at the stake of Joan-of-Arc. Nevertheless, in essence I would agree with Wood that equal work demands equal pay, and accepting that premise, starting salaries should be the same for women and men alike, if the qualifications are the same.

There are, however, mediating factors, which even Wood admits to, such as "the natural loyalty . . . expected . . . and stays with the particular institution." Such an expectation deserves more than a mere mention, and, if fact, lies at the heart of what is often interpreated as discriminatory or sexist practices.

An employer wants to know, indeed, has the right to know, if a woman applicant is married, intends of have children, will move with her husband if he relocates, etc. After all, this simply reflects a trust is the basic family structure and its integrity. (I presume it is still honorable to believe in that.) Such information indicates an expected longevity of service. This is important in determining the potential value of prospective employee has to a department or institution. A considerable amount of on-the-job training is invested in each employee.

The quote from the Wall Street Journal must be either inaccurate or incomplete. I cannot believe that the 1.7 million increase since 1974 in the labor force is **all** women; further, saying "the average female college graduate earned less last year than the average high-school dropout" sounds like a militant libber's battle cry and says nothing about such probabilities as some of those college graduates electing marriage and home-making, or temporary or part-time employment, instead of careers in the labor force.

The figures Wood present are also incomplete and misleading. They do not account for the numbers entering and leaving the field nor at what times. Is there not also an element of election to be considered? Certainly not many women desire to become engineers and some of those who do may very well do so for simply scholarly reasons.

Personally, I see no Catch-22 system involved in discriminating amoung job applicants. I can see (usually) when a female is a female and this brings to mind certain questions which have a sound basis.

The Supreme Court, however misguided some will think them to be, recently delivered a very sound judgement, in my opinion, in denying sick pay for maternity leave, thereby reaffirming what biologists have known all along — that the human male cannot arbitrarily decide to get himself pregnant!

I know that I am not occupying a popular position; rather, I no doubt will be labeled a male chauvinist pig by some. Nevertheless, I cannot help but believe that evoking this argument of sex discrimination has elements of a straw man to it. There are some left-handed ways of doing it also, to wit: the 19 November issue of SCIENCE carried an article entitled "Carrying Behavior in Humans: Analysis of Sex Differences." It dwelled on how males and females CARRY THEIR BOOKS! The authors state, "social modeling may explain the regular increase in adult female type of carry among pre-adolescent females," but they also could not deny a genetic predisposition involving anatomic differences (Thank God!) But, their implication is that social **remodeling** could erase the observed differences! Needless to say I was relieved to see that no public funds supported this so-called research.

If one studies the figures closely presented by the National Center for Education Statistics of last September it will be discovered that more women that men **entered** the academic ranks last year, which would tend to drive **average** salaries for women lower than that for men. However, at the full Professor rank women showed an increase in salary of 6.5% compared to 6.3% for men. And, the number of women at the rank of Associate Professor increased by a factor of 6.1% compared to 4.3% for men.

Frankly, I am rather pleased that I can readily detect the differences between male and female and I will continue to do so when applicants come before me. The starting salaries I will leave to the Personnel department, which tells me what I can and cannot offer anyway.

As a final note, I would say to Wood when the swell and cacophony of sexist charges are heard, relax, Joe, and eat the popcorn.

— WARD KISCHER

TSEM — POLYMATH

Not everyone can be a polymath, and unfortunately, there aren't a whole lot of them around. A group of individuals with widely divergent interests, however, can develop an organization which is basically a polymath society. The TSEM comes close.

When using the term "polymath", I will admit that the first time I ever heard such an individual was in an essay "Demise of the Polymath" written by Dr. Irvine H. Page and appearing in "Modern Medicine" in 1967. Dr. Page noted in his essay that the term polymath "had nothing to do with mathematics but is a person with broad and variegated knowledge." He also noted that with the massive expansion of knowledge, a true polymath is indeed a rarity, but is even more important than ever before in order to rise . . . above the jungle of facts so that he may see their relationships."

So what has this got to do with the TSEM? A Lot!

There may not be many individuals in this group that are polymaths, but the composition of the society is such that any member may assemble widely divergent information from other members and see a relationship.

The interests in the TSEM range the spectrum from pure physics to pure biology and with all shades of the spectrum in between. This creates a climate for extensive cross pollination. The active participation of students is especially fortuitous as that have not learned all the answers, (some of which are erronious), and are willing to ask questions that are candid and to the point. It is a fact that it is somewhat embarrasing for an expert in a particular field to ask what he may consider a stupid question but he is often happy to have a student ask the questions in order to acquire a fact, or an acknowledgement of ignorance from another expert. Since my own field of "expertise" is Pathology, I can speak with some authority in this respect — especially since a pathologist is usually considered the judge in the court of last appeal in Medicine. One of the more psychologically traumatic occurances for a medical student is the first time that he discovers that there actually may be disagreement between pathologists; and for a new pathologist a similiar traumatic experience may be his first tumor conference where it becomes obvious that there are not only differences of opinion as to the classification of a tumor, but even as to whether it is benign or malignant. (It may be possible that the specialists in the non-medical fields also have differences of opinion).

In the TSEM there is a blending of information since students ask everyone questions and authorities in one field aren't ashamed to quiz authorities in a field unrelated to their own. A biologist may have the problem of staining a particular enzyme in a cell and calls on the Chemist, or a physicist may have an idea that is looking for a biological application. Ideas are conceived and hypotheses may be born.

A problem with hypotheses and polymaths incidentally is the torrent of hypotheses that a polymath can generate. Unfortunately there are few scientific meetings that will consider a "hypothetical" presentation and the number of medically oriented scientific journals in the United States that will publish a "non-expert's" hypothesis is infinitely small or absent. Fortunately for nascent polymaths there is a new, unique publication, "Medical Hypotheses." The editor, Dr. David Horribin states, "I shall publish some ideas that seem improbable and perhaps even faintly ridiculous . . . I follow Karl Popper in seeing the virtue of improbability."

The Editorial Offices of this publication are listed as follows: Department of Physiology, University of NewCastle (England), Published by Eden Press of Canada and England, and the editorial advisory board includes the following: Sir MacFarlane Burnet, Sir John Eccles, Arthus C. Guyton, M.D., Linus Pauling, PhD, and Sir Karl Popper.

As an organization, the TSEM fits the definition of a polymath and there may be individuals of this rare breed as members. Our society is small enough so that we can still talk easily with each other, but is varied and large enough to have access to brains, not necessarily operating on the same wave length as our own. The possibilities are enormous.

J. C. STINSON, M.D.
Scott and White Clinic

References: (1) Page, I.H.: Demise of the polymath, Modern Medicine. April 10, 1967, pp. 87-89. (2) Horrobin, D.F.: The probable to be tested. Journal of the American Medical Association. **233**:467, 1975.

Editor's Comments

As your new editor of the TSEM newsmagazine, I would personally like to thank all of you who contributed to the last issue which was distributed at the Fall '76 Temple meeting. This fall issue of the newsletter had many new features and additions to it with many more new ideas to come. I has been strongly suggested we get a library congress number as well as a copyright number so that our newsletter could also be distributed to many libraries throughout Texas and the rest of the country.

I feel that as members of the TSEM we can be very proud of our newsletter which can be attributed to the many efforts and hard work of our past editors. We've come a long way in 12 years with nothing but a bright horizon ahead of us.

I would like to encourage all of you to continue to support your TSEM newsletter by sending me scientific articles, brief notes, interesting micrographs for the cover page.

Thanks to all of you again for supporting TSEM and its newsletter.

Robert A. Turner
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Dept. of Pathology
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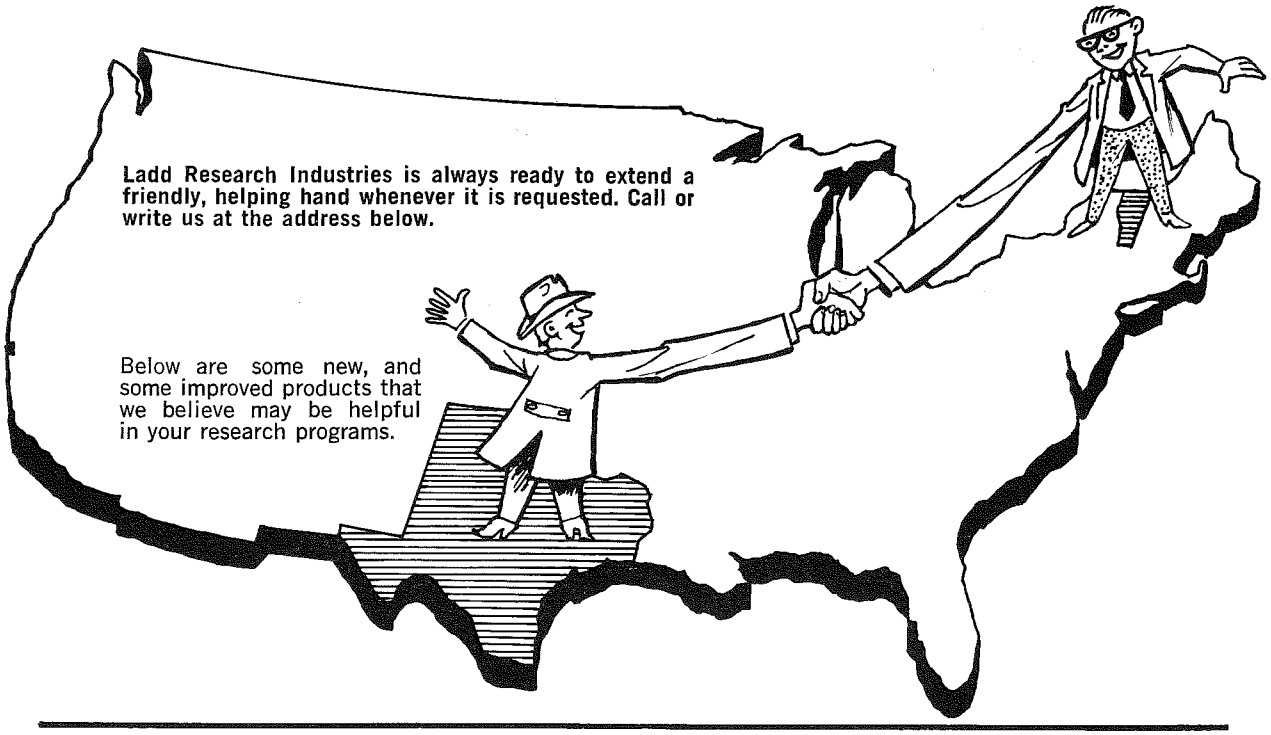

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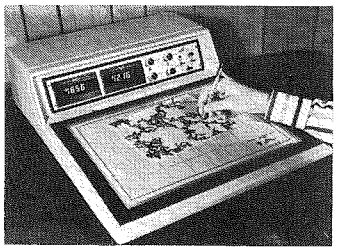

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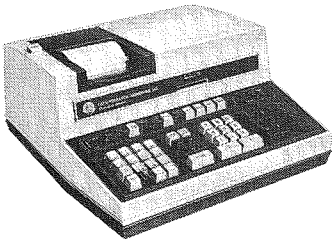

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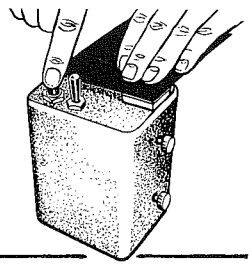

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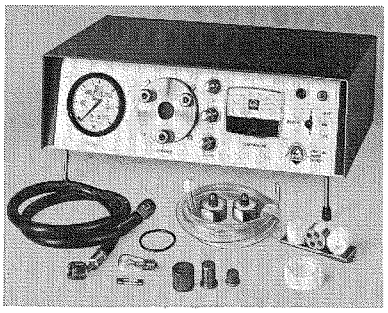

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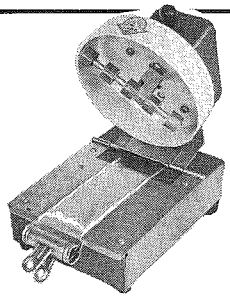

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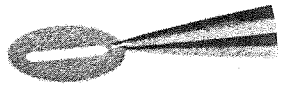

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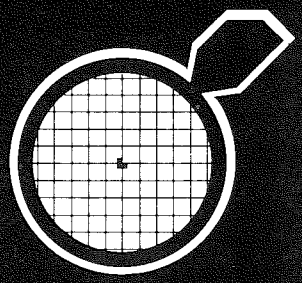

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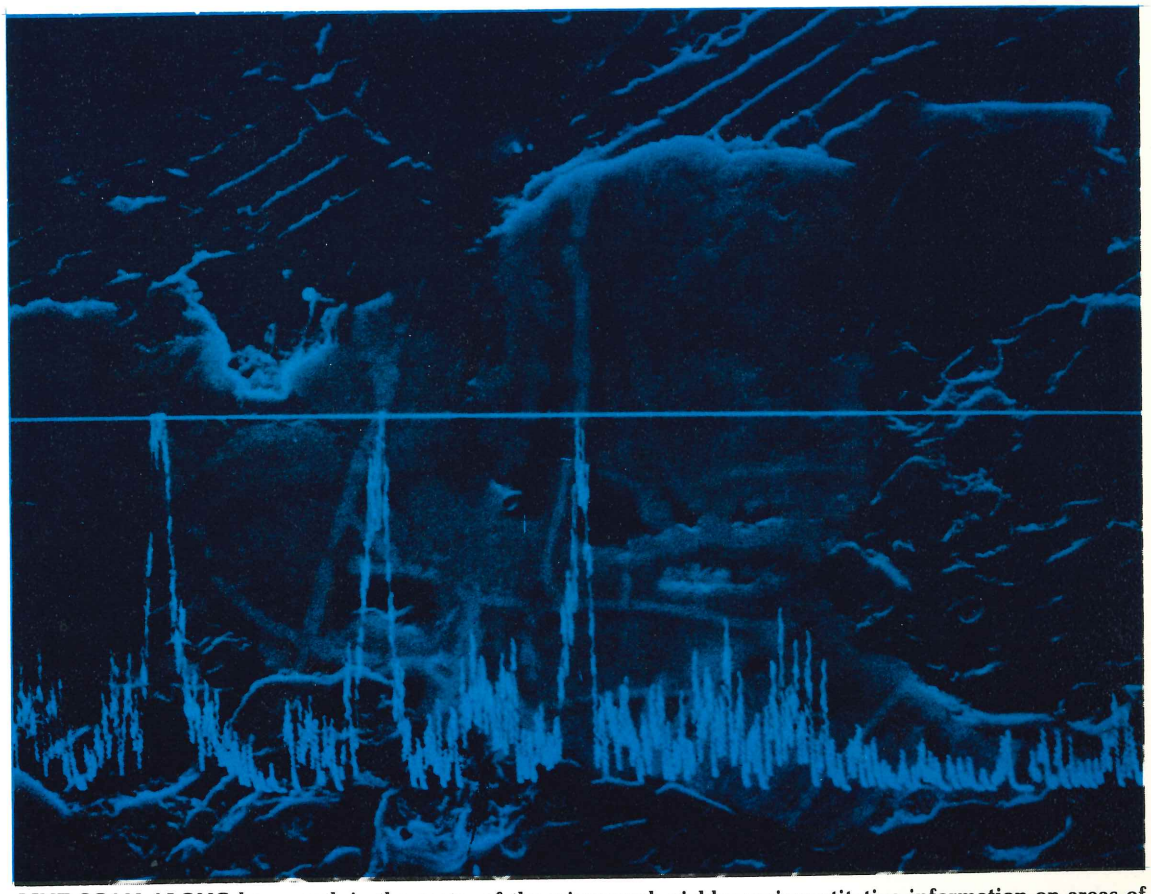
SEM/EDX: a versatile analytical combination for nondestructive testing

By GLENN W. MEYER



LINE SCAN ALONG beam path in the center of the micrograph yields semiquantitative information on areas of antimony content.

In recent years, the price of scanning electron microscopes (SEM) has decreaed to the point where almost any company can justify the purchase of one. The average price of an instrument with 100A resolution is $20,000 to $30,000.

This price, couples with the lowered prices of energy dispersive x-ray (EDX) spectrometers, now priced at $15,000 to $35,000, offers the potential user a package price of $35,000 to $50,000 — approximately half of what a scanning electron microscope alone would have cost a few years ago.

These price decreases, along with an increase in the ease of operation and interpretation of data, have made the SEM/EDX systems affordable and usable to anyone. These low prices include features such as TV scanning, dynamic focusing, 100A resolution, and automatic vacuum systems.

It has been said that the most important advantage of the SEM/EDX combination is that is in nondestructive. There is no need to preselect the elements to be analyzed; the energy dispersive spectrometer "looks" at all the elements at the same time and indicates their position by means of peak identification markers, sometimes called MLK or KLM makers. Other methods, such as atomic absorption or chemical techniques require prior dissolution of the sample.

The qualitative aspect of x-ray analysis is probably the most intriquing. For example, the distribution of an element or elements can be shown by means of an x-ray map. In addition, semi-quantitative information can be obtained when one makes a graph of count rate for a particular element, as in a line scan. The information here is a combination of element concentration and geometry.

The semiconductor industry uses a scanning electron microscope for examining, for step converage, in looking for microcracking, etc. With the addition of an EDX system, one can see also the elemental corrosion products;

Glenn W. Meyer is an engineer for International Scientific Instruments, Inc., Mountain View, California. A graduate of the University of Nevada, he has performed postgraduate work in scanning electron microscopy, transmission electron microscopy, and gamma ray spectroscopy. He specializes in applications for SEMs and energy dispersive analysis.

one can make measurements of coatings and measure allow ratios such as the SiAl alloy with considerable accuracy.

The environmental fields also have found the SEM/-EDX system useful in analyzing and identifying asbestos fibers in the lung. Shape and chemical composition of particulates can be identified. This type of particle analysis also can be carried out on filter paper as is done with air particulate samples.

Particle analysis

This analysis works better with a scanning electron microscope than with an x-ray tube excited system because the entire energy of the beam can be concentrated on a single particle. The result is a higher peak-to-background ratio, improving the accuracy and detectability limits.

When only a few particles are present, there may be no analysis results because the particle intercepts such a small percentage of the x-ray flux. The fluorescent x-rays are lost in the background when a tube-type x-ray system is used.

Environmentalists conduct fly ash composition and heavy element analyses. The latter of these would work the best with the bulk mode analyzer described later.

Forensic laboratories have found the positive identification of gun powder residue based on form and chemical composition to be most helpful. And they have used the technique to identify paint chips and other particulates.

Ppm detections with SEM/EDX

Metallurgists can measure the thickness of layers of materials and the migration of one element into another at boundaries of metal to metal joints by using the line scan technique. This method gives a graphic representation of element distribution along the X axis. It is possible also to measure the kinds and amounts of elements present (Na and above), as well as inclusion identification, plating thickness, etc.

There are similar applications in geology. For instance, one can make a tentative identification based on morphology and confirm this by elemental composition.

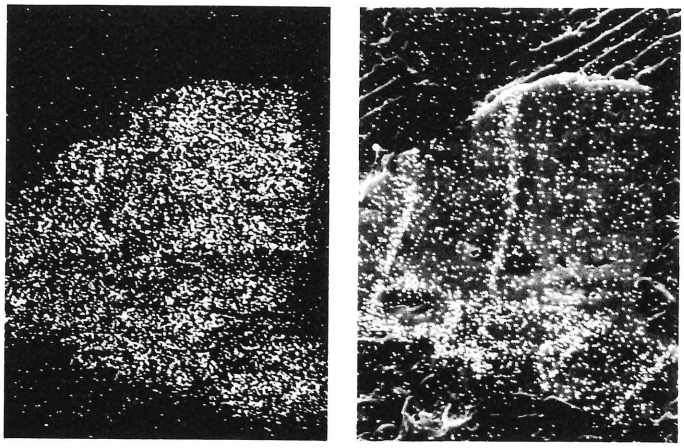
Recently, a new technique has been introduced on commercial SEM/EDX systems, called "bulk mode analysis." According to Hans Zulliger of NSI Corp., the electron beam in the column is converted to an x-ray beam by placing a thin foil, such as molybdenum, in the path of the electron beam. The electrons fluoresce characteristic x-rays from the foil material; these x-rays, in turn, fluoresce the sample.

Most of the *Bremsstrahlung* generated by the deceleration of the electrons is filtered out by the foil, thereby reducing the background radiation. This technique is complementary to electron excitation in the sense that spatial resolution is traded for better sensitivity.

For example, an x-ray beam spot of 1 x 6 mm can detect less than 10 ppm of lead in 500 sec in an organic matrix. The bulk mode excitation technique works particularly well for elements having x-ray lines between 5 and 15 keV.

Since the electron beam does not hit the sample itself, sublimation of elements from the sample and contamination of the surface are avoided. In some cases, analysis of a larger specimen volume can be of advantage if the average elmental concentration is of interest.

Since the x-rays penetrate the specimen more deeply (up to 2 mm in low Z materials, as opposed to 5 to 10 μm for 25 kV electrons), subsurface elements can be detected.



X-RAY MAP FOR iron (left) shows uniform distribution. Similar map for antimony (right) shows concentration of antimony in veins.

Using this method, it is possible to analyze liquids that are sealed in thin-window containers.

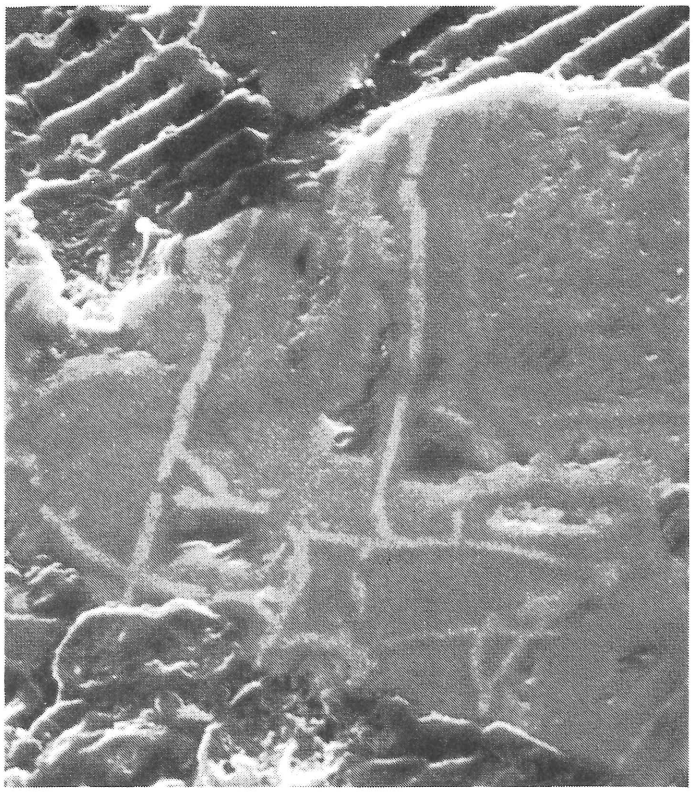
Often it is important to estiamte the detectability limits for various elements. This is difficult, since detectability depends greatly on the sample matrix, the accelerating voltage, and the magnification range or size of the raster. As a rule of thumb, a detectability limit of 10^{-15} g applied to transition elements for an analysis time of about 1,000 sec.

One also can use the beam penetration, x-ray generation, and absorption to measure the thickness of layers of material. For example, with a 1 μm thick layer of A1 over a Si base, a line scan could be performed for either Al or Si. Changes in count rate would indicate changing thickness of the Al layer.

In mating an SEM with EDX, a key feature to look for is Z motion capability. This capability is required to adjust the sample height in respect to the x-ray detector. This movement, coupled with the tilt of the sample and the x-ray detector position, will define the take-off angle. A high take-off angle is desirable because it lessens the amount of self-absorption in the sample. In turn, this minimizes matrix effects and improves count rate.

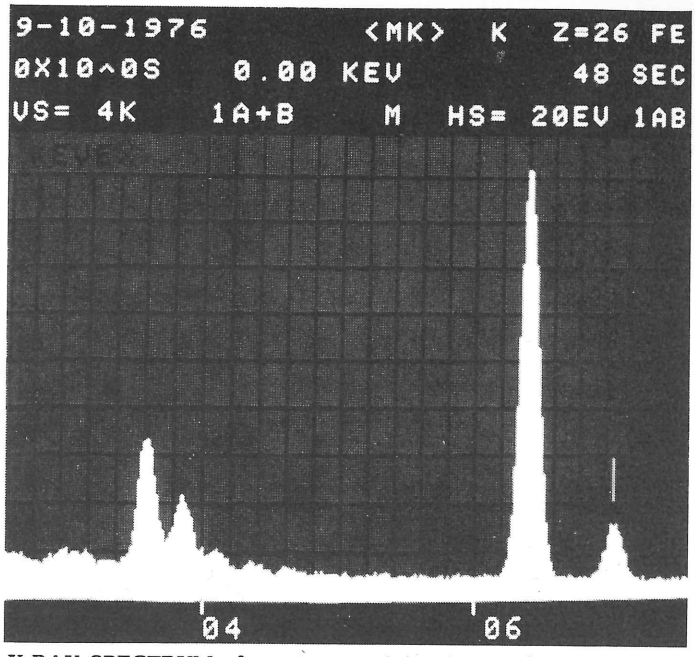
Dynamic focusing allows the use of a large aperture (200μm). This enables the operator to achieve high x-ray counting rates and still maintains edge-to-edge sharpness at high tilt angles and low magnifications.

We want a means of driving the beam across the sample at a variable but slow rate. Under these conditions, line scans are possible without extensive modification of the scanning electron microscope. The spot mode is useful for analyzing small areas or particles. This spot should be controllable in X and Y directions.



WITH THE WINDOW set on the region of interest, the analyzer outputs all pulses coming into this window to the SEM CRT input. The x-ray events appear on the SEM as dots (previous page) in locations the same as on this secondary image.

BACKSCATTER electron image of sample showing element differences more clearly than the secondary electron micrograph (above). The backscatter electron image is atomic-number dependent.



