TSEM Texas Society for Electron Microscopy NEWSLETTER

Fall 1977

Contents

Volume 8, Number 4
Fall, 1977

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

ON THE COVER

Stinging nettle plant (urtica pillulifera) from Art Sowers, Electron Microscopy Center, Texas A&M University, College Station, Texas

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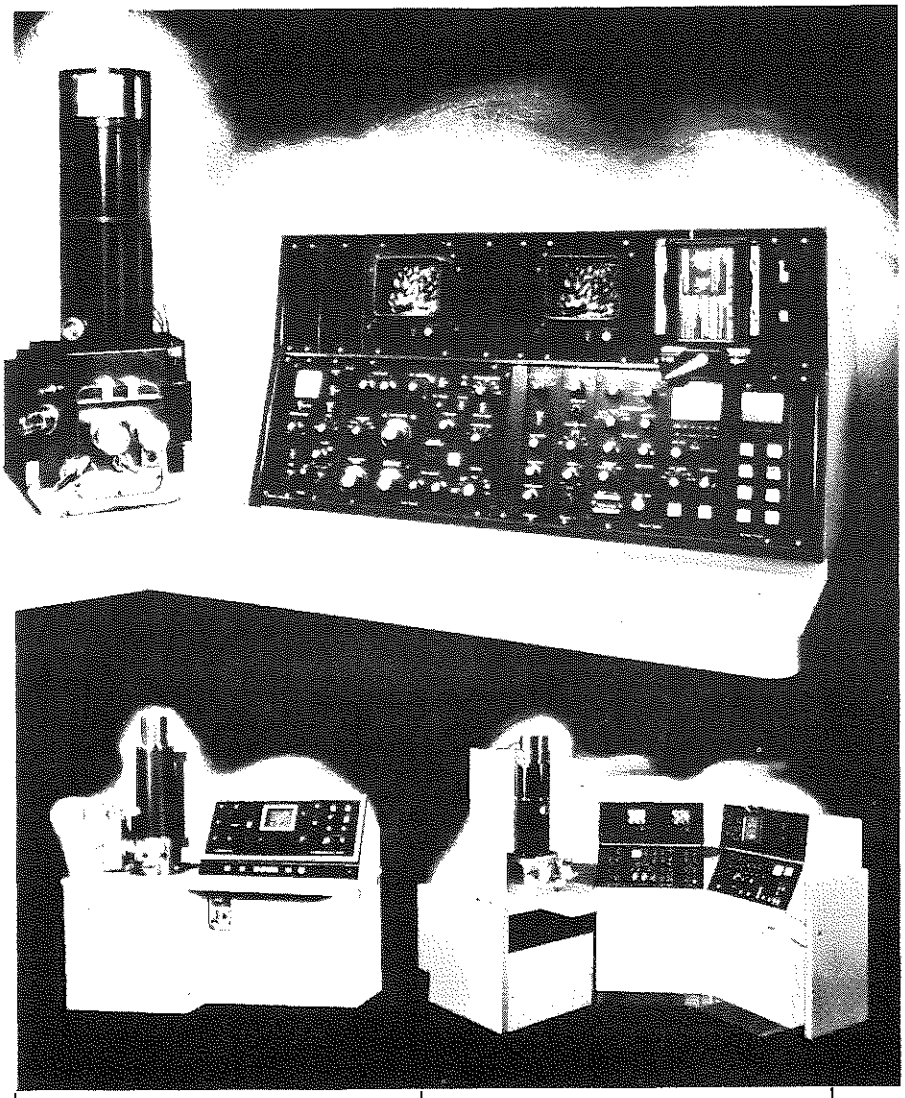

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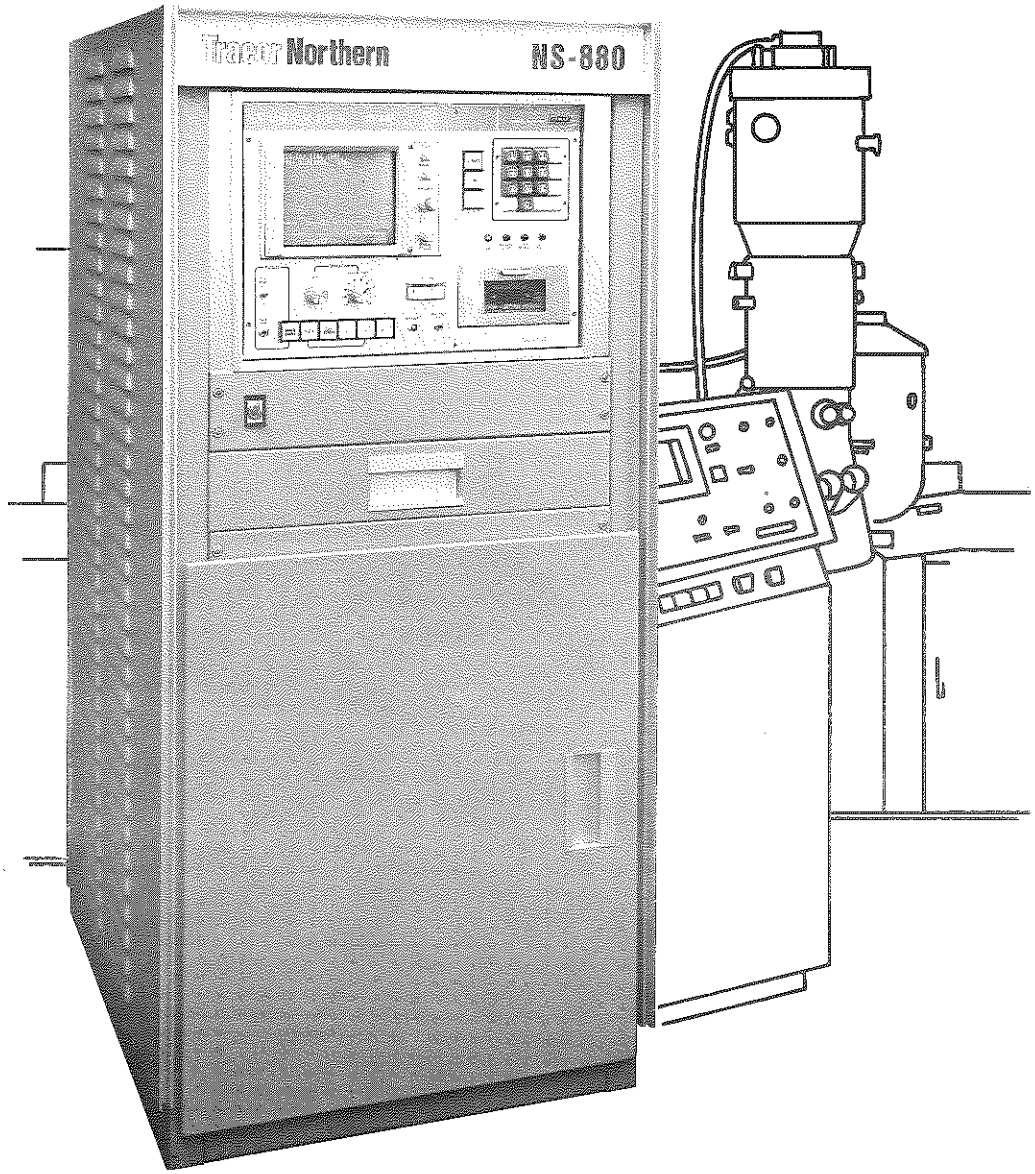

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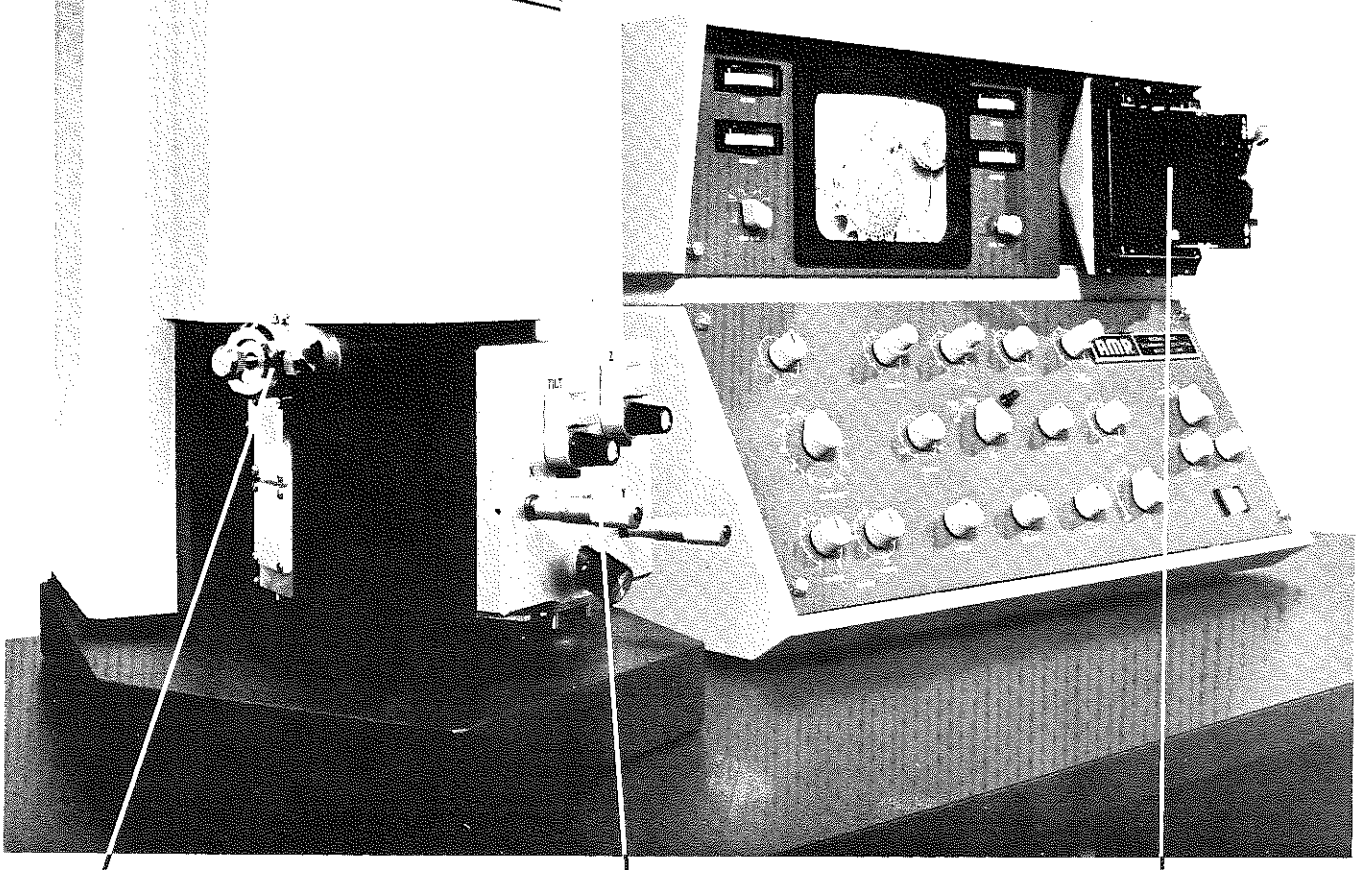

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President's Message

I should like to take this opportunity to officially thank last years officers. In particular, the Society owes a debt of gratitude to Larry Thurston who so capably guided us the past year. As a result of Larry and other individuals both before and with him, this Society is in exceedingly good shape and I am able to look forward to the coming year with a great deal of pleasure.

Our three meetings this year will be held in Arlington, San Antonio, and Lubbock. The Stereology Workshop in Arlington looks like a sure-fire winner and the annual Joint Symposium with L.S.E.M. (in San Antonio) is in very capable hands and will hopefully have the quality that we have come to associate with this event. The dark horse is Lubbock and we are still working on a West Texas style meeting.

I return now to the state of the Society. As good as we are it occurs to me that we have a problem. Specifically, I think all of us should consider this Society for what it is, i.e., a place for the free exchange of scientific information. To this end, I believe too few of us present papers at meetings. I realize a lot of information gets transferred in the halls and various places, but I would like to see us double the number of contributed papers at our meetings. And I don't just mean graduate student presentations although nothing is wrong with such papers and I would like to see these increase in number. But I'd also like to see more senior and established members on the platform. In my opinion, this would most enhance our Society right now. Thus, my major goals for this coming year will be (1) to increase the number of contributed papers from all of our members and (2) to get as many senior people as I can on the programs of contributed sessions.

Y'all come cause y'all welcome.

JERRY D. BERLIN
President

TSEM FINANCIAL REPORT

Period Ending August 1, 1977

Total Assets as of March 25, 1977		$ 7,566.40
Certificate of Deposit (University Bank No. 4470)		1,215.22
Certificate of Deposit (Fannin Bank No. 17864)		1,000.00
Saving Account (Fannin Bank No. 12-0900043)		3,230.89
Balance in checking account March 25, 1977		$ 2,120.29
RECEIPTS		
Corporate dues	$ 477.50	
Regular dues	244.50	
Secretary (Hillman) transfer	278.34	
Corporate donations (Austin meeting)	475.00	
Registration receipts (Austin meeting)	1,823.00	
Savings account closure (Fannin Bank)	3,348.34	
Total Income	$ 6,646.68	$ 6,646.68
		$ 8,766.97
DISBURSEMENTS		
Student travel	$ 321.00	
Speaker honorarium and travel (Austin)	488.00	
Meeting expenses, Austin	1,388.56	
Deposits for San Antonio meeting	150.00	
Newsletter expenses (Turner)	109.47	
Secretary expenses (McCombs)	1,000.00	
Display boards (Baur)	86.62	
Banking expenses	14.70	
Savings account deposit, University National Bank, Galveston, No. 01-7420-3	4,000.00	
Total Expenses	$ 7,508.35	$ 7,508.35
Balance in Checking Account August 1, 1977		$ 1,258.62
SAVINGS ACCOUNTS		
Certificate of Deposit (University Bank No. 4470)		$ 1,252.22
Certificate of Deposit (Fannin Bank No. 12-0900043)		1,000.00
Savings Account (University National Bank No. 01-7420-3)		4,000.00
TOTAL ASSETS August 1, 1977		**$ 7,510.84**

Application/Nomination For Membership

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

Name of institution applicant or corporation nominee person _____

P. O. Address _____

_____

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

_____

_____

_____

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

One year's dues in the form of a check or money order should be sent with the application for Membership form. (Regular $5.00, Student $1.00, Corporate $50.00)

Signature of one Member making the nomination:

_____

Dated _____ 19 _____

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

_____

Presented to the Council at _____ meeting. Date _____

Action _____

Remarks _____

Send Application to: Bill McCombs, Secretary
Department of Microbiology
Scott & White Clinic
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On the same stud, 1000 mesh copper screening is positioned to one side. This screening calibrates specimens at magnifications from 500X to 1000X.

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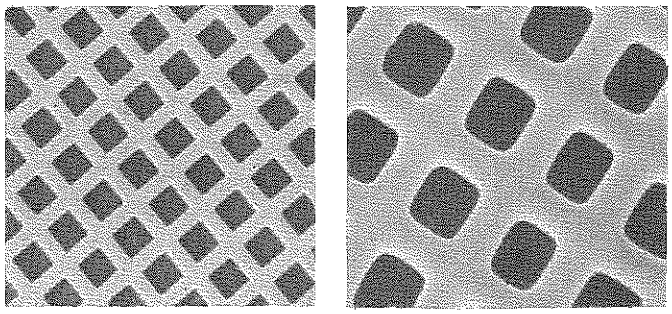

100x 500x

Cat No. 31550
Grating — 15,240 lines per inch

Made specifically for use in scanning electron microscopes. This replica is taken from a ruled diffraction grating with 15,240 lines per inch.

Very useful for magnification determination in the range 1000X to 15,000X.

Measurement chart and instructions included.

Cat. No. 31650 — Grating — "Waffle" Type — A dependably accurate, easy to use specimen, specifically made for use in scanning electron microscopy. The original replica is taken from a diffraction grating with two sets of lines ruled at right angles to each other.

Very useful over a magnification range of 10,000X to 50,000X. It is also used for detection and measurement of lens distortion and determination of the degree of fore-shortening in an image, as a function of specimen tilt.
Measurement chart and instructions included

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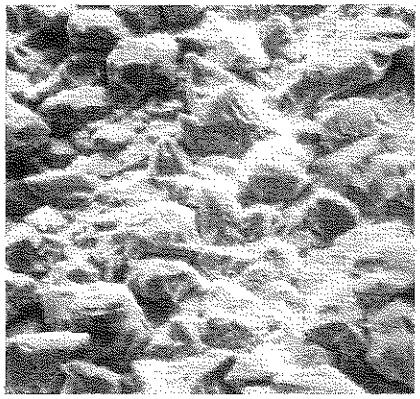
This composite specimen is both a time and space saver.
Measurement charts and instructions for use included



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STEREOLOGY:

A TECHNIQUE FOR OBTAINING QUANTITATIVE INFORMATION FROM ELECTRON MICROGRAPHS

Fagerberg, W. R. and H. J. Arnott
Department of Biology, University of Texas at Arlington
Dean of Science, University of Texas at Arlington

Have you ever wondered whether a specific organelle type, let's say mitochondria, have undergone any change in your developmental or experimental system? Perhaps you were frustrated with your inability to clearly and quantitatively detect such changes. Stereology could provide your answers. Have you ever wanted to know what volume of a specific cell type or series of cell types is composed of mitochondria, nuclei, chloroplasts, golgi, vacuoles, etc. Stereology can provide some real quantitative answers to these and many other cellular problems. It is important for the reader to distinguish between stereology and stereography: Stereology (the subject of this article) is the technique of converting two dimensional data into three dimensional data whereas stereography is a technique using two stereo pictures to recreate a three dimensional picture by parallax enhancement.