X-RAY SPECTRUM of an ore containing iron and antimony. The EDX looks at all the elements at the same time. MLK marker is used to indicate peak of major component; Window can be set on Region of Interest, and smaller windows are set on each side or the ROI for background correction.

SEM features

Most people find that TV scanning speeds sample searching. It also is consistent with normal x-ray analysis, thus improving operator efficiency by overcoming limitations of slow scan modes or even reduced-raster, rapid scanning. A working distance factor meter is important for determining accurate maginfication and for proper sample orientation with respect to the x-ray detector.

For reproducible analyses in terms of absolute amount it is desirable also to have a means for measuring the specimen current. This usually is accomplished by an electrically isolated stage which is grounded through a microammeter.

Accelerating voltage potentials are important and can be determined by using the rule that the kV of the accelerating voltage should be 2 to 3 times the keV energy one wishes to detect. Therefore, for low atomic number elements, one can apply the "times 3 rule" of 15kV or higher for Ti 4.5 ke V and 25 k V for Au and PbL lines at 9 to 12 keV.

Since only two sets of lines must be identified, such as M and L or L and K, the author recommends the use of the lowest voltage which looks promising. The higher voltages increase beam penetration into the sample (frequently undesirable) and raise the background to the detriment of the peak-to-background ratio, adversely affecting the minimum detectable limits. Indeed, there are times when 2 kV will be useful for SEM operation *e.g.* when looking at voltage-sensative materials that tend to develop surface charging.

X-ray spectrometer features

One of the most vaulable and valid of the x-ray spectrometer functions (in preparing this article, I used x-ray

spectrometers at Kevex Corp. and at ISI Corp.) is the yield of qualitative data. These data often are compared to other data taken either on a different part of the same sample or to a spectrum acquired at another time. This ratio comparison requires at least a 1,024-channel memory, with spectra being stored in each of its halves or quarters. If mass storage of spectra is available, so much the better.

After acquiring a spectrum and perhaps comparing it to another, the use usually wants to identify the peaks observed. This is most-easily accomplished with markers that show the element and its associated peak patterns. In this way, the MLK markers show not only the position, but also the relative heights.

Once a spectrum has been identified, the location of the element is question or its integral x-ray counts can be found by setting a "window" on the peak of interest. A "window" also is called a Region of Interest.

The spectrometer analyzer then will output all pulses coming into this window to the SEM video input. The x-ray events appear on the SEM screen as dots, whose location is the same as seen on the secondary image.

The count rate from a window can be coverted by a ratemeter to a varying voltage which will move the spot in the Y direction on the SEM cathode ray tube. This produces a graph of element concentration.

Data reduction

The subject of data reduction has many ramifications. Some users want quantitative answers; some what qualitative answers; and others want both — or somewhere in between.

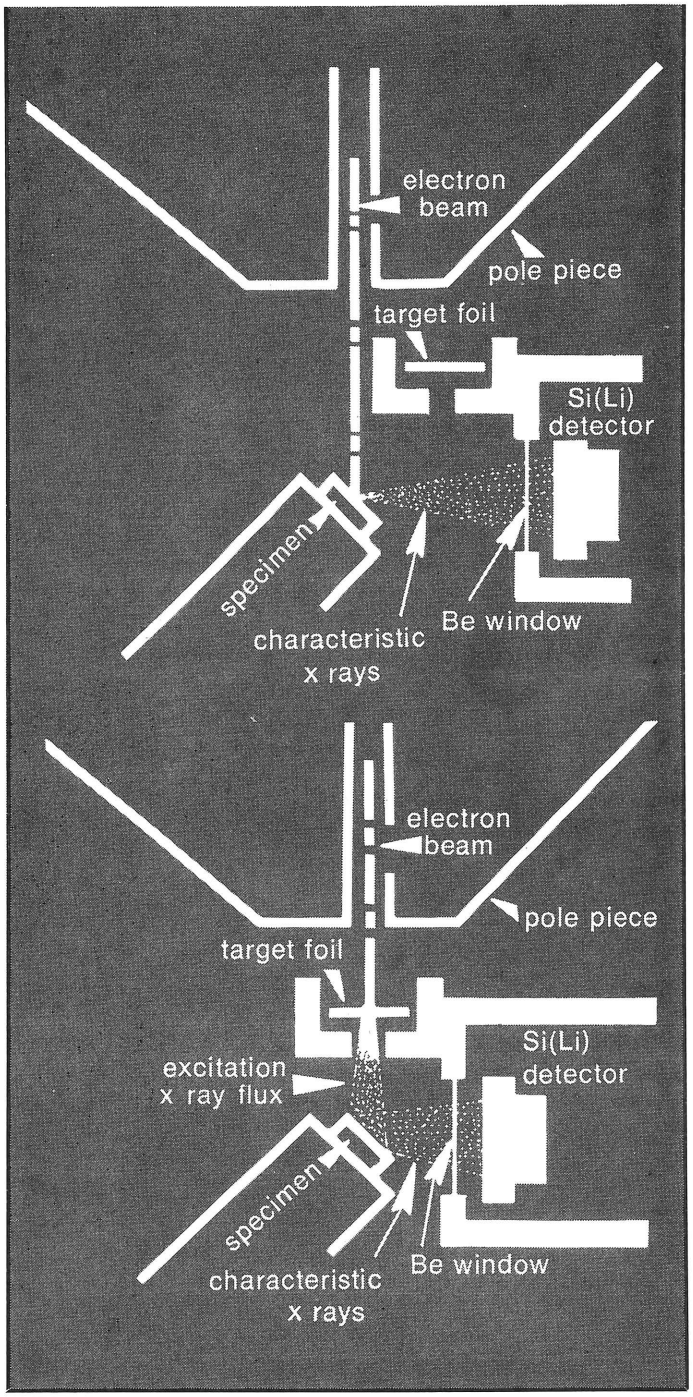
In reality, the degree of accuracy for quantitation depends of the quality of the sample — it must be polished flat and homogeneous. Yet qualitative and semiquantative results can be obtained on almost any sample with ease. Times for such analyses range from 20 sec to 5 min.

There has been much discussion regarding the resolution of x-ray detectors. How much resolution is necessary is difficult to answer — and to some extent, depends on the samples analyzed. But it is safe to say that when resolving peaks that are close together or when looking for L line patterns around silver, the better the resolution, the easier the identification and integration above background will be.

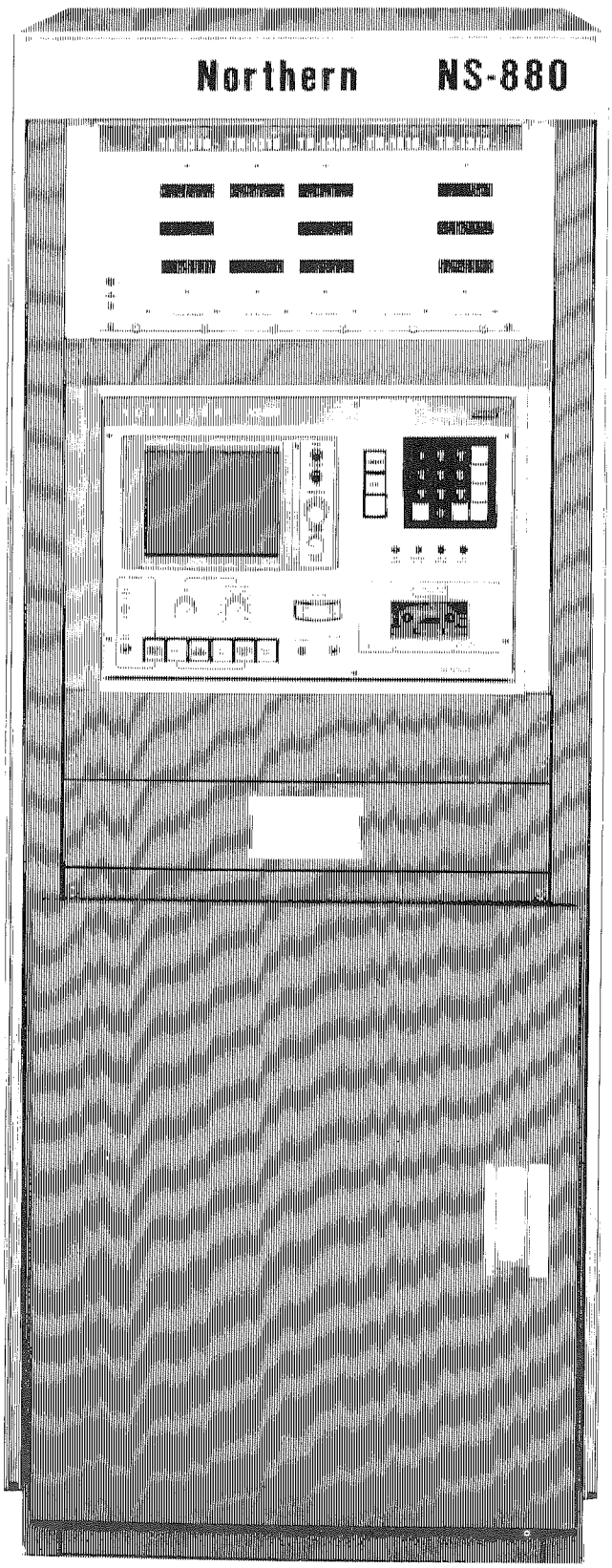
Most x-ray detectors today are of the so called variable geometry design. They move in and out or to and from the specimen. This motion, coupled with tilt and Z motion from the scanning electron microscope stage, allows predetermination of the take-off angle and solid angle regardless of sample size.

A high resolution scanning electron microscope and energy-dispersize x-ray system that is convenient to use and will achieve accurate, reliable results can be purchased for about $45,000. Previous to this time, such an instrument would have cost $150,000 or more. It would have been difficult to use and would have required a dedicated operator/technician.

The potential applications for the newly designed SEM/EDX systems are many. Now the lowered cost of the combined instruments has reached an attractive level. I am sure that, as more people become acquainted with SEM/-EDX systems, their uses will be limited only by the imagination of the users.



DIFFERENCES IN mode of operation of the micro-made (upper) and the bulk-mode (lower) surface analysis. In the micro mode, electrons striking the target cause x-ray fluorescence characteristic of the lements in the specimen. In the bulk mode, a thin metal foil converts the electron beam to x rays. These characteristic x rays in trun fluoresce the specimen. The bulk mode trades spatial resolution for better sensitivity.



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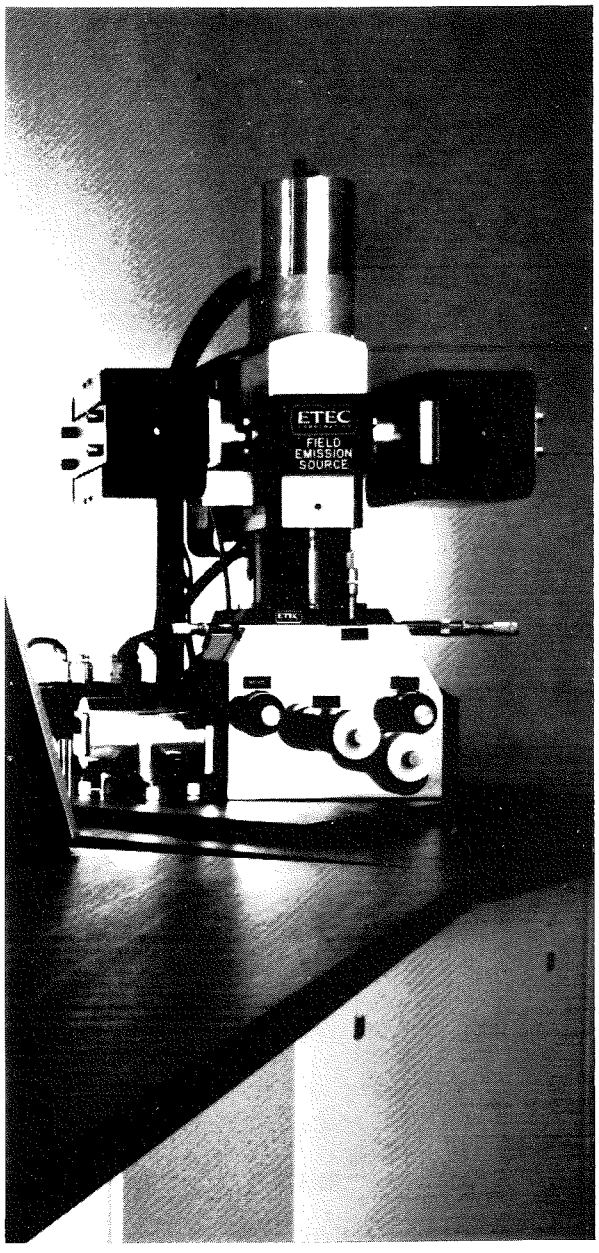

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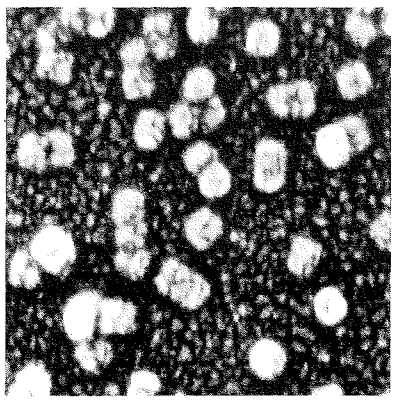

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Scanning Electron Microscope For Living Specimens

Outline of Design Requirements

By ARTHUR E. SOWERS
Electron Microscopy Center, Texas A&M University, College, Station, Texas 77843

This paper has three objectives: (1) To briefly review the problem of examining living specimens by SEM, (2) To mention some of the reasons one would want to look at living specimens, and (3) To suggest that SEMs designed along lines different from those commonly used today would greatly facilitate this type of microscopy.

Many fundamental unsolved problems in biology could be better approached if it were possible to visualize the test organism or model system with SEM quality images. Indeed many problems can only be tackled if a model system can be observed in real time. The following examples indicate possible areas which would be benefited if SEM of living specimens were possible:

1) Determination of kinetics of growth and change is surface details on the same specimen as the changes are taking place.

2) Detection and location of structures in specimens at very early stages of development for sampling for later conventional TEM unltrastructure studies.

3) Micromanipulation/microsurgery/microtransplantation.

4) Inducing specifically localized and controlled electron beam damage for studies involving regeneration and response to injury (SEM equivalent of Laser microscopes).

5) Microdeposition of hormones, inducers, growth substances, antibiotics, inhibitors or other small molecule controlling compounds (eg. cyclic AMP or ppGpp) onto specific locations on specimen.

6) Follow progress of pathogen invasion of host, ie. nematode-plant systems.

In general, the prospect of examining living specimens by SEM breaks down into two problems: (1) How well the specimen tolerates the expore to the hypobaric environment of the specimen chamber, and (2) how well the specimen tollerates the irradiation by the electron beam. It has been known for some time that many species of living insects survive direct examination by SEM and even reproduce (Pease and Hayes, 1966). It has also been known for some time the sprouting seeds can be examined directly in the SEM (Pease and Hayes, 1968). Just recently the author reported that plants in highly advanced stages of growth continued to grow and differentiate after repeated examination with SEM (Sowers, 1976). It was found that if the lowest accelerating voltages and specimen currents are used, the limitation in the repeated examination of the species studied was vacuum damage. Results from work on cotton, garden bean, and sorghum show that while there is a spectrum of responses in plant growth to short repeated exposures to SEM vacuums, it was concluded that, in general, quite a large number of species of plants should make excellent living specimens for direct SEM. The details of this work will be reported elsewhere. Recent reports (Lyon, Kirsh, and Parsons, 1974 and Lyon, Kraus, Kirsh, and Parsons, 1976) indicate that mammalian cells in culture survive moderate vacuums for a few minutes and therefore suggest that SEM of this type of system is also possible. Systems which are very soft will be beyond the capacity of present technology to provide us with useful SEM images, such a system is the planarian which does not survive out of water pressures of about 0.1 Torr (approx.) for more than 20 seconds. The results indicate, however, that many systems can be found which will permit many useful kinds of experiments to be performed using as SEM designed for use on living specimens.

In the course of work on epidermal cell differentiation into trichomes and emergences in certain plants it occureed to the author that the examination of living specimens would be better facilitated if SEMs were designed along somewhat different lines than are conventional at present. The two fundamental restrictions taken into consideration are (1) Minimum exposure to the vacuum required for SEM, and (2) Minimum exposure to the electron beam. The former is probably more limiting that the latter and may be alleviated to some degree by providing the microscope with a differential pumping system so that the gun chamber can be operated in the 10^{-4} Torr range while the specimen can be under a 10-100 Torr pressure (Robinson, 1976). However, to what extent a given specimen can better survive for a given exposure time in a 10 to 100 Torr atmosphere instead of the 10^{-3} Torr pressure* has not yet been demonstrated. This author feels, however, that shor-

tening the exposure to the hypobaric pressure is more important than making the environment for the specimen less hypobaric. Hence the author stresses the distinction between the approach to the examination of living systems by the employment of environmental chambers versus the approach to the examination of living systems by use of the absolute minimum exposure to the microscope specimen chamber conditions consistent with obtaining visual information according to what present technology can deliver. There does remain the possibility that design of an environmental chamber which will accomodate exposure periods equally as brief as the non-environmental-cell protected specimen chamber which the author envisions will further reduce the magnitude of the temporary disturbance which the specimen will suffer during the brief examination by SEM.

From the above discussion, the following mechanical and electrical specifications would be desirable for a SEM designed for the examination of living specimens:

a) **Micrography:** Micrography done by motor advanced 35mm camera set for exposure times of about one second of images generated at TV rates with operator simultaneously observing images to detect artifact movements or other short-time-interval chages which would require re-photography of the given area.

b) **Vacuum System/Specimen Exchange:** Very small specimen chambers and much higher capacity roughing pump and/or ballast tank assited pre-pumping so that specimens can be placed into the scope at one atm pressure and be at an operating pressure within 10 seconds (or 30 seconds for specimens which have very high outgassing rates).

c) **Area "Adressing" on Specimen:** The conventional approach to viewing different areas on a specimen is accomplished by varying the specimen position with an expensive, complicated, andcumbersome mechanical stage under the raster. The center of the raster always remains in the same place in this system. In the SEM for examining living specimens, it is suggested that the position of the center of the raster on the specimen by variable in the x and y coordinates over a fixed position specimen by superimposing a direct current offset bias into the raster generating magnetic field. The raster position would be controlled by a "joystick" on the front panel which would be manipulated with one hand while the shutter release, and other minor adjustments, are done with the other hand. Tilt would be motor driven and controlled by twisting the axis of the "joystick."

d) **Programmable Microprocessor:** A microscopist would have the option of releasing control of filament saturation, specimen current, focus adjustment, and photography, and x and y position selection according ot a plan preprogrammed into the microprocessor for optimum signal-to-noise ratio, and minimum examination time in the SEM.

The type of experiments that this SEM would be used for would not require resolutions or magnifications quite as high as a conventional SEM (typical upper limits might be 0.5 μm and 10,000 X respectively). Less expensive construction would be required in many subassemblies of the unit. Futhermore, accelerating voltages need not be higher than 10-15 kv, which further simplifies the construction of this type of SEM. Such a scope might be designed in modular fashion such that the specimen chamber and column parts would be sufficiently interchangeable with other, more conventional, subsystems that the "grade" of the microscope could be easily modified for examination of fixed specimens at higher magnifications or resolutions should the direction of future research in a particular laboratory make such modifications appropriate.

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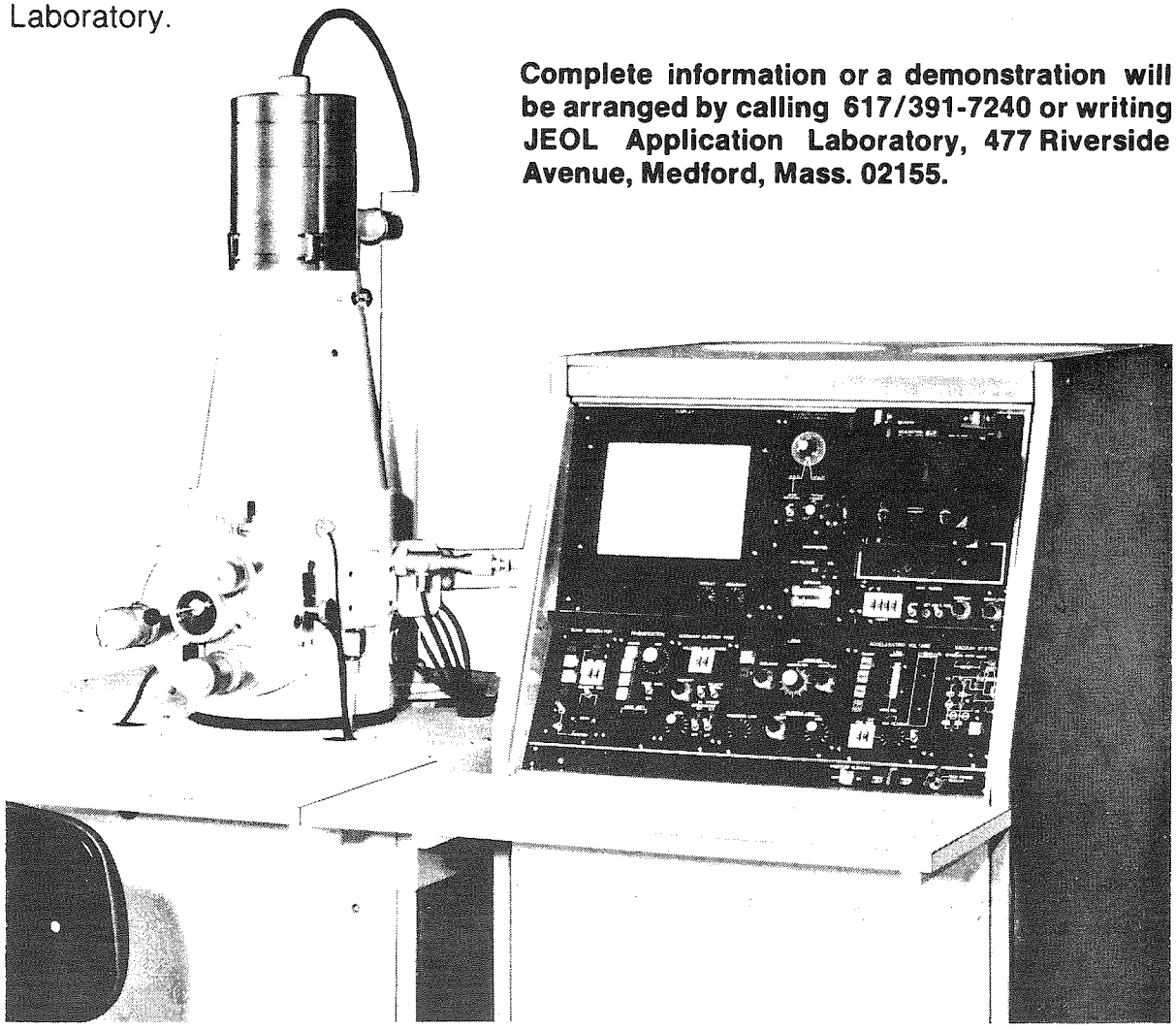

Abstracts

ZOOSPORES, ZOOSPOROGENESIS AND MULTILAYERED STRUCTURES IN THE PARASITIC GREEN ALGA Cephaleuros (CHROOLEPIDACEAE). Russell L. Chapman, Department of Botany, Louisiana State University, Baton Rouge, Louisiana, 70803.

Within terminal sporangia, repeated (apparently semi-closed) mitoses produce eight or more nuclei. Formation of bilaterally keeled flagella (like those present in the motile cells of **Phycopeltis** and **Trentepohlia)** precedes the completion of cytoplasmic partitioning. The flagella are most often observed to be in groups of four and are in some cases closely appressed. Some nearly mature uninucleate zoospores contain two distinct sets of spline microtubules which extend posteriorly from the flagellar bases beneath the plasmalemma on opposite sides of the cell. Multilayered structures comparable to those seen in **Trentepohlia, Phycopeltis,** and perhaps other alga taxa are present at the flagellar bases. The rather unique arrangement of the four flagella and the presence of two multilayered structures per cell suggest the possibility that the general association of one multilayered structure per pair of flagella is constant and that the two multilayered structures observed in **Trentepohlia** were present in quadriflagellate cells.

Most of the development of zoospores can occur in unreleased zoosporangia, but the data available do not indicate whether or not maturation and release of zoospores can occur in unreleased sporangia. It is apparent that not all of the sporangial cytoplasm is utilized in production of the zoospores. The distinct morphology of the flagella may be a useful taxonomic parameter at the family level and the morphology of the multilayered structures may be an important indicator of phylogenetic affinities among advanced chlorophytes, bryophytes, and lower tracheophytes.

SCANNING AND TRANSMISSION ELECTRON MICROSCOPIC STUDIES OF EMBRYONIC CHICK HEART FIBROBLASTIC CELL CULTURES. D.H. Bernanke, R.R. Markwald and J.M. Krook, Department of Anatomy, Texas Tech University School of Medicine, Lubbock, Texas 79409.

Distinctly fibroblastic cells grown in culture from explants of embryonic chick heart cushions have been studied utilizing both high resolution scanning and transmission electron microscopy. Explants free of epicardium (visceral pericardium) were taken by microdissection from sites of valvular and septal primordia (endocardial cushion pads) in 96-hour embryonic check heart and placed between glass coverslips in 35mm polystyrene petri dishes containing medium 199 with Earles salts and HEPES buffer with 10% fetal calf serum and 25U/ml penicillin and 25mcg/ml streptomycin. Some cultures were grown on 0.2-micron Nucleopore membrane filters for ease of handling for TEM processing. The cultures were incubated at 37°C in 5% CO_2 for 24 hours, at which time, the coverslips were separated, the explants themselves removed, and the medium replaced with fresh medium. Representative cultures were taken at 24 hour intervals up to 14 days. The cultures were washed with medium without fetal calf serum and fixed in 2% glutaraldehyde in 0.067M Na-cacodylate, pH 7.2-7.4, for 2 hours, post-fixed in osmium tetroxide, dehydrated and critical point dried for SEM, or, alternatively, embedded in low viscosity medium or EPON for TEM. SEM examination was made with a Hitachi S-500 scanning electron microscope at 15 keV. For TEM, the cultures were sectioned transversely and examined with a Zeiss EM-10 transmission electron microscope. The cells obtained by this explanting method were believed to be endocardial cushion tissue cells, a cell type which takes on a distinctly fibroblastic morphology **in vivo,** very similar to that seen in these cultures. The migrated cells never exhibited contractile behavior when examined by phase interference light microscopy prior to fixation, even in the presence of a contracting explant. The cells presented an extremely flattened morphology, with fine filopodia and lamellapodia at what appeared to be the leading edges of these migratory cells. The TEM picture of these cells was also very similar to that obtained from endocardial cushion tissue cells **in vivo.** No contractile elements of the type seen in myocardial cells were observed. This explanting method seems to provide a homogeneous cell population, which, at least by morphological criteria, can be identified as endocardial cushion tissue cells.

THE FREEZE-FRACTURE-WASH PROCEDURE: AN APPROACH TO MEMBRANE MOLECULAR ARCHITECTURE. J. David Robertson, M.D., Ph.D., Department of Anatomy, Duke University Medical School, Durham, North Carolina, 27710.

During the course of studies on the purple membrane of **Halobacterium halobium,** (in preparation for publication with Werner Schreil) an extension of the technique recently developed by Knute Fisher (Science **190:** 983 (1975)) for studying erythrocyte half membranes has been applied. This has resulted in the production of tangential fractures through the purified purple membranes which are normally factured only transversely.

The tangential fractures proceed along at least two different levels in the planes of the membranes. One of these involves the leaflet attached directly to the polylysine-glass surface. Another proceeds between the lipid bilayer including the lattice of bacteriorhodopsin (Henderson and Unwin (1975). Nature **257:** 28-32.), and an external layer. Occasionally fractures occur externally to this part of the leaflet and reveal a thin amorphous layer that covers the bacteriorhodopsin-lipid lattice. We have not yet obtained fractures through the center of symmetry of the membrane by this method. In whole organisms the fracture plane preferentially tends to follow the center of the bilayer except at the purple membrane patch where it tends to follow the external surface of the membrane.

Occasionally, however, it may proceed through the center of symmetry of the purple membrane patch and reveal the bacteriorhodopsin lattice. In this case the protein molecules of the lattice are revealed by a decoration phenomenon. The lattice shows up as arrays of ∫2.5 nm wormlike decorations. In the purified purple membrane fraction, when the fracture plane proceeds along the surface of the lipid-protein lattice after attachment to glass, the decoration effect has not been seen but particles about 10nm in diameter have been revealed.

These are sometimes closely packed in a regular hexagonal lattice but in some places the lattice is disrupted and the particles are loosely packed and appear scattered. These particles are too large to represent the individual bacteriorhodopsin molecules and it appears that they may represent a lipid-protein complex. We have devised a variant of Fisher's method to study those particles further. In this approach the purple membrane is

attached to polylysine-coated glass, covered with a copper disc, immersed in liquid nitrogen and fractured. The glass is then removed, thawed, and subjected to various washing and extraction procedures.

Preliminary results support the view that the 10nm particles seen are at least partly lipid. This technique is currently being applied to the general problem of determining the nature of intramembrane particles.

NOTES ON THE ALGA Phycopeltis epiphyton (CHLOROPHYTA, CHROOLEPIDACEAE). B.H. Good and R.L. Chapman, Department of Botany, Louisiana State University, Baton Rouge, Louisiana 70803

Phycopeltis epiphyton is an epiphytic green alga that is foliicolous and supracuticular on numerous higher coriaceous plant hosts in Louisiana. The alga forms a small discoid, monostromatic thallus which is composed of laterally appressed, branching filaments. Light microscopy revealed that **P. epiphyton** does not induce a host-wound respouse as has been described for a related alga, **Cephaleuros virescens.** Electron microscopic studies have shown that the cell walls of **P. epiphyton** contain a densely staining material which is a sporopollenin. The different amounts of this substance create two zones in the cell wall. The cross walls of this alga contain intercellular pit-regions which enclose plasmodesmata 40-50 mm in diameter and radial divisions appear to occur by an "infolding" of terminal cell walls rather than an ingrowth (furrowing) which has been described at the light microscopic level.

P. epiphyton reproduces asexually by the formation of biflagellate zoospores in abscissed, wind-borne zoosporangia and sexually by biflagellate gametes formed in sessile gametangia (modified vegetative cells). The ultrastructure of both gametes and zoospores revealed that spline microtubules and multilayered structures are present, as are two lateral keels associated with each flagellum. This is comparable to what has been observed in **Trentepohlia** and **Cephaleuros.** Thus far, the presence of more than one multilayered structure in each motile cell has not been observed. These observations may be useful in taxonomic and phylogenetic studies.

RAPID PROCESSING OF BIOLOGICAL TISSUES BY THE DMP-HXSA/VCD METHOD Joe A. Mascorro, Department of Anatomy, Tulane University School of Medicine, New Orleans, Louisiana 70112

Rapid dehydration and infiltration of muscle, epithelial and nervous tissues was performed for electron microscopy by **combining** 2,2-dimethoxypropane (DMP) instant chemical dehydration (Muller and Jacks, J. Histochem. Cytochem., 23: 107-110, 1975) with ultralow viscosity embedding in n-Hexenyl succinic anhydride (HXSA)/vinyl cyclohexene dioxide (VCD) (Mascorro et. al., EMSA Proceedings, 34: 346-347, 1976). The procedure was as follows: (a) usual fixation(s) and buffer rinse(s), (b) 2 washes in distilled water, 2-3 minutes each, (c) acidified DMP, 5 minutes, (d) infiltration in 1:1 DMP-HXSA/VCD, 5 minutes, (e) infiltration in full HXSA/VCD, 10 minutes, and (f) polymerization in 70°C oven, preferably 24-36 hours.

Acidified DMP was prepared by adding 1-2 drops concentrated HCL to 50 ml DMP. The embedding medium was prepared gravimetrically by combining 0.5 part VCD (epoxy resin), 1.0 part HXSA (anhydride hardner), 0.05 part Araldite RD-2 (modifier) and 1.0% by volume dimethylaminoethanol (catalyst). Tissues examined with the electron microscope generally displayed characteristics associated with good fixation. Damage attributable to faulty dehydration (i.e., vacuolization due to protein extraction) was not obvious. Infiltration with HXSA/VCD was flexible (from a few minutes to several days) and complete. The plastic showed good trimming and sectioning qualities. Thin sections resisted the electron beam well and were not subjected to undue creeping, burned spots, sublimation, etc. Maximum plastic strength was evident after 36 hours of polymerization, but blocks could be cut following overnight or 24 hour curing. En block uranyl acetate straining was desirable to alleviate the inherent low contrast of ultralow viscosity epoxies. DMP-HXSA/VCD eliminates strong organic intermediates (propylene oxide) and significantly abbreviates the usually lengthy dehydration and infiltration procedures of electron microscopy.

BIOPHYSICAL AND STEM ANALYSIS OF SYNTHETIC ORGANOMETALLIC PHOSPHOLIPIDS THAT MIMIC THE NATIVE SYSTEM. S. Brian Andrews and Russell J. Barrnett, Section of Cytology, Yale University School of Medicine, New Haven, Connecticut, 06510.

An organometallic analog of palmitic acid, 12,12-dimethyl-12-stannahexadecanoic acid (I), was chemically synthesized. **In vitro** coupling of **I** to egg lysolecithin yielded **II,** an organotin analog of egg lecithin. The biophysical characteristics of **II** are substantially similar to native egg lecithin, as measured by vesicle formation, nuclear magnetic resonance, agarose gel filtration, and x-ray diffraction. However, the average aggregate diameter of sonicated dispersions of **II** differed from that of egg lecithin vesicles (390 A and 230 A, respectively), and the size distribution of **II** vesicles was substantially broader. The **in vivo** incorporation of significant amounts (40-45%) of **I** into phospholipids and glycolipids of the cell membranes of **Acholeplasma laidlawii** further supports the utility of this probe. This was accomplished by culturing the organisms, which cannot synthesize fatty acids, in a medium in which **I** was the only fatty acid provided. The potential utility of these probes for investigations of membrane structure by STEM was subsequently investigated.

A small drop of ghosts isolated from **Acholeplasma** which incorporated **I** was micro pipetted onto a handmade, clean carbon plate, and air dried at room temperature. For x-ray microanalysis the electron image was observed with a JEM 100C fitted with a goniometer stage, an ASID-4 device and an EDAX-707B energy dispersive x-ray microanalyzer system. The electron image was taken as a scanning secondary image (SEM) and for the purposes of the x-ray microanalysis, samples were tilted at 30-45° to the electron beam so as to optimize x-ray counts from the specimens. In the x-ray experiments accelerating voltage was typically 10 KeV with a beam current of 10^{-11}A; spot size was 70 nm in diameter. An "Sn window", centered at 3440eV, was selected, and this allowed the accumulation of characteristic Sn-Lα emissions from control and experimental ghosts. More significantly x-ray line pulse analysis data were obtained, superimposed on SEM-images.

Results clearly indicated that statistically significant Sn-Lα x-ray counts could be detected in 100 seconds in ghosts of the experimental specimens, but not in controls. The line pulse data indicated an absolute correlation between density of Sn-Lα counts and images of ghosts. The results suggest that appropriately labeled heavy metal probes can be used to investigate surface topology of isolated membrane systems and can be extended to other specific constituents of membranes.

ULTRASTRUCTURE AND HISTOCHEMISTRY OF BRUNNER'S GLANDS OF THE SYRIAN HAMSTER. John M. Shackleford and Walter H. Wilborn, University of South Alabama College of Medicine, Mobile, Alabama 36688.

Glands of Brunner exhibit a combination of acidic and neutral mucosubstances. Surface portions of the glands are in-

tensely stained with alcian blue (AB) at pH 2.6 and deeper segments are AB-negative. All parts are periodic acid Schiff (PAS) positive. Brunner's glands of male hamsters show a greater affinity for AB than do females. Visual impressions of this sexual difference were confirmed by atomic absorption spectrophotometry (AA) which gave values for isolates of Brunner's glands of 2.3 for females and 4.4 for males. The isolates were specifically stained for sialomucins in 0.1M AB according to the critical electrolyte concentration method. The AA values (expressed as μg Cu/mg tissue) depend on the fact that copper is a constituent of AB and is bound to the tissue in proportion to the degree of AB staining.

Ultrastructurally, the cells of Brunner's glands are rich in rough endoplasmic reticulum (RER) and Golgi membranes. RER profiles tend to converge toward the Golgi apparatus where they exhibit budding and degranulation. Golgi saccules show progressive stages of secretory granule formation. Surface cells contain secretory granules with filamentous densities while the secretions of deep cells exhibit amorphous densities. Preliminary data indicate that secretory granules which contain filamentous densities are the source of acidic mucosubstances while those with amorphous densities are the source of PAS-positive, AB-negative granules.

Additional features of the gland include well developed intercellular tissue spaces and microvilli at luminal surfaces. Mitochondria and lysosomes are scattered throughout the gland cells. Free ribosomes, cytoplasmic filaments, and various types of vesicular structures are also present. Intercellular canaliculi are absent. Brunner's glands show ultrastructural characteristics of exocrine glands which produce a purely mucous secretion.

PREVENTION OF ISCHEMIC CONTRACTURE OF THE LEFT VENTRICLE DURING AORTIC CROSS CLAMPING: AN ULTRASTRUCTURAL EVALUATION Larry J. Tillman, Alan B. Weckerling, and George L. Zumbro, Anatomic Pathology and Cardiothoracic Surgery Services, Brooke Army Medical Center, Fort Sam Houston, Texas, 78234.

Irreversible myocardial damage continues to be a significant cause of death after aortic valve replacement and coronary artery bypass. There have been numerous studies attempting to prevent ischemic damage to the myocardium, including hypothermic techniques and coronary perfusion with various agents. No suitable experimental model of ischemic contracture of the myocardium existed until Armstrong recently developed a reproducible canine model of ischemic contracture of the left ventricle (stone heart). Reul, et al, have recently presented clinical data suggesting that propranolol administered to patients before cross clamping the aorta resulted in a lower incidence of stone heart after aortic valve replacement.

The myocardial protective effect of propranolol during aortic cross clamping was evaluated by controlled experimental studies on 21 mongrel dogs. On a double blind basis, 12 dogs under normothermic conditions and 10 dogs under hypothermic conditions received either 5cc of saline or a 5cc solution containing 20 micrograms/kg of propranolol intravenously five minutes prior to cross clamping the aorta. Left ventricular epicardial biopsies were taken prior to beginning bypass and immediately after removing the aortic cross clamp. Ultrastructural studies were conducted to evaluate mitochondrial changes and cellular depletion of glycogen. As previously documented, the overall myocardial preservation was found to be superior in the hypothermic specimens. However, propranolol treatment did not appear to preserve myocardial ultrastructure or prevent stone heart of the left ventricle.

INTRANUCLEAR ZOOFLAGELLATE INFECTION OF THE CILIATE MACRONUCLEUS IN Euplotes. John J. Wille, Jr., and Earl Weidner. Department of Zoology and Physiology, Louisiana State University, Baton Rouge, LA 70803

Preliminary light and electron microscopic studies have revealed infections of **Leptomonas** sp. exclusively in the macronucleus of the hypotrich ciliate, **Euplotes** sp. Wild populations of **Euplotes** were sampled from ponds near the LSU campus over a six-month period. One pond showed a routine 90% incidence of infection in **Euplotes.** Although infections resembled those reported in **Paramecium trichium** (1), **Paramecium** sp. and **Oxytrichia** sp, present in the same samples as **Euplotes,** were not infected. The number of leptomonads varied from a few to several hundred per nucleus. Heavily-infected macronuclei were packed with quiescent leptomonads in various stages of cell division. Upon liberation from the macronuclei, the leptomonads were activated into an ineffectual forward motion generated by the posteriorly-directed flagella.

The results of our clonal analysis of single cell isolates of infected populations of **Euplotes** demonstrates that infected cells are capable of at least two, possibly three, cell divisions. Heavily-infected cells eventually die due to the severe macronuclear disruption; however, lightly-infected **Euplotes** apparently can outgrow the infection. Replication bands were not observed in heavily-infected cells; however, leptomonads were seen in actively dividing nuclei. Attempts to transmit infections by exposing uninfected organisms to highly-infected **Euplotes** were unsuccessful. The natural mechanism of parasite transmission is a mystery but is under current investigation.

1. Reference: C. Gillies and E. D. Hanson. J. Protozool. 10(4), 473 (1963).

THE EFFECTS OF BAPN IN THE MARGINAL ZONE OF INDUCED MYOCARDIAL INFARCTS IN RATS. Michael D. Lahey, Department of Anatomy, Texas Tech University School of Medicine, Lubbock, Texas, 79409.

The effects of BAPN were studied with respect to these particular points of interest in the marginal zone of myocardial infarcts produced by coronary artery ligation in rats: 1) The effects of BAPN on scar tissue formation, 2) The effects of BAPN on "marginal myocytes", 3) The effects of BAPN on the proliferative response of ventricular myocytes (currently being studied using H^3-thymidine and light microscopy).

BAPN given intraperiotoneally (25 mg/daily) produces loosening of the connective tissue in the marginal zone. The dense collagen fibers normally present are no longer seen after BAPN administration as it is a specific inhibitor of the lysyl oxidase enzyme. The collagen is unable to cross-link, hence the inability to form a dense connection tissue scar. The connective tissue appears to be loose, with a moderate number of fibroblasts present.

The marginal myocytes are no longer surrounded by the dense connective tissue following BAPN administration and appear as disorganized myofibers, with some degree of separation apparent between adjacent myofibers. The myocytes show accumulation of lipid droplets, which decreases in a gradient fashion as the distance from the infarct increases. The marginal myocytes show some degree of disintegration of myofibrils and prominent nucleoli. The characteristics have been recognized as being present in dedifferentiating tissues.

Autoradiography currently being undertaken will determine the proliferative response of ventricular myocytes in response to myocardial infarction with and without BAPN administration.

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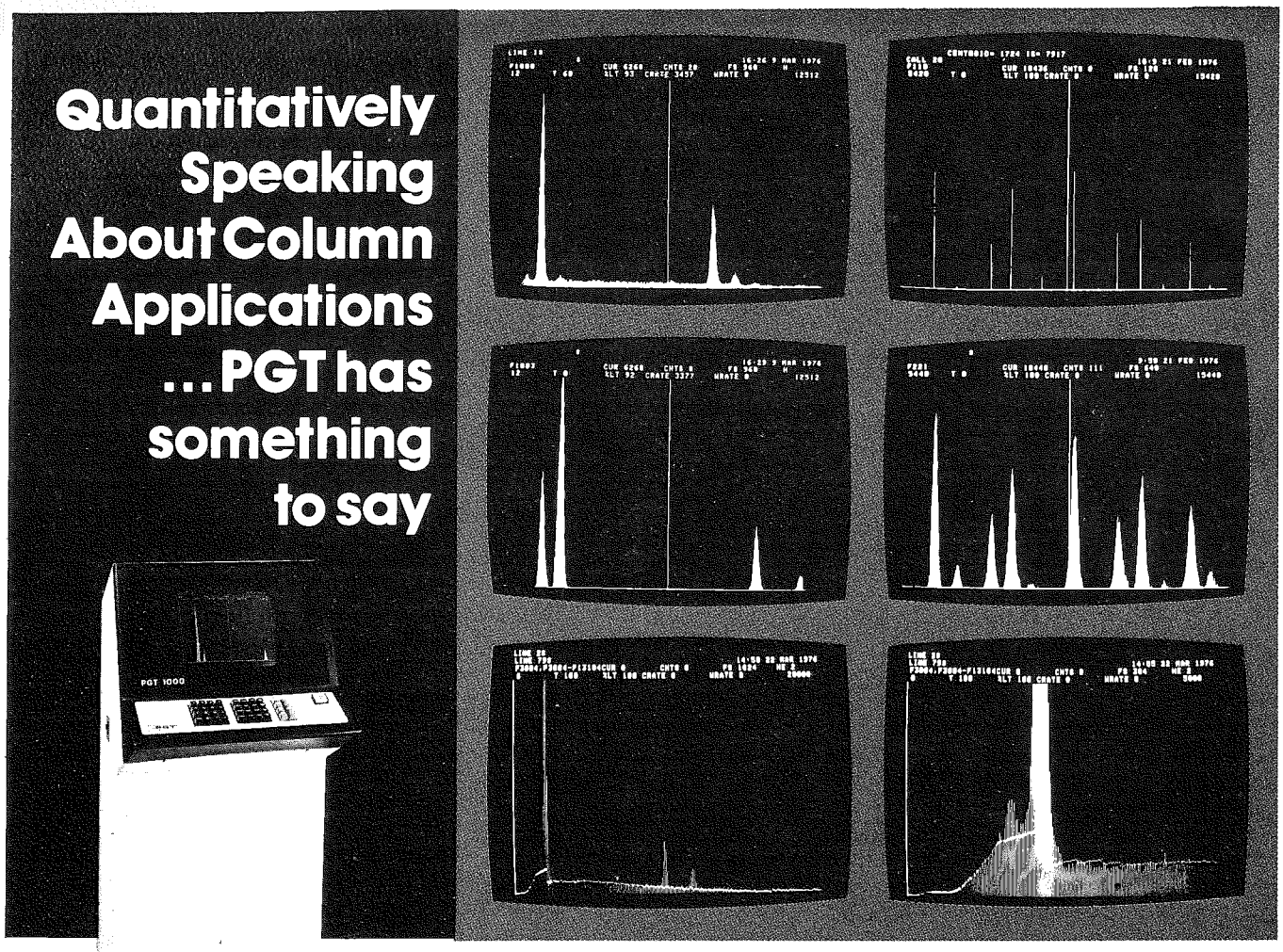

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SPONTANEOUS ATHEROSCLEROSIS IN THE WHITE CARNEAU PIGEON. John T. Hansen, Department of Anatomy, The University of Texas Health Science Center, San Antonio, Texas, 78284.

The atherosclerotic lesions, associated with the celiac intimal smooth muscle cushions, of four and five year old White Carneau pigeons were studied with the light and electron microscopes. Light microscopic examination of the spontaneous lesions demonstrated large intimal cushions composed of smooth muscle, collagen, foam cells and cholesterol crystal clefts.

Ultrastructural examination of the intimal atheroma revealed dilatations between apposing endothelial cells which contained a flocculent material, similar to that observed in the subendothelial space. The subendothelial compartment contained abundant collagen, extracellular lipid and vesiculated material. In addition, fibroblast-like interlaminar cells were often observed. These cells had morphological characteristics similar to the myofibroblasts seen in granulation tissue associated with wound healing. Numerous intimal smooth muscle cells were observed which displayed varied morphology. Foam cells were also present within the intimal atheromas.

The presence of atherosclerotic lesions in preexisting intimal smooth muscle cushions suggests that hemodynamic factors may be important in the progression of these spontaneous lesions. Endothelial cell dilatations may provide an important route of transport for circulating elements which may accumulate within the subendothelial space. Morphologically, it appears that the smooth muscle cells undergo modification and may represent the precursors of foam cells in this species.

Supported by a General Research Support Grant from NIH and The University of Texas.

ULTRASTRUCTURAL CHANGES IN MOUSE HEPATOCYTES AFTER MORPHINE PELLET IMPLANTATION. A. Thureson-Klein, J.R. Wang-Yang, I.K. Ho, and Eva Martenson, Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, Mississippi, 39216.

One, three or nine days after mice had been implanted with placebo or morphine containing pellets (75 mg) they were anesthetized with ether and perfused with fixative. Small portions from the same area of their livers were removed and processed for electron microscopy. One day after implantation with the morphine pellet the liver appeared pale yellowish in comparison with the brownish red livers of the controls. The color of the livers of the morphine treated animals showed a reversal towards normal three and nine days after pellet implantation. When viewed under the electron microscope the hepatocytes were found to contain numerous lipid droplets. The saccules of the Golgi apparatus were filled with particles ∫300-500 A believed to represent very low density lipo-proteins. Also the cisternae of the endoplasmic reticulum contained some particles. Similar particles were also prominent in the space of Disse. There were no obvious changes in other cell organelles. Probably because of its high lipid contents the tissue was difficult to post fix in the osmium tetroxide even though 2% solutions were used. Three days after pellet implantation the number of round lipid droplets had decreased but instead the hepatocytes contained larger irregular lakes of translucent material. Hepatocytes from animals exposed to the morphine for nine days (receiving a new pellet every three days) still contained more lipid droplets than controls but less than those from one and three days of implantation. The hepatocytes of nine days implanted mice also had more aggregates of filamentous material than after one and three days. (Supported by USPHS Grants DA-01310 and GM 15490).

ADRENOMEDULLARY EFFECTS OF ACUTE TREATMENT OF RATS WITH KEPONE®: AN ULTRASTRUCTURAL AND BIOCHEMICAL STUDY. Richard L. Klein, Jack McC. Baggett, Asa Thureson-Klein, Harihara M. Mehendale and Eva Martensson. Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, MS 39216.

Rats weighing 200g were treated with the pesticide Kepone for 8 days at 200 ppm in the diet. In an earlier investigation, a marked alteration in adrenomedullary cell ultrastructure was noted such that the majority cell population contained mostly spherical granules largely depleted of matrix material while the minority cell population contained elongated granules which retained an electron dense matrix, after prefixation by paraformaldehyde-glutaraldehyde perfusion, uranylacetate staining **en bloc** and OsO_4 postfixation. Biochemical analysis showed the total catecholamine content per adrenal gland to have decreased about 50%.

Subsequent experiments have been designed to determine if the pesticide had differential effects on the epinephrine- and norepinephrine-containing cells by utilizing analytical methods to discriminate between the two catecholamines together with more specific staining procedures. It was found that Kepone caused a selective 64% decrease in the epinephrine content from 15.6 ± 2.8 to 5.6 ± 1.8 ug/gland, while norepinephrine content actually increased 42% from 6.2 ± 0.9 to 8.8 ± 1.4 ug/gland. Similar changes occurred if the data were based on protein content. The relative norepinephrine content more than doubled from 28% to 61% of the total catecholamines. For ultrastructural study, the rats were perfused through the ventricles with glutaraldehyde-phosphate buffer followed by potassium dichromate and OsO_4 (Wood method) or with paraformaldehyde-glutaraldehyde containing chromatedichromate buffer followed by OsO_4 (Tranzer method). The results confirmed the earlier impression that presumptive epinephrine granules in the majority cell population became depleted while the minority cell population became more prominent and the presumptive norepinephrine granules maintained mostly elongated shapes with densely stained matrix after Kepone treatment. (Supported by USPHS Grants GM 15490 and GM 00359)

AN ULTRASTRUCTURAL STUDY OF PYELONEPHRITIS IN NON-HUMAN PRIMATES. T.W. Smith Jr., James A. Roberts, B.J. Martin, A Co-operative Study of the Delta Regional Primate Center of Tulane University School of Medicine and the Department of Biology, University of Southern Mississippi, Hattiesburg, Mississippi, 39401.