The electron microscope has been used by biologists for nearly 30 years to study cells and tissues. During this time many techniques have been developed, improved and utilized to explore the fine structure of cells and cell components. The use of glutaraldehyde and the better resin mixtures greatly improved the electron microscopist's ability to accurately describe cell fine structure. Progress in this field has continued until the present. At about the same time glutaraldehyde was first being used as a standard EM fixative, stereology introduced to biologists a set of techniques which enable them to describe the excellently fixed cells in a quantitative way. Stereology did not receive wide use at the time but has recently gained wider recognition as a potentially powerful tool for the analysis of electron micrographs. Using these techniques the uniqueness of the fine structure of a particular cell or tissue can be measured and the components of various cells related to each other in an objective way.

Attempts to accurately characterize cells and tissues ultrastructurally and relate this information to other measured parameters without the use of stereology has only been partially successful. Primarily this is because electron microscopists deal with two dimensional representations of three dimensional objects and each two dimensional micrograph contains only a very small fraction of the total contents of the cell. This means that single micrographs are very poor representatives (even median sections) of the totality of the cell from which they are taken and thus will not directly allow accurate characterization. Additionally, investigator bias brought on by the natural tendency of the investigator to select for study tissue sections which display the maximum number of components in esthetic arrangement, also introduces substantial inaccuracies in attempts to describe and characterize cells and tissues. Finally, information concerning tissue and cell characterization often takes on a relative or subjective nature, e.g. more mitochondria, more abundant E.R., fewer vesicles, etc. These judgements provide little meaning when trying to compare or relate studies performed by separate investigators. Thus there are a number of problems concerned with the accurate characterization of cells and tissues that must be dealt with before meaningful data can be obtained:

1. Single micrographs are poor total representatives of cells from which they are derived.
2. Investigator bias in selection of certain features in a studied material result in a loss of randomness essential to the accuracy of the study.
3. The subjective nature of the data which results from most studies makes comparisons with other studies very difficult.

Stereology provides a set of techniques which go a long way to solving the problems mentioned above.

What is stereology?

As already indicated the term stereology has created some amount of confusion. The natural tendency is to associate the word stereology with a study of stereo

pictures which is not the case. Stereology involves uniaxial viewing and has nothing to do with parallax enhancement. Rather, stereology involves the interpretive reconstruction of the three-dimensional structure of cells. At the first ISS (International Society for Stereology) conference (1961) Elias defined stereology in the following way: "Stereology, **sensu stricto**, deals with a body of methods for the exploration of three-dimensional space, when only two-dimensional sections through solid bodies or their projections on a surface are available. Thus, stereology could also be called extrapolation from two- to three-dimensional space."

Cells, tissues and organs are three-dimensional objects composed of an array of subcomponents and are often studied by two dimensional representations (micrographs). Fortunately, a quantitative relationship exists between the three-dimensional volume of cellular components and their profiles as seen in two-dimensional micrography (Weibel and Bolender, 1973). When certain sampling conditions are satisfied the profiles of an organelle in a section or micrograph are quantitatively representative of that organelle as it is contained in a unit of volume (the cell in this case), therefore measurements from micrographs can be used to derive three-dimensional structural dimensions by relatively simple means (Weibel and Bolender, 1973). It is important to point out here that the section planes which are cut from cells and tissues are three-dimensional planes and there are some problems which arise because of this during projection onto the two-dimensional micrograph surface (Underwood, 1970; See Holmes effect: Weibel and Bolender, 1973; Weibel, 1975). Thus stereology can provide us with several fundamental and important understandings:

1. A relationship exists between the area of the profile (created on the micrograph) and the volume of the solid from which it was derived.

2. A relation exists between the profile boundary which may often be composed of (membranes) and the area they enclose or occur within etc.

3. The true shape and size distribution of particles can be determined from profiles on a two-dimensional surface. (Underwood, 1975).

The relationships which allow definition of these parameters lie in mathematics and particularly the mathematics of integral solid geometry and geometric probability theory. These relationships form the basis of stereological thought.

The information derived in stereology is quantitative and thus lends itself to confidence predictions and other statistical measurements as well as the ability to interrelate with other kinds of data obtained by other means. Stereology is a means of converting pictorial data into mathematical information.

The concept of using two-dimensional representations to describe three-dimensional structure is not new as a science or to light and electron microscopy. Cytologists have long used techniques of serial sectioning to describe the nature of certain cells and tissues. However, this technique is laborious and sometimes frustrating. Stereological methods of describing three-dimensional structure are not new either, geologists have been using these techniques for over 120 years, mineralogists for over 40 years, and biologists for approximately 15 years. These methods have been proved, expanded and improved through such use. It has only recently begun to enjoy wide attention by a large number of biologists.

Historical perspective.

The mathematical foundations of stereology are well established in usage and in theory. The relationship between two-dimensional representation and three-dimensional structure was first proved by the French geologist Delesse in 1847. Using a number of random sections of rocks Delesse showed that by measuring the areas of various components on the surface of the sections he could predict their volumetric content in the rock. The Delesse principle forms the basis of stereology and has been proven mathematically many times (Underwood, 1970). The ease and accuracy with which this principle can be applied to biological specimens depends upon the size and shape of the component phase to be measured as the predictive capabilities of the principle are dependent upon geometric probability theory. Much of the innovative, technique research currently underway will establish correction factors for more accurately measuring structures of widely differing shapes (Cruz-Orive, 1975; Hilliard, 1975; Lindberg, 1975; Weibel, 1975).

How are measurements made?

In biological work profiles of cellular components (the α phase) are measured from micrographs and compared to the profile of the cell in which they reside (the β phase) in much the same way that Delesse measured the surfaces of rocks. Once we are confident that areal measurements can be related to the geometric volume of a component this leaves us with a number of relatively simple methods of obtaining information from micrographs. The most commonly used method of sampling the micrograph is

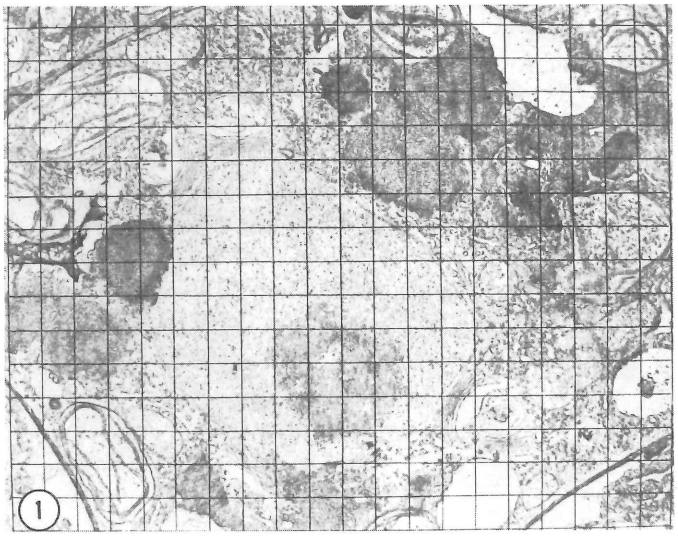

FIGURE 1 — *An electron micrograph with a point grid overlay. The points are generated by the intersection of two lines. In this sample micrograph 67 points fall over the nucleus while 13 fall on the nucleolus. In this case the nucleolus occupies 19.4% (13/67) of the area of the nucleus where as the latter occupies 19.9% (67/336) of the area present in the micrograph.*

through the use of a grid array of equally spaced points or intersecting lines (Figure 1). This is simply placed over the micrograph and the number of points falling within the α phase is divided by the total number of points falling in the β phase (the areas of n number of α phases can be determined in this manner). A second commonly used test system consists of an array of straight lines of known length, the intersection of the α phase profiles with these lines is related to the total line length within the β phase (Weibel and Bolender, 1973). A third method consists of tracing the boundary of the α phase particle with a planimeter which converts the information to an area in user units. The area of the α phase is divided by the area of the β phase to yield the standard V_V relationship. This type of measurement can be accomplished more accurately and rapidly by electronic digitation which requires an X-Y point generator and a desk top computer. Finally, automatic image analysis techniques are being developed which utilize television cameras and computers to analyze areal components on micrographs. This list of test systems is not complete as many other systems may find use in special situations.

Types of data.

Because of the nature of stereology a number of kinds of data can be obtained from micrographs. However, only six kinds will be listed here as those which might be used most often by biologists.

1. V_V = The % of total (cell β phase) volume a particular component (organelle α phase) occupies.
2. S_V = Surface to volume ratio. The surface area of a boundary or enclosed surface (membrane) per unit of volume of the enclosing or enclosed structure, e.g. cristae mm^2/mito mm^3, E.R. sq. in./cell cu. in., etc.
3. Ω = The orientation of the axis of the rotation of a component (α phase) to the axis of rotation of the cell (β phase).
4. N_V = The number of particles (α phase) per unit volume (β phase).
5. L_V = Length of a line in units per unit volume of the β phase. e.g. length of SER or RER mm/cell mm^3.
6. S_a = A measurement of spacial association between α phase profiles. e.g. Are mitochondria associated to a greater degree with the plasma membrane or nuclear membrane?

By applying one of several types of test systems to micrographs and making counts under the appropriate circumstances these data can easily be obtained and used to describe the three-dimensional nature of the cells.

There are a number of things that need careful attention if one is to achieve maximum accuracy in sampling. The first requirement for good measurement of a specimen is to insure that the two-dimensional samples were derived in a random or unbiased way. Basically, the tissue section must represent a random section plane through the sample and cytological structure of interest must be randomly distributed in reference to the test (grid) system. These criteria can generally be satisfied by following several procedures during tissue preparation: 1) the cell (tissue) orientation within the block should be random in reference to any inherent axis of the tissue; 2) blocks must be selected and trimmed with as little investigator bias as possible, e.g. any cell within a particular tissue must have an equal chance of being sectioned and the plane of section must pass through the cell at non-selected depth; 3) micrographs and areas of sectioned material selected for micrographs must not depend upon biased investigator selection but must occur in a non-subjective way. (For methods of overcoming this problem, see p. 273, Weibel and Bolender, 1973). There are special cases in which some of the above conditions may require modification and the authors suggest for further information concerning these cases and techniques involved that the reader consult Underwood (1970); Weibel and Bolender (1973); Eisenberg, et al. (1974); James and Meek, (1975); Fagerberg (in press).

Another feature that must be considered is that the number of samples taken will adequately represent the tissue under study. Since each individual EM section through a cell represents such a small part of the total cell, there is a direct relationship between the size, shape and distribution of a structure within a cell and its probability of appearing in the plane of section. Randomness of section plane is important here but the investigator must also be aware of his (her) ability to extrapolate the three-dimensional relationship of the α phase, which at least must be present in measurable quantities in each sample. This may involve a considerable amount of work and a large number of samples that must be analyzed if the α phase particles are of rare occurrence, small in size, or highly polarized in distribution. A number of methods have been derived to predict the number of sections (micrographs) that must be analyzed to achieve an investigator selected confidence interval (Carpenter and Lazarow, 1962; Weibel and Bolender, 1973; Nicholson, 1975; Weibel, 1975; Hilliard, 1975; Fagerberg, in press).

The information derived from stereological analysis results in the description of an "average" or representative cell for a given tissue and if stereology is the only method of study employed much valuable information derived from individual cells (micrographs) will be lost. How well an "average cell" can fulfill the needs of a particular investigation must be carefully evaluated by the investigator. In tissue comparisons, for example, if individual cells of a tissue are distinctly different from others in the same tissue this would cause serious problems with the "average cell" concept. In this regard it is important that the distinctive or "essential" characteristics of a cell (tissue) are the ones being measured in a particular situation if the results are to be meaningful. Some of the basic information of this sort is best obtained by good descriptive observation so essential to a well planned stereological study. There are limitations to the usefulness of the "average cell" concept. However, it is this concept that enables us to carry out studies that would not otherwise be possible and it is the average cell or cell reaction concept that physiologists, biochemists and geneticists have employed so successfully over the years.

Sample problem.

The different ways in which stereological analysis can enhance the quality and type of information gained from

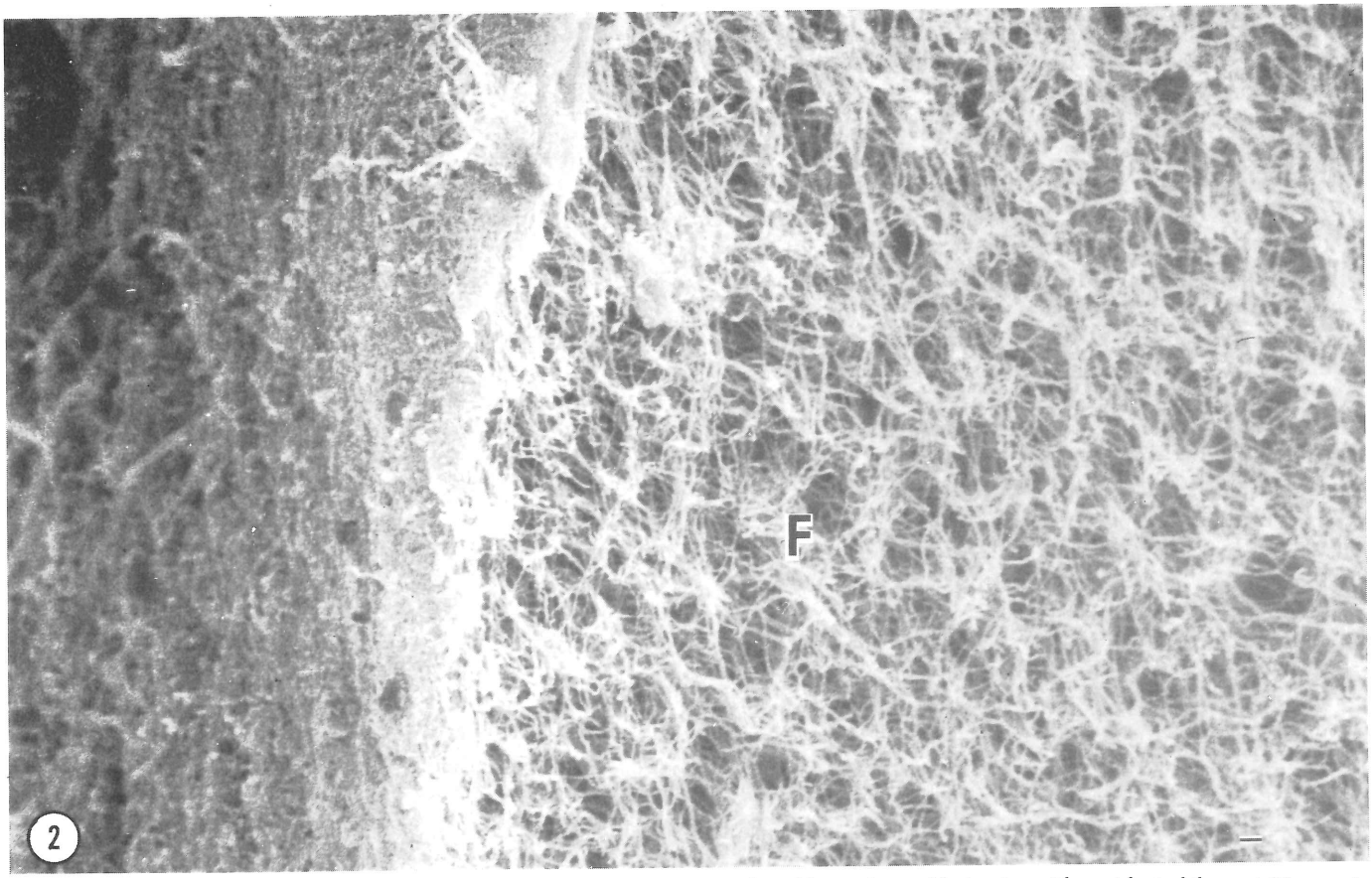

FIGURE 2 — *A scanning electron microscope of a Blue-Green algal/Bacterial mat found in geothermal hotsprings. The cut fact of the mat (F) reveals the complex structure of the mat. Grid bar 1 μm. The surface of the mat is seen at left.*