The present paper describes preliminary results of an ultrastructural study of chronic pyelonephritis in non-human primates: **Macaca arctoides** (stumptail monkey) and **Macaca mulata** (Rhesus monkey). The nature of the pathology observed, the role of vesicoureteric reflux, and the use of immunosuppression by cyclophosphamide are demonstrated. The infection was induced in these experiments in a retrograde fashion by means of a uni-lateral catheterization of the ureter whereby an inoculum of 10 cc of broth containing approximately 2 billion **E. coli**-04 per cc, radio-opaque dye, and ^{125}I were injected under pressure (mimicing vesico-ureteric reflux). Fake infections were also given whereby the bacteria were omitted from the inoculum. In each of these 2 groups of test animals the infection (fake or real) was administered following 1 week of immunosuppression with cyclophosphamide. A third group of animals were not immunosuppressed and given a single fake infection under high pressure (approximately 150 mm Hg). No evident pathology was seen here, supporting other proposals that scarring may be produced with repeated high pressure reflux. Several abnormalities are demonstrated ultrastructurally in the

immunosuppressed real-infected animals. In particular, bacteria within the tubular lumen and interstitium, tubular loss or atrophy, and iniltration of leukocytes into the tubular lumen and interstitium can be seen.

RESPONSE OF THE RAT ADENOHYPOPHYSIS TO PHENOTHIAZINES. T.G. Sarphie, Department of Anatomy, College of Medicine, University of South Alabama, Mobile, Alabama 36688.

The introduction of phenothiazine derivatives into the therapeutic management of psychiatric disorders has resulted in the elucidation of their rather unique and varied neuroleptic actions. Extensive research over the past two decades has demonstrated that phenothiazines are capable of inducing a variety of not only behavioral but metabolic changes in both animal and man. Systemic bioassays have revealed that approximately 50 to 75 percent of all daily doses are excreted in the urine and feces with the remaining aliquot accumulating in various body tissues such as the hypophysis and hypothalamus.

Bearing this in mind, the specific objective of this study of the phenothiazine's mode of action is to observe for any morphological alterations in the trophic hormone-secreting cell types in the adenohypophysis of rats treated with a number of commercially available phenothiazine derivatives via electron microscopic comparisons of both control and experimental animals. The response of different cell types to each phenothiazine derivative administered is emphasized in an effort to associate individual pharmacological characteristics with either known or suspected endocrine dysfunctions.

The adenohypophysis of the rat contains at least five, possibly six, recognizable types of differentiated secretory cells which possess reasonably reliable characteristics such as size, shape, position with respect to blood vessels, secretory granulation, and cytoplasmic fine structure all of which provide suitable criteria for their morphological identification. Normal ultrastructural features of the various cell types were utilized as an index for comparative studies of the experimental groups to facilitate the recognition of variances from the typical architectural pattern.

EVALUATION OF BONE INGROWTH INTO IMPLANTED POROUS BIOMATERIALS USING SCANNING ELECTRON MICROSCOPY. Jo Ann C. Shively, Department of Veterinary Anatomy, Texas A&M University, College Station, Texas, 77843.

Many different biomaterials are currently under evaluation for use as orthopedic implants for repair or replacement. Routine histology is often unsatisfactory as an evaluation procedure because the biomaterials are difficult to section. Scanning electron microscopy has been known to be a rapid method for evaluating the extent and morphology of bone ingrowth into implanted porous biomaterials and x-ray analysis is helpful in determining calcium:phosphorous ratios in association with implanted materials.

Samples of canine femur implanted in the diaphyseal region with porous vitreous carbon for 6, 7, 8, 10, and 12 weeks were sectioned with a diamond saw, dehydrated, and critically point dried. The sections were then coated with carbon or gold palladium and examined with the scanning electron microscope. Tissue structure in the internal pores of the carbon implants resembeled bone morphologically and X-ray analysis confirmed that the ingrowth was of similar elemental composition when compared to surrounding bone. The ratio of calcium to phosphorous was found to be much higher in bone ingrowth compared to bone surrounding the implant in all five groups.

FINE STRUCTURE OF THE HOST-PARASITE INTERFACE IN THE CEDAR-APPLE GALL RUST DISEASE. Charles W. Mims, Department of Biology, Stephen F. Austin State University, Nacogdoches, Texas, 75962.

Eastern red cedars infected with the rust fungus **Gymnosporangium juniperi-virginianae** produce spherical galls often exceeding 2 inches in diameter. These galls consist of both host cells and fungus hyphae. Intercellular hyphae are often closely appressed to the plant cells. Some hyphae actually appear to be surrounded by the cell wall of the host. The fungus is thought to absorb nutrients from host cells by means of specialized structures called haustoria. Haustoria penetrate the wall of the host cell and invaginate the cell membrane. The host cell membrane is separated from the haustorial wall by a sheath or encapsulation zone. Except for the presence of haustoria, the host cells appear normal and contain organelles characteristic of a typical plant cell.

A SCANNING ELECTRON MICROSCOPE STUDY OF GLASS ADHERENT CELLS FROM THE LUNGS OF SMOKERS AND NONSMOKERS. J.R. Dardano, B. Sue Criswell, Glenn Warr, and Stephen L. Kimzey, Cellular Analysis Laboratory, Johnson Space Center, Houston, Texas.

Leukocytes, predominantly macrophages, were classified into three categories based upon surface morphology and extent of spreading on glass coverslips by scanning electron microscopy. Class cells showed definite spreading on the surface of the coverslip and had a smooth veil of membrane extending for a minimum of 2-3μ from the central area of the cell. Cells included in Class II also exhibited spreading, but were often ruffled in appearance and the membrane spread 1μ or less from the central area of the cell. Cells included in class III showed no spreading, were smooth or had ruffled surfaces, and often were adherent to the glass only by means of several long filopodia.

Lung mononuclear cells from two groups of volunteers, either smokers or nonsmokers, were obtained by lavage, coded, allowed to settle onto coverslips for 45 min., and processed for study. Smokers had significantly higher percentages of Class I cells (60±8 vs 31±8) than nonsmokers; while nonsmokers had significantly higher percentages of Class III cells (41±4 vs 17±4) than smokers. Class II cells remained unchanged. The smoker and nonsmoker cells were compared for phagocytic ability when exposed to high multiplicities of **Candida krusei.** Smoker cells showed contact with the yeasts but not complete engulfment of particles. Nonsmoker cells showed both contact as well as complete engulfment. In conclusion, scanning electron microscopy has proved useful in differentitating these two groups of cells and may yield further information in understanding changes that occur as a result of smoking at the cellular level in the lungs.

THE PARACNEMID GLAND OF Bufo alvarius. M. Samuel Cannon,[1] Darlene C. Brindley[2] and Manley McGill[2], [1]Department of Human Anatomy, Texas A&M University, College Station, Texas, 77843 and [2]Section Biological Ultrastructure, The American National Red Cross Blood Research Laboratory, Old Georgetown Rd., Bethesda, Maryland, 20014.

The gross and microscopic anatomy of the venom-producing paracnemid gland of **Bufo alvarius** has been studied by light and electron microscopy. Histochemical reactions for the presence of venom constituents were performed. The paracnemid gland, located on the limbs of **B. alvarius,** are composed of many individual simple acinar glands, each having a duct which opens onto the skin. Each acinar gland is a unit with a lumen surrounded by a double cell layer. The outer cell layer resembles myoepithelial cells and contains filaments, some mitochondria,

the latter often in close association with granular endoplasmic reticulum, ribosomes and occasional pinocytotic vesicles. An intercellular (intervillous) space separates the outer and inner cell layers. The inner cell layer contains some granular and agranular reticulum, membrane-bounded particles, vacuoles and abundant mitochondria. Moreover, the inner cell layer appears continuous with the nearly homogenous secretory product. The cell layers and the secretory product give negative chromaffin and Schultz (for steroids and cholesterol and its esters) reactions. The intercellular space contains periodic acid-Schiff positive material, while the cell layers are negative; the secretory product is moderately PAS positive. The outer cell layer may be contractile and probably assists in discharge of the secretory product; furthermore, the outer and inner layers are probably involved in venom synthesis. The paracnemid gland resembles an immature venom-producing parotid gland. Perhaps because of its caudal location, the paracnemid gland need not be as physiological efficient as a defense mechanism as the anteriorly-situated parotid gland.

Supported in part by the American National Red Coss, Contribution No. 368.

ANTITUMOR ACTIVITIES OF PLANTINUM-THYMINE ON SARCOMA — 180 ASCITES In Vitro: ELECTRON MICROSCOPIC STUDIES. Gustav A. Ofosu, Department of Biological Sciences, Delaware State College, Dover, Delaware 19901.

Platinum-pyrimidine complexes have proven to be antitumor agents against a broad spectrum of different host systems. Sarcoma — 180 Ascites cells cultured in Eagles' basal medium (BME) treated with Platinum-thymine blue (60ug/ml) were analyzed ultrastructurally for morphological changes, if any. Cultured cells at varying time intervals were fixed in 1.5% buffered glutaraldehyde and 1% osmium tetroxide. The cells were dehydrated in acetone series and embedded in Araldite. Exposure of cells between 15 and 30 minutes induce microfilaments which migrate to form distinct band around the nucleus. Between 30 and 45 minutes treatment show increase in cellular volume. Disruption of the cytoplasm and increased nuclear clumping with nucleolus segregation marked treatment after 60 minutes. Cytoplasmic organelles move close to the nucleus and after 2 hours treatment the cells show sloughing off of cytoplasm. Mitotic activity is inhibited after 24 hours of treatment.

EFFECT OF PROTEIN SYNTHESIS INHIBITORS ON GASTRIC ACID SECRETION. K.S. Carlisle, C.R. Reagan and S.J. Hersey, Department of Physiology, Emory University, Atlanta, Georgia 30322.

Isolated frog gastric mucosa was employed as a model for a combined physiological, biochemical and ultrastructural study of the role of protein synthesis in the acid secretory process. It has been previously shown that stimulation of acid production by theophylline results in a 10-fold increase in plasma membrane surface area of the oxyntic cell. The origin of this greatly enlarged surface area has been questioned, **i.e.**, if **de novo** membrane synthesis is required or if the cell reorganizes and recycles existing cytoplasmic membranous components. Since membrane synthesis should require protein synthesis, the influence of the inhibitors cyclohexamide (CHM) and puromycin (Pur) on acid production, oxygen consumption and incorporation of ^{3}H leucine was examined. A one hour treatment with Pur (80ug/ml) or CHM (5ug/ml) reduced leucine incorporation by 90-95% compared to control tissues. However, neither inhibitor had any significant effect on spontaneous acid secretion or basal oxygen consumption. Moreover, stimulation of respiration and acid production by theophylline in the presence of the inhibitors was similar to that of control tissues. Electron microscopic examination of non-secreting and secreting tissues showed changes in oxyntic cell surface area which were similar for control tissues and those treated with the protein synthesis inhibitors. These studies show that **de novo** protein synthesis is not required for the oxyntic cells to undergo the biochemical and morphological changes which accompany the transition from the resting to secreting state and support the hypothesis that there is conservation and recycling of membranous components of the cell dependent upon the functional state. Supported in part by NIH Grant No. AM 14752.

THE VALUE OF ELECTRON MICROSCOPY IN THE STUDY OF GLOMERULAR CAPILLARY WALLS IN RENAL BIOPSIES. Ben. O. Spurlock[1'2] & R.N. Rao, M.D.[2], Departments of Anatomy[1] & Pathology[2], Medical College of Gorgia, Augusta, Georgia 30902.

Renal biopsies are routinely processed through paraffin and the sections stained by Hematoxylin-Eosin. In pathological states, the capillary walls, as seen by the stain, often appear thickened. Such a thickening may be due to alterations in the basement membranes, the related epithelial and endothelial cytoplasm, or due to the presence of protein deposits on either side or in the basement membrane. The Hematoxylin-Eosin stain does not differentitate these various components, and this may also be impossible with the many special staining procedures available. Electron microscopy for this purpose is of great value. Determination of the cause of the capillary wall thickening is of great importance, not only in the diagnosis and classification of the lesions, but also for the evaluation of therapy and prognosis. This paper illustrates some examples of the value of electron microscopy in membranous glomerulonephritis, systemic lupus erythematosus, malignant hypertension, thrombotic thrombocytopenic purpura, membranoproliferative glomerulonephritis, and chronic post-streptococcal glomerulonephritis.

AN ELECTRON MICROSCOPE STUDY OF THE MORPHOGENESIS OF NORMAL PRONEPHROS AND HERPESVIRUS-INDUCED PRONEPHRIC TUMORS IN DEVELOPING Rana pipiens LARVAE. Myron Tanenbaum and Merle Mizell, The Champman H. Hyams III Laboratory of Tumor Cell Biology, Department of Biology, Tulane University, New Orleans, Louisiana 70118.

Zonal sucrose gradient fractions of purified Lucke tumor herpesvirus (LTHV) particles obtained directly from the winter phase Lucke renal adenocarcinoma were injected into developing frog embryos. Differentiating pronephric and mesonephric kidney tissues are targets of this oncogenic LTHV fraction.

In **Rana pipiens,** the pronephric anlage appears in the 70-hour neural tube stage as a thickening in the somatic mesoderm. In the tail bud stage (3mm) the cells of the forming pronephros are arranged radially along an anterio-posterior axis. Tubule formation is noted in the muscular response stage (4mm). By the heart beat stage (5mm), the developing pronephric duct has reached the cloaca. Further studies have indicated that fluid excretion by the pronephros is initiated shortly after the union of the pronephric duct and the cloaca. Under normal conditions, the pronephros continues to grow and develop caudally for approximately 35-40 days, which corresponds to Taylor-Kollros metamorphic stage VIII. At this time, the organ has reached its maximum size. Degeneration is first seen in the anterior region of the pronephros at approximately 70 days and then can be traced caudally.

Many ultrastructural characteristics of the pronephros

change as the leopard frog undergoes metamorphosis from TK I through TK XVIII. In this study, changes in such ultrastructural parameters as: microvilli, basal and intercellular infoldings of the plasmalemma, yolk, mitochondria, lymphatic activity, and the integrity of the pronephric lumens were noted.

This study also reports on the ultrastructural appearance of virus-induced pronephric tumors.

MULTIPLE ANALYTICAL TECHNIQUES ON A SINGLE SPECIMEN. W. K. Paull, Department of Anatomy, Medical College of Georgia, Augusta, Georgia 30902

In an effort to study the uptake and transport mechanisms of bioactive molecules within the endocrine hypothalamus, we have developed a modification of the Wickham and the Worthen technique. In addition to SEM, LM, and TEM analysis of a single sample, we have found that we could localize radio labelled compounds by means of light autoradiography.

Five and fifteen minutes following the infusion of 10μ1 of H^3-dopamine into the cerebral ventricular system of adult male rates, the animals were sacrificed by means of cardiac perfusion with a 2% glut./2% paraformaldehyde fixative. The median eminence and basal hypothalamus were dissected out and processed for SEM analysis. Following critical point drying and gold coating, the ependymal surfaces were evaluated. After SEM, the specimen was removed from the stub, immersed in propylene oxide, embedded in Spurrs or Araldite, and sectioned at both 1μ and 60mμ. 1μ sections were then prepared for autoradiography by dipping the slides in Kodak NTB-2 emulsion (emulsion thickness $\int$ 2μ) and exposed for 6, 8, or 10 weeks. The emulsion was then developed with Dektol and the sections stained with methylene blue and azure II. The 60mμ sections were stained with uranyl acetate and lead citrate and their ultrastructure evaluated with a TEM.

This technique has enabled us to evaluate large surface areas with SEM, subsequently section the tissue for LM and TEM, and localize a labelled neurotransmitter all within the same piece of tissue. Although we were interested in the localization of neurotransmitters within the hypothalamus, this series of techniques could be utilized in a variety of studies where the up-take, incorporation, transport, or localization of molecules within tissues is important.

Stem Spermatogonium of The Nine-Banded Armadillo. Dr. Frank. J. Weaker, Department of Anatomy, The University of Texas Health Science Center at San Antonio.

This unusually looking cell is believed to be the stem spermatogonium of the nine-banded armadillo. The nucleus is irregular in shape with deep infoldings and frequently displays bizarre configurations. It contains finely packed chromatin granules, which are homogeneously dispersed, as well as one or two prominent nucleoli. The cytoplasm of the stem cell is less opaque than the other germ cells and contains a paucity of organelles with the mitochondria being the most prevalent. Although not shown, the Golgi is usually small and consists of several crescent shaped saccules and a few small vesicles.

CYTOLOGIC CHANGES IN THE PONY CORPUS LUTEUM (CL) DURING THE LUTEAL PHASE OF THE ESTROUS CYCLE. P. A. Maxwell, P.J. Ives, N.H. McArthur, and G.G. Stott. Department of Veterinary Anatomy, Texas A&M University, College Station, Texas 77843.

Spontaneous prolonged corpus luteum syndrome is an important factor contributing to infertility in the mare. In order to more fully understand the cytological changes in this and other infertility problems in the Equine, the normal corpus luteal morphology was studied.

The equine CL differs from that of other domestic animals in that it is deeply rather than superficially located in the ovary. Luteal cell development and regression were correlated with the stages of the estrous cycle. The observation of these changes began with lutenization (postovulation or early diestrus) and were followed through the luteal cell development to regression during the subsequent stages of the estrous cycle.

Luteal cell development was characterized by the formation of large, actively secreting cells with well defined nuclei and an increasing number and size of cytoplasmic granules. This development began at early diestrus and extended through diestrus into proestrus. Luteal cell regression began during late diestrus and continued through the succeeding estrous cycle. The regression was characterized by irregularly shaped and smaller nuclei with peripherally clumped chromatin and darker staining cytoplasm with increased lipid accumulation.

During luteal development, mitochondria cristae were tubular in shape, smooth endoplasmic reticulum (SER) was abundant and densely packed, and lipid inclusions were sparse and small.

Degeneration of the luteal cell was characterized by fewer mitochondria with dense matrices and lamellar cristae, a reduced amount of SER, and an increase in size and number of lipid inclusions, which were often associated with residual lysosomes. By the end of one full estrous cycle, luteal cells were extremely degenerated with large lipid inclusions and a paucity of cell organelles.

ULTRASTRUCTURAL CHARACTERISTICS OF THE FOLLICULAR CELLS IN THE Cebus apella, MONKEY THYROID. P.J. Ives, N.H. McArthur, and C.L. Tompkins, Department of Veterinary Anatomy, Texas A&M University, College Station, Texas 77843.

The present study was conducted to examine the ultrastructural morphology of the thyroid gland from immature female, intact and castrated male and female, and tapazole treated female monkeys.

The striking feature of the thyroid follicles in the intact animals was the cytological polarity of the follicular cells. One pole of the follicle had cuboidal to low columnar follicular cells containing abundant rough endoplasmic reticulum (RER), a few electron dense vesicles (EDV) and some short unevenly distributed microvilli on the luminal surface. The opposite pole had flattened squamous appearing follicular cells with numerous lysosomal bodies and autophagic vacuoles.

Thyroid follicles from castrate animals exhibited a decreased synthetic activity. There was a decrease in flocculent materials within the cisternae of the RER and the presence of numerous lysosomal bodies. The follicles from the immature animals contained mostly uniform cuboidal to low columnar follicular cells. The follicles varied only slightly in colloid content. The follicles showed slight or no uneven cell height or polarity. The follicular cell generally showed evidence of great activity with dilated RER, and large numbers of EDV.

The tapazole treated animals exhibited a marked proliferation of mitochondria, dilitation of the cisternae of the RER, a prominent Golgi area and numerous lysosomes. The follicular cells had a marked increase in size and contained numerous microvilli on the luminal surface. Follicular polarity was absent in the tapazole treated animals.

SCANNING ELECTRON MICROSCOPY OF THE EPENDYMAL AND SUPRAEPENDYMAL CELLS OF THE 3rd VENTRICLE OF THE ARMADILLO, Dasypus novemcinctus L. P.J. Ives, and N.H. McArthur, Department of Veterinary Anatomy, Texas A&M University, College Station, Texas 77843.

The ependymal lining of the 3rd ventricle of the adult armadillo was examined by scanning electron microscopy. The presence and polymorphism of different cell types on the ventricular surface of the various mammalian species has been reported in the literature. The activity of these different cell types in phagocytic or autoregulatory mechanisms involving the synthesis and/or transport of releasing hormones or biogenic amines has been speculated.

The dorsal wall of the 3rd ventricle was densely ciliated. In the rostral portions of the wall, ventral to the interthalamic adhesions, there was intermittent folding or pitting of the ependymal surface. The ventral wall had scattered clumps of cilia and numerous microvilli on the surface. In this sparsely ciliated area, numerous supraependymal cell (SEC) processes could be seen traversing the ependymal surface. Numerous SEC were present on the ventral wall as it reflected onto the ventricular floor or into the supraoptic and mamillary recesses. In this area, there were numerous spherical blebs which occurred singly and in clusters protruding from the ependymal cells. SEC were most numerous on the surface of the rostral portion of the median eminence. Most SEC were restricted to the hypophysiotrophic area. SEC configuration varied from bipolar to multipolar with many being stellate in appearance. Their cell processes were either closely associated with the underlying ependymal cells, or free on the surface, either suspended on ependymal microvilli or entangled by them. Two basic types of cells were present. Type I cells had a smooth or rough surfaced cell body with well defined, distinct processes. The Type II cells had an irregular, folded cell body with large pseudopod-like processes that extended into thin sheets of cytoplasmic membranes.

A MODIFIED THIOCARBOHYDRAZIDE BINDING TECHNIQUE OF BOTANICAL SPECIMENS FOR SCANNING ELECTRON MICROSCOPY. Michael T. Postek and Shirley C. Tucker, Department of Botany, Louisiana State University, Baton Rouge, Louisiana, 70803.

The ligand osmium binding technique utilizing thiocarbohydrazide has been a successful alternative to evaporative or sputter coating of botanical specimens. A modification to this technique will be presented which has been used successfully in our laboratory on a variety of botanical specimens. This modification permits the reduction of the overall quantity of osmium needed in the binding steps thus reducing cost. Further, this technique also permits a reduction of specimen handling by the complete chemical dehydration of the tissue by the use of 2-2 dimethoxypropane.

RESPONSE OF THE RAT PAROTID GLAND TO CYSTIC FIBROSIS SERUM. Walter H. Wilborn, Department of Anatomy, University of South Alabama, College of Medicine, Mobile, Alabama 36688.

The purpose of this study was to evaluate the effects of serum from patients with cystic fibrosis and of normal human serum on the parotid glands and pancreases of rats. Three groups of rats were injected daily (0.2 ml/day, intraperitoneally) for 2-9 days as follows: Group I, normal saline; Group II, control serum (CS); Group III, cystic fibrosis serum (CFS).

CFS caused pronounced structural alterations of the parotid gland, resulting in acinar cell atrophy, degranulation, and an increase in lysosomes, autophagic vacuoles, and mitotic activity. These changes were observed after CFS had been injected for only 2 days. Reduction in Golgi membranes and the sparsity of electron lucent secretory granules indicated that new granule formation was greatly impaired. These same kinds of effects were observed after CS, but they were greatly reduced in magnitude. Parotid amylase levels were significantly lower and serum amylase levels were higher in rats injected with CFS than in rats receiving CS or saline. The pancreas was not affected as judged by electron micrographs.

Since the responses of the parotid to CFS were considerably more marked than those elicited by CS, the rat parotid may have potential usefulness in assaying for the presence of the cystic fibrosis factor(s) in human serum.

ULTRASTRUCTURE OF LAYER IV OF THE PRIMARY AUDITORY CORTEX OF THE SQUIRREL MONKEY (Saimiri sciureus). Diane E. Smith and Norman Moskowitz, Department of Anatomy, Louisiana State University Medical Center, New Orleans, Louisiana, 70112, and Daniel Baugh Institute of Anatomy, Jefferson Medical College, Philadelphia, Pennsylvania, 19107.

Studies of the ultrastructure of layer IV of the primary auditory cortex in the squirrel monkey were initiated to provide additional information on the morphology of the granule cell columns previously identified in Nissl sections (Jones and Burton, J. comp. Neur. 168: 197-248, 1976).

Young male squirrel monkeys (600-800 gms) were injected intramuscularly with propromazine hydrochloride (0.2mg/kg) 30 minutes before administration of sodium pentobarbital (20mg/kg). The moneys were then perfused transcardially with a warmed paraformaldehyde — gluteraldehyde solution in phosphate buffer. After overnight storage in the refrigerator, representative segment of cortex were cut at right angles to the gyrus and processed for examination with the electron microscope.

Layer IV is characterized by beads of granule cells oriented at right angles to the pial surface. In agreement with Jones and Powell (Phil. Trans. Roc. soc. B. **257:** 1-11, 23-28, 1970), medium to large myelinated axons are characteristically observed ascending vertically throughout layer IV. The granule cells are round to oval in shape and display a thin rim of cytoplasm surrounding a deeply indented nucleus. Close apposition of adjoining perikaryal membranes is frequently observed. Subsurface cisterns may be present, but tight junctions have not been noted. The most prevalent synaptic complex is the asymmetric Gray's Type I contacting the dendritic spines. Axosomatic synapses are encountered less frequently, but when present make multiple contacts with the perikaryal surface.

This work is supported by NIH grants NS 09275, NS13155 and NS 10806.

ULTRASTRUCTURAL STUDIES OF NORMAL AND BRACHYPOD EMBRYONIC MOUSE LIMBS. Jackie C. Duke, Biology Department, Emory University, Atlanta, Ga. 30322.

Brachypodism, an autosomal mutation of mice, affects the differentiation and development of the skeletal limb elements. These studies were carried out on limbs from 12-day embryos to ascertain the effect of the mutation on cell contacts during blastema formation in the limb.

Cells of both genotypes appear to be typical prechondrogenic cells with a high nucleocytoplasmic ratio. The cytoplasm contains numerous polysomes along with some distended channels of rough endoplasmic reticulum. Occasional small stacks of flattened Golgi cisternae are seen.

In sections from normal limbs, there is a great deal of intercellular space, and contacts between cells are primarily by filopodia, although there are some areas of broader contact.

The intercellular spaces in sections from brachypod limbs are not as great as those in normal limbs, and brachypod cells appear to have more filopodial processes than do normal cells. Also, tight packing of cells is found in some regions in

brachypod limbs, often in the same sections with more loosely packed cells.