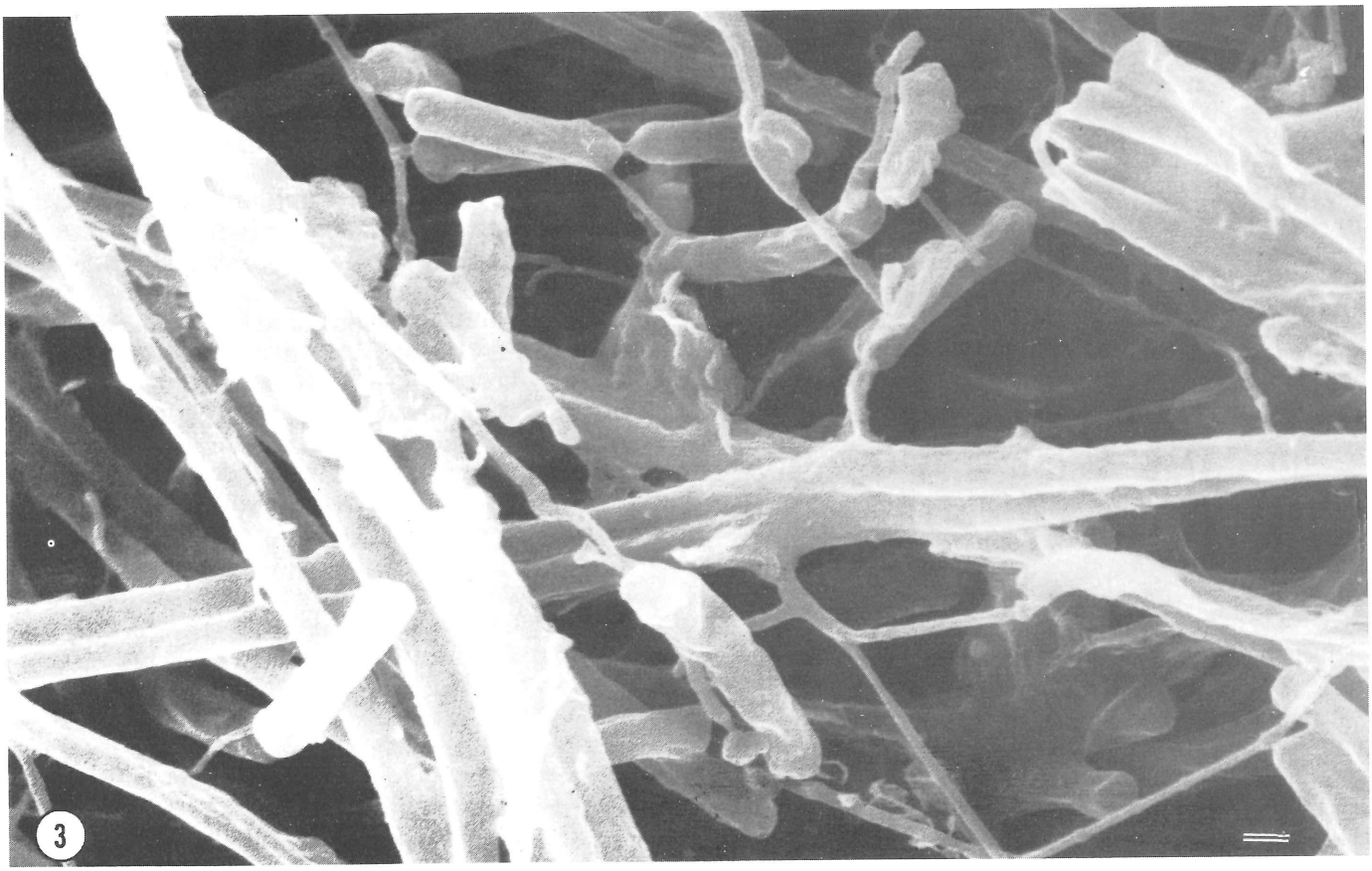

FIGURE 3 — *An enlargement of the algal mat as viewed with the SEM. Note the complex associations between organism. Grid bar 1 μm.*

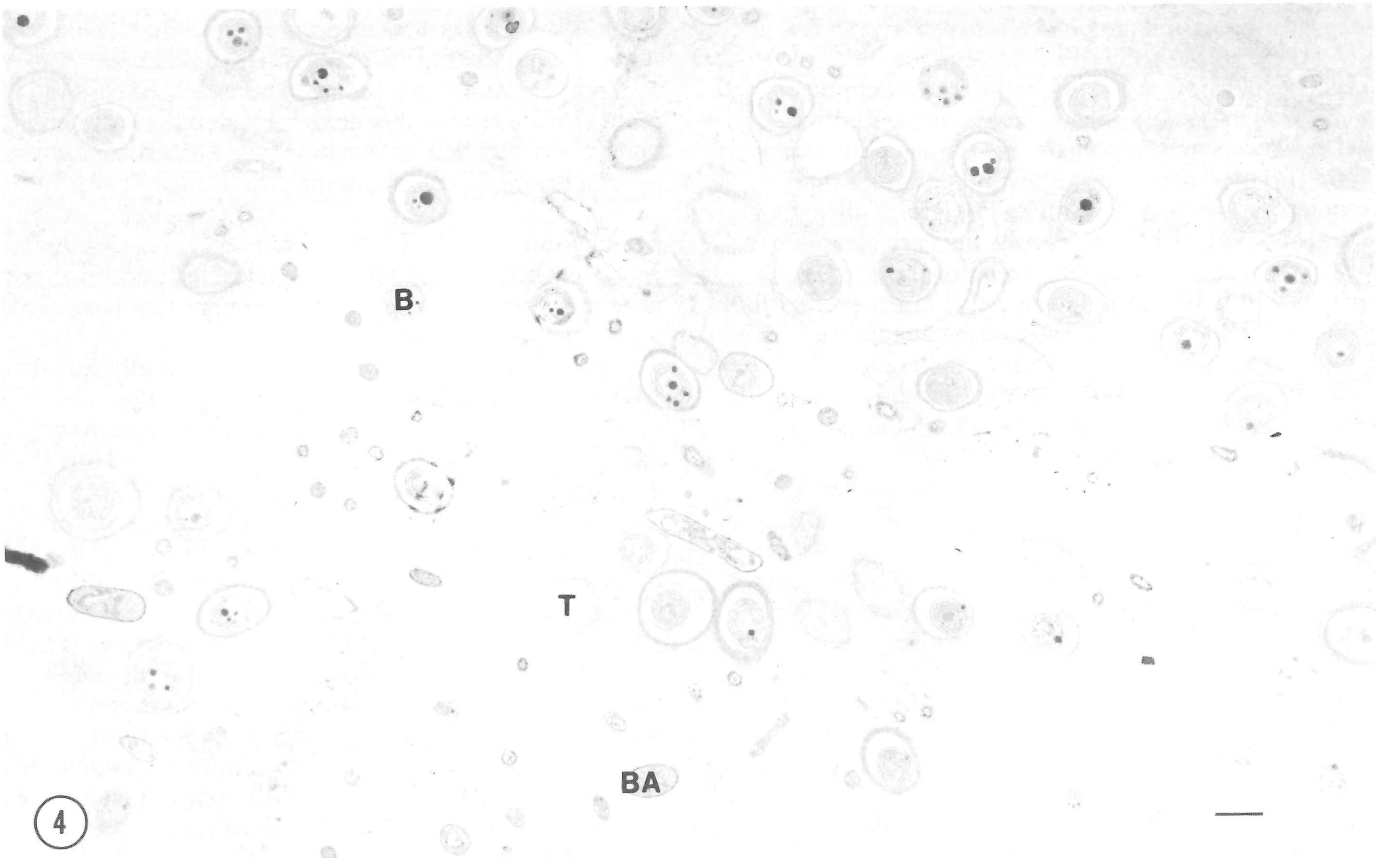

FIGURE 4 — *A transmission electron micrograph of the algal mat. Note the numbers and diversity of organisms; Blue-Green alga (B), Bacteria (BA), Tubes (T) through which organisms migrate. Grid bar 1 μm.*

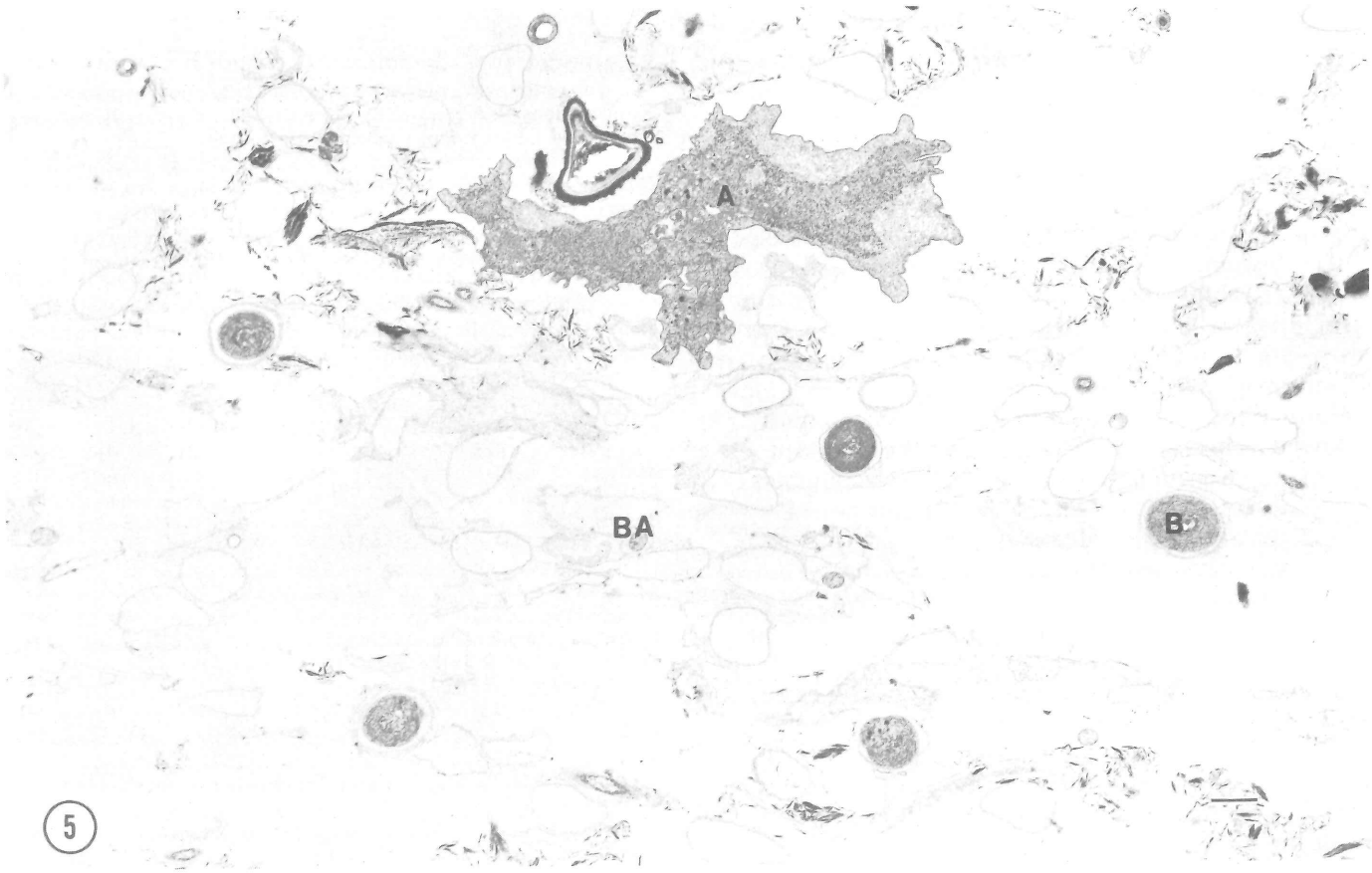

FIGURE 5 — *A transmission electron micrograph showing the variability of the organization of the organisms within the mat. Blue-Green alga (B), Bacteria (BA), Amoeba (A). Contrast this region with that of Fig. 4. Grid bar 1 μm.*

cytological studies are numerous. In fact many studies would be impossible without such techniques. As an example of such a situation the authors have employed these techniques to study species dynamics with Blue-Green algal bacterial mats associated with geothermal hotsprings. These mats present several problems that make microecological studies of this type very difficult. The first of which is how to describe the very complex nature of these mats and the interrelationships of the organisms within them (Figures 2 and 3) in a meaningful way. Secondly, the very small size of the organisms which occur within the mat (Figures 4 and 5) dictate that the electron microscope be used to analyze these systems. This would result in hopeless confusion without methods for quantitatively handling the data.

In this study three stereological parameters were used to describe the algal mats: 1) V_V (% volume of the mat occupied by different organisms), 2) N_V (number of organisms per unit volume of mat), and 3) S_V (the surface to volume ratio of the photosynthetic membranes of the various algae within the mat). In addition, because the data was quantitative we were able to utilize many standard ecological parameters in defining these systems such as "important values" of the major groups and measures of the mat structural complexity and stability via a "species diversity" and "evenness" index.

The sampling procedure for this system consisted of dissecting whole mats into $1mm^3$ pieces in fixative and of the 200 or so resulting pieces 20 were selected, randomly, to comprise the sample. These were trimmed and a section from each block was used in a similar way to a classical ecological quadrat. These micrographs (quadrats) were used in the stereological analysis. In thin sections the organisms within the mat lost the identifiable morphological features used in classical taxonomic designation, therefore each organism was assigned a number based on an ultrastructurally recognizable feature and designated as a morphological species. The volume of the mat occupied by each of these "species" was calculated as well as the number per unit volume. One can easily appreciate the ability of stereology to accumulate data into a useful format when trying to relate the information from Figure 4 with that of Figure 5 and then consider trying to relate information from 20 such samples.

Information gained through quantitative techniques has allowed us to describe the structure of these mats in terms of volume occupied by algae and bacteria, numbers of each species present, and through the application of various classical ecological relationships to make a predictive statement about the stability and energy dispersal capability of the mat. These studies have provided us with a baseline of information upon which to construct laboratory experiments to test the effect of critical environmental factors on the structure of these systems.

In this example we have not created an "average cell" but rather an "average quadrat" which is representative of the mat structure as a whole. We can use this example to illustrate the limitations of the "average cell" concept because the vertical and horizontal distribution of species are not recorded in this data for but rather "quadrat" assumes a homogeneous structure for the mat. (This is the same assumption the "average cell" would make concerning all cells in a tissue). This is not the case in reality but it serves when an overview of the entire mat is needed. Information on zonation could have been obtained by simply altering the sampling procedure.

Conclusion.

Data derived through stereological techniques allows us to raise some very important questions that have, up til now, been impossible to answer.

1. How closely does cellular structure really correlate with reported function?

2. How consistent is the cytology of a tissue in different samples under similar conditions (i.e. How accurate is the predictability of ultrastructure?

Stereology involves a considerable amount of time both in preplanning, the repetitive work of analyzing micrographs and dealing with the data once acquired. These techniques do not represent a quick or easy way to study cells, quite the opposite. Special requirements for fixation of tissues, tissue manipulation, sample size considerations and data manipulation require considerable effort, but the information gained is not practically available by any other method. In addition, stereological procedures are setting the standards upon which much of the future ultrastructural work will be judged. However, the question "Will stereology provide useful information in this particular study?" should be carefully asked before undertaking such a volume of work.

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The Healing Of Burn Wounds

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When the word "burn" is mentioned, "scar" often comes to mind. This is because the healing process of a moderate to severe burn most often results in the regeneration of the integument and the concomitant formation of a disfiguring scar. However, if the burn injury lacks severity, the resultant regenerated tissue is so nearly perfect that it could not be called a scar.[27] The burn injury does not appear to be much more complex, with respect to healing, than are other injuries (abrasive, mechanical, excisional, etc.) to the integument. Likewise, the phases of burn wound repair, the cellular populations involved, the methodology of tissue reconstructions, and the regaining of integumentory tensile strength are similar, if not identical, to those observed in other large wounds to the skin.[23, 31, 35-39] The course or length of the healing process of a severe burn wound is by definition a lengthy process because the resultant malformed scar continues for years to attempt replacement, modification, and/or remodeling to a more normal configuration.[2-4, 20, 23, 24] Even though not completely understood, many facets of the burn wound repair process are being brought to light by

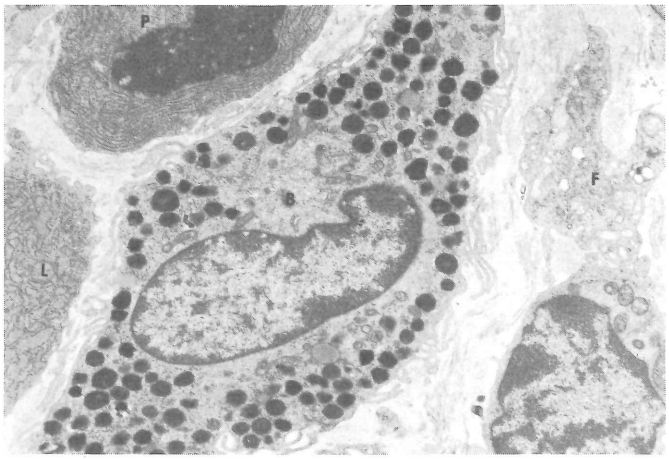

FIGURE 1 - *A basophil (B), plasma cell (P), fibroblasts (F), and small lymphocyte (L) observed by TEM within granulation tissue that was biopsied 9 days post burn. Numerous types of circulating leukocytes can be observed in burn wounds during the early days of granulation (13-year-old white female).×9500.*

* *All of the skin/scar materials seen in this study were initially fixed 2 to 6 hr at room temperature in a 0.1 M Pipes (piperazine N-N' bis (2 ethanol sulfonic acid)) buffered (pH 7.4) 3 per cent glutaraldehyde solution with an osmolarity of 510 Mos.*[7, 18] *Following fixation an additional 24 to 36 hours at 0-4°C in the same solution, the biopsies were sliced in half with one portion being processed for transmission electron microscopy (TEM) and the remainder prepared for light microscopy (LM) and/or scanning electron microscopy (SEM).*

The LM and SEM samples were fixed an additional 24 hours in a 10 per cent formalin solution, dehydrated in a graded series of ethanol solutions, cleared in xylene, and embedded in paraffin. Sections (5 to 6μ thick) were cut, mounted on slides, and deparaffinized with xylene. Some of these sections were stained for LM with hematoxylin and eosin for general cell morphology. Other sections selected for SEM study were critical point dried following their deparaffinization and were coated with gold (300A) prior to their examination on a Cambridge S4-10 scanning electron microscopy operated at 30kv. The samples processed for TEM were rinsed several times in Pipes buffer, post fixed in Pipes buffered (0.1M, pH 7.4) 1 per cent osmium tetroxide, and rinsed several times in distilled water. Dehydration, infiltration, and embedment in an epoxy resin was followed by thin sectioning on a Porter-Blum Mt-2 ultramicrotome. The sections were stained with uranyl acetate and lead citrate and examined on a Philips 201 or 300 transmission electron microscope.

publications devoted solely to that topic.[2-4, 7, 16-20, 24, 25, 49] Reviews devoted to the healing of burn wounds have not been plentiful due primarily to the general similarities of all types of wounds. Fortunately, the burn wound appears to be only slightly complicated by the trauma itself, which will be discussed later.

The burn wound is variable with respect to surface area involved and depth of the injury. Because of their size, burn wounds are usually allowed to repair by secondary intention.[32] Like all wounds, the primary concern of a burn wound appears to be the effectual restoration of the epidermis barrier which prevents fluid loss and/or microbial invasion.[47] In order to maintain the repaired epidermis, the healing phenomenon is likewise directed towards the restoration of a supportive dermis with adequate vascular supply and tensile strength to resist tears or rents.

The Trauma

Burn traumas are more commonly induced in tissues by the sudden application of excessive thermal energy or by caustic chemicals. The immediate effects of this trauma are the cessation of capillary flow and the coagulation of cellular and tissue components.[49] If coagulation (essentially denaturation of proteins) occurs through the entire skin (a full-thickness third degree burn), an eschar will form and if a somewhat less destructive trauma occurs, a vesicle or blister (a partial-thickness second degree burn) will be produced.[32] In those areas of the skin adjacent to or underlying the wound the initial vasconstriction occurs seconds after the onset of trauma and lasts for only a few minutes. Vasodilation then follows and maximizes within 10 minutes. Several hours later leukocytes infiltrate the tissue which accompany vascular stasis and occasional local hemorrhage.[41] The inflammatory response that follows the trauma is probably a response to the excessive quantities of devitalized and/or necrotic tissues or by microbial contaminants which are both characteristic of burn wounds.[41]

Granulation Phase of Repair

The granulation tissue that is formed after a burn wound has stabilized consists of a complex tissue which is composed of fibroblasts, capillary networks, and other infiltrating cells (Figure 1).[27, 41] The initiation of the granulation phase of repair occurs at the time of trauma although the first visible signs of granulations do not appear for 2 or 3 days postburn.[32, 41] The first indications are the proliferation of the capillary networks at the base and/or margins of the wound which result in the bright red coloration of the tissue[27, 41] Little is known about the factors that stimulate this vascular growth. It has been postulated that reduced blood pressure, low PO_2, changes in the ground substances, metabolic changes, and mast cell stimulation all might be factors that promote the response.[41] In any case, the vascular production is accompanied by fibroblasts migrating into the wound tissue and producing new extracellular materials (collagen principally with some mucopolysaccharides initially.)[9, 41] Likewise macrophages from the blood migrate through the area and remove the debris. Experimental data have been presented to indicate that granulation tissue has contractile capacity somewhat similar to smooth muscle. Its requirement for oxygen in order to contract suggests that a cellular process is involved.[9] The presence of myofibroblasts (discussed later) in this tissue may account for its contractile nature.[2-4, 9, 40]

Collagen filaments found in burn granulation tissues are characteristically irregularly and angularly shaped with a mean diameter of 440 A. The mean filament diameter of collagen filaments observed in normal skin has been estimated at 1000 to 1050 A.[16] Moreover, most of the filaments within some of the fibers seen in granulation tissues and early scars are extracted during electron microscopic processing so that their images appear washed out in the micrographs (Fig. 2). This extractability is a feature of neutral or new collagen which is soluble in dilute salt solutions.[42]

During the latter stages of granulation, the forming

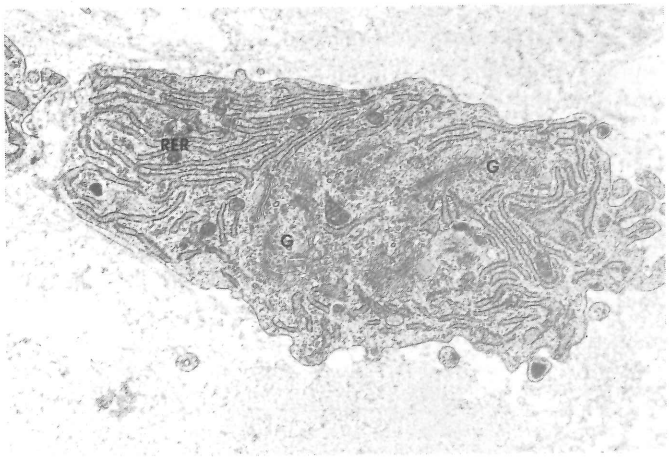

FIGURE 2 - *A synthetic fibroblast found in an early hypertrophic scar (5 months post burn) and observed by TEM. A vast array of rough endoplasmic reticulum (RER) with dilated cisternae and containing an electron dense material and extensive Golgi bodies (G) are clearly obvious. Filaments comprised of new collagen (washed out by the dilute salt solutions of the preparative baths) can be observed surrounding this very active cell (5-year-old white female).×31,500.*

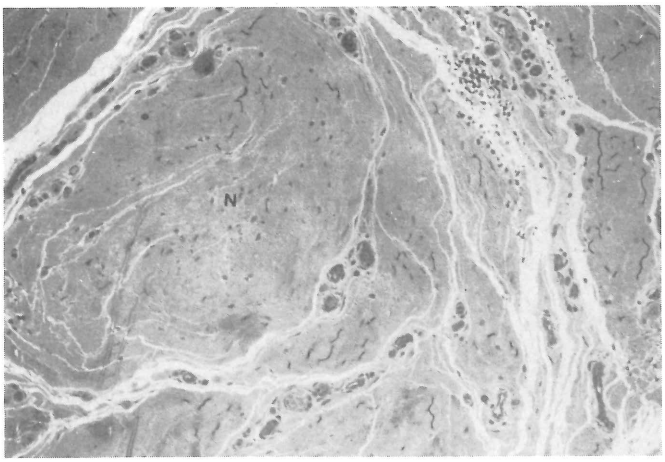

FIGURE 3 - *The characteristic nodules (N) of a 4-year-old hypertrophic scar can be seen in this LM view. These nodules are composed of a few cells and heavily solidified masses of collagen and its associated ground substances. The resolution of these solid masses into fiber-like patterns can be observed at the margins of each nodule. This fiber reformation is a characteristic feature of a remodeling (natural or induced by pressure wraps) hypertrophic scar (9-year-old white female).×120.*

dermal components can be observed to assume bizarre configurations of collagen.[25] These patterns continue to enlarge so that in early scar formations bundles or tracts of collagen begin to assume a whorl-like arrangement. As the scar continues its development characteristic nodules of fused collagen filaments and cells are observed in the deeper layers of dermis (Fig. 3).[2, 3, 17, 25] The genesis and subsequent degradation of these nodules, which are characteristic features of hypertrophic scars and keloids, will be discussed later.

Restoration of the Epidermis

The restoration of the epidermis is effected by the mitotic activity of basal cells located in the stratum spinosum of undamaged normal epithelium.[47] The cell division occurs at the wound margins and/or from identical areas of skin appendage (hair follicles or sweat glands) remnants located within the wound itself.[27] The zone of mitotic activity seldom exceeds one millimeter in width back from the edge of the wound,[27] and appears to be unaffected (with respect to size) by the extent of the trauma or its severity.[47] The epidermal mitotic activity becomes apparent within 42 hours after trauma with hair follicles appearing to be the most important source of epidermal cells.[27] The mitotic activity of the regenerative epidermis appears to demonstrate a diurnal rhythm with the greatest activity during rest and sleep.[47] This could account for the rarity of mitotic figures that can be observed in burn wound biopsies of tissues. After cell division new daughter cells appear to detach, flatten considerably, and migrate over the wound surface until they cover the entire wound.[47] If a crust or blister is present, the cells migrate through the base of the blood and fibrin material of the crust or over the stratum reticularis of the dermis within the vesicle.[27, 47] The production of fibrinolytic or proteolytic enzymes by these cells allows them to migrate through the base of the crust material.[27] Within the blister of a second degree burn the rate of epithelization appears to be twice that seen in similar desiccated areas.[27,32] The migration of the epidermal cells and their eventual coverage of the wound does not appear to depend upon the presence of a basement membrane which is later reformed by the epidermis irrespective of dermal activity. (Fig. 4).[13, 31] The differentiation of the migrated epidermal cells then reforms the scar epidermis.[27, 47]

If the entire integument has been destroyed (third degree burn) re-epithelization must await the reformation of the proper substrata (vascular beds, new collagen fibers, etc.) produced by granulation tissue. As new epidermal cells can only migrate an estimated one centimeter from the site of cell proliferation (mitosis), then the large full-thickness burns that lack skin appendages must be provided with sources of epidermal cells.[27] This is usually accomplished by grafting procedures which are fruitful only after the vascular beds have been adequately reformed or earlier by excision of burn tissue to viable tissue.

Repair of the Dermis

The repair of dermis is mediated primarily by the very numerous fibroblasts observed in wound healing tissue (Fig. 5)[27, 41, 45] This large (up to 100μ) polymorphic cell is characterized by fusiform or stellate configurations with extensive processes surrounding or situated between identifiable collagen filaments and/or fibers.[45] These cells have been exhaustively studied.[26, 34-36, 38, 39] Cytoplasm is often scarce in the main body of the cell which is filled with rough endoplasmic reticulum (RER) whose cisternae are markedly dilated and interconnected. The dilated RER, which may be the distinguishing feature of fibroblasts, seldom extend into the processes and usually contain a medium dense homogeneous substance. Small 50 to 70 A filaments are often observed in the cytoplasm and at the cell border. These filaments have never been observed to penetrate the cell membrane.[35] Free ribosomes and a well developed Golgi zone are also common features of these cells. Pinocytotic vesicles are commonly seen on or near the cell membrane. The nucleus is normally ovoid, indented, and contains only a slight amount of heterochromatin.[7, 11] Several nucleoli are commonly observed.

Fibroblasts synthesize the collagen, glycoproteins and

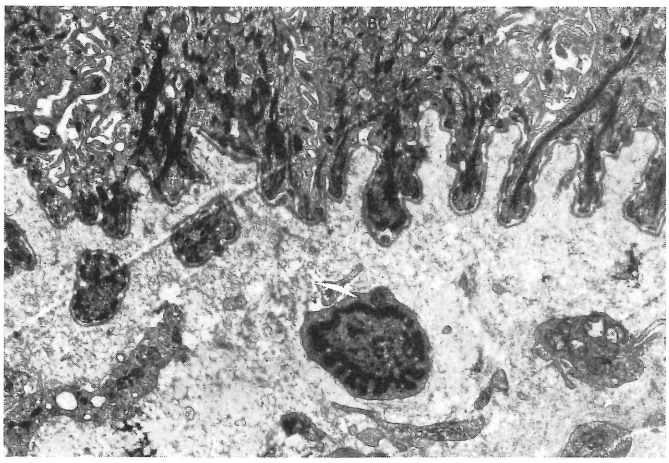

FIGURE 4 - *The epidermal/dermal junction in a 2-year-old scar. The reestablished basal lamina, hemidesmisomal junctions, and normal appearing basal cells (BC) can all be observed in this view (5-year-old black female).×33,000.*

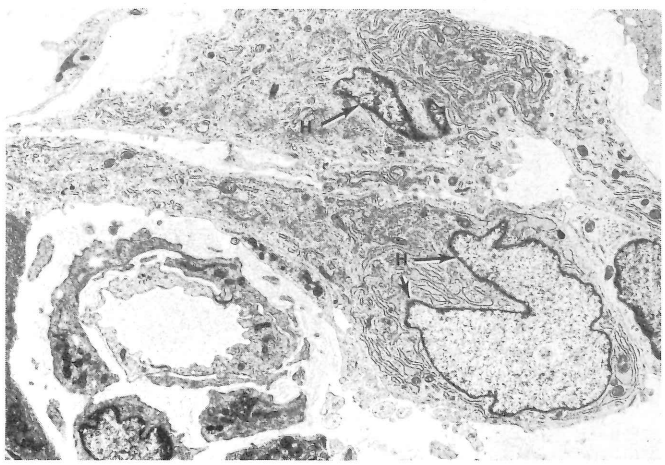

FIGURE 5 - *Two synthetic fibroblasts can be seen in close proximity to an adjacent capillary in this TEM view. This biopsy is from a granulation tissue collected 4 days post burn. The slight amount of heterochromatin (H) suggests that these are very active (synthetic) cells and are not fibrocytes (15-year-old white male).×18,000.*

mucopolysaccharides, that comprise the dermal fibers which provide tensile strength to the integument.[10, 27, 35, 41, 45] During the early stages of repair fibroblasts grow through the wound on fibrin strands found within the crust or clot but the fibrin network does not seem to be essential for this movement.[41] Later in the repair process the fibroblasts appear to move through the tissue on a network of collagen filaments synthesized by other fibroblasts. Although they resemble smooth muscle cells in configuration, fibroblasts do not have an associated basement membrane (basal lamina).

The origin of fibroblasts remains somewhat speculative but it appears that they do not originate from large monocytes and/or macrophages which enter the wound from the blood. It is more probable that they are derived from resting fibrocytes (more or less dormant fibroblasts) that are found in the adjacent loose subcutaneous tissue, the adventitia of small blood vessels, or cells in fatty tissue.[26, 41, 45] Long (years) after the wound repair process has terminated, the density of fibroblasts that can be observed in the scar is similar to that seen in normal skin. It has been postulated that the reduction in cell number is accomplished in burn wounds by cell death.[20] Indeed, vacuolated cells, cell ghosts, and debris can be observed in scars past the stage of highest synthetic activity, usually a year or more after the trauma, which tends to support this concept.[4] Likewise, this slow attenuation in cell numbers can account for the changes that occur within the scar that are reflected in its loss of coloration, rigidity, thinning, etc.

Myofibroblasts, first reported in wound tissues of animals by Majno[28] and by Baur et al.[3] in burn wound tissues of humans, are similar to fibroblasts with respect to size and shape.[29] They appear to have little or no RER during the latter stages of wound repair, but during the early stages the cells have a large amount of the RER and are obviously involved in protein synthesis. (Fig. 6)[2-4] The myofibroblast is different from the fibroblast only in that the former cells contain sizable bundles of contractile elements within the cytoplasm. The unidirectional bundles often extend the entire length of the cells into the filamentous processes and are comprised of numerous actin filaments 60 to 80 A in diameter associated in a parallel array.[2] During the stage of wound repair when contraction or a contracture occurs (to be discussed) the cells and thus their contractile bundles are observed to be aligned with the direction of the scar movement. The contractile bundles in the cells are occasionally noted to bifurcate but the overall direction of the two or more bundles appears to be similarly oriented (see Fig. 6). Extracellular fibrillar-like material (30 to 50 A in diameter) can often be observed on the surfaces of myofibroblasts in the older granulation tissues, wound contractions, scar contractures and in active hypertrophic scars (see Figs. 6 & 7). This material is similar in structure to that first reported by Gabianni and called the stroma by him.[9] This material, which appears to be proteinacious, might well be the anchoring factor of these contractile cells to juxtaposed collagen fibers and/or other cells. The predominance of this material on the cell surfaces which were adjacent to or overlying contractile bundles terminations in the cytoplasm has straightforward implications. Cellular contraction could not be reflected in tissue contraction and/or eventual hypertrophy if the cells were not tightly attached to other tissue components such as the collagen fibers. Otherwise the myofilament foreshortening would only result in cellular contraction and the cells would simply slide within the tissues to assume more fusiform configurations. We have proposed that the material be called myofibroblast anchoring substance (MAS). The apparent reduction and/or the rearrangements of the MAS in pressure treated tissues suggests that the loss or disruption of cell to fiber unions may be the foci of the therapy-induced contraction abatement discussed later.[2]

The myofibroblasts are first observed in second and third degree burn granulation tissues 3 to 5 days after the trauma and represent only a small fraction of the total fibroblast population at that time. Their numbers continue

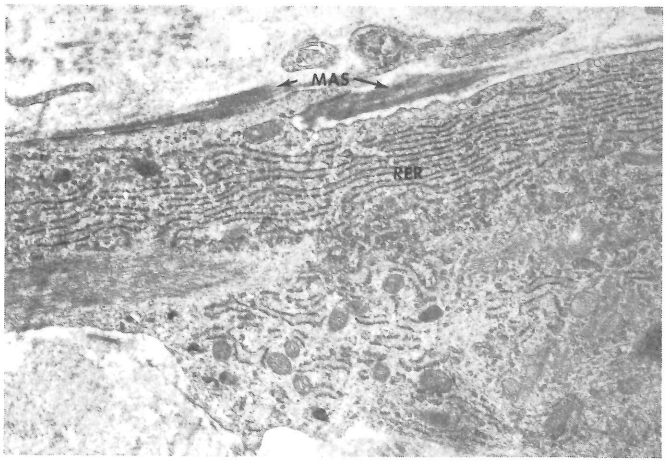

FIGURE 6 - *In this TEM view the myofibroblast anchoring substance (MAS) can be seen on the cell surface in intimate contact with the cell membrane of this myofibroblast. For cellular contraction to be reflected in tissue contraction the cells must be tightly attached or anchored to the passive dermal components. A sizable quantity of RER is also seen in this cell from a 4-month-old hypertrophic scar (1-year-old Latin American male).×20,000.*

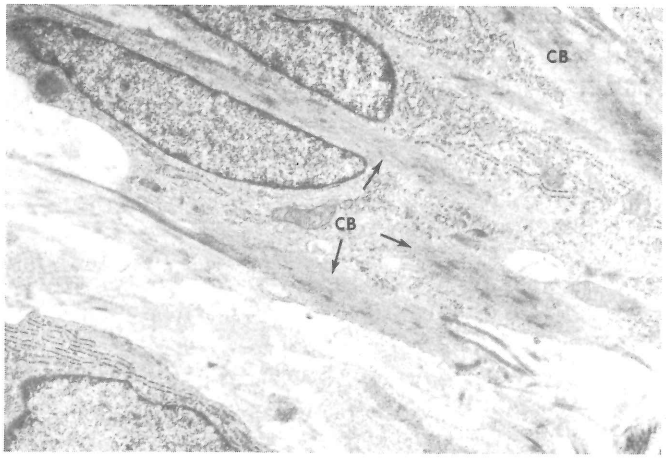

FIGURE 7 - *In this TEM view a single myofibroblast contains what appear to be four or five distinct contractile bundles (CB) within the cytoplasm. One bundle bifurcation can be observed in this cell that in this view lacks significant quantities of RER and Golgi. Myofibroblast anchoring substance appears at the border of the cell as a dense fibrillar substance. This sample is from an early hypertrophic scar (4 months post burn).×14,700.*

to increase during the course of wound repair so that at the height of the wound contraction phase of repair and up to 120 days post burn their numbers may comprise most (50 to 75 per cent) of the fibroblasts in the tissue. At the peak of a scar contracture formation they may account for 100 per cent of the total fibroblast population.[2]

Although no data exist to prove the origin of myofibroblasts, they are most probably formed from existing fibroblasts within the wound tissue. Due to their unexplained frequencies and their lack of correlation with the migratory nature of the fibroblasts, these cells may have the propensity to disassociate their contractile bundles into individual actin filaments which are known to be a part of the cytoskeleton and return to a synthetic fibroblast configuration.