The tighter packing and decrease in intercellular space observed in sections from brachypod limb buds is consistent with data from rotational reaggregation studies which show that brachypod cells are more adhesive than normal cells.

HIGH VOLTAGE ELECTRON MICROSCOPY: APPLICATION TO THE STUDY OF THICK BIOLOGICAL MATERIAL. Jerome J. Paulin, Department of Zoology, University of Georgia, Athens, Georgia 30602.

Through grants obtainable from the Biotechnology Resources Branch of The National Institutes of Health, biologists have the opportunity to use high voltage electron microscopes located at Monroeville, Pennsylvania; Madison, Wisconsin, and Boulder, Colorado. Due to the high accelerating voltage (>1,000 KV), thick sections of plastic embedded material (1-2 μ thick) and dried whole cells can be viewed with adequate resolution and contrast. Tilting stages on these instruments can be utilized for a stereopair analysis of specific cellular organelles to reveal their three-dimensional structure. Stereopair analysis and reconstruction of organelles from serial thick sections are means by which three-dimensional information can be obtained.

In our laboratory we have been able to demonstrate the unitary structure of the chondriome of three species of typanosomatid flagellated protozoa. The reconstructed models of the mitochondrion of each species were obtained from cellulose acetate overlays of serial thick sections (section thickness between 0.25-0.50 μ). Stereo-pair analysis of a specific section in a series was also utilized to eliminate overlap of structures which may confuse the image.

Whole cells dried by the critical point method were also examined and the nucleus, kinetoplast and flagellar axonemes clearly revealed. Some indication of the subpellicular microtubular system was also evident.

Negatively stained undischarged and discharged trichocysts of **Paramecium** were examined. The paracrystalline lattice of these unique organelles was resolvable in these intact isolated organelles.

SCANNING ELECTRON MICROSCOPIC OBSERVATIONS OF NATRIX DORSAL SCALES. Susan Caster and Ellen Mattingly, Department of Zoology, University of Georgia, Athens, Georgia 30602.

Dorsal scales were removed from freshly killed Natrix and fixed for either light or electron microscopy. Glutaraldehyde-tannic acid fixed scales were processed routinely, critically point dried, and observed with the scanning microscope.

Light microscopic observations confirmed the presence of a surface Oberhautshen lying over a layer of β-keratin. Between the β-keratin layer and the stratum germinativum is a multilayered band of α-keratin.

We have used the scanning electron microscope to extend our observations on the structure of the Natrix scale. Low-power observations on the surface of the scale show a series of irregularly parallel ridges tending to converge toward the keel. These ridges cover the entire surface of the scale except for the twin "pit" areas at the tip of the scale.

Observations at a much higher power show a pattern composed of V-shaped elevations tending to lie parallel with the ridges. At the point at which two adjacent ridges fuse, one and more frequently two pits are seen.

We are presently extending our studies of the relationship of these surface structures to the underlying layers by the analysis of freeze-fractured scales with the scanning microscope, and are attempting to relate these observations to structures previously described by light microscopists.

FLAGELLAR DYSGENESIS IN MALE-STERILE MUTANT MICE. John H. D. Bryan, Department of Zoology, University of Georgia, Athens, Georgia 30602.

Male mice homozygous for the recessive, pleiotropic, mutation hydrocephalic-polydactyl (hpy) are sterile due to a failure to produce complete spermatozoa. For the ultrastructural investigations reported here, seminiferous tubules from mutant and wild-type littermates were fixed and processed as described by Bryan and Wolosewick 1973 (Z. Zellforsch. 138: 155-169). No mutation-induced perturbations of spermatogenesis were noted until after the completion of the second division of meiosis. A principal defect seen during spermiogenesis was the failure of flagellar development. Because this could result from an absence of centrioles (or the presence of highly defective ones), centriolar ultrastructure was investigated in detail.

Centriole structure appeared normal and several distal centrioles appeared to be initiating axoneme assembly. However, only a few partial axonemes were encountered. In some just central-pair components were present, often only one member of the pair was recognizable. In others, all members of the 9 + 2 complex were present but their appearance was atypical (A- and B-tubules looked alike) and the A-tubules appeared to lack dynein arms. Such axonemes were usually surrounded by a thin cylinder of spermatid cytoplasm devoid of mitochondria or other flagellar components. In many spermatids loosely organized groups of outer coarse fibers together with mitochondria occupied the cytoplasm posterior to the nucleus. Released "gametes" usually consisted of a distorted nucleus with its adherent acrosome enveloped in a tight fitting plasma membrane. Ciliary structure is also abnormal in **hpy**-homozygotes (see paper by D. Chandler for details).

THE DEVELOPMENT OF SUPPORTING CELLS AND THEIR SECRETORY ROLE IN THE FORMATION OF THE TECTORIAL MEMBRANE. A.J. Siegel, C.D. Fermin, and G.M. Cohen, Department of Bvlogical Sciences, Florida Institute of Technology, Melbourne, Florida 32901.

In the avian lagena supporting cells alternate with the shorter hair cells, and both are closely aligned along their vertical axes. Supporting cells, which are pseudostratified and exhibit irregularly positioned nuclei, extend from the basilar membrane to the apical lumen.

By day 8 the apical ends of supporting and hair cells are already coupled by tight junctions that are fortified intracellularly by cytoplasmic anchors. Microvilli have already emerged from supporting cells' apical surfaces; centrioles have migrated to supranuclear regions. By day 11 mitochondria are considerably smaller than those in adjacent hair cells but are similarly shaped. By day 12 organelles are highly polarized along their vertical axes; multivesicular bodies appear in the supranuclear region by day 13.

By day 8 the presumptive tectorial membrane (TM) first becomes evident as a thin veil. By day 9 rough endoplasmic reticulum (rER) egins to fill with protein as it forms an extensive tubular network throughout the supporting cell, which by day 11 is retained only basally. More centrally, tubular rER transforms into round or oval cisternae during the migration to the neck, clustering directly below the microvilli. Between days 11-12 microvilli become conspicuously enmeshed with the nascent TM by strands of amorphous material. The developing TM arches completely over the hair cells and forms vaulted canopies into which mature stereociliary tips may later embed. By day 13 the TM condenses slightly in its upper face but remains

amorphous underneath. At day 14 as TM formation reaches its climax, the cisternal population peaks but shortly thereafter virtually disappears; by day 19 cisternae are largely replaced by Golgi apparatuses.

LOOSE AND COMPACT MYELIN FORMATION AROUND AXONS THAT INNERVATE LAGENAR HAIR CELLS. C.D. Fermin and G.M. Cohen, Department of Biological Sciences, Florida Institute of Technology, Melbourne, Florida 32901.

The axons and cell bodies of adult spiral and vestibular ganglia are covered by a combination of loose and compact myelin sheaths, which is an uncommon myelin pattern. In loose myelin, unlike its compact counterpart, the cytoplasm persists within the sheath. In the present study we traced the formation of these untypical myelin sheaths in ears of embryonic chicks from days 8-20.

At day 8 in Schwann cells, a halo of granular cytoplasm surrounds a prominent nucleus that occupies almost the entire somal volume; rough endoplasmic reticula (rER) that is dilated with protein clusters within the cytoplasmic processes, a consistent processal feature at all developmental stages and suggesting a secretory function. The processes from several adjacent Schwann cells form a boundary around the groups of naked axons that are growing towards the basilar membrane. Presumptive fibroblasts, characterized by coils of tubular rER, populate the area; a few collagen fibrils meander closely by the axons and Schwann cells. By day 11 processes of Schwann cells encircle groups of 10 or more axons, even before completion of afferent innervation of hair cells by day 12. Strands of collagen are increasingly abundant and lace through the sparcely filled region below the basilar membrane. By day 14 Schwann cell processes tightly clutch groups of axons. By day 16 processes insinuate between individual axons to divide the axonal groups into smaller bunches. The first evidence of compact and loose myelin occurs but only intermittently, since some Schwann cells are less developed. By day 19 ensheathed axons are characterized by loose myelin in the outer and innermost lamellae sandwiching about a dozen lamellae of compact myelin, which at its opposite poles often transforms to loose myelin.

THE DEVELOPMENT OF HAIR CELLS IN THE EMBRYONIC CHICK LAGENA. C.D. Fermin and G.M. Cohen, Department of Biological Sciences, Florida Institute of Technology, Melbourne, Florida 32901.

In birds little is known about the structure of lagenar hair cells, which are the auditory receptors. In the present study we followed hair cell differentiation from days 8-21 (hatching). We used eggs from white leghorn chickens.

During day 8-11 hair cells transform from cuboidal epithelium into either presumptive cylindrically-shaped tall hair cells (THC) or pitcher-shaped short hair cells (SHC); by day 13 hair cell types are unambiguous. "Hairs" (stereocilia) and a kinocilium first sprout from the apical surface by day 8 and resemble the adult pattern by day 13. Internally, the cytoplasm at day 8 is already dense because of the abundance of organelles, which then being to migrate to specific regions as synthetic activities accelerate. The culticular plate (CP), which anchors stereociliary rootlets, is first recognizable by day 11 and completes its formation shortly after hatching. Relative nuclear volume declines from 60% to 30% from days 8-19 because of cell growth; the nucleus is positioned centrally in THCs but basically in SHCs. Nucleoli are prominent but become inconspicuous in adults. Long and short mitochondria succeed the oval ones; some cluster beneath the nascent CP; later many encircle the nucleus once elevated synthetic activities subside. By day 13 multivesicular bodies appear but are confined to the supranuclear region. After day 16 large numbers of microtubules traverse the cell's long axis; ER and Golgi are also similarly polarized. By day 11 sensory nerves regularly touch hair cell bases, though no evident synaptic specializations occur until day 12 when synaptic bars and plasma membrane densities form. Efferent nerves contact the hair cell bases by day 13; only then do terminals begin to fill with vesicles.

MICROSCOPIC EVALUATION OF THE PREPARATION OF DECAPITATED PLASMALEMMAE-FREE, MITOCHONDRIA-FREE BULL SPERM FLAGELLA. Eleanor B. Smithwick and Leona G. Young, Department of Physiology, Emory University School of Medicine, Atlanta, Georgia, 30322.

Sperm motility depends on the ability of flagellar ATPase to convert the chemical potential energy of ATP into mechanical movement. To isolate the movement-related ATPase(s) of mammalian sperm free from contamination by membrane and/or mitochondrial ATPases, we developed a rapid and reproducible procedure for the preparation and isolation of decapitated, plasmalemmae-free, mitochondria-free bull sperm flagella. Incubation of washed sperm in dithiothreitol and collagenase B followed by brief sonication effects removal of the plasmalemmae, partial disruption of the mitochondrial sheath and decapitation. Freed flagella are isolated on a discontinuous sucrose gradient. Already partially disrupted, essentially all the mitochondria are removed from the flagella by incubation in dithiothreitol and Triton X-100 at alkaline pH. Throughout the development of this procedure, the alterations in sperm morphology caused by each treatment were monitored by light microscopy, and TEM of negatively stained samples confirmed quickly at higher resolution the degree of mitochondrial removal. Finally, by TEM of thin sectioned samples, we evaluated the effect of each treatment on the internal flagellar ultrastructure and confirmed that after the mitochondria are gone the outer dense fiber/axonemal complex, including those structures implicated in motility, usually remained intact. From these plasmalemmae-free, mitochondria-free flagella, we are able to extract an active ATPase. (Supported by grants from Emory University, USPHS HD-06491, and NRS Award HD-00493 to EBS.)

DISTRIBUTION OF ANIONIC SITES ON THE OVIDUCT CILIARY MEMBRANE. Cheryl E. Hein and Richard G. W. Anderson, Department of Cell Biology, The University of Texas Health Science Center at Dallas, 5323 Harry Hines Boulevard, Dallas, Texas 75235

Polycationic ferritin (PCF) was used to detect anionic sites on the rabbit oviduct ciliary membrane. The binding of PCF to the membrane was dependent upon the concentration of PCF in the incubation media. At low concentrations (0.08% - 0.16 mg/ml), PCF bound only to the tip of the cilium. At higher concentrations (0.32 - 0.64 mg/ml), PCF was bound not only to the tip, but also to the base of the cilium around the transition region, with scattered clumps of PCF occasionally bound to the remainder of the cilium. PCF binding was associated with special surface modifications of the ciliary membrane. Tip binding was associated with the filamentous glycocalyx termed the ciliary crown. Base binding was associated with a set of five or six ridge-like formations, approximately 245 A apart which encircled the base of the cilium at the transition region. Treatment with proteases and neuraminidase blocked PCF binding, indicating that the PCF binding sites may be negatively charged mucopolysaccharides or glycoproteins.

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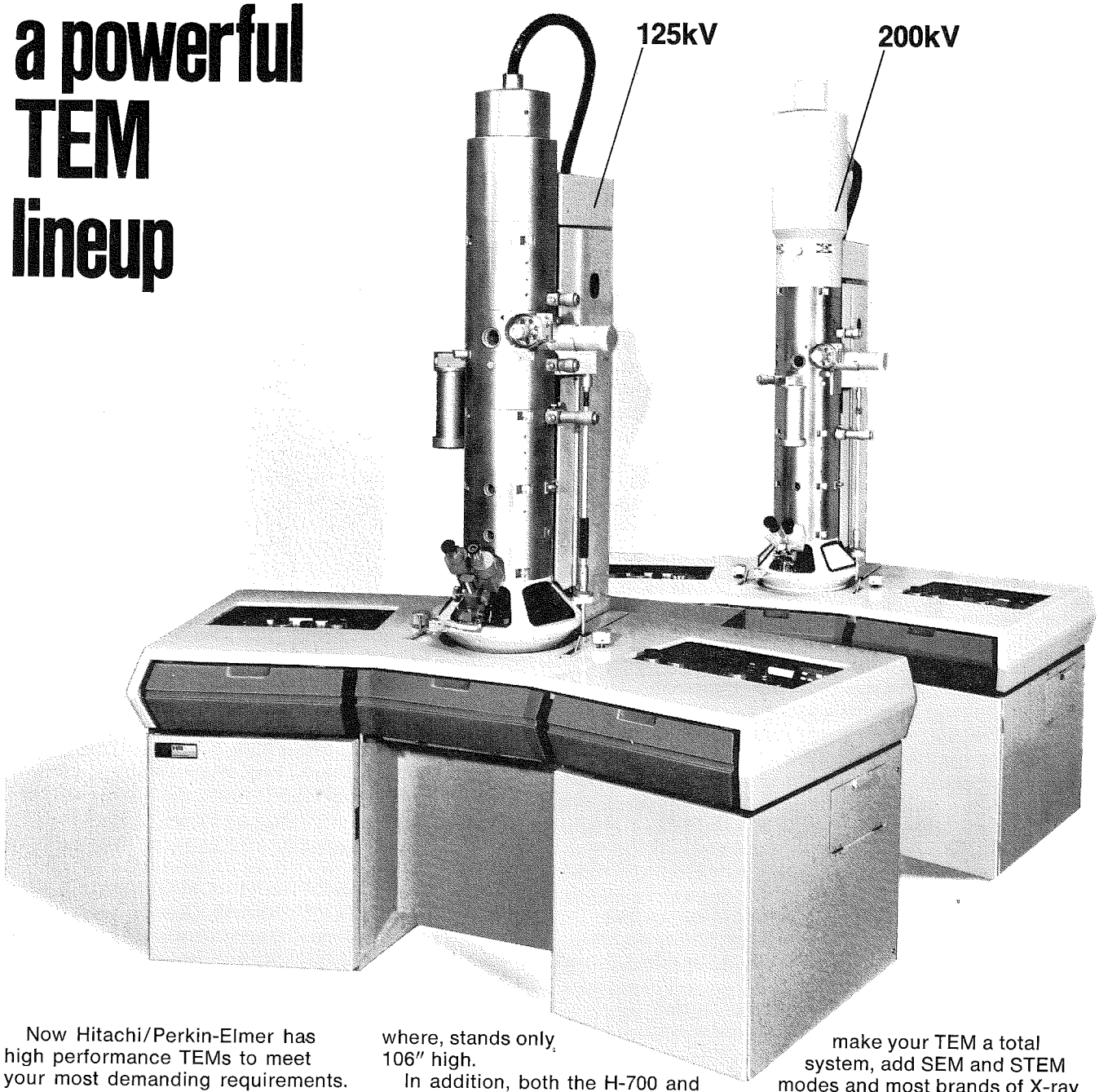

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A SCANNING AND TRANSMISSION ELECTRON MICROSCOPIC COMPARISON OF PUROMYCIN AMINONUCLEOSIDE INDUCED NEPHROSIS TO HYPERALBUMINEMIA INDUCED PROTEINURIA WITH EMPHASIS ON KIDNEY PODOCYTE PEDICEL LOSS. Peter M. Andrews, Department of Cell Biology, Southwestern Medical School, University of Texas Health Science Center, 5323 Harry Hines Blvd., Dallas, Texas 75235

Ultrastructural changes in the visceral epithelium and proximal tubules of rats were studied by scanning and transmission electron microscopy during the onset and progression of Puromycin aminonucleoside nephrosis (PAN) induced proteinuria. These changes were compared with those which occur during a similar degree of proteinuria induced by intraperitoneal injection of albumin. With the onset of proteinuria and oliguria, PAN rats exhibit loss of podocyte pedicels and podocyte major processes, an increase in pinocytotic activity, and an accumulation of cytoplasmic vacuoles and granules of variable size, shape, and electron density.

Loss of podocyte pedicels involves a gradual decrease in pedicel high beginning at the pedicel tip and progressing down the pedicel arm, formation of nub-like protrusions and interpedicel microbridges (35-45 nm in width and 40-60 nm in length) along the pedicel's base, the merging of microbridges to form more extensive regions of interpedicel contact, and gradual broadening and retraction of pedicels. In response to hyperalbuminemia induced proteinuria (HIP), kidney podocytes exhibit an increase in pinocytotic activity and an accumulation of protein absorption droplets.

Unlike during PAN, however, the podocyte pedicels, slit pores, and major processes of HIP rats remain discrete. The loss of pedicels and major processes during PAN therefore apparently results from the effects of Puromycin aminonucleoside **per se** rather than from the proteinuria associated with this disease. HIP rat proximal tubules exhibit the same characteristic changes as PAN rat proximal tubules (i.e., loss of brush border, dilated lumina, abnormally thin walls, and accumulation of PAS-positive electron dense luminal casts and cytoplasmic protein absorption droplets).

AXO-AXONIC SYNAPSES ON INITIAL SEGMENTS OF HIPPOCAMPAL PYRAMIDAL CELLS. R. B. Chronister, Department of Anatomy, and R.W. Sikes and L.E. White, Jr., Neuroscience Research, College of Medicine, University of South Alabama, Mobile, Alabama 36688.

The existence of synapses onto initial segments of axons within the central nervous system has been documented in the prepiriform cortex. These synapses occur on spinelike processes of the axons of prepiriform cortex neurons. These spinelike processes are visible in both light and electron microscopy. Golgi impregnations of the hippocampal formation reveal similarly appearing spinous processes. For this reason, the hippocampal formation was examined with transmission electon microscopy to ascertain the presence of initial segment axo-axonic synapses.

Adult rats were perfused with Peters' modification of Karnovsky's fixative. Following fixation, small blocks were cut from precisely localized regions of the hippocampus and postfixed in 1% osmium tetroxide. The blocks were then stained **en bloc** in uranyl acetate and embedded in epon. One micron thick sections were cut and examined to further substantiate localization. Silver-gray sections were mounted on grids, stained per usual with lead citrate, and examined on a Phillips 301 transmission electron microscope. Initial segments were recognized by the thickening of the membrane and the bundling of the microtubules.

The spinous processes were of two basic types. The most common spine was sessile although pedunculated spines were also encountered. The pedunculated spines had vacuolated structures similar to those of dendritic spines while the sessile spines appeared to be devoid of these structures. In addition to the vacuolated structure, the pedunculated spines also contained fine filaments.

Both types of spines were postsynaptic. Presynaptic boutons often contained flattened vesicles and made type II synapses upon the spines. Similar type II synapses also were found on the shafts of the axons. On the dendrites of these neurons, type I synapses were present on the dendritic spines. The significance of these findings will be discussed.

FINE STRUCTURES OF THE SPORE SURFACE OF SOME ANTAGONISTIC Streptomyces SPP., ISOLATED FROM THE SOIL. Shelat Harnath, G.B. Howze, and S.O. Fadulu, Department of Biology, Texas Southern University, Houston, Texas 77004.

Several new species of the antagonistic **Streptomyces** have been isolated from the soil of tropical countries (India and Nigeria). These newly isolated species differ from the already known species of **Streptomyces** on the basis of their sugar fermentation. Out of the twenty active strains isolated ten of them show broad spectrum antibacterial activity on **Mycobacterium tuberculosis, Mycobacterium kansisii, Salmonella paratyphi, Bacillus subtilis, Escherechia coli,** and **Staphylecoccus aureus.**

Some workers have successfully used electron microscopy as an important aid in the classification of the **Streptomyces.** This method utilized the surface cenfiguration of the spores which is a remarkable and constant species characteristics. Thus making electron microscopy a reliable and useful taxonomic aid. In general surface architecture or configuration of the **Streptomyces** species is classified into five distinct groups of rough and smooth. The rough surfaced spores are in addition catagorized into those that are spiny or hairy or warty. Surface configuration plus color of the aerial mass and sugar fermentation will be used to set up the taxonomic species identification of the newly isolated strains.

We will present electron micrographs of a number of species of **Streptomyces.** We will in addition present our conclusion on the taxonomy of our own newly isolated species based on electron micrograph studies, aerial mass color and sugar fermentation.

LIGHT AND SCANNING ELECTRON MICROSCOPIC OBSERVATIONS ON TWO SPECIES OF THE GENUS Stephanodiscus (BACILLARIOPHYTA). Edward Theriot, Department of Marine Sciences, and Russell L. Chapman, Department of Botany, Louisiana State University, Baton Rouge, Louisiana, 70803.

In samples taken from a bayou in the Atchafalaya River Basin, two diatoms of the genus **Stephanodiscus** which are easily confused with other previously described species were encountered regularly. Light and scanning electron microscopic observations were made to determine specific affinities. It was concluded that these two diatoms may be new to the literature and may deserve specific ranking.

Both taxa closely resemble other previously described species of **Stephanodiscus** and at the light microscopic level are virtually indistinguishable from their cogeners. The taxonomy of the subjects of this paper is based on ultrastructural details.

ANALOGUES SUBSTITUTE FOR NATURALLY OCCURRING PRE-SILICA MONOMERS IN Urtica pillulifera STINGING CELL WALLS. Arthur E. Sowers and E.L.

Thurston, Electron Microscopy Center and Department of Biology, Texas A&M University, College Station, Texas 77843.

Silicification in biological systems results from the polymerization of silicic acid ($Si(OH)_4$) monomers. Silicification in the cell wall of the stinging cell of **Urtica pillulifera** (common name: Stinging Nettle) results in two types of silica deposit. At the ultrastructure level, they have the following appearance: 1) roughly spherical electron dense particles (called Silica Bodies) independently distributed and having a spongy texture, and 2) a solid matrix appearing to be made of fused Silica Bodies. The spherical particle type of deposit, in turn, has two classes of characteristics (a) high electron density, low range of diameters, and low variability in shape, and b) low electron density, high range of diameters, and high variability in shape. The mechanism of silica deposition in this system and how it is regulated is unknown. To learn more about the silica deposition process, plants were grown in hydroponic solutions containing, in addition to nutrients, various silicic acid analogues in the hopes of disturbing in some way the deposition process. We previously reported (EMSA Proceedings, 1976, p. 38-39) that the analogues germanic acid ($Ge(OH)_4$), trimethyl silicic acid ($Si(OH)(CH_3)_3$), and dimethyl silicic acid ($Si(OH)_2(CH_3)_2$) do not substitute for silicic acid. New experiments show that if silicic acid is added along with dimethyl silicic acid, then the shape of the one class of spherical particle is replaced with rod-like branching structures. Adding methyl silicic acid ($Si(OH)_3(CH_3)$) or ethyl silicic acid ($Si(OH)_3(C_2H_5)$) to the hydroponic solution does result in stinging cell walls with electron dense analogue Silica Bodies similar to those found in normal plants. Ethyl silicic acid produces Silica Bodies which are nearly normal in diameter, but less electron dense and coarser in spongy texture than in normal Silica Bodies. Methyl silicic acid produces Silica Bodies which are much smaller in diameter and more numerous than normal.

ULTRASTRUCTURAL CHANGES IN THE THYMUS OF PRE-LEUKEMIC MICE AFTER THE ADMINISTRATION OF THE LEUKEMOGEN, 7, 12-DIMETHLBENZ (ALPHA) ANTHRACENE. Donella J. Wilson and Dr. John J. Session, Department of Biology Texas Southern University, 3201 Wheeler, Houston, Texas 77004

Mice of the ICR strain were administered 160 micrograms of 7, 12-Dimethylbenz (alpha) anthracene (DMBA) subcutaneously within 48 hours after birth. Seventy-six per cent (76%) of these mice developed thymus lymphosarcomas when allowed to survive one-hundred fifty (150) days after treatment. When the DMBA treated mice were sacrificed at various time intervals after injections (12, 55, and 100 days) a high incidence of karyological and immunological abnormalities were observed in samples. Ultrastructural changes were observed in sections obtained from the thymuses of DMBA treated mice and compared with those of the sham treated controls. How such ultrastructural changes might be involved in neoplastic transormation will be discussed.

MORPHOLOGICAL STUDIES ON THE ENUCLEATION OF COLCHICINE-TREATED MAMMALIAN CELLS. Jerry W. Shay, Department of Cell Biology, The University of Texas Health Science Center at Dallas, 5323 Harry Hnes Boulevard, Dallas, Texas, 75235.