Other cells commonly observed in normal skin and in burn wound tissue, especially in hypertrophic scars and keloids, are the mast cells.[4, 18] Although scarce in granulation tissues their numbers increase significantly as healing progresses. These cells appear to be ubiquitous throughout the body and are found mainly in loose connective tissues.[1, 41] The cells appear to increase in number during a chronic inflammatory reaction which is a persistant feature of a healing burn wound and its subsequent scar formation.[41] Likewise the mast cell appears to be directly involved in wound healing.[41] This dendritic connective tissue cell contains basophilic granules that are comprised of sulfated mucopoly-saccharides (heparin, heparin sulfate, the chondroitin sulfates, etc.), histamine, etc. (Fig. 8).[11, 18]

Degranulating mast cells configurations have been observed in active hypertrophic scar and granulation tissues.[4] This release can be induced by an edematous environment which is a characteristic feature of a burn wound.[1] In fact the water content of granulation tissues has been estimated at 87 per cent while that of an active hypertrophic scar has been reported at 82 per cent. Both values are noticeably elevated when compared to the 64 and 67 per cent values of normal skin and mature scar respectively.[19] The released mucopolysaccharides could then form a mucinous ground substance by the hydration of the acid glycosaminoglycans which may in turn provide the matrix for new collagen formations.[1] This histamine that is released appears to accelerate wound healing and vasodilation. This vasodilation of the wound or scar capillaries could account for the chronic inflammation of the tissues. In fact, the inflamed appearance of the tissue is the major clinical sign of active healing and/or scar formation. Thus mast cell activities (degranulation and release of ground substances—histamine release and vaso-dilation) are both key features of overt scar formations and when one function is lost the other appears to be likewise halted.

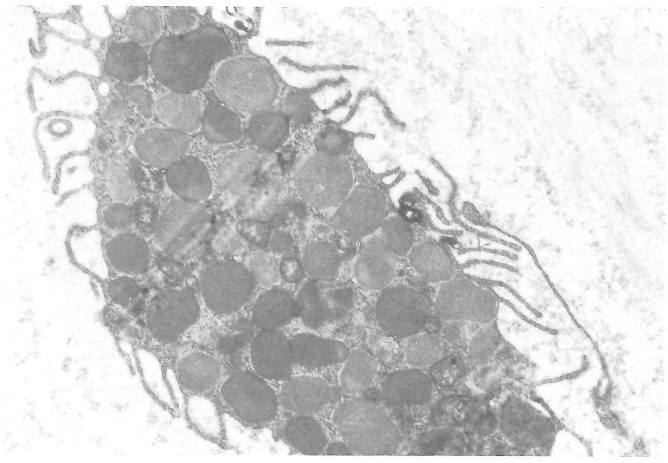

FIGURE 8 - *Mast cells are common cellular components of hypertrophic scars. Many such mast cell configurations give evidence of recent degranulation, especially in very active hypertrophic scars. From a 4-month-old hypertrophic scar (4-year-old white female).×30,000.*

Wound Contraction and Scar Contractures

When the margins of a wound move inwards to effect closure the process is known as scar contraction.[27, 46] However, burn wounds are usually too extensive to be closed by this activity even though the phenomenon does occur to some extent.[32, 46] Many possible explanations have been proposed for this movement with two being the most plausible. The first suggests that active cells within the margin of the wound migrate inward pulling on the material within the margins of the defect. This is known as the "picture frame theory." However, another explanation might be the "pull theory" in which material (collagen fibers, cells, etc.) within the defect pull on the wound margin.[46] We favor the latter concept due to the extensive distribution of myofibroblasts in the margins of wound tissue when scar contraction is at its peak. Any adjacent skin that is stretched by wound contraction is thinned but is later restored to full thickness by a process known as intussceptive growth.[46]

Burn wound contractions often continue, with respect to time, beyond the actual closure of the wound. This closure may either be effected by normal repair and/or split-thickness and mesh graftings. When this happens the scar becomes a disabilitating and/or disfiguring "scar contracture," especially if joints underlie the tissue. Thus scar contractures are in actuality unabated wound contractions.

The contractile myofibroblasts appear to be responsible for both wound contraction and scar contractures. As mentioned, at the height of contracture the cells may for the most part comprise as many as 100 per cent of the observed fibroblasts within the scar which are aligned with the axis of scar fore-shortening. Likewise these cells seem to be tightly attached to adjacent collagen fibers by the MAS as previously described with gap junctions appearing to join adjacent cells. The cells are predominantly observed in the base of the scar. The myofibroblasts observed in contractures are characteristically shorter than those seen in granulation tissues and/or old scars. They likewise appear to be attached to adjacent sinusoidal arrays of collagen. It is our contention that cellular contraction, effected by the actin filaments, results in tissue foreshortening by nature of the intimate attachments between the cells and the passive tissue components. As the cells shorten the tissue shortens and results in the relief of stress upon the collagen filaments, fibrils, and fibers. This relief then in turn forms sinusoidal patterns and/or supracoils of increasing amplitude due to the helical nature of the collagen

molecule.[3] Thus the foreshortened collagen fibers are thereby increased in thickness and the tissue becomes elevated. Subsequent fusion of these components into a solid mass by synthesized mucopolysaccharide ground substances accounts for the rigidity of the integument which is a characteristic feature of contractures. Increased production of the ground substance material in hypertrophic scars has already been reported by Shetlar and his co-workers.[42, 43]

If myofibroblasts are found in an area where excess skin or scar exists the cells do not appear to be able to align themselves in any ordered direction but instead distribute themselves in an omnidirectional pattern primarily partitioned in the base of the scar. The cells' subsequent contractions and the concomitant collagen fiber disfigurements lead to the elevations or puckering or the overlayered dermis which results in the formation of a hypertrophic scar or a keloid. If prime cellular contraction originates in the deeper dermal areas of the scar, subsequent nodule formation should also be greatest in this zone, as is the case. Thus cellular contraction and subsequent fiber fusion both appear to play an important role which in all probability differs only quantitatively in the formation of the contractures and both disfiguring types of scars.[3]

The process by which these cells are induced to contract is not known. Hunt et al.[12] and Niinikoski et al.[30] and their colleagues reported that the PO_2 of the dermal areas of scar tissue varies, with the highest oxygen levels and the most active fibroblasts found in proximity to the capillary beds which, in the case of granulation tissue, are located in the deeper areas.[3] Other studies indicate that large numbers of myofibroblasts located in close proximity to the vessels are found in these areas in the granulation tissues and early hypertrophic scars. Kapanci and his co-workers[15] already demonstrated a sensitivity of this type of cell to PCO_2. Therefore, these cells may contract in response to either PCO_2 or PO_2 present in these areas and this contraction is responsible, at least in part, for the elevation of the overlying scar tissue.[3]

An observation has been made that the myofibroblast configuration may be only a reflection of cellular mobility. This is a possibility, for fibroblasts in culture demonstrate these contractile bundles when they are actively migrating over the substrate surface. However, in burn wound tissue when cell migration is highest (granulation phase of repair) the myofibroblast population may account for only a small fraction of the total fibroblasts whereas when migration has subsided within the wound (3 to 4 months post trauma) and contracture is greatest, they may comprise the entire population.

Scar Formation

Nearly every severe burn wound results in the formation of a scar due to the imperfect architecture of the replaced integumentary tissues. As mentioned, the epidermis is replaced but primarily lacks the rete-pegs which interdigitate with the dermis. Likewise the dermis of burn wound healing tissue is malformed with respect to its microarchitecture. Instead of normal (size and shape) collagen fibers distributed uniformly throughout the depth of the mature burn scar, tremendous accumulations of fused collagen in the form of matts are observed.[25] During the early stages of scar formation, the collagen filaments are apparently laid down in omnidirectional arrays and increase in diameter to an estimated mean of 600 A.[16] This meshing network of collagen is then apparently fused into the solidified matter by the production of abnormal levels of mucopolysaccharide ground substances. It has been demonstrated that there is a redistribution of the synthetic patterns of these mucopolysaccharides with dermatan sulfate and chondroitin-4-sulfate being producced disproportionately.[42, 45] As the ground substances fuse the meshwork of collagen filaments the desposition accrues at the intercepts of each collagen filament (Fig. 9). Thus the dermis of a forming burn scar when viewed by scanning electron microscopy (SEM) appears as a fenestrated array of tissue. Later as synthesis of mucopolysaccharides continue, these fenestra fill in which results in the formation of a fiberlike structure comprised of solidified collagen and ground substances (Fig. 10). If this abnormal production or hypertrophy of dermis continues long after

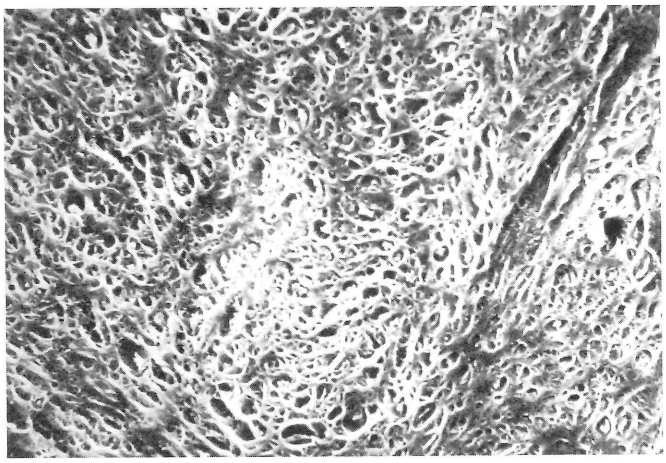

FIGURE 9 - *A SEM of a forming hypertrophic scar demonstrating the omnidirectional arrangements of the collagen filaments which are being fused into a solidified nodule. The solidification is initially observed as a fenestrated pattern. The fenestra are later occluded by material assumed to be ground substances. This view was observed in the dermis of 5-month-old scar (14-year-old white female).×400.*

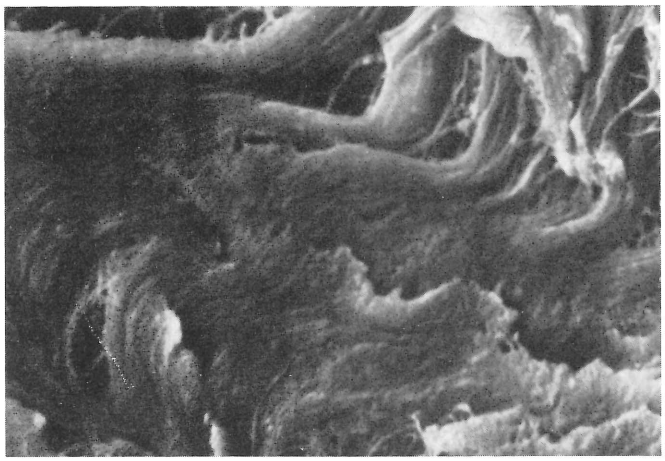

FIGURE 10 - *A scanning electron micrograph (SEM) of a fused collagen mass found within a nodule of hypertrophic scar biopsied 1½ years post burn (13-year-old white male).×5800.*

the epidermis has been replaced then the tissue will form either a hypertrophic scar or keloid. A hypertrophic scar is apparently an overgrowth of dermal components (collagen, cells, vascular elements, etc.) within the boundary of the original wound. A keloid is similar to the hypertrophic scar differing only in that its overgrowth invades surrounding tissues (exceeds the dimensions of the original scar).[14] Although not unique responses to burn wounds, these scars are often formed following the repair of a moderate to severe second degree burn. Likewise, donor sites, from which very thick skin grafts have been taken, also form hypertrophic scars or keloids if the patient has the propensity to do so. The ability of the graft donor site to hypertrophy precludes the thermal trauma as a factor in the scar production and likewise demonstrates the control intact epidermis has upon the synthetic activity of underlying dermis. If dermal appendages have survived (slight second degree burn or split-thickness graft donor site) and the epidermis is quickly replaced the scars do not hypertrophy. Likewise, if epidermis is replaced at a trauma site by grafting, hypertrophy of the underlying dermal tissue will cease, diminish, or be prevented. Thus the quick replacement or reformation of the epidermis from appendage remnants appears to be the common denominator. In both types of scars the epidermis is not thickened or does not in any way structurally contribute to the depth of the lesion.[27] However, biochemical factors produced by the epidermis seem to play a role in suppressing overgrowth or hypertrophy.

The formation of hypertrophic scars or keloids, which are both favored in dark-skinned victims,[14] has been postulated by Koonin to be due to some aberration of the melanophore stimulating hormones.[21] Children show a tendency to hypertrophy which may be due either to their greater skin tension or their actively growing (collagen synthesis) integument.[14]

Remodeling

The use of pressure bandages has been successfully employed as a prophylactic measure to prevent the development of hypertrophic scars. The treatment also prevents successive hypertrophy if the therapy is used after the scar has been initiated.[22, 23] This technique appears not only to arrest or suppress the production of additional hypertrophic tissue but also appears to enhance the natural remodeling process that normally occurs long after the initial injury.[2, 4, 20, 24] The overall effect of pressure is an acceleration of the remodeling process which in the absence of pressure bandaging occurs a year or more after the formation of the hypertrophic scar. The therapy involves the wrapping of the scar tissue with elastic bandages in such a manner than the enforced pressure (24 mm Hg) exceeds the inherent capillary pressure. This results in an immediate blanching of the tissue and a reduction in its thickness. After several days the consistency of the originally raised and rigid mass changes, with shrinkage and scar softening commonly observed. To be effective, this therapy must be applied continuously (up to 1 year or more) until the scar loses its inflamed appearance and no longer is inclined to hypertrophy.[2, 4, 20] If the therapy is interrupted at any time prior to these clinical signs, and the scar allowed to relax, the tissue will immediately reinitiate contraction and/or hypertrophy. Thus the capacity to contract or hypertrophy is of long duration and those structures responsible for these functions must remain intact in spite of the therapy.[2, 24] The steadfast population of myofibroblasts observed in this tissue, even after successful pressure treatment, reinforces the suggestion that the entity responsible for contraction is the cells and that the capacity for scar contraction, and thus hypertrophy, is maintained as long as sizable myofibroblast populations exist. In view of the obvious clinical differences between pressure and nonpressure-treated scars, few, if any, dissimilarities can be noted between their cell populations during the early stages of treatment. The amount of endoplasmic reticulum observed in the non-contractile fibroblasts and some myofibroblasts seen in naturally remodeling scars and those treated with pressure infers a high level of synthesis on the part of the cells. The nature of the products of this synthetic activity is, of course, speculative but it has been shown that both of these scars are involved in a remodeling process at an accelerated pace, which ultimately results in a tissue closely resembling that of normal skin.[2] It can be assumed that some of these enzymes required for nodule degradation, namely collagenase, hyaluronidase, etc., may be major products of this overall synthetic activity undertaken by the fibroblasts, and that the pressure treatment alters the distribution and/or effectiveness of the enzymatic products to favor nodule degradation. This degradation is first noted as a fenestration of the fused collagen masses found within the scar.[2, 4, 20] This fenestration is probably due to the enzymatic degradation of the mucopolysaccharide ground substances that commonly bind the collagen fibers into mats and/or nodules.[2, 4, 20] Similarly noted are the alterations of the omnidirectional collagen arrays within the mass into fibers that approach near-normal configurations. This alteration would be in direct contrast to the more typical enzymes and cellular products found in developing scars which unquestionably are involved in nodule formation and/or tissue enlargement. In the work of Eisen et al.,[8] the activity of in vivo collagenase was suppressed by the presence of serum, in particular in alpha[1] and alpha[2] M globulin component. In fact, the presence of tissue collagenase had to be demonstrated by means of in vivo immunologic reactions for the presence of even slight concentrations of the α-globulins during any phase of the extraction procedures inhibited the action of the enzyme and rendered it ineffective. These observations were further substantiated by Cohen et al.[5] Several observations of serum proteins in burn patients have demonstrated an increase in the level of circulating blood α_2-globulins in patients as long as 60 days post burn.[7, 33] Thus, the nonpressure-treated wound area may not be able to undertake collagenase mediated remodeling due to the high levels of the α-globulins. Pressure treatment on the other hand undoubtedly reduces the blood flow into the scar.[4] Concomitantly the reduction in blood volume also reduces the availability of α-globulins. Thus any collagenase mediated remodeling could occur at a more rapid rate in pressure-treated areas

due to the lower levels of α-globulins. The enzymes required for degradation of the mucopolysaccharides found in the hypertrophic scars may similarly be affected. Pressure treatment likewise reduces edema in the scar. This loss of tissue water could play an important role in the production and/or release of mucopolysaccharides by the mast cells. Thus, it appears that hypertrophic scar maturation, either accelerated by pressure or natural, involves in part the altered production, degradation, and/or alteration of the dermal components (collagen and ground substances).[4]

Cosmetic Features

Unfortunately, the epithelium of a scar resulting from the repair of a wound more severe than one caused by a moderate second degree burn seldom regains a natural or skin-like configuration. We have examined the epidermal layers of numerous burn scars and it has been our observation that the differentiated epidermal cells reform an epidermis that appears to be complete with respect to: size or thickness, tissue layers, cell populations, cell fine structure, cell junctions, etc. Likewise at the termination of repair the scar dermis is eventually comprised of components such as collagen fibers, elastin, capillaries, cells, etc., that differ but slightly (size and orientation) from those observed in normal skin. Apparently the simple lack of rete-pegs,[47] the stratum papillaries, and the formation of a straight epidermal-to-dermal junction are architectural alterations that render the new epithelium cosmetically abnormal with respect to its color, hue, friability, and texture. Occasionally pseudo rete-pegs are formed in the tissue but they lack the configurational and periodic aspects of those seen in normal skin.[32, 47]

Summary

Animal models of burn wound healing have not been established to any extent due to the complexities of establishing a "standard trauma" even though studies of wound repair in animals have been extensive.[21, 35-38, 46] Moreover, the wound healing of a human subject appears to differ somewhat from similar wounds of any animal model, which may be a simple anatomic difference.[32] This can be best illustrated by the fact that animals do not appear to form hypertrophic scars, keloids, and/or many deforming scar contractures. Likewise the cosmetic features of a healed wound in any animal appears to be better than that achieved by man. Thus the human burn wound healing process can best be studied only in man. Because of this, the pertinent cytologic, biochemical, and physiologic research cannot be conjured or planned but must depend upon the availability of victims willing to participate in research endeavors. Such research is undoubtedly warranted because of the frequent occurrences of burn injuries and their high incidence of morbidity. Moreover, burn victims and their physicians realize that the healing process is seldom cosmetically successful.

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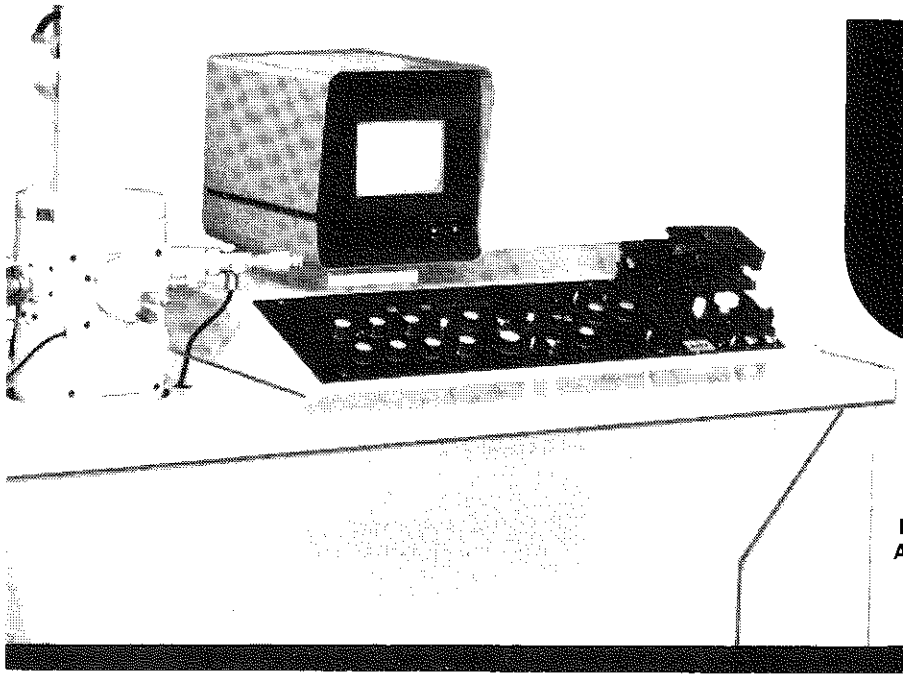

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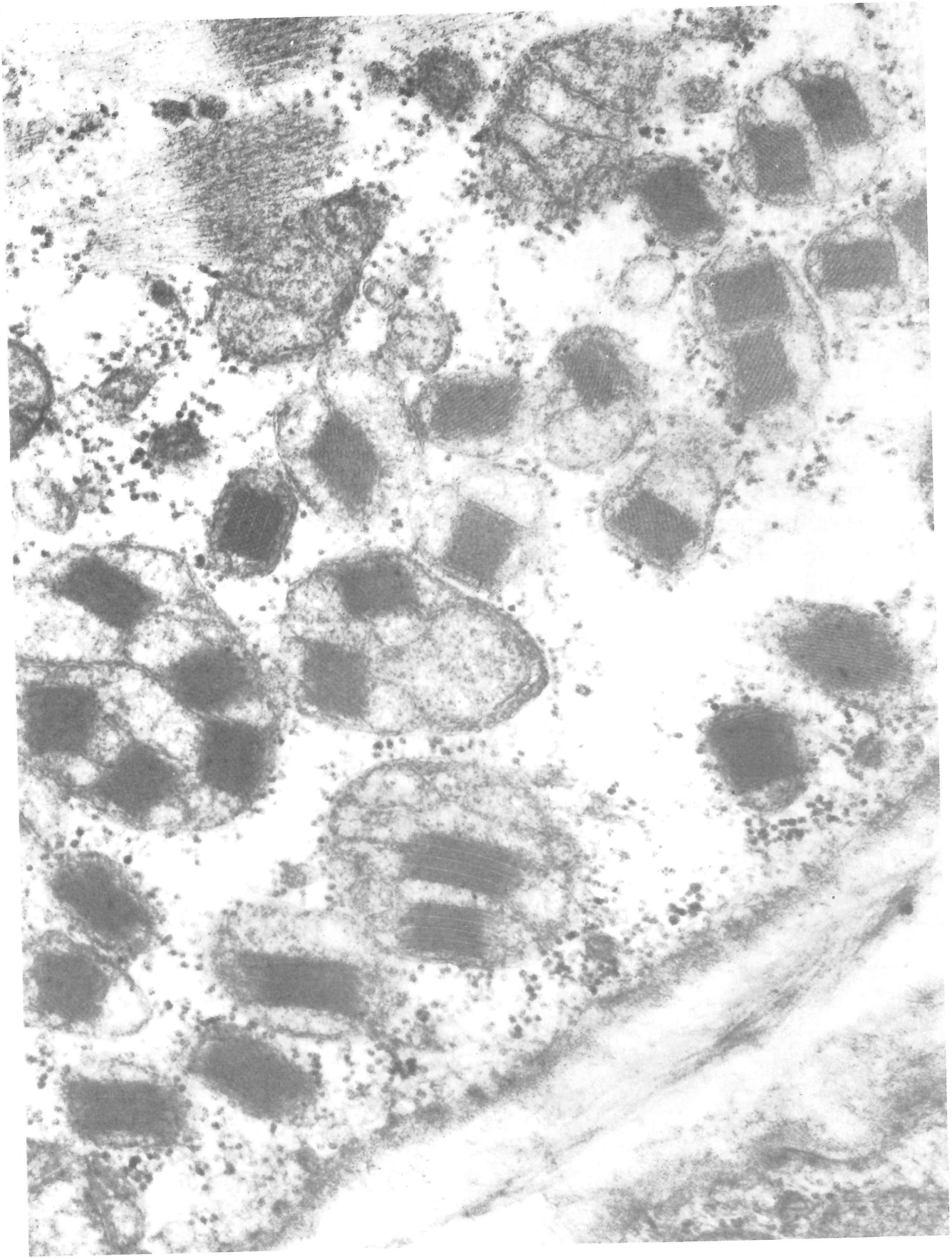

MITOCHONDRIAL MYOPATHY

Paracrystalline inclusions within mitochrondra are found abundantly in this biopsy from a case of progressive opthalmoplegia. The micrograph is from the Department of Neurology, Baylor College of Medicine, with the scientific collaboration of Dr. Bernard M. Patten and Lena Wai-Fong Chu

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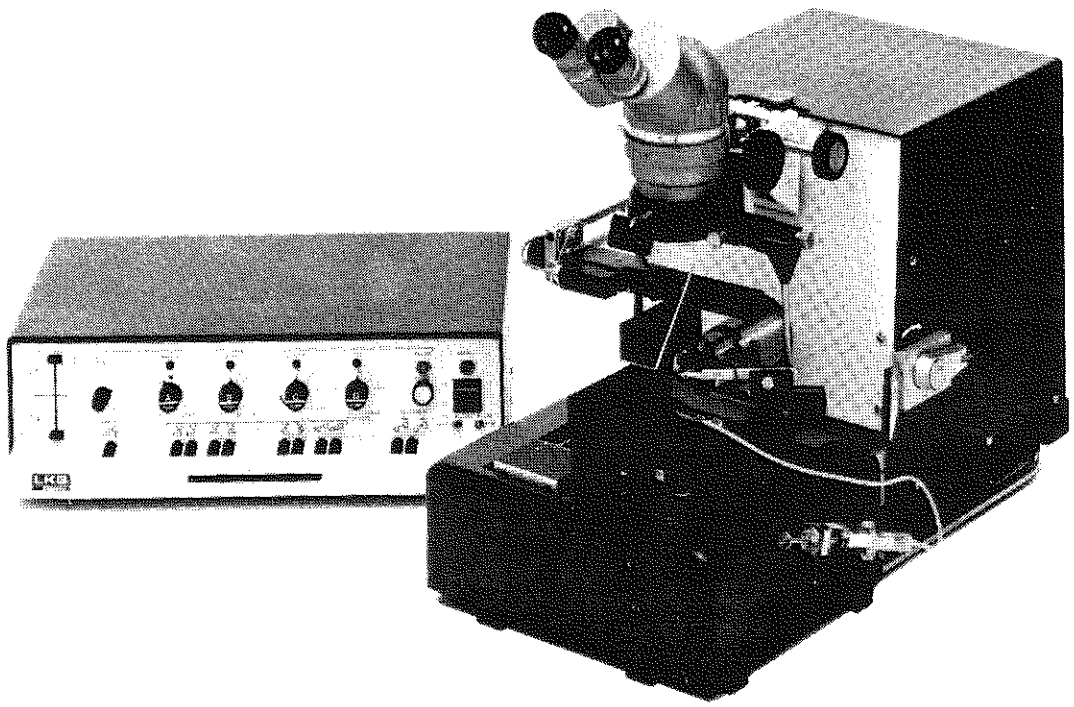

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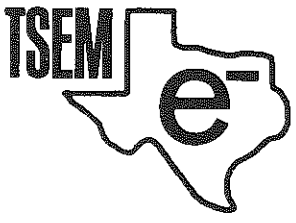
Situation Wanted — Eslie M. B. Sorensen, Ph.D. 1974 (Zoology). Currently post-doctoral appointee with Div. Biol. and Med. Res. Argonne Nat'l. Lab., Argonne, Ill. 60439. (312) 739-7111 (Ext. 5418). Primary research interest: toxicity, accumulation, and sebcellular transport and localization of heavy metals; experience in therapeutic decorportation of metals from mammals. Analytical techniques used: neutron activation analysis and radioisotopic labeling. Experience in TEM, SEM, and XES, as well as autoradiology, semiquantitative ultrastructural analysis, contact radiography, histology, and histochemistry. Desires position in research and/or teaching. Reference and curriculum vita are available.

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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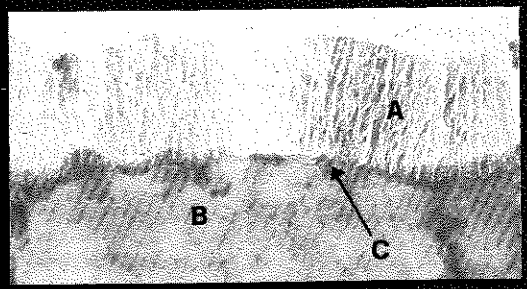
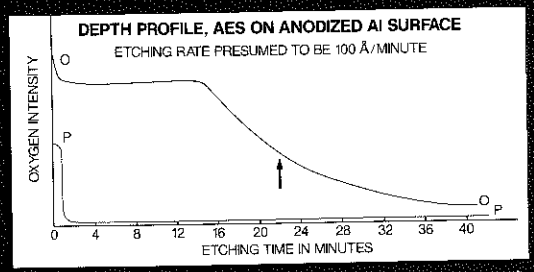
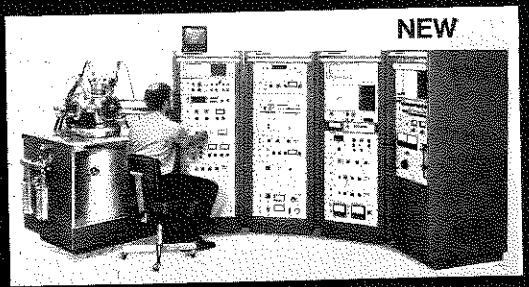


TEM thin-section of anodized Al surface, made specifically to reveal the thickness of the anodized layer, its structure and morphology, and to gain some insight into its uniformity. From TEM results, the image thickness is measured to be about 2000 Å. Arrows denote (A) Anodized layer, (B) substrate aluminum, and (C) interface.



Auger electron spectroscopy (AES) depth profile into anodized aluminum layer using etching rate previously calibrated and presumed to be 100 Å/minute in Al_2O_3. The time for oxygen to drop to one-half its original intensity was 22 minutes, or an implied 2200 Å, which was in good agreement with the TEM results. Broad interface region can be clearly attributed to surface roughness and variation in anodization thickness. P is confined to top 100 Å or less.



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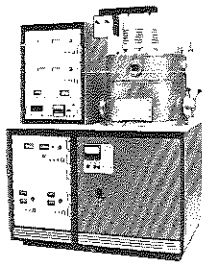

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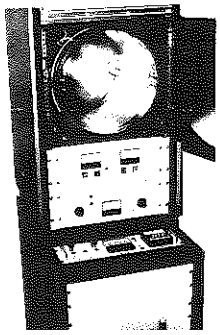

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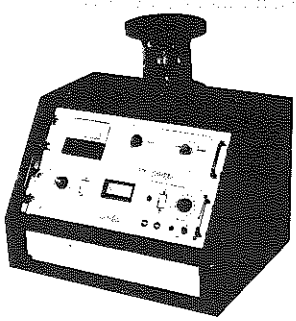

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Abstracts

THE ULTRASTRUCTURE OF VEGETATIVE MITOSIS IN THE DIPLOID GENERATION OF CLADOPHORA FLEXUOSA. Ruth E. Lewis, Electron Microscopy Center, Texas A&M University, College Station, Texas.

Vegetative mitosis in the diploid generation of **C. flexuosa** was studied with the electron microscope as part of a larger research effort aimed at deducing the phylogenetic affinities of this species. Nuclei of these coenocytic cells display asynchronous division within closed spindles. The nucleolus, although persisting throughout the cycle, becomes increasingly diffuse from prometaphase through early anaphase and regains a more compact structure throughout the remainder of the cycle. Paired protecentrioles, unique to metaphase, are observed slightly displaced from the poles. Anaphase nuclei reveal distinct trilaminar kinetochores and elongation of the interzonal spindles as chromosomes are pulled to the poles. The mitotic process culminates in the separation of the two daughter nuclei from the interzonal spindle.

SOME EFFECTS OF CROWDING STRESS ON HEXACHLOROBENZENE TOXICITY, Hilton H. Mollenhauer, Marcel H. Elissalde, and Donald E. Clark, Veterniary Toxicology and Entomology Research Laboratory, ARS, USDA P. O. Drawer GE, College Station, Texas 77840.

The effects of social stress (crowding) on hexachlorobenzene (HCB) toxicity in rats were evaluated by a morphometrical analysis of adrenal cortex mitochondria. Eighty male rats were placed individually in molded plastic cages with 1000 cm^2 of floor space per rat for 28 days. The food for one-half of the animals (40) was supplemented with 250 ppm HCB. On the 29th day, one-half (20) of the rats from each diet group were placed, 4 rats per cage, in molded plastic cages with 100 cm^2 of floor space per rat. On days 30, 31, 33, 36, 39, 4 rats from each group were killed and the adrenal cortices processed for electron microscopy. No gross structural aberrations were observed in any of the tissues. No significant differences in mitochondrial volume, surface area, or cristae area were observed in HCB treated or in stressed animals as compared to the controls. Significant increases in total mitochondrial volume and diameter were observed only in those animals simultaneously exposed to HCB treatment and crowding. The data show that even a simple social stress such as mild crowding may significantly alter a physiological response to toxicant exposure.

INTERFERENCE WITH CELL WALL ASSEMBLY IN THE URTICA PILLULIFERA STINGING CELL BY DIMETHYL SILICIC ACID. Arthur E. Sowers and E. L. Thurston, Electron Microscopy Center, Texas A&M University, College Station, Texas 77843.

Despite much research, the specific physiological role(s) of silicon in higher plants is still open to debate. Our approach to the problem utilized hydroponically grown plants fed with chemical analogs of silicic acid (SA), the form of silicon generally believed to be absorbed by all plants. Experiments were conducted using the silica-depositing stinging cell of the Stinging Nettle plant **Urtica Pillulifera**. In this study a total of seven analogs were added, either alone or along with the normal substrate (SA), to the hydroponic solution and the effect on the ultrastructure of the cell wall and cytoplasm observed by TEM. In certain combinations with SA, one of these analogs, dimethyl silicic acid, caused (a) unusual alterations in OsO_4 affinity in the stinging cell wall and (b) two new osmiophillic structures to accumulate in the cytoplasm and cell wall. One of the new structures is fibrilar and the other is spherical. The fibrilar structures are about 30-40A in diameter and up to 1000A in length. The spherical structures were either hollow or structured internally and measured about 1000A in diameter. The internally structured spheres have a constant diameter and appear to be made up of subunits in a regular array while the hollow spheres have a variable diameter. The alterations of OsO_4 affinity are accompanied by a range of structural alterations, on several levels of organization, that display components with structural uniformities and regularities. These results indicate not only that a silicon-containing compound plays a critical role in cell wall assembly but also that dimethyl silicic acid may be a chemical useful for the study of cell wall assembly mechanisms and processes.