Treatment of mammalian cells that are growing in monolayer culture with 2.0 μg/ml of colchicine or podophyllotoxin for 36-48 hours produces fragmentation of the nucleus, a process termed micronucleation. If these treated cells are then centrifuged in medium containing 10 μg/ml of cytochalasin B, the individual karyomeres are removed in a single strand. Once removed, each karyomere and its associated cytoplasm is called a microkaryoplast (J. Ultrastruct. Res., Jan., 1977, Shay and Clark). Each microkaryoplast contains a small amount of decondensed chromatin surrounded by a nuclear envelope, and a small amount of cytoplasm containing ribosomes and mitochondria enclosed by an intact plasma membrane. Microkaryoplasts do not usually survive in isolation longer than 24 hours. However, these microkaryoplasts are uniquely packaged such that for a short while one can fuse them to other whole cells using PEG (polyethyleneglycol) or inactivated Sendai virus, resulting in their subsequent integration and viability. It is also possible to produce cell fragments containing reduced genetic complement by partial enucleation, and such a methodology may provide useful information on nuclear-cytoplasmic interactions. Experiments helping to elucidate fundamental mechanisms of cell transformation utilizing these techniques will be discussed.

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EM COMPARISON OF LYTIC AND PERSISTENT MEASLES VIRUS INFECTION IN HEp-2 CELLS. S.E. Miller, L.L. Wright, E.C. Hayes and H.J. Zweerink. Microbiology and Immunology Department, Duke Medical Center, Durham, North Carolina 27710.

A persistent infection of human epithelial cells was established with the Edmonston strain of measles virus as a model system for studying chronic central nervous system diseases, e.g., subacute sclerosing panencephalitis. Our biochemical studies have shown that there is a host restriction of the virus since parental virus from lytically infected (LI) cells plaques on both VERO and HEp-2 cells, while virus from persistenly infected (PI) cells plaques only on VERO cells. Comparison of macromolecular species shows some differences in viral glycoproteins and RNA's from PI and LI cells. The PI virus is temperature sensitive and appears to contain defective interfering particles. By fluorescence microscopy, all PI cells appear to contain viral antigen. By transmission electron microscopy, few differences can be discerned between viral nucleocapsids or surface antigens of PI or LI cells. Light and scanning electron microscopy show similar morphology of PI or LI cells but differences in the number and size of giant cells. Antiserum against purified viral components has now been prepared and will be used in immunoelectron microscopy.

STRUCTURES THAT RESEMBLE DICTYOSOMES ARE PRESENT IN GUINEA PIG SPERMATOCYTES. Hilton H. Mollenhauer and D. James Moore, Veterinary Toxicology and Entomology Research Laboratory, ARS, USDA, P.O. Drawer GE, College Station, Texas 77840 and Departments of Biological Sciences and Medicinal Chemistry, Purdue University, Lafayette, Indiana 47907.

Structures resembling dictyosomes are present in guinea pig spermatocytes but absent from spermatogonia and from spermatids in early and middle stages of development. The dictyosome-like-structures (DLS) are composed of stacks of 2-17 saccules. Most saccules are about 200 A in thickness (membranes and lumena) and 0.4 μ in diameter. The saccules are usually separated from one another by about 120 A. Bridging elements cross the intersaccular space and function to hold the saccules together. The membranes of the DLS are tripartite in appearance but are not as intensely stained as the membranes of the endoplasmic reticulum or of the Golgi apparatus. Also, the inner, or luminal, leaflet of the trilamellar membrane is often discontinuous or globular in appearance. The DLS are different

from other cellular components and may possibly be considered as a new organelle unique to certain stages of spermatogenesis.

ULTRASTRUCTURE OF CONIDIAL FORMATION IN Entomophthora floridana. G.R. Carner, Dept. of Entomology and J.S. Hudson, Electron Microscope Facility, Clemson University, Clemson, S.C. 29631

E. floridana, a fungal pathogen of the 2-spotted spider mite developes as rod-shaped hyphal bodies in the hemocoel of its host. Shortly after the host dies, the fungus forms resistant hyphal bodies immediately below the cuticle of the mite. These hyphal bodies remain inactive but viable as long as humidity remains low. When the humidity is raised to 100%, conidiophores and conidia are produced within 2 hours. Each hyphal body produces a single conidiophore which begins as a small protuberance at the apex of the hyphal body and pushes up through the cuticle. A single conidium is formed at the tip of each conidiophore.

When each conidium reaches its maximum size, a septum is formed and separation between the spore and the conidium occurs at this point. Fine structure of this conidial formation was studied using scanning and transmission electron microscope techniques.

MORPHOLOGY OF A NAPHTHALENE-INDUCED BRONCHIOLAR LESION. Harvey Bank, David Mahvi and Russell Harley, Department of Pathology, S.C. Medical University of S.C., 80 Barre Street, Charleston, South Carolina 29401.

Nonciliated bronchiolar epithelial (Clara) cells are selectively damaged by interperitoneal administration of naphthalene. We examined these changes using light microscopy, transmission electron microscopy and scanning electron microscopy. This bronchiolar lesion may be a useful model for studying the physiology of the bronchioles as the changes in both the Clara cells and ciliated cells are transitory with the recovery period proportional to the dose. This lesion is characterized by a dilation of the Clara cells with a loss of apical projections followed by an exfoliation of Clara cells from large areas of the bronchioles. Rapidly following Clara cell loss, abnormalities appear on the surface of the ciliated cells. Upon regeneration of the Clara cells the ciliated cells gradually return to their normal appearance. This indicates that the Clara cell secretions directly affect the physiological state of the surrounding ciliated cells. We interpret these abnormalities as resulting from the increased viscosity of secretions in contact with the ciliated cells.

RESPONSES OF THE INTESTINES TO TOTAL INTRAVENOUS NUTRITION. Ivan L. Cameron and Thomas B. Pool, Department of Anatomy, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284

Total intravenous feeding is finding wide clinical application. Because it is difficult to do controlled studies on humans we have turned to studies on i.v. fed rats to find out how the intestines adapt to the absence of intralumenal food. Our earlier work showed that one week of i.v. feeding reduced the weight of the small and large intestine by 25 to 40 percent, respectively. We have now looked at the morphology and cell proliferation activity of the intestines of these rats. Scanning electron microscopy and light microscopy reveal the leaf-shaped villi of the ileum to be thinner and packed more closely together in the i.v. fed rats. The number of goblet cells per unit surface area is increased in both the ileum and colon of these i.v. fed rats. The intestinal folds and crypts are also packed more closely together in the colon.

The rats were injected with tritiated thymidine (lμci per gm body weight) one hour before being killed. Portions of ileum and colon were used for 1) radioautography, for 2) analysis of the tissue DNA content and for 3), specific activity of the DNA. The DNA content of ileum and colon is decreased 3 fold while the specific activity of DNA is increased more than 4 fold in the ileum and colon of the i.v. fed rats. The number of labeled cell nuclei per ileum crypt is significantly decreased from 23 to 16. The intestines adapt to i.v. feeding by decreasing weight and DNA content which is accompanied by a reduction of cell proliferation activity per crypt. Because the amount of DNA per cell is known to be constant we conclude that the overall cellularity of the intestines decreases about 3 fold in the i.v. fed rats and that cell proliferation in the crypts, although significantly decreased, is still supporting an active epithelial renewal process.

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EFFECTS OF ISOPROTERENOL ON PORCINE PAROTID GLANDS. Jerry L. Boshell, Dept. Oral Biology — Anatomy, Medical College of Georgia, Augusta, Georgia, 30901.

The purpose of this investigation was to determine the effects of chronic administration of isoproterenol (ISO) on pig parotid glands. A comparison of parotid glands of adult, isoproterenol treated, and control pigs was made.

Six pigs, each eight weeks of age at the beginning of the experiment, were used. Four animals were injected daily with ISO for eight days, while the two control pigs were injected at corresponding times with normal saline. The pigs were sacrificed on the tenth day and the parotid gland tissue was processed for histochemistry and electron microscopy.

A previous study by this investigator has shown that adult parotid glands have many unusual features. Acini stain little with periodic acid-Schiff (PAS) and not at all with alcian blue at pH 2.6 or 0.5 which indicates a paucity of neutral mucins and a lack of sialo-or sulfomucins. Histologically, acinar cells have vacuolizations which correspond with large electron lucent secretory granules seen with the electron microscope. Secretory granules also contain electron dense bodies and lipid droplets. Pig parotid glands differ histochemically and ultrastructurally from typical serous cells and should be classified special serous.

In parotid glands of pigs chronically treated with ISO, acinar cells contain more PAS-positive material than control or adult animals. Ultrastructurally, the PAS-positive substance corresponds with large secretory granules filled with flocculent material. Ribosomes and rough endoplasmic reticulum (RER) are increased in comparison to control or adult animals.

In conclusion, ISO stimulates production of large granules which contain PAS-positive, flocculent material and increases the quantity of ribosomes and RER.

CYTOLOGIC PATHOLOGY IN AN ANIMAL MODEL OF HEREDITARY MOTOR NEURON DISEASE. John F. Munnell and Linda C. Cork, Department of Anatomy and Radiology, College of Veterinary Medicine, University of Georgia, Athens 30602 and Department of Pathology, School of Medicine, Johns Hopkins University, Baltimore, Maryland 21200.

Infantile spinal muscular atrophy, in its various forms, and amyotrophic lateral sclerosis are examples of human motor neuron diseases which are primarily characterized by loss of motor neurons and subsequent denervation atrophy of muscle. The neuronal loss has been termed an abiotrophy suggesting the cause of the degeneration to be a defect of vital endurance. The pathogenetic mechanisms leading to this degeneration are unknown. Work has been limited by the lack of an animal model closely resembling the human disease. Such a model of motor

neuron disease has recently been identified in a family of brittany Spaniel Dogs. The cytopathology of affected neurons and muscle of a 9 month old male pup from this family is the subject of this presentation. The dog was presented exhibiting progressive paresis and muscular atrophy. Neurons of the ventral horn of the spinal cord and certain cranial nerve nuclei were markedly chromatolytic. Large boutons packed with synaptic vesicles were noted in contact with the neuronal cell soma. Increased numbers of inclusions resembling lipofuscin were scattered in the neuronal cytoplasm. Ballooned axons containing disoriented neurofibrillar material were numerous. Purkinje cells of the cerebellar cortex were also affected. Many multilamellar membranous arrays were seen in the soma and more numerously in the dendrites. Muscle changes closely resembled those of the neurogenic atrophy in human motor neuron disease. Such observations will be discussed in reference to various theories as to the mechanisms involved in the abiotrophy.

SCANNING ELECTRON MICROSCOPY OF HUMAN SEMINIFEROUS TUBULES. Fannie E. Smith, J. Franklin Bailey, and Jerry D. Berlin, Department of Biological Sciences, Texas Tech University, Lubbock, Texas 79408

The three-dimensional relationship between germinal and Sertoli cells in the seminiferous epithelium is frequently difficult to visualize from transmission electron micrographs. However, the arrangement and interaction of these cells can be seen in whole mounts of normal human seminiferous tubules employing scanning electron microscopy.

Within the tubules, the ramdom arrangement of the cellular associations in the epithelium is exemplified by the discontinuity of advanced spermatids in the areas of tubular cross sections. Young spermatids appear as rounded cells connected by cytoplastic bridges. In early stage of spermatid development, the head is bured in the Sertoli cell cytoplasm and only the tails are seen projecting into the lumen. As spermiogenesis progresses, more of the head and neckpiece protrude from the Sertoli cell cytoplasm. Sertoli cells show extensive cytoplasmic overlapping and areas of fenestrated cytoplasm, similar to the morphology of rat Sertoli cells grown **in vitro.**

EFFECTS OF A RADIOPAQUE CONTRAST AGENT ON THE BLOOD-BRAIN BARRIER. Gregory T. Kitten, Herbert Janssen, Irene Bradley, Shannon Halloway and Robert L. Casady, Departments of Anatomy and Physiology, Texas Tech University School of Medicine, Lubbock, Texas 79409.

Ventriculo-cisternal perfusions with artificial cerebrospinal fluid (CSF) were conducted on thirty-one English spotted rabbits. A sample of effluent cerebrospinal fluid (CSF) was collected over a 3-minute period, followed by an injection of 10% Evans Blue (1 cc/kg) via a femoral vein cannula. Following a 1-minute interval, allowing for circulation and distribution of the dye, a second 3-minute sample of CSF was taken. At this point 0.25 cc/kg of a radiopaque compound (Hypaque 50, Winthrop Laboratories) was injected into the carotid cannula for pasage directly to the left side of the brain. Consecutive 3-minute samples of CSF were then collected for 24 minutes (8 samples); another samples of CSF were then collected for 24 minutes (8 samples; another sample was taken from 32-35 minutes, and a final sample was taken from 43-46 minutes. The samples were analyzed for concentration of Evans Blue using a Beckman Acta C III Spectrophotometer. The presence of Evans Blue in the samples of CSF was used as evidence for a contrast agency-induced alteration of the blood-brain barrier and the brain-CSF barrier.

Controls that were conducted include (1) 4% saline instead of Hypaque 50 to control for the osmolarity of Hypaque 50 (approximately 1270m0s/1); (2) 0.9% saline (isotonic with respect to the blood); and (3) no injection, to control for injection pressure and the temporary replacement of blood within the vessels.

Hypaque 50 injections resulted in significantly higher levels of CSF Evans Blue concentration in all but the first and last samples when compared to both the 0.9% saline injection and the "no injections". Hypaque 50 levels were also significantly higher than 4% saline in all but the first and the last three samples. The 3 groups of controls were all approximately the same with no significant differences found between any samples collected over the same time interval.

DEGENERATIVE CHANGES IN THE AREA POSTREMA OF THE CYNOMOLGUS MONKEY (M. FASCICULARIS) AND SQUIRREL MONKEY (SAIMIRI SCIUREUS) FOLLOWING SYSTEMIC INJECTION OF 6-HYDROXYDOPAMINE. K.R. Brizzee, P. Klara and C. Palazzo, Delat Regional Primate Research Center and Department of Anatomy, Tulane Medical School, New Orleans, La.

The neurotoxic agent 6-hydroxydopamine (60HDA), was administered via the saphenous vein in two cynomolgus and two squirrel monkeys on two successive days. On the first day, the cynomolgus monkeys received 150 mg/kg and the squirrel monkeys recieved 50 mb/kg. On the second day, the cynomolgus monkeys received 200 mg/kg and the squirrel monkeys received 100 mg/kg. All doses were calculated as the free base. One animal of each species was sacrificed by intracardiac perfusion with mixed aldehydes two hours after the last injection; the second animal of each species was sacrificed in the same manner 24 hours after the last injection. Two additional animals of each species received I.V. injections of the diluent (saline (0.85%) ascorbic acid (0.1%)) on the same time schedule as the experimental animals.

Evidence of dystrophic alterations and degenerative changes were observed throughout the Area Postrema (AP). No evidence of regional selectivity within a particular region of the AP could be discerned. Some of these bodies exhibited an irregular whirled or lamellated structure and many appeared collapsed. In other areas, masses of collapsed membranes were seen; some enclosing a moderately dense homogenous substance and others, definite clear spaces. The amounts of glycogen granules in neuroglia processes of the treated animals appeared much greater than in those of control animals. Also, phagocytic cells containing a considerable number of lareg homogenous dense bodies and other cellular debris were observed in the perivascular spaces. the suggestion of increased intercellular space was also present and may represent edema. While the morphology of the cynomolgus monkey AP resembled that already reported for the squirrel monkey, the reaction of the AP to systemically administered 60HDA appears to be somewhat less substantial than that seen in the rat. The appearance of 60HDA degeneration, however, is demonstrable in both species of monkeys and in both dosage regimens. Discussion will include a review of primate AP morphology as well as consideration of the involvement of adrenergic elemtns in AP function. Supported by NASA NCA_2-0R800 NIH RR00164-13

ULTRASTRUCTURE OF MUSCLE AND NEUROMUSCULAR INTERRELATIONSHIPS IN TRYPANORHYNCHID TAPEWORMS AS A MODEL SYSTEM. Richard P. Wyeth and D.W. Fredericksen, Department of Biology Tulane University, New Orleans, Louisiana 70118.

Trypanorhynch tapeworms are unique in that they possess three distinct morphological types of muscle. Their subtegumental muscle is typical of that found in other flat worms, but in addition to this muscle type tow other forms have been observed

that are not typically found in platyhelminths. Once such muscle type is organized into four discrete bulbs which effect hydrostatic eversion of independently operated tentacles. Untrastructurally, tentacle blub muscle is more sophisticated than any other muscle type that has been described as occurring in platyhelminths. A second district muscle type is located anteriorly in the head region or scolex, and is apparently involved with its operation. whereas this muscle type does not show striations as seen in tentacle blub muscle, it does demonstrate associations with unusually well developed mitochondria. Because of a well developed and easily discerned nervous system associated with these unique muscle types, tapeworms of this type may be a good candidate for studying neuromuscular interrelationships in platyhelminths.

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LIGHT AND SCANNING ELECTRON MICROSCOPY OF Diplodia gossypina ON COTTON BOLL. M.G. Sachdev and J.P. Snow, Departmen of Plant Pathology, Louisiana State University, Baton, Rouge, Louisiana, 70803.

Light and scanning electron microscopic observations of cotton bolls (Gossypium hirsutum) revealed a uniform coating of wax deposited on the boll tissue except on the stomata and the multicellular epidermal hairs. Amount of the wax on the boll surface was directly related to the boll age. Young bolls had lesser amounts of wax on the surface compared to the older bolls. Diplodia gossypina infection the boll directly through the wax-covered epidermal layer, through unwaxed multicellular epidermal hairs and through open stomata. No infection peg, appresorium or haustoria were observed.

ULTRASTRUCTURAL ABNORMALITIES OF CILIA IN MALE STERILE MUTANT MICE. David B. Chandler, Department of Zoology, University of Georgia, Athens, Georgia, 30602.

Recently, Afzelius (Science 193:317-319, 1976) reported that sperm flagella and epithelial cilia of infertile men lacked dynein arms. Because male mice homozygous for the recessive, pleiotropic, mutation hydrocephalic-polydactyl (hpy) are sterile due to the lack of sperm flagella, I have been investigating ciliary structure in these mice to see if this inborn error parallels the condition found by Afzelius in men.

Tracheae of mutant and wild type littermates were exposed and the epithelia fixed by irrigation with 2% glutaraldehyde in 0.1M cacodylate buffer (pH 7.2), postfixed in 2% osmium tetroxide in the same buffer, washed in buffer, dehydrated in an ethanol series followed by propylene oxide and infiltrated and embedded in Epon-Araldite. Some samples were stained en-block with 3.5% uranyl acetate in 70% alcohol (during dehydration). Silver sections were stained with Reynolds' lead citrate and examined in an electron microscope at 80 KV.

Two obvious abnormalities of ciliary ultrastructure were noticed. First, only the outer members of each pair of dynein arms could be recognized and in many cases this arm appeared shorter than normal. Second, several ciliary cross sections showed the presence of an extra pair of central tubules (9+4). Experiments to determine whether these cilia are motile are in progress.

MULTIVESICULAR BODY FORMATION IN TRYPSINIZED BHK21 CELLS. Randy L Moses* and John J. Biesele, Department of Zoology, University of Texas, Austin, Texas 78712.

Multivesicular bodies were found to form on the surface of BHK21 cells treated with .25% trypsin. They were present after 30 seconds of trypsinization, and their frequency increased until 10 minutes after treatment. Genesis of these structures began with an accumulation of vesicles under the plasma membrane and a loss of cortical microfilaments in the area of vesicular accumulations. The plasma membrane in this area then became distended, and the vesicles moved into and eventually became sequestered in this outpocketing of loosened membrane. The base of the distention then became contricted giving rise to the final morphology of the organelle: a collection of 5 to 50, 50 x 200 nm vesicles enclosed by a plasma membrane and attached to the cell proper by a microvillus-like stalk. Multivesicular structures were exceptional in their lack of cortical filaments and the close approach of the vesicular and plasma membranes without fusion. At least some of the vesicular membranes exhibited a typical trilayered structure in cross-section. Multivesicular bodies could be a means of externalizing excess internal membrane or enzymes as a consequence of trypsinization.

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INTESTINAL CALCIUM LOCALIZATION IN NORMAL, RACHITIC AND VITAMIN D-TREATED CHICKS. Mary C. Schunder* and J.L. Matthews, Department of Microscopic Anatomy, Baylor College of Denistry, Dallas, Texas. *Present address, Department of Anatomy, Texas College of Osteopathic Medicine, Denton, Texas, 76203.

Transmission electron microscopy was used to study calcium localization with glutaraldehyde-potassium pyroantimonate technique in the duodenal mucosa of normal, rachitic, Vitamin D_3-treated and 1,25-dihydroxycholecalciferol (1,25-DHCC)-treated chicks. The anti-monate precipitate was further characterized by microincineration, revealing a high mineral content for the antimonate precipitate with an organiz component associated with the intracellular calcium localizations. Chelation was also done, using EGTA, which is highly specific for calcium. Chelation of the microvillar and plasma membrane associated calcium deposits was accomplished, but the intracellular calcium deposits did not chelate, possibly due to binding to the organic component noted on microincineration.

The calcium localizations noted in this study correlate well with known physiological data for intestinal response to Vitamin D therapy. There was a paucity of intracellular calcium localizations in rachitic animals; initial appearance of calcium precipitate in the Golgi profiles and vesicles is at three hours post-treatment with 1,25-DHCC, approximating saturation of the chromatin binding sites for this metabolite, with an increase in the amount of calcium precipitate at the nine to twelve hour period, corresponding to the known lag time for maximal calcium transport resonse. The data for the Vitamin D_3-treated animals was similar to that for 1,25-DHCC with the exception of a longer lag period.

POSITIONAL RELATIONSHIPS OF PERI PHERAL NERVOUS SYSTEM MYELIN PROTEINS. Ronald W. Gruener and Richard G. Peterson, Department of Neurobiology and Anatomy, University of Texas Medical School, P.O. Box 20708, Houston, Texas 77025.

By combining biochemical and morphological techniques, methods have evolved which permit effective solubilization and/or localization of PNS myelin proteins.

Utilization of a Triton X-100 ammonium acetate system has lead to the solubilization and localization of the basic myelin proteins P1 and P2. Electrophoretic studies revealed that the P1 and P2 components of PNS myelin were selectively removed.

Parallel high resolution electron microscopy showed consistent splitting within the main period band, while the intraperiod band remained unchanged. Control specimens soaked only in the ammonium acetate exhibited normal morphology. This data suggests that the main period band is the morphologically normal location of the basic myelin proteins.

Lactoperoxidase has also been used to study localization of PNS myelin proteins. In concurrent studies, horseradish peroxidace was used to demonstrate the availability of the intraperiod band to proteins. Lactoperoxidase iodination revealed that most of the ^{125} was incorporated into the major myelin protein P0, and the X protein. Iodination of disrupted myelin resulted in the labeling of all the proteins. When the iodination reaction was carried out without the lactoperoxidase, there was no labeling of proteins. Material exposed to horseradish peroxidase shows intense density in intraperiod band area, including many area where the surface of the membranes are completely exposed. The control material, which was treated identically except that horseradish peroxidase was excluded, exhibited normal density in the intraperiod areas.

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AN IN VITRO MODEL TO STUDY ENDOSTEAL BONE CELLS: A MORPHOLOGICAL AND PHYSIOLOGICAL STUDY. G. W. Miller and J. L. Matthews, Dept. Cell Biology, Univ. Texas Health Science Center, 5323 Harry Hines Blvd., Dallas, Texas, Dept. Pathology, Baylor Univ. Med. Center, 3500 Gaston Ave., Dallas, Texas.

The purpose of this investigation was two fold: (1) to establish the validity of a new **in vitro**incubation system for studying endosteal lining cells, (2) to describe the normal **in vitro**surface morphology of endosteal lining cells using scanning electron microscopy (SEM). This layer of bone cells and their communication with more deeply buried osteocytes has been theorized to form a "functional syncytium" in bone separating bone extracellular fluid from general extracellular fluid. It is hypothesized that such a membrance could function to regulate cell-to-cell communication as well as ion fluxes in bone and ultimately control calcium and phosphate homeostasis. The SEM observations revealred the normal surface of the **in vitro** cells to be comparable to that of normal **in vivo** endosteal cells for periods of up to six hours. During the first six hours the cell of this "pavement epithelium" demonstrated microvilli, blebs, some small surface depressions and filopodia which appeared to attach one cell with another. After six hours there was a noticable decrease in surface activity and an overall change in surface morphology. These changes in surface morphology after six hours corresponded with data acquired from biochemical analysis, i.e. O2consumption, CO2combing power, alkaline phosphatase activity and calcium and phosphate concentrations.

SYNAPTOGENESIS IN THE RAT VENTROBASAL COMPLEX. M. A. Matthews, Department of Anatomy, LSU Medical Center, New Orleans, Louisiana 70119.

Electron microscopy has been employed to analyze morphological alterations of components of synaptic complexes of the developing ventrobasal (VB) nuclear complex of the rat at 7, 12, 15, 20 and 60 days postnatal (dpn). Dendrites of immature VB neurons seen at 7 dpn are characterized by a thick, irregular shaft with large bulbous expansions from which filopodia are elaborated. Synaptic development is minimal at this stage, but occasional boutons are found in association with both the shaft and filopodia of dendrites. By 12 dpn dendritic maturation is considerably advanced and specialized post-synaptic structures begin to appear as synaptic populations increase and diversify. Synaptic boutons form both simple (single junction) and compound (multiple junctions) complexes with dendritic elements of varying size. Lemniscal afferents can be distinguished during this period on the basis of a large pre-synaptic element forming multiple juctional specializations with one or more dendritic spinous protrusions and partially ensheathed with a glial lamella. With further development (15 dpn and older) terminals of lemniscal afferents increase in size, complexity and numbers of junctional contacts with the post-synaptic component. Some populations of smaller boutons increase further in density in temporal association with the maturation of intrinsic circuitry and descending cortical and subcortical afferents. Based upon the appositional relations and spatial arrangement of the pre- and post-synaptic members, synpatic complexes were classified and counted to analyze developmental alterations in populations density, and these date, together with a qualitative analysis of synaptogenesis in the developing VB, are briefly discussed in relation to somatosensory impulse propagation and local integration.