SEM OF BONE AND TEETH IN THE STUDY OF PRIMATE EVOLUTION. Robert W. Rice, Ordean J. Oyen, and Allen Walker*, Dept. of Human Anatomy and Anthropological Research Laboratories, Texas A&M University, College Station. (* Dept. of Anatomy, Harvard University)

Anthropological and paleontological studies of the origins and evolution of man traditionally evaluate the hard tissues capable of withstanding the passage of time, i.e. bones and teeth. These analyses, however, characteristically generate only static measurements of lengths, surface areas or volumes. Statistical analysis of these values determines their validity within a population or reveals comparative features among related forms. From such data hypotheses have been developed concerning the morphological evolution of modern man. Recently interest has developed in interpreting the functional determinants of that evolution as recorded in the fossil remains. Patterns of locomotion, dietary habits and nutrition, state of health and behavioral features (e.g. aggressive tendencies) are some of the lifestyles mirrored by the anatomical organization of bones and teeth. However, the structural manifestations of these activities are sometimes appreciated only over large areas and/or at high magnification. Conventional light microscopy presents a thin section or a small area at low magnification. Thus the methodology of scanning electron microscopy offers great promise as an analytic tool in reconstructions of the emergence of man. This presentation will demonstrate examples of SEM identification of biological and behavioral processes in extinct and extant forms. Such data will likely generate the formulation of new and the reevaluation of old hypotheses concerning the continuity of structure and function between our forebears and ourselves.

SCANNING ELECTRON MICROSCOPE STUDIES ON THE BIOLOGY OF LARVAE AND EGGS OF THE IMPORTED FIRE ANT, SOLENOPSIS INVICTA BUREN. Ronald S. Petralia, Department of Entomology, Texas A&M University.

The scanning electron microscope was used to examine chemically fixed and live larvae and eggs of the imported fire ant, **Solenopsis invicta**, in an attempt to define optimum morphological preservation. Living specimens were free from artifacts present in chemically fixed specimens.

Larval development can be characterized into four stages based on mandible and labial denticle morphology, and by the frequency and structure of body hairs. The morphological changes in larval development can be correlated with observed changes in larval-worker interactions in the colony, and in larval feeding behavior. Thus, the various types of curled body hairs of the third and fourth stage larvae hook larvae together in piles to fascilitate the transport and storage of larvae by workers in the colony. The adhesive coating of the eggs has a similar function. The specialized straight hairs of the ventral anterior body region of fourth stage larvae form a feeding basket for holding solid chunks of food, as they have become specialized for feeding on solid food, whereas earlier stages have a semi-solid diet.

STRUCTURAL AND FUNCTIONAL CHANGES IN CHROMATIN AS VISUALIZED BY ELECTRON MICROSCOPY OF THIN SECTIONS. Ivan Cameron, Thomas Pool, Frank Weaver and James Jeter, Jr., Department of Anatomy, The University of Texas Health Science Center at San Antonio and Tulane University Medical School.

Isolated chicken erythrocyte nuclei and tissues from several vertebrate and invertebrate species were fixed, block stained with uranyl acetate (UA), sectioned and stained with UA and lead citrate. The isolate nuclei were extracted sequentially with 0.15 N NaCl, 0.25 N HCl, phenol, and hot 5% SDS in addition to being water swollen during each step. Measurements of fiber sizes were made on such chromatin as well as on dispersed and condensed chromatin **in situ** and during spermatogenic cell stages in armadillos. The native chromatin fiber is 20 +nm in diameter in all chromatin. Fibers of 7 to 10 nm are created by swelling of nuclei. A 2 to 4 nm omnipresent fibril is present as the basic structural unit which is packaged into the larger chromatin fibers. The 20 +nm fiber increases in size in dispersed or euchromatin due to a looser packing of the same number of 2 to 4 nm fibril, whereas, the increase in 20 +nm fibers seen during spermatogenesis is due to an increase in number of fibrils. These observations will be related to the nucleosome concept of chromatin organization. Supported by USPHS grant no. CA16831.

RHABDOMYOSARCOMA, B. Mackay, A. G. Ayala, W. W. Sutow, M. D. Anderson Hospital and Tumor Institute, Houston, Texas.

Rhabdomyosarcoma is relatively common among the sarcomas, particularly in younger patients; but the diagnosis is often made without acceptable histologic evidence. Pleomorphic, alveolar, and embryonal forms have been identified. However, many supposed pleomorphic rhabdomyosarcomas in older patients are malignant fibrous histiocytomas, an alveolar pattern may be presented by a variety of neoplasms, and other small round cell tumors must be considered in the differential diagnosis of embryonal rhabdomyosarcoma. Demonstration of skeletal muscle myofilaments is the principal criterion for the identification of rhabdomyosarcoma, but the myofilaments must form myofibril fragments of moderate size to be resolved by light microscopy.

When a sufficient number of rhabdomyosarcomas is studied by electron microscopy, it is realized that myofilament formation is commonly sparse and frequently absent, even in tumors which can be classified by light microscopy. Ultrastructural demonstration of myofilaments may, nevertheless, be the only means whereby the diagnosis can be achieved. Selected cases from a series of 80 rhabdomyosarcomas will be shown to demonstrate the spectrum of fine structural appearances and illustrate problems in differential diagnosis. The findings from this study support the view that rhabdomyosarcoma is frequently misdiagnosed, and reveal that primitive forms of other mesenchymal neoplasms also occur in the pediatric age group. Correlated light and electron microscopy is consequently advisable in all cases of suspected rhabdomyosarcoma.

REFLECTORS IN THE LIGHT ORGAN OF ANOMALOPS (ANOMALOPIDAE, TELEOSTEI). M. Watson, E. L. Thurston and J. A. C. Nicol, Texas A&M University, College Station and The University of Texas, Austin, Texas.

The suborbital light organ of **Anomalops** contains luminous bacteria which are housed in tubules perpendicular to the outer surface. It has two relfectors, one is opaque and lies behind the tubules, and the other is differentially transparent and lies in front of the tubules. The internal reflector forms a concave backing to the organ and is covered by a thick pigmented skin. It is a thick layer of reflecting cells containing stacks or packets of hexagonal guanine crystals. Light is reflected in a diffuse fashion.

The external reflector lies at the lower ventral edge of the outer face of the organ and consists of a layer of cells containing a row of thin crystals lying oblique to the surface. It is a specular reflector which directs the luminescence outwards and upwards and dims light emission ventrally.

In both reflectors the crystals form a system of quarter wavelength films, reflecting light by constructive interference. The crystals are enclosed in membrane-bounded chambers which lie in crystal sacs. These and other features of the light organ are compared with those of other luminescent fish.

LIGHT AND ELECTRON MICROSCOPY OF THE UROPYGIAL GLAND OF THE EASTERN BOBWHITE QUAIL COLINUS VIRGINIANUS VIRGINIANUS. Ken E. Hannah, Department of Biology, Stephen F. Austin State University, Nacogdoches, Texas 75962.

The uropygial gland (oil gland) of birds is a bilobed sebaceous gland located dorsally and medially on the rump of the bird. In **Colinus virginianus virginianus** the gland is separated into two lobes by the muscular and connective tissues of the isthmus. Each lobe contains numerous secretory tubules which surround and empty into a large central cavity. A dark staining layer of basal cells lines each tubules. Basal cells are characterized by the presence of rough endoplasmic reticulum. Basal cells give rise centripetally to secretory cells which migrate toward and eventually empty their contents into the lumen of the tubule.

Several different secretory stages can be identified on the basis of ultrastructural changes occurring as the cells migrate toward the lumen of the tubule. Secretory cells are characterized by the presence of numerous strands of smooth endoplasmic reticulum with dilated cisternae. Changes in the perinuclear cisternae were also observed in these cells. As the cells move closer to the lumen oil droplets appear and become progressively larger until the cellular organelles become tightly packed between the droplets. Secretion is accomplished when the cell membrane disintegrates releasing the secretion products and cellular debris into the lumen of the tubule.

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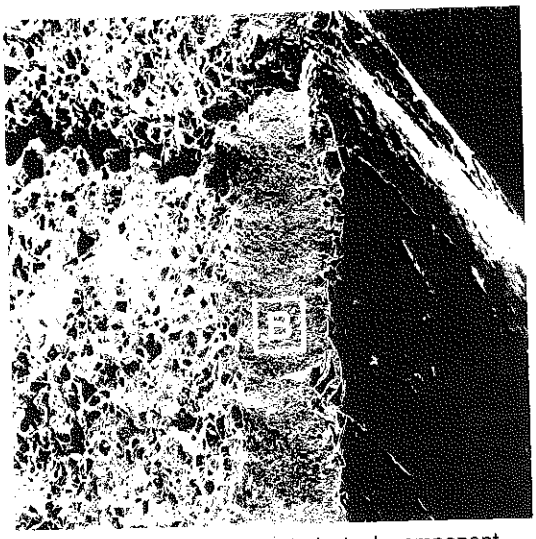
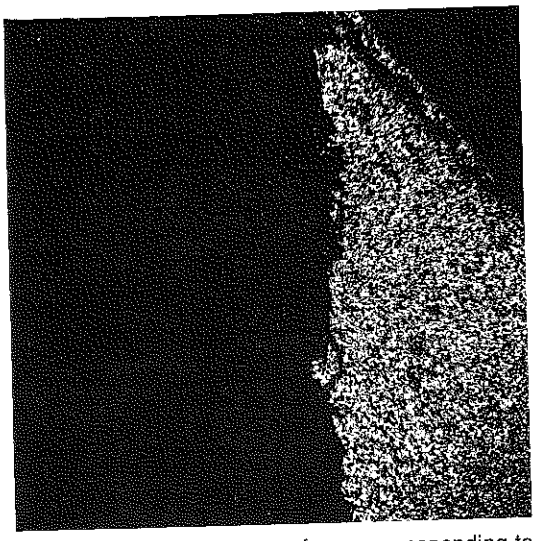
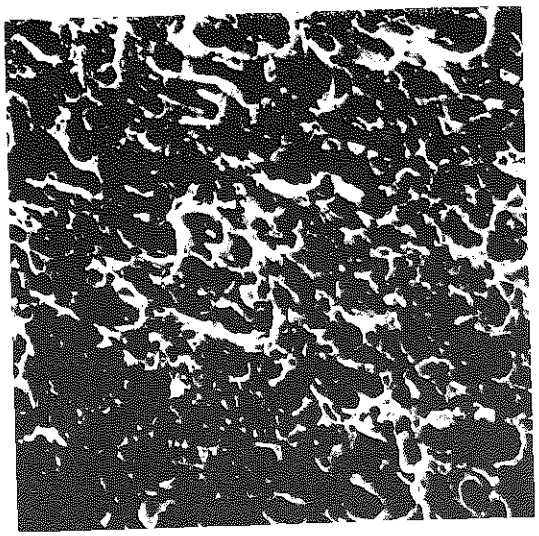


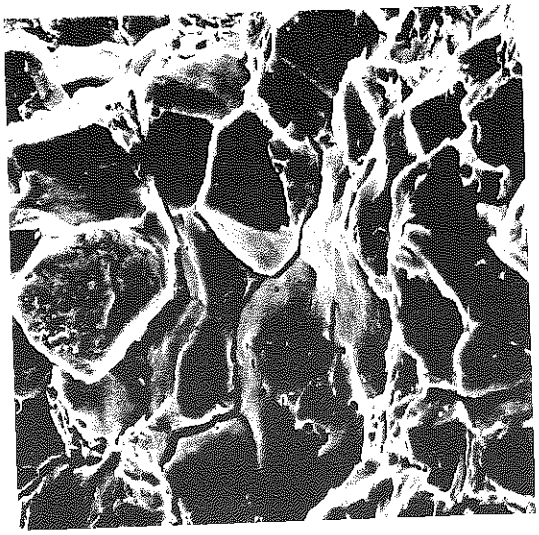
Fracture topography of plated steel component — area A failed in brittle fracture while area B failed in a ductile manner. 100X



Zinc x-ray distribution map of area corresponding to plating. 100X



Closeup of area B showing dimple rupture of plating. Note spherical second phase particles located within some dimples. 2000X



Closeup of area A shows intergranular cracking due to hydrogen embrittlement caused by improper bakeout after plating. 1600X

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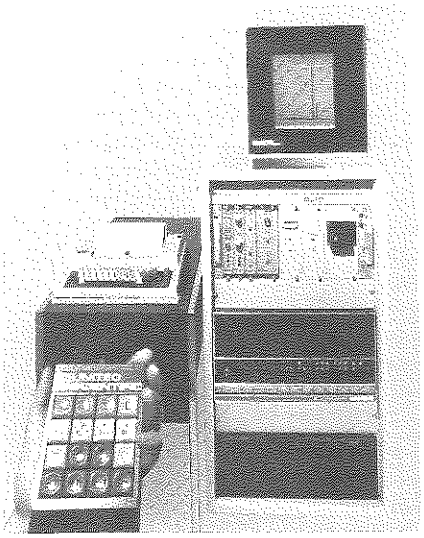

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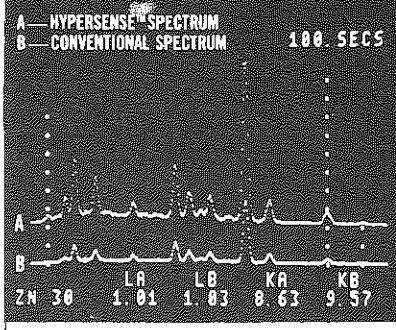

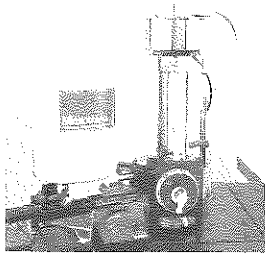

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UNITED SCIENTIFIC
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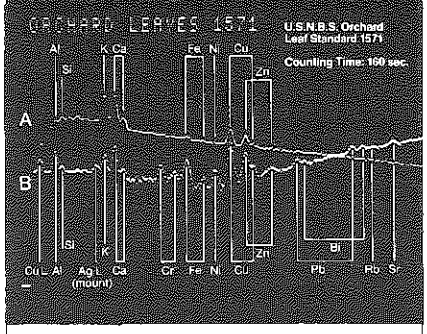
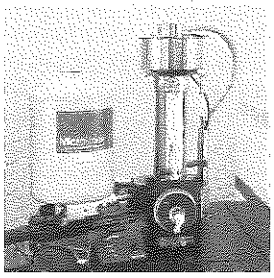
Six hidden elements revealed by new X-ray analyzer





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Regional News

NEW ORLEANS: Tulane Anatomy.

Dr. I-Li Chen has recently received a Schleider Foundation grant. Dr. Chen is pursuing ultrastructural studies in the area of the male reproductive system as well as the production of erythropoetin by the kidney.

The Department of Anatomy recently added a new faculty member, Dr. Frank Olivito. Dr. Olivito is an electron microscopist/neuroanatomist interested in the effects of protein deficiency on the central nervous system.

Joe A. Mascorro is now a graduate student in the Department of Anatomy at the LSU School of Medicine. He is pursuing a PhD degree in Anatomy but still maintains a part time position as Instructor at Tulane Anatomy. (The LSU people say they expect great things from the Super-Mex.).

Dr. Robert D. Yates, Professor and Chairman, recently edited a book published by Masson Publishers. The book is entitled "The Male Reproductive System," and is co-edited by Mildred Gordon from the New York State University at Buffalo.

Recent publications: "The anatomical distribution and morphology of extraadrenal chromaffin tissue (abdominal paraganglia) in the dog." Joe A. Mascorro and Robert D. Yates. Tissue & Cell. 9: 449-462, 1977.

"Morphological characteristics of the abdominal paraganglia in the adult Rhesus monkey." Joe A. Mascorro, Peter M. Klara, and Robert D. Yates. EMSA Proceedings, In press. (Abstract).

LUBBOCK: Texas Tech University School of Medicine, Department of Anatomy.

Members of the Department of Anatomy are saddened at the resignation of Dr. J. Richard Hillman from his faculty position at Texas Tech. Dr. Hillman recently obtained his M. D. degree, and has chosen to enter a residency in the Department of Pediatrics at the University of Colorado Medical School. Since he personally has been responsible for a large portion of the current facilities available within the Department of Anatomy, it is with much regret that we see him go, but we wish him success in his future endeavors.

Dr. Roger Markwald recently received a grant from the Texas Heart Association and was presented the Lyndon Baines Johnson Research Award: to quote from the award, "In recognizing the outstanding research project in Texas towards the eradication of cardiovascular disease." Dr. Markwald attended an awards dinner in his honor on June 11, 1977, at which he was recognized for his achievement in heart research. Specifically, Roger's grant received the highest rating of any of the grants submitted for this year in the state of Texas.

Dr. Robert L. Casady was recognized for outstanding achievement in teaching by the graduating class of 1977. He was given the award for being the outstanding professor in the basic science years.

Mrs. Judy Land received her Master's Degree in Anatomy and is currently enrolled in the freshman medical class at the University of Texas at San Antonio. We wish her success in her future endeavors towards a career in medicine.

Research Awards: Dr. Roger Markwald was recently awarded a grant from the Texas Affiliate of the American Heart Association. His grant is entitled "Macromolecular Ordering in Cardiogenesis."

Dr. John A. Yee has been awarded a continuation of his grant from the National Institute of Health for his grant entitled "Cytodifferentiation of PDL Fibroblasts."

Publications: Hutson, J. C., P. J. Gardner and G. C. Moriarty. 1977. Immunocytochemical localization of a follicle stimulating-like hormone in the testis. J. Histochem. Cytochem.

Hutson, J. C. 1977. Effects of various hormones on the surface morphology of testicular cells in culture. Amer. J. Anat.

Casady, R. L. and J. R. Hillman. 1977. The relevancy of anatomy and other basic sciences to the practice of medicine. J. Med. Educ. 52:210-211.

Dalley, B. K., F. F. Bartone, and P. J. Gardner. 1977. An Autoradiographic study of smooth muscle hyperplasia in the swine ureter. Invest. Urol. 14:478-481.

Markwald, R. R., T. P. Fitzharris and F. J. Manasek. 1977. Structural development of endocardial cushions. Am. J. Anat., 148:85-120.

SAN ANTONIO:

Grants Awarded: Dr. John Hansen, Department of Anatomy, American Heart Association Grant-in-Aid, "Innervation of the Aortic Bodies" (3 years).