SCHISTOSOMA MANSONI: BODY SURFACE FINE STRUCTURE AND FUNCTIONAL CORRELATES. Francis M. Gress and Richard D. Lumsden, Department of Biology, Tulane University, New Orleans, Louisiana 70118.

Growing appreciation of the importance of surface phenomena in biological systems and the dependence of these activities upon the molecular architecture of the cell surface have stimulated inquiries into the chemical, structural, and functional dynamics of the cell surface membrane and the cell periphery. The importance of surface phenomena to the biology of parasites and to the general ecology of the host-parasite relationship is unequivocal; specializations vital to the parasitic life style are often expressed and effect their functions at the body surface.

For many parasites, the surface membrane defines a digestive and absorptive interface across which essential nutrients of host origin must be assimilated. Frequently it is against this surface that host defense mechanisms are directed. As the body wall of many parasitic platyhelminths comprises a membrane limited cytoplasmic syncytium, attempts to correlate surface functions and chemical properties with recognizable structures contained therein have contributed substantially to the understanding of parasitism from the perspective of cell biology. In this regard the mammalian schistosomes provide a particularly interesting system for study. The different life cycle stages of **Schistosoma mansoni** occur in blood plasma (schistosomulum and adult), fresh water (miracidium and ceracaria), and tissue fluids of the snail intermediate host (mother and daughter sporocysts). Examples are presented which suggest that changes in body surface fine structure and topochemical properties constitute important adaptations permitting this variability in life style.

AREA POSTREMA EPENDYMA: MODIFIED STRUCTURE AND FUNCTIONAL IMPLICATIONS. P.M. Klara and K.R. Brizzee, Tulane Anatomy Department, 1430 Tulane Avanue, New Orleans, LA 70112.

The ependyma covering the area postrema (AP) in the IV ventricle is morphologically quite different from the surrounding mural ependyma. In contrast to mural ependyma, AP ependyma lack kinocilia, possess numerous microvilli on the ventrucular surface, demonstrate intercellular interdigitation, and share both ventrucular and perivascular surface.

While these morphological features are not present in mural ependyma, they do appear in modified ependyma in ventricular areas of known neurosecretory activity. Horstmann identified ependyma with these characterictics as tanycytes. Recently con-

ducted ultrastructural studies in several mammalian species demonstrate that modified AP ependyma consistently share both morphological and cytochemical similarities with tanycytes. Since the function of the AP has not been clearly demonstrated, the morphological and cytochemical evidence collected in this study are of value in determining the plausibility of current hypothetical functions of the AP.

The most conclusively proven function of the AP concerns chemorecption for the emetic reflex. Other well supported functions include a receptor site for angiotensin binding and blood flow control as well as a center for synchronization of slow wave sleep via a serotonergic mechanism. With the recent display of a periventricular serotonergic plexus and the absence of a blood brain barrier for the AP, the demonstration of tanycytic ependyma on the AP take on added significance. It has been demonstrated that median eminence tanycytes contain serotonin and are integrally related to median eminence functions. Our study demonstrated the morphological and cytochemical similarities between AP ependyma and tanycytes and thereby opens the way to future studies concerning AP ependyma and serotonergic mechanism.

DIAGNOSTIC ELECTRON MICROSCOPY. E. O. Huffman, M.D., J. Coover, M.T. and K. Armstrong. Laboratory Service, V.A. Hospital and Pathology, L.S.U. Medical Center, New Orleans, LA 70146.

The invaluable help of the electron microscope in resolving selected diagnostic problems in surgical pathology is very well known by the few pathologists using this equipment as a diagnostic tool. Unfortunately, there is limited knowledge among some other pathologists, potential providers of these selected material, on the uses and limitations of this method.

The purpose of this paper is to demonstrate how EM studies have helped to change light microscopic diagnosis and reach an accurate final diagnosis in 12 selected cases using not only optimally processes tissues but also tissues recovered from formalinized and paraffin embedded material. LM, FM and EM pictures with concise descriptions will accompany each of the following cases.

Light Microscopic Diagnosis, Electron Microscopic Diagnosis. (1) Kidney: Hyperacute rejection, Sickle cell crisis (2) Abdominal skin, Metastasis Ca of stomach, Lymphoma (3) Kidney: Minimal change disease, Amyloidosis. (4) Vagina: Undifferentiated Carcinoma, Melanoma. (5) Lung: Sarcoma, Spindle cell carcinoma. (6) Kidney: Normal, Early membranous G.N. (7) Lung: Undifferentiated tumor, Adenocarcinoma. (8) Lymph node: Hyperplasia, Metatasis Ca prostate. (9) Kidney: Normal, Minimal change disease. (10) Liver: Non-specific hepatitis, Chronic persistent hepatitis. (11) Small intestine: Chronic inflammation, Whipple's disease. (12) Abdominal mass: Liposarcoma, Rhabdomyosarcoma.

Regional News

TEMPLE: Scott & White Clinic, Department of Microbiology.

Recent Publications: Giovanella, B.C., Stehlin, J.S., Santamaria, C., Yim, S.O., Morgan, A.C., Williams, JR., L.J., Leibovitz, A., Fialkow, P.J., and Mumford, D.M.: Human Neoplastic and normal cells in tissue culture. Il Cell lines derived from malignant melanomas and normal melanocytes. JNCI, 56:6, 1131-42, June 1976.

Lectures: Dr. Wm. B. McCombs recently presented a series of lectures in Microbiology and Immunology at the Temple Veterans Administration Hospital School of Allied Health Sciences.

Meetings: Albert Leibovitz, Kenneth Mazur, and Debbie Mabry attended the December meeting of the Gulf Coast Area Branch of the Tissue Culture Association in Houston.

Department of Pathology: John Frank Greene, Jr., M.D. recently joined the staff of Scott & White's Department of Surgical Pathology, Section on Anatomical Pathology. Dr. Greene earned his M.D. degree at Loma Linda University in California. He served his internship and part of his residency at Charles F. Kettering Memorial Hospital, Dayton, OH, then completed his residency at Letterman Army Medical Center, San Francisco, CA.

DALLAS: University of Texas Health Science Center at Dallas, Department of Pathology.

New Equipment (...and baby makes three.) We have a new addition to our family. In June the JEOL JEM 100-S was added to the JEM 100-C and the JSM 35 to complete our set of electron microscopes. All are doing well.

Meetings: The EMSA — MAS meeting held in Miami, Florida in August was attended by Herb Hagler, Mickey Glass, Bruce McCarty and Donna Rainey.

Dr. L.M. Buja presented a scientific exhibit at the International Academy of Pathology held in Washington, D.C. in October. The exhibit entitled "Pathophysiologic Basis for the Scintigraphic Detection and Sizing of Acute Myocardial Infarcts", is one example of the successful work being done on our X-ray analysis equipment which is now in the capable hands of Dr. Herb Hagler.

On November 5 we held an informal meeting for UTHSCD faculty and staff interested in electron microscopy. We discussed briefly the up-coming national and state meetings and then watched the film "Biological Procedures in Electron Microscopy". On Dec. 6 a film entitled "The Penetrating Eye" will be shown.

16l10.5ARLINGTON: The University of Texas at Arlington.

Lectures: Dr. Harry T. Horner Jr., Department of Botany and Plant Pathology, Iowa State University, presented a seminar entitled "The Bacterial Leaf Nodule Symbiosis — Fact and Fiction."

Publications: Butler, James K., 1976. "An Illuminator for Ultramicrotome Knife Orientation and Block Approach" Stain Techn. 51: 241-243. Stovall, Randall H., 1976. "Observations on the Micro-and Ultrastructure of the Visual Cells of Certain Snakes" J. Herpetol. 10: 269-275.

New Equipment and Faciltiies: Dr. Howard J. Arnott's laboratory has obtained an ISI M-7 Scanning Electron Microscope, a Hummer Junior Sputter Coater, and a Sum-

magraphics X-Y data table which is interfaced with a Hewlett Packard 9815A calculator and a 9871A impact printer. The latter system is being used to obtain and process stereological data from electron micrographs.

E.M. Education: Currently six students are enrolled in the graduate level Biological Electron Microscopy course and twelve students are enrolled in the Ultrastructure Interpretation Course. Students from the UT Arlington and Texas Christian University EM courses have exchanged visits as part of the laboratory work in the respective courses.

DALLAS: Southern Methodist University, Department of Biology.

New Equipment: SMU has recently installed a new Siemens 102 Electron microscope in the Fondren Science Building.

Publications: Kelsoe, G., Ubelaker, J.E. and Allison, V.F. Fine structure studies on spermatogenesis of Hymenolepis diminuta (Cestoda). Zeit. Parasitenk (in press).

McAlister, L.E., Allison, V.F., Jeter, J.R. and Nations, C. High density induction of a quiescent cell state in Physarum polycephalum. J. Cell Sci. (in press).

Sohal, R.S., Peters, P.D. and Hall, T.A. Fine structure and X-ray microanalysis of mineralized concretions in the Malpighian tubules of the housefly, Musca domestica. Tissue & Cell 8, 447-458, 1976.

Sohal, R.S., Peters, P.D. and Hall, T.A. Origin, structure, composition and age-dependence of mineralized dense bodies (concretions) in the midgut epitheium of the adult housefly, Musca domestica. Tissue & Cell (in press).

DENTON: Texas Woman's University, Department of Biology.

New Equipment: The Department of Biology has installed an AMR 1200 scanning electron microscope. We have the Denton vacuum evaporator and a Polaron critical point drying apparatus.

New Faculty: Dr. Paula Pendergrass is a new faculty member in Biology on the Denton campus. Her research interest is on the mechanism of ovalation at the ultrastructural level.

FORT WORTH: Texas Christian University.

Publications: Barcellona, Wayne and Marvin L. Merstrich, Ultrastructural integrity of mouse testicular cells separated by velocity sedimentation, Journal of Reproduction and Fertility (accepted for publication).

General News: Wayne Barcellona attended a national symposium on Target Organ Toxicity-The Gonads, sponsored by the National Institutes of Environmental Health Sciences and the Socity of Toxicology held in Nashville, Tennessee December 1-3. Ernest Couch returned to T.C.U. this fall after spending his sabbatical year at the Institute of Endocrinology at Gunma University Medical School in Maebashi, Japan. He was a long-term visiting scientis supported by the U.S.-Japan Cooperative Science Program of N.S.F. and worked with Professor K. Kurosumi and Dr. Zen Itoh. The class in electron microscopic techniques taught by Ernest Couch (6 sutdents) exchanged visits with Dr. Jim Butler's EM class at the University of Texas at Arlington in the latter part of the Fall Semester. The experience was enjoyed by all.

EL PASO: The University of Texas at El Paso, Department of Biological Sciences.

Publications: Ellzey, Joanne Tontz and Elaine Huizar. Synaptonemal complexes in antheridia of Achlya ambisexualis E 87. Archives of Microbiology. (In press.)

New Members: Ms. Elaine Huizar began work as an Electron Microscope Technician supported by MBS funds on August 1, 1976. Mr. Micufl Tobin and Ms. Joyce Carson began graduate work in the Ultrastructure Laboratory in September, 1976.

New Building: In June, 1976 we moved into a spacious new laboratory designed by Dr. Joanne Tontz Ellzey. Our facilities include two electron microscope rooms, tow negative darkrooms with Kreonite equipment, a print darkroom, photofinishing room, microtone room and tissue preparation laboratory. Visitors are welcome!

E.M. Courses: During the Fall, 1976 semester Analytical Cytology was offered for graduate students. During the Spring, 1977 semester Biological Ultrastructure Interpretation and Methods in Ultrastructure will be offered for seniors and graduate students.

Meetings and Presentations: 27th Annual AIBS Meeting, Tulane University. Ellzey, Joanne Tontz and Elaine Huizar. Fine-structural observations of gametic meiosis in Achlya. June 4, 1976.

EL PASO: The University of Texas at El Paso, Electron Microscopy Laboratory, Anatomical Pathology Service, Department of Pathology, William Beaumont Army Medical Center.

Staffing: Chief, Electron Microscopy, Bernhard E.F. Reimann, Dr. rer. nat. (ScD); Associate, Walter Daniell, M.D., MAJ, M.C.; Technician, presently unoccupied; Trainee (SER/CETA), Raul Alvarado.

Facility: The Laboratory is a self-contained entity, located in the otherwise abandoned old Hospital Area. Planning of an integration with the new Hospital Building is underway. The laboratory possesses two transmission electron microscopes: RC EMU-4 (purchased in 1967 and since this time in operation) Siemens Elmiskop 101 (purchased in 1971) Microtome: LKB Ultrotome III, Knifemaker. The laboratory is fully equipped with instruments which make an independent operation possible: Stereo and compound miscroscopes for preparation and dissection, osmometer, conductivity bridge, water purification facility (filter — deionizer — still), various thermostats, low temperature bath (-125°C), freeze-etching vacuum stand (Bendix-Balzers), 2 vacuum evaporators (Kinney and Norton with facility to evaporate pure metals on a cooled target and ionic sputtering (beam) facility). Independent photo room with automatic negative processer and scanning (LogEtronic) enlarger. Microdensitometer (Joyce-Loebl), stereo evaluation equipment, polishing equipment.

Mission: Primary mission of the laboratory is to provide histopathology support at the ultrastructure level for the Anatomical Pathology Service. In addition, locally and estramurally funded research projects are supported. The Chief, Electron Microscopy provides classes for pathology residents and staff in normal and pathological human ultrastructure.

Affiliations: The Chief, Electron Microscopy holds by special appointment a position as Associate Professor, Biology, Department of Biology, New Mexico State University. Research projects are done in affiliation with Dr. owen Weeks in biochemical microbiology. Research projects are done in affiliation with the New Mexico Institute of Mining and Engineering, Socorro, New Mexico. Dr. Eleanor Duke, Professor, Department of Biological Sciences, University of Texas at El Paso, is Co-investigator on several research projects.

Projects: (1) Ionic etching of biological membranes, development of a methodology. (2) The ultrastructure of human decidua in relation to labor. (3) Localization of thromboxanes, prostagladins, their intermediates and precursors in human fetal

membranes. (4) Collaboration in case reports and smaller projects.

COLLEGE STATION: Texas A&M

Lectures: Dr. Russell Barrnett from the Yale University Department of Anatomy delivered two seminars on ultrastructural histochemistry and cytochemistry at Texas A&M University in October. His presentations were extremely interesting and very well received.

New Equipment: The TAMU Center for Electron Microscopy has a new cold sputtering module for SEM coating.

New Staff Members: New staff members on board include Ruthie Lewis, technician I, at the TAMU EM Center and Christy Tompkins, technician I, in the EM center at the TAMU Veterniary School.

Nominations: Dr. E.L. Thurston has been nominated for Director of the Electron Microscopy Society of America.

AUSTIN: The University of Texas at Austin.

Cell Research Institute: Dr. W. Gordon Whaley attended during the summer a seminar on cell membranes and carcinogenesis sponsored by the Given Institute of Pathobiology and held is Aspen, Colorado. He also attended the 12th International Conference on Embryology held in Holland, and presented a paper on the involvement of the Golgi apparatus in cell transformation at the Fifth International Congress of Histochemistry and Cytochemistry held in Bucharest, Romania.

Presentations at the First International Congress on Cell Biology, Boston, MA: M. Dauwalder, W.G. Whaley. The Golgi appartus and multivesicular bodies in spermatogenesis. W.G. Whaley and M. Dauwalder. Differentiation of the Golgi appartus as a critical aspect of cellular differentiation. T.P. Leffingwell and W.G. Whaley. Modifications or morphology and functions of the Golgi apparatus in celles at stages of virus-induced transformation.

Department of Zoology and Cell Research Institute: J. Skipper, T.H. Hamilton and Ruben Ramirez-Mitchell. Regulation of egg yolk phosphoprotein synthesis presented at The Conference on Regulation of Egg Yolk Phosprotein Synthesis sponsored by the National Cancer Institute/National Institutes of Health and held in Washington on Sept. 13-15.

Department of Zoology: Elsie Sorensen — Ultrastructural changes in the hepatocytes of green sunfish Lepomis cyanellus (R.), exposed to solutions of sodium arsenate. Journal of Fish Biology 8: 229-240. — Toxicity and accumulation of arsenic in green sunfish, Lepomis cyannelus, exposed to arsenate in water. Accepted by Bulletin of Environmental Contamination and Toxicology. — A population study of the isopod, Armadillidium vulgare, in Northeastern Texas (with R.D. Burkett). Accepted by the Southwestern Naturalist. — Morphometric analysis of the hepatocytes from fish exposed to arsenic. Presented at the meeting of the Electron Microscopy Socity of America, Miami Beach, Florida, 9-13 August.

Department of Botany: Sidra B. Stabler from Terry Hoage's laboratory is now working as an electron microscopist under Garry T. Cole. Garry T. Cole (with M.N. Guentzel, L. Field and L.J. Berry). The localization of Vibrio cholerae in the ileum of infant mice. Accepted for the Scanning Electron Microscopy Symposia/IITRI, Chicago, 1977. — Conidium ontogeny in marine hyphomycetous fungi: Asteromyces and Zalerion. In print Marine Biology 38: 3467-3471, 1976. — Conidiogenesis in pathogenic hyphomycetes. I. Sporothrix, exophiala, Geotrichium and Microsporum. Sabouraudia 14: 81-98, 1976. — New research project: Examination of intestinal candidiasis in infant mice. Correlation with chemotheraphy based on immunodepressants.

Yasuo Kitajima, Takashi Sekiya and Yoshinori Nozawa. 1976. Freeze-fracture ultrastructural alterations induced by filipin, pimaricin, nystatin and amphotericin B in the plasma membranes of Epidermophyton, Saccharomyces and red blood cells. A proposal of models for polyene-ergosterol complex-induced membrane lesions. Biochim. Biophys. Acta. 445: 452-256.

Charles E. Martin, Kayoko Hiramitsu, Yasuo Kitajima, Yoshinori Nozawa, Lars Skriver and Guy A. Thompson, Jr. 1976. Molecular control of membrane properties during temperature acclimation. Fatty acid desaturase regulation of membrane fluidity in acclimating Tetrahymena cells. Biochemistry, in press.

Reiko Kasai, Yasuo Kitajima, Charles E. Martin, Yoshinori Nozawa, Lars Skriver and Guy A. Thompson, Jr. 1976. Molecular control of membrane properties during temperature acclimation. Membrane fluidity regulation of fatty acid desaturase action. Biochemistry, in press.

Yasuo Kitajima and Guy A. Thompson, Jr. 1977. Electron microscopic evidence that Tetrahymena strives to maintain the fluidity interrelationships of all its membranes constant in a changing environment. J. Cell Biol. in press.

SAN ANTONIO: The University of Texas, Health Science Center at San Antonio.

Grants — Weaker, F.J., "Duration of the Cycle of the Seminiferous Epithelium in the Nine-Banded Armadillo", institutional research.

Adrian E. "Cell Proliferation in Injured Nervous Tissue", nih

Articles: Hansen, J.T. 1976, "Morphometric Study of the Airtic Body Type I Cell." Experientia (in press).

Herbert, D.C., Cisneros, P.L. and Rennels, E.G. 1976 "Morphological Changes in Prolactin Cells of Male Rats after Testosterone Administration." Endocrinology (in press).

Weaker, F.J. 1976 "The Fine Structure of the Interstitial Tissue of the Testis of the Nine-Banded Armadillo." Anat. Rec. (in press).

News Briefs: Dr. Ivan L. Cameron has been appointed to a three-year term on the American Cancer Society National Institutional Reserach Grants Committee.

Dr. Rusty Pool gave a short course on tissue preparation and instrumentation for SEM in October 1976. The course was attended by over 30 faculty and staff members of the Health Science Center.

SAN ANTONIOL Southwest Research Institute

Articles: "Fatigue crack tip plastic zones in low carbon steel" D.L. Davidson, J. Lankford, T
Yokobosi and K. Soto. International Journal of Fracture 12(4) 579-585 (1976)

"Environmental Alteration of Crack Tip Dislocation Cell Structure and Mode of Growth during Fatigue Crack Propagation in Ferritic Steel" James Lankford and D.L. Davidson, International Journal of Fracture 12, 775-76 (1976)

"Microstructural Control of Fatigue Crack Growth in a Brittle, Two-Phase Polymer," James Lnkford, William J. Lankford and Marc A. Asher, Journal of Materials Science, 11, 1624-1630 (1976).

"Rotation between SEM micrographs and Electron Channeling Patterns," David L. Davison, J. Phys. E., 9, 341-343 (1976).

"Application of Electron Channeling to Materials Science," D.L. Davidson, EMSA proceedings, 1976, p. 402-403.

NEW ORLEANS: tulane Anatomy.

Abstracts Presented: Gravis, C.J., Light and electron microscopic localization of magnesium activated ATPase in epineshrine induced testicular degeneration in the Syrian

hamster. American Association of Anatomy, 89th Annual Meeting, April, 1976.

Mascorro, J.A. and R.D. Yates, A study of the distribution and morphology of abdominal paraganglia in the young rabbit, cat and dog. American Association of Anatomy, 89th Annual Meeting, April, 1976.

Palazzo, M.C., Studies on the functional and ultrastructural development of the Area Postrema. American Association of Anatomy, 89th Annual Meeting, April, 1976.

Klara, P.M., Kostrzewa, R.,. and Brizzee, K.R., Destructive action of systemically administered 6-hydroxydopamine on the rat Area Postrema. American Association of Anatomy, 89th Annual Meeting, April, 1976.

Kirby, G.S., Ultrastructural organization of microfilaments in Amoeba proteus. American Association of Anatomy, 89th Annual Meeting, April, 1976.

Miscorro, J.A., Ladd, M.W., and Yates, R.D. Rapid infiltration of biological tissues utilizing n-Hexenyl succinis anhydride (HXSA)/vinyl cyclohexene dioxide (VCD), an ultra-low viscosity embedding medium. 34th Annual EMSA Meeting, August, 1976.

Gravis, C.J., Chen, I-Li, and Yates, R.D., Alkaline phosphatase localization in testicular degeneration induced by epinephrine treatment (an invited presentation), 34th Annual EMSA Meeting, August, 1976.

Chen, I-Li, Gravis, C.J., and Yates, R.D., Fine structure of lipid droplets in Sertoli cells (an invited presentation), 34th Annual EMSA meeting, August, 1976.

Klara, R.M., and Brizzee, K.R., Comparative scanning electron microscopy of the Area Postrema in the squirrel monkey, cat and dog. Socienty for Neuroscience, 5th Annual Meeting, 1976.

Publications: Mascorro, J.A., Yates, R.D., and Chen, I-Li. A glutaraldehyde/potassium dichromate tracing method for the localization and preservation of abdominal extraadrenal chromaffin tissues. Stain Technology, 50: 391-396, 1976.

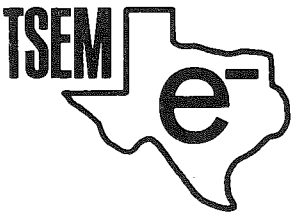
Chen, I-Li, Mascorro, J.A., and Yates, R.D. Morphology and functional considerations of the carotid body and paraganglia. Chapter 22 in Chromaffin, Entero-chromaffin and Related Cells, R.E. Coupland and T. Fujita, Editors. Elsevier Scientific Publishing Company, 1976.

Klara, P.M., Kostrezewa, R.M. and Brizzee, K.R. Destructive action of systemically administered 6-hydroxydopamine on the rat Area Postrema. Brain Research, 104: 187-192, 1976.

Area News: The following researchers represented Tulane Anatomy and presented papers at the 34th Annual EMSA Meeting in Miami Beach: Robert Yates, Peter Klara, I-Li Chen, Curtis Gravis, and Joe Mascorro. In addition, Gravis, Klara, Mascorro, and Yates served as Session Chairmen at the meeting.

The Distinguished Scientist Lecture Series at Tulane Anatomy recently hosted Dr. Burton L. Baker, Professor of Anatomy at the University of Michigan, and Dr. Robert Day Allen, Chairman of Anatomy at Dartmouth College. Dr. Baker lectured on the functional role of the pituitary pars tuberalis while Dr. Day discussed the molecular basis of amoeboid movement.

The following LSEM members served on the 1975 EMSA Program Committee: Robert D. Yates, program chairman; Joe Miller, LSU; Robert Dyer, LSU; John Ruby, LSU; Billy Goyner, USDA; Robert Morris, Tulane; and Joe Mascorro, Tulane Medical School.



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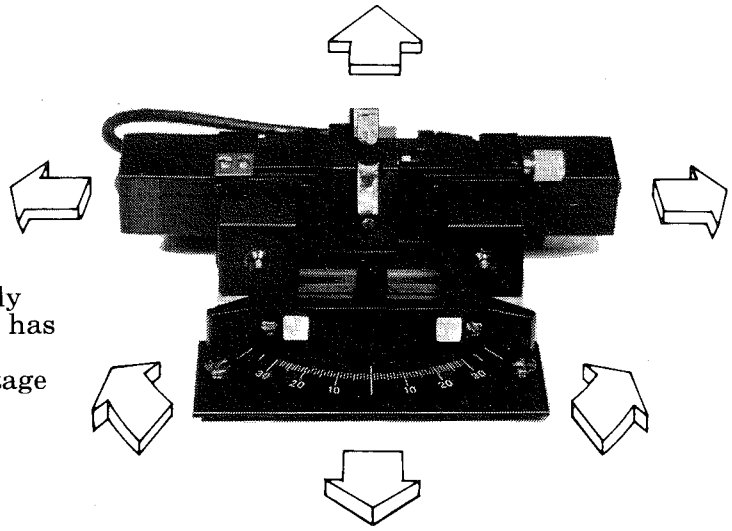

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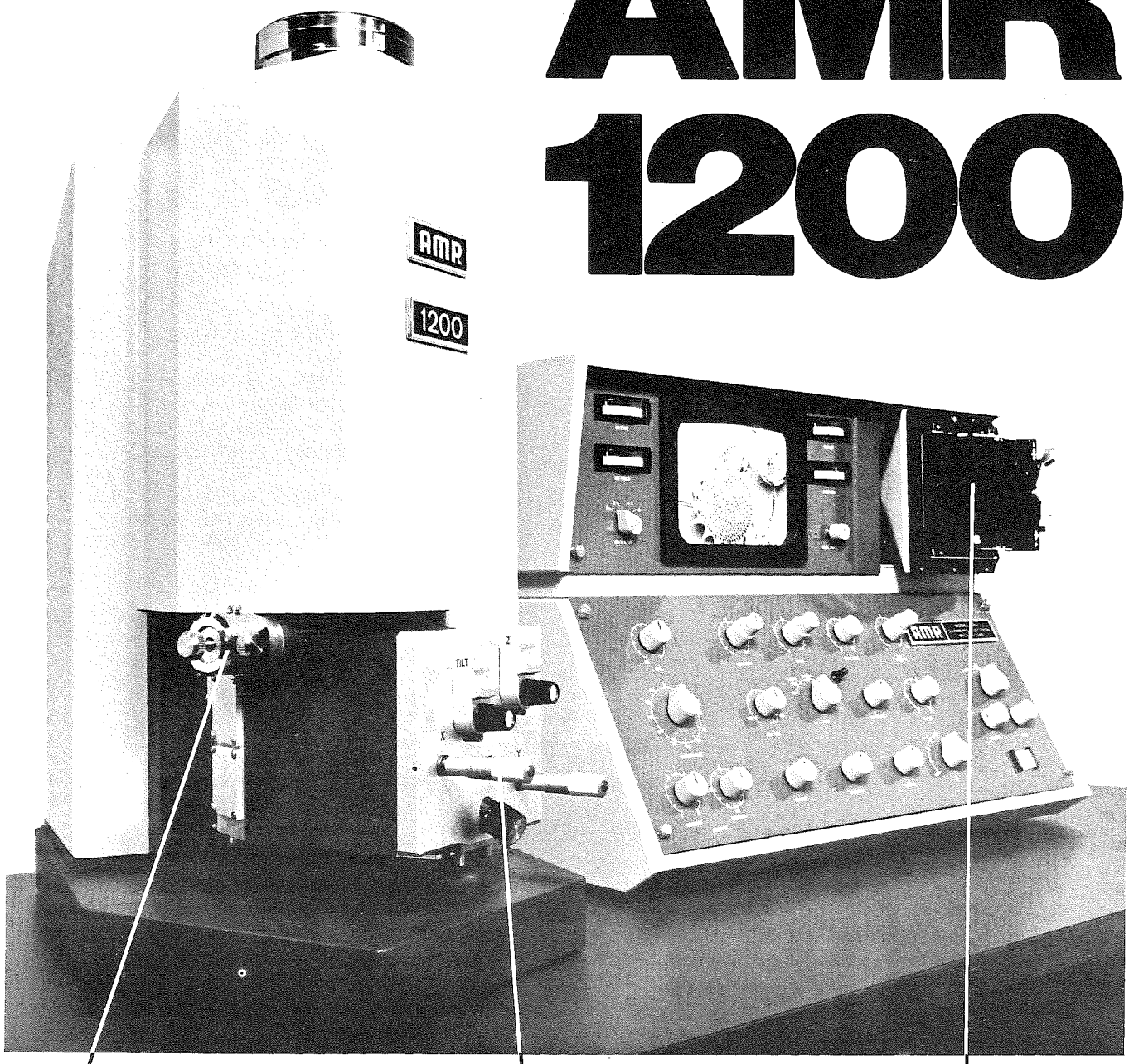

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TSEM Minutes

The business meeting of the Texas Society for Electron Microscopy was called to order at 12:30 p.m. at the Ponderosa Motel in Temple, Texas on the 2nd of October, 1976. The meeting was called to order by President Thurston who introduced the individuals at the head table.

1. The Report of the Secretary was dispensed with.

2. The Treasurer's Report was given by D. Peterson. It was approved as read.

3. The Newsletter report was given by B. Turner who noted that the Newsletter would be given a Library of Congress number and that page numbers were used in the recent newsletter.

**4. A motion was made by R. Scott of Texas A&M that the Program Chairman wear a T-shirt with the program on it.

**5. Ivan Cameron moved that programs be sent out to members before meetings if this is possible. The motion passed.

6. L. Thurston announced the upcoming meetings of TSEM:

a. The Joint meeting with LSEM-SEEM will be held in New Orleans, Louisiana February 3rd through the 5th, 1977.

b. The Spring meeting of TSEM will be held in Austin May 5th through the 7th, 1977.

7. Thurston announced that the EMSA educational materials booklet is available.

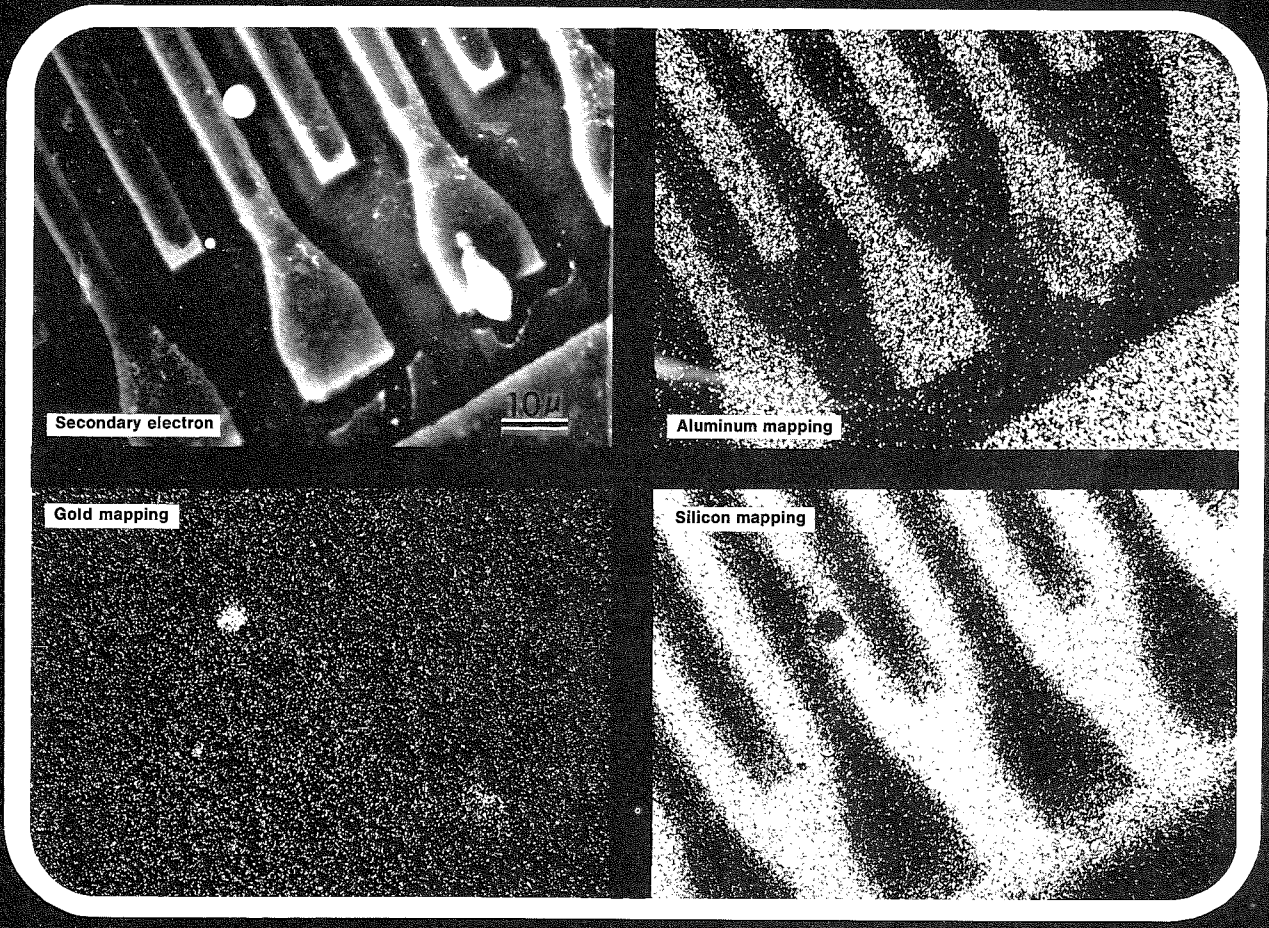
The meeting was adjourned at 12:50 p.m.

FINANCIAL REPORT

Period Ending December 8, 1976

Total assets August 23, 1976		$ 7340.08
Certificate of deposit (University Bank No. 4470)		-1179.31
Certificate of deposit (Fannin Bank No. 17864)		-1000.00
Savings account (Fannin Bank No. 12-0900043)		-3114.25
Balance in checking account August 23, 1976		2046.52
RECEIPTS		
Corporate dues	$ 75.00	
Member dues	279.50	
Registration (Fall)	465.00	
Corporate Contributions	645.00	
Total Income	$1464.50	
		+1464.50
		3511.02
DISBURSEMENTS		
Presidential travel	$ 100.00	
Temple meeting	1287.53	
Other	2.08	
Total Expenses	$-1389.61	-1389.61
Balance in checking account December 8, 1976		2121.41
SAVINGS ACCOUNTS		
Certificate of deposit (University Bank No. 4470)		1197.13
Certificate of deposit (Fannin Bank No. 17864)		1000.00
Savings account (Fannin Bank No. 12-0900043)		3172.30
TOTAL ASSETS of TSEM December 8, 1976		**$ 7490.84**

Analytical Research Services



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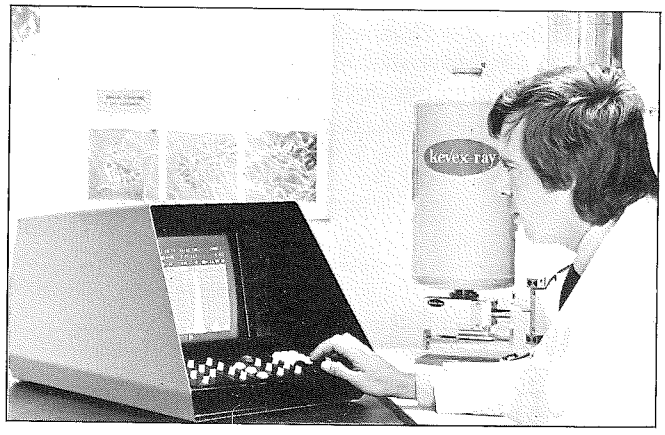

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Situation Wanted — Raul Joseph Alvarado, 5300 Tropicana, El Paso, TX 79924. (915) 751-0691. Single, 5-10, 180 lbs, born July 24, 1951. Wants career in medical field as a Laboratory Technician. Majored in Microbiology at El Paso Community College, GPA 3.6 on a 4.0 scale. Presently employed as Bio-Lab aide, electron microscopy, Dept. of Pathology, William Beaumont Army Medical Center, El PASO, TX. Has been recommended by Bernhard E.F. Reimann, Dr., rer. nat., Chief, at William Beaumont Army Medical Center. Dr. Reimann is in the process of training Mr. Alvarado and will be available for full-time job on Jan. 21, 1977. Other references and a complete resume are available.

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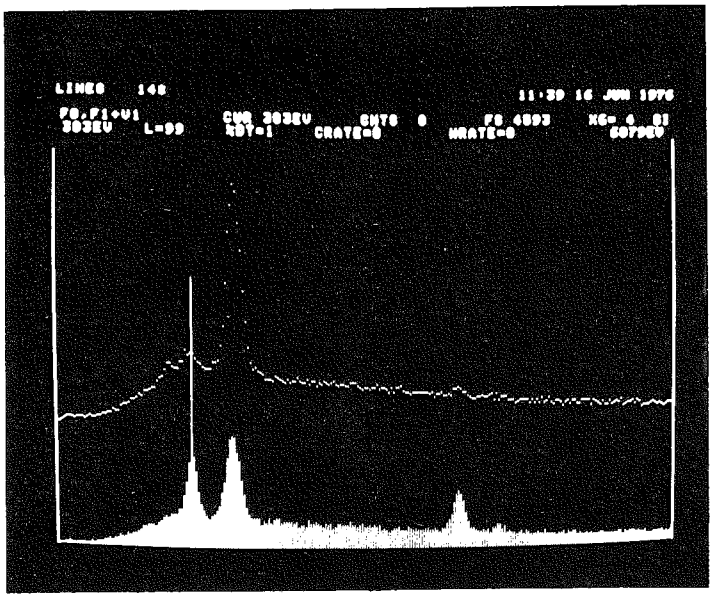
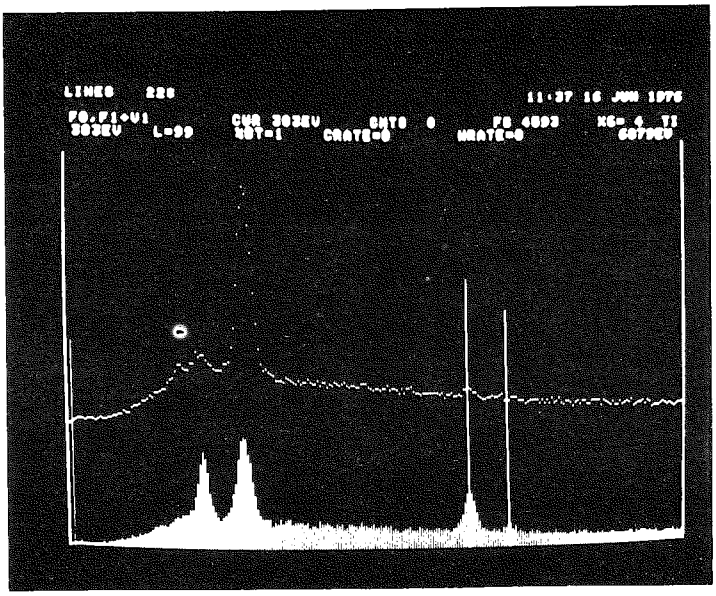


Spectra with Si K Line Markers



Spectra with Ti K Line Markers

FIGURE 1: Spectra acquired from Black Flecks (represented by bars) and from Noral Polyester Area (represented by dots). The large unmarked peak is the Au M line due to the Au sample coating.

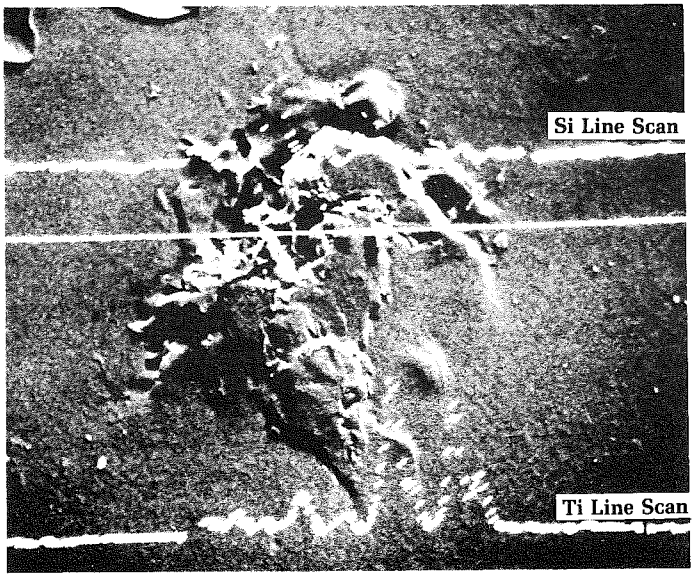
Analysis of Black "Flecks" In Polyester Film

A sample of polyester thin film (labeled C552, 17-1, 5-11-76), exhibiting black defects ranging in size from 0.1 to 0.8 mm, has been analyzed using the PGT-1000 Energy Dispersive X-Ray Analysis System.

The analysis showed these defects to contain high concentrations of both Si and Ti when compared with the normal polyester film.

The sample was mounted and coated with a thin layer of evaporated gold to make it conductive to the electron beam. Spectra were collected from the black defect and from a normal area of the film. These spectra (Figure 1) show the defect (spectrum with solid bars) to contain significantly more Si and Ti than the normal area (spectrum with dots). It should be noted that the large peak appearing in both spectra is the Au M line from the sample coating.

Figure 2 shows a defect with the corresponding Si and Ti line scans superimposed. In this mode of analysis, the electron beam is driven across the sample on one line (the analysis line) while X-ray intensities for particular energy regions are monitored (in this case Si and Ti K αlines). The result is a plot of X-ray intensity for a particular element as a function of position on the anaylsis line. As can be seen in Figure 2, significant concentrations of Si and Ti correspond directly to the area of defect.



100X 100 μm

FIGURE 2: Micrograph of Black Fleck in Polyester Thin film with corresponding Si and Ti Line Scans.

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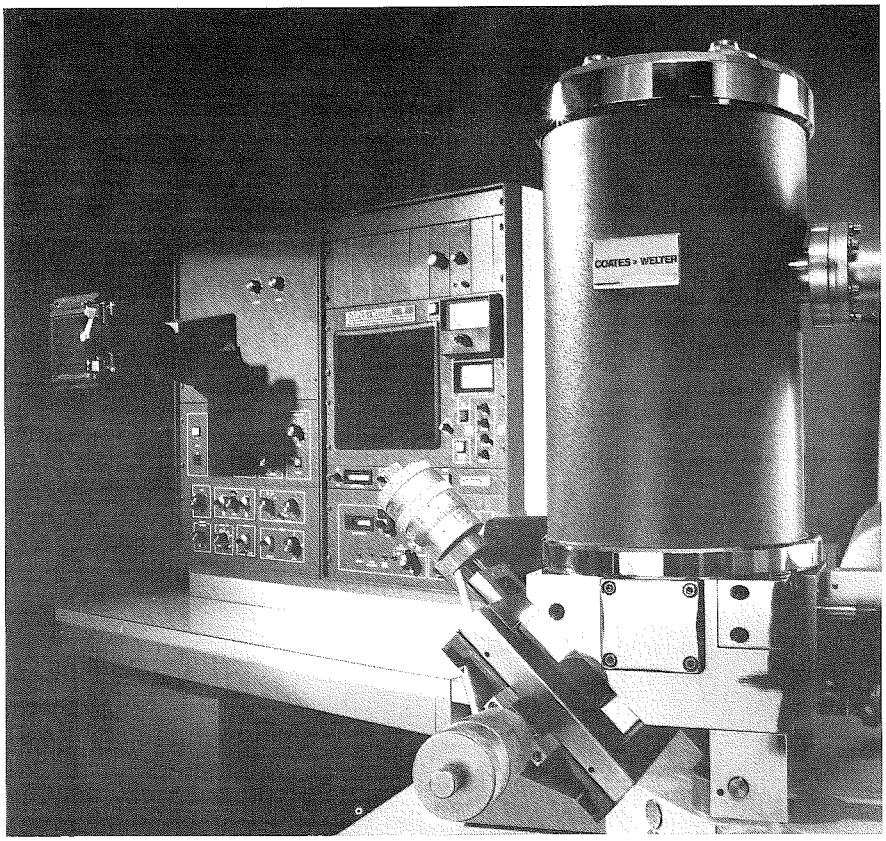

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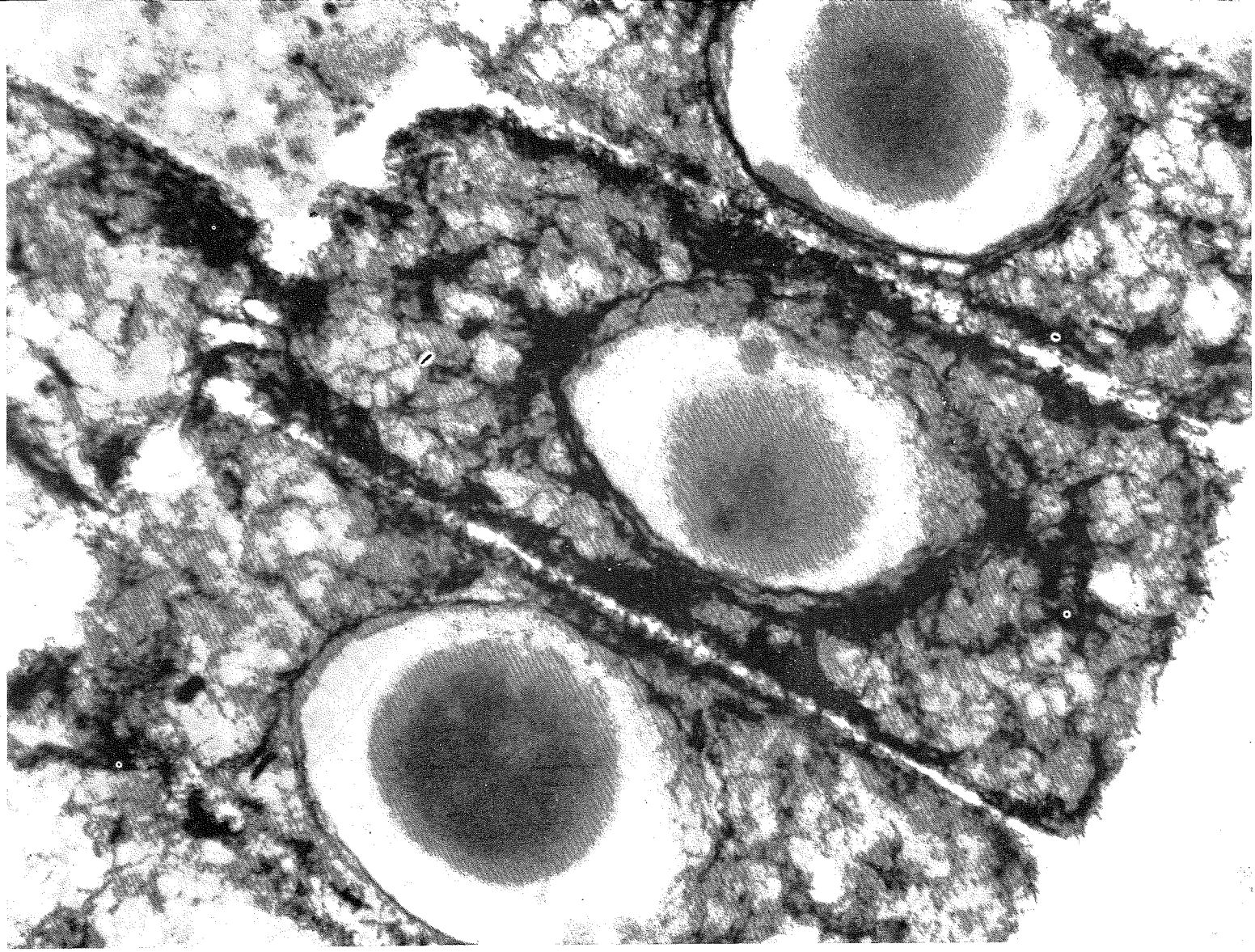

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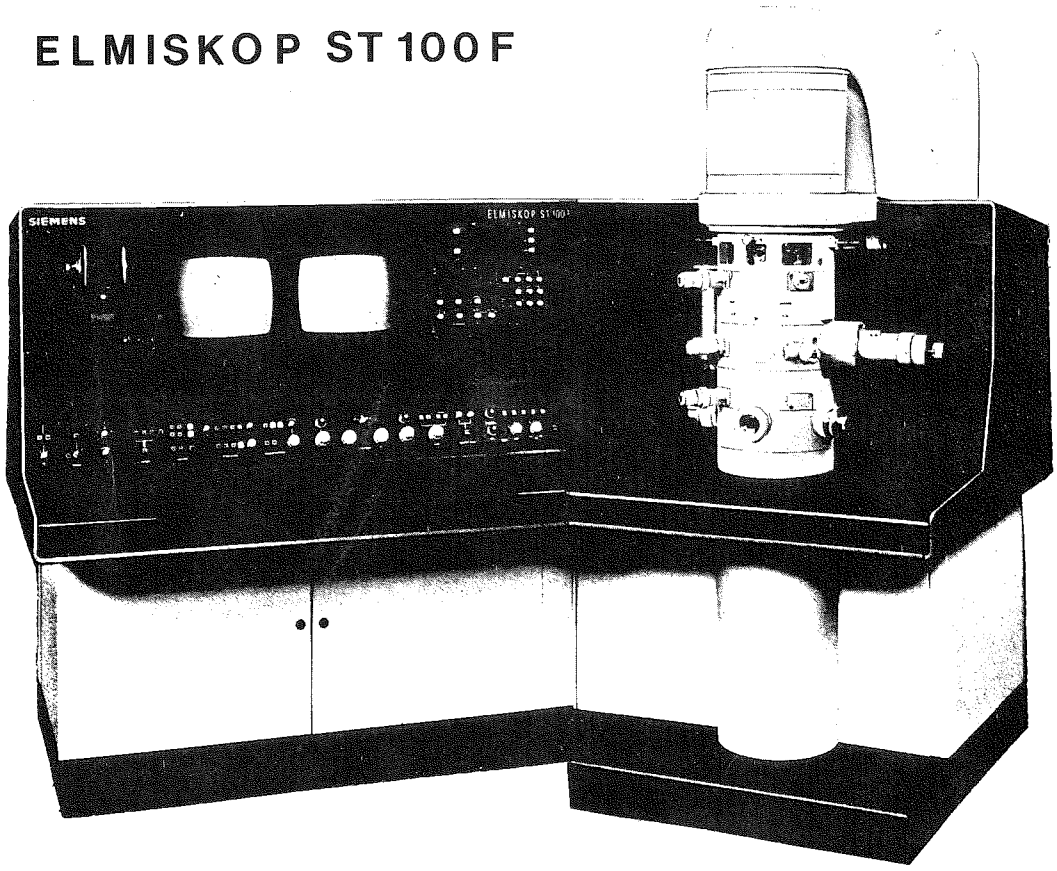

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