Mr. David Heitman, Department of Anatomy, "Control of cell proliferation in epithelium of normal colon and in induced carcinomas of the colon."

Dr. Thomas B. Pool, Department of Anatomy, Institutional Grant, "Role of nutrition in structure and function of submandibular salivary gland of the rat."

Articles: Weaker, F. J., 1977. Spermatogonia and the cycle of the seminiferous epithelium in the nine-banded armadillo. Cell Tiss. Res. 179:97-109.

Herbert, D. C., J. Schuppler, A. Poggel, P. Gunzel and M. F. El Etreby, 1977. Effect of cyproterone acetate on prolactin secretion in the female rhesus monkey. Cell Tiss. Res. (In Press).

Rennels, E. G. and D. C. Herbert, 1977. Stimulation of prolactin secretion by estrogen and androgens in PMS-HCG treated immature rats. Biol. Reprod. (In Press).

Cameron, I. L. and T. B. Pool, 1977. Chromatin fiber differences between condensed and dispersed chromatin: a comparative ultrastructure study. Cytobios. (In Press).

Cameron, I. L., W. J. Ackley and W. Rogers, 1977. Responses of hepatoma bearing rats to total parenteral hyperelimination and to **ad libitum** feeding. J. Surgical Res. (In Press).

Ishikawa, H., M. Shiino, A. Arimura and E. G. Rennels, 1977. Functional clones of putuitary cells derived from Rathke's pouch epithelium of fetal rats. Endocrinol. 100:1227-1230.

Ishikawa, H., M. Shiino and E. G. Rennels, 1977. Effects of fetal brain extract on the growth and differentiation of rat pituitary anlage cells (39840). Proc. Soc. Exp. Biol. Med. 155:511-515.

Shiino, M., H. Ishikawa and E. G. Rennels, 1977. **In vitro** and **in vivo** studies on cytodifferentiation of pituitary clonal cells derived from the epithelium of Rathke's pouch. Cell Tiss. Res. (In Press).

Hansen, J. T., 1977. Spontaneous atheroslcerosis: An ultrastructural study in the White Carneau pigeon. Virchows Arch. A. Path. Anat. Histol. (In Press).

Southwest Research Institute.

Presentations/Publications: D. L. Davidson and J. Lankford. "The Influence of Water Vapour on Fatique Crack Plasticity in

Low Carbon Steel." Fourth International Conference on Fracture, Waterloo Ontario, June 22, 1975. Paper also published in the proceedings.

J. Lankford. "Twinning and Microcrack Nacleation During Indentation of Aluminum Oxide." Scripta Metallurgia 11. 1977, P. 349.

News Briefs: Dr. Alexis Burton has relinquished his teaching responsibilities within the Department of Anatomy but will continue his film making activities as an Adjunct Professor of Anatomy. Dr. Burton also received a CINE Eagle certificate award for his teaching film "Early Development of the Embryo."

The Anatomy Department presented and/or co-authored 27 papers at the Annual Meeting of the American Association of Anatomists in Detroit. The number of papers presented was the most by any department in the country.

GALVESTON: University of Texas Medical Branch.

Department of Anatomy — Dr. Donald Duncan was recently awarded a two year grant from the Institute of Neurological Diseases and Stroke of NIH. The grant was funded at $37,000 and is entitled "Fine Structure of the Substantia Gelatinosa in the Cat."

Division of Cell Biology — Dr. Paul S. Baur has received a second year renewal for his grant "Structure of Baro-Receptors in Hypertension." The amount awarded was for $33,606 from the Institute of Heart, Lung and Blood of the NIH.

Dr. Jeffrey P. Chang was awarded a research contract from Rohm and Haas for a study of the "Low Level Effect of Ethylenethiourea and Related compounds."

Department of Microbiology — Dr. Pat Davis has been invited to speak at the International Symposium of Microbial Ecology. The UNESCO sponsored meeting will be held on August 22-26 at Dunedin, New Zealand. The title of Dr. Davis' talk is "Normal Flora of Dogs."

Publications: Yokoyama, M., Chang, J. P., Hom D. and Wilson, Sandra L., An EM cytochemical study on concanaval in A binding sites and their mobility in normal and cystic fibrosis fibroblasts **in vitro**, Pediatric Research, 11: 765-769. (1977).

Moller, P. C., Yokoyama, M., and Chang, J. P., Ultracytochemical localization of Glucose-6-Phosphatase in Chang rat hepatoma **in vivo** and **in vitro**. J. Natl. Cancer Inst., 58:1401-1405. (1977).

Cox, Susan M., Baur, P. S., Haenelt, B., Retention of the glycocalyx after cell detachment by EGTA. J. Histochem. Cytochem. (In Press).

Baur, P. S., Brown, A. M., Rogers, T. D., Brower, M. E. 1977. Lipochondria and the light response of **Aplysia** giant neurons. J. Neurobiology, 8:19-42.

Wiktorowicz, J., Srivasta, S. K., Baur, P. S. 1977. Introduction of liposomesequestered human hexosaminidase A and ferritin into lysosomes of mouse machrophages. Cytobiologie, 14:401-412.

HOUSTON: University of Texas Health Science Center.

The Neurobiology and Anatomy Department at the University of Texas Medical School at Houston has moved from the Center Pavilion Hospital to the John H. Freeman Building. With this move has come many changes. Dr. Richard Peterson, Associate Professor, will be leaving August 31 to join the Department of Anatomy, Indiana University School of Medicine, Indianapolis, Indiana. Leslie Arwin, Teaching Assistant, will be leaving the Department August 9, to become a Freshman at the University of Michigan Medical School in Ann Arbor. Susan Meixner, presently Administrative Assistant, has accepted the faculty position of Teaching Assistant left vacant by Ms. Arwin's move.

Neurobiology and Anatomy Department has had three visitors and all have accepted faculty positions for this coming Fall. Leonard D. Aldes, Ph.D., from Colorado State University, Department of Anatomy, presented a seminar on "Cerebellar Control of the Primate Tongue" on June 20. Michael N. Oberdorfer, Ph.D., from the University of Wisconsin, Madison, Anatomy Department, presented a seminar on "Normal and Misrouted Visual Pathways in Mink" on June 2. Jo Ann McConnell, Ph.D., from the University of Arizona, College of Medicine, Department of Anatomy, presented a seminar on "Early Neurogensis in the Mouse and Chick" on June 16. We all welcome and look forward to their coming this Fall.

Dr. S. J. Enna presented papers May 13th to the Texas Medical Association Research Forum in Houston, and to the Texas Pharmalogical Society in Galveston. Between May 28th and June 3rd he presented a symposium lecture at the American Chemical Society meeting in Montreal, a lecture at the National Institute of Health in Washington, D. C., and he consulted on research projects at Johns Hopkins Medical School in Baltimore and at Merck Sharp & Kohme Research Laboratories in West Point, Pennsylvania. On June 21, Dr. Enna gave a lecture at ICI-USA Pharmaceuticals, Inc., in Wilmington, Delaware and he has been invited to make a presentation and chair a panel discussion in GABA receptors at the NATO Advanced Study Institute Symposium to be held in Synnfjell, Norway, August 14 to 19. He has also been asked to lecture on GABA pharmacology at Hoffman-LaRoche in Basel, Switzerland on August 12.

Dr. Yvonne Clement-Cormier presented papers in New Orleans, Louisiana at the Third International Conference on Cyclic Nucleotides, July 17 through 22. She attended scientific meetings at the University of Talahassee, Florida on July 27 through 29 and will present papers at the Fall meeting of the American Society for Pharmacology and Experimental Therapeutics August 21 through 25.

Dr. Richard Peterson presented a paper in Duluth, Minnesota on June 1 and 2, and June 6 through 9 he prepared a monograph for the Solvent Abuse Conference, a monograph symposium, in San Francisco, California. Dr. Peterson presented papers in Indianapolis, Indiana, on June 13 and 14 and in Arlie, Virginia on August 11 through 14 he will attend the Peripheral Nerve Study Conference.

Dr. Dianna A. Redburn received a Research Career Development Award from the National Eye Institute. This is awarded for five years and will pay her salary plus indirect costs. The purpose of this award is to support young investigators with high potential for a career in biomedical research and is available only to those investigators who already have an active research grant from the National Eye Institute. This award will allow Dr. Redburn to be relieved of some of her administrative and teaching duties and to devote more time to her research in Characterization of Neurotransmitters in Retina.

Dr. Richard Wiggins attended the American Society for Neurochemistry meetings in Denver, Colorado March 13 through 18 where he presented a report on "Regional Brain Labeling by Intracranial or Intraperitorial Injection." He also attended the American Society for Microbiology meetings in New Orleans, Louisiana May 10 and 11, and participated in a round table discussion on "Current Advances in Liquid Sintillation Countries." Dr. Wiggins also attended the American Association of Anatomists Meetings in Detroit from May 1 through 5.

Dr. Joe G. Wood, Chairman of the Neurobiology and Anatomy Department, attended the American Association of Anatomists Meetings in Detroit from May 1 through 5. Dr. Wood also attended the annual board meeting of the Anatomical Board in Galveston on May 13.

COLLEGE STATION: Texas A&M University.

Grants Awarded: Walter M. Kemp, Department of Biology, Edna McConnel-Clark Foundation-"Studies of Immunoglobulins Associated with the Tegument of **Schistosoma mansoni**" $86,000. and W. M. Kemp, Department of Biology, Institutional Biomedical Research Grant from NIH. "Studies of Antigens Shared Between **Schistosoma mansoni** and its Intermediate Host, Biomphalaria Pfeifferi," Total $48,000.

Presented Papers: McArthur, N. H. and P. J. Ives, Department of Vet. Anatomy, "Transmission and Scanning Electron Microscopy of the Proximal Neurohypophysis (PN) of the Armadillo **(Dasypus novemcinctus L.)**". Ninetieth Annual Meeting of the American Association of Anatomists, Detroit, Michigan.

Droste, Theresa E., Department of Entomology, "The Morphology of the Ventral Eversible Gland of 5th Instar Walnu Caterpillar **(Lepidoptera notodontidae)**." Joint Meeting of S. W. & Pacific Branches of Entomological Society of America and Ento. Soc. Mexico, Guadulujara, Mexico.

Ingham, E. R., Department of Biology, "Limited **In vitro** Culture of Cristispira, an Oyster Symbiont," Annual Meeting of the American Society of Microbiology, New Orleans, Louisiana.

Rosier, J. G., Department of Biology, "Comparative Morphology of the Tegument of **Schistosoma mansoni, S. japonica,** and **S. haen** as viewed with SEM and TEM," S. W. Association of Parasitologists, Oklahoma City, Oklahoma.

Taranto, M. V., Department of Food Science, "Microscopic Structure of Textured Fortified Cottonseed Flours" and "Texturized Soy and Cottonseed Flours: A Microscopic Analysis" both co-authored by G. F. Cegla and K. R. Bell, Annual Meeting of the Institute of Food Technologists, Philadelphia, Pennsylvania.

Invited Papers: W. M. Kemp, Department of Biology-U. S.-Japan Cooperative Medical Science Conference, Washington, D. C.

E. L. Thurston, Department of Biology, 41st Annual National Convention for Medical Technologists, McMasters University, Hamilton, Ontario, Canada, and Tri-Beta Regional Meeting, Lake Texoma, Oklahoma.

Published Papers: D. J. Morre and H. H. Mollenhauer. 1976. "Interaction Among Cytoplasm, Endomembranes, and the Cell Surface" in Encyclopedia Plant Physiology. New Series. Vol. 3: Transport in Plants III. Ed. C. R. Stocking and U. Heber, Springer-Verlag.

H. H. Mollenhauer, B. S. Hass, and D. J. Morre. 1976. "Membrane Transformation in Golgi Apparatus of Rat Spermatids. A Role for Thick Cisternae and the Two Classes of Coated Vesicles in Acrosome Formation" J. de Microscopie et. du Biologie Cellulaire, 27, 33-36.

H. H. Mollenhauer and D. J. Morre. (in press) "Dyctyosome-Like Structure with Cylindrical Intersaccular Connections (microtubules?) in Guinea Pig Spermatocytes" Am. J. Anatomy.

S. M. Meola, H. H. Mollenhauer, and J. M. Thompson, 1977. "Cytoplasmic Bridges Within the Follicular Epithelium of the Ovarioles of Two Diptera-**Aedes aegypti** and **Stomoxys calciprans**" J. Morphology, 153: 81-86.

W. M. Kemp, R. T. Damian, and N. D. Greene. 1976. "Immunocytochemical Localization of IgG on Adult **Schistosoma mansoni** Tegumental Surfaces" J. Parasit. 62:830-832.

W. M. Kemp, R. T. Damian, N. D. Greene, and W. B. Lushbaugh. 1976. "Immunocytochemical Localization of Mouse Alpha 2-Macroglobulinlike Antigenic Determinants on **Schistosoma mansoni** Adults" J. Parasit. 62:413-419.

W. M. Kemp, 1977. "Studies on the Isolation, Characterization, and Immunohistochemical Localization of Human Soul," J. Irreproducible Results, 23:22-24.

J. A. Shively, and D. C. Van Sickle. 1977. "Scanning Electron Microscopy of Equine Synovial Membrane" Am. J. Vet. Res. 38:681-684.

EMSA Presidential Scholarship Recipients: Ives, P. J., Department of Vet. Anatomy, "The Armadillo Median Eminence: Correlative Scanning — Transmission Electron Microscopy and Cytochemistry."

Arthur E. Sowers, Department of Biology, "Silicic Acid Analogs Affect Silica Body Assembly and Cell Wall Deposition Mechanisms in **Urtica pillulifera** Stinging Cells."

News Briefs: The Veterinary Anatomy Department recently completed expansion and remodeling of its Electron Microscopy Facility. A near doubling in floor area was accompanied by the aquisition of a new AMR-1200 Scanning Electron Microscope, a Bomar SPC-1500 Critical Point Dryer, a Denton DV-502 vacuum stand and DSM-5 cool sputtering device, a Calumet EM film processor model BW 950, and a Talos Systems Model 611 Cybergraph — a digitizing tablet for stereological and quantitative EM. Arrangements are also being made to add an RCA EMU-4 transmission electron microscope to the facility. In charge of these changes was Mr. P. J. Ives.

Dr. Thayne Dutson, Department of Animal Sciences, recently acquired a JEO1. Model 100S transmission electron microscope for his research.

E. L. Thurston was chairman of a session "Teaching Scanning Electron Microscopy" at the IITRI/SEM Meeting in Chicago, and taught a short course in Scanning Electron Microscopy at Woods Hole Biological Laboratory, Mass.

The Texas Veterinary Medical Diagnostic Laboratory has been operating for nearly one year now using a newly acquired Phillips 301 electron microscope and peripheral equipment for ultrastructural diagnostics under the leadership of Dr. Eugster.

Acting Regional Newsletter Editor for this issue of the Newsletter was Mr. Arthur E. Sowers.

TEMPLE: Scott & White Clinic, Division of Pathology.

Publications: A. Leibovitz, W. B. McCombs, III, C. E. McCoy, K. C. Mazur, N. D. Mabry and J. C. Stinson: "Human Colorectal Cell Lines as a Source for Carcinoembryonic Antigen (CEA)" in Cell Culture and Its Applications, Academic Press, 1977.

"Preparation of Medium L-15" by A. Leibovitz in the Tissue Culture Manual.

News Briefs: Col. Leibovitz has been appointed to a Manuscript Review Committee for the Tissue Culture Association Journal **In Vitro.**

A collaborative study by A. Leibovitz of the Scott & White Clinic and J. M. Quarrels and N. R. Morris of the Microbiology and Immunology Department of the Texas A&M Medical School on "Human Tumor Cultured by Capillary Devices" was approved by the Scott & White Research and Education Committee and funded for pilot studies.

Dr. J. C. Stinson recently attended a week long session at Baylor Medical School in the Department of Neuromuscular Diseases to study the technical aspects of muscle preparation.

The Department of Surgical Pathology recently established liasion with Dr. Gerald Beathard of Austin to process kidney biopsies for electron microscopy on a fee basis. Dr. Beathard has also agreed to give lectures in the future on various aspects of kidney disease.

In addition to his duties at Scott & White, Dr. D. A. Jutzy has been appointed Director of Clinical Pathology at Santa Fe Hospital.

The section of Electron Microscopy is collaborating with Dr. A. A. Trowbridge, Department of Hematology, on morphological studies of serial sections of red blood cells from a

patient infected with letospira.

W. B. McCombs has agreed to be a referee for a review article, Carcinoembryonic Antigen and alpha-Fetoprotein: Methods and Clinical Significance, in Critical Reviews in Clinical Laboratory Science.

Marie Morgan, who recently completed her work at the University of Texas at Austin, has accepted a position in the Cellular Immunology, Tissue Culture and Virology Section.

Presentations: Dr. McCombs presented a lecture, Fluorescent Antibody Techniques and Their Clinical Applications, in the Department of Microbiology at the University of Texas at Austin.

Col. Leibovitz was on the program of the Tissue Culture Association Annual Meeting in New Orleans.

Robert A. Turner was a guest speaker at the Northwest Ohio Electron Microscopy Society on September 9 in Bowling Green, Ohio.

DALLAS: The University of Texas Health Science Center.

Department of Cell Biology. Appointments: Dr. Jerry Shay has been appointed adjunct member of the Alton Jones Cell Science Center (Tissue Culture Associations Headquarters) at Lake Placid New York for 1977 & 1978. He will participate as a faculty member this year in the "Cell Hybridization and Chromosome Maping Course" and next year will be the director of a course entitled "Cell Hybridization Techniques in Cell Biology."

Dr. Shay has also been elected President of the Southwestern Medical School Sigma Xi Club.

New Staff Members: Mr. Tom Kagan, a recent graduate of the University of New Mexico, has joined the laboratory of Dr. Jerry Shay; and Dr. John Fuseler will be coming September 1 from the University of Texas Medical Branch in Galveston.

Department of Pathology. Presentations and Publications: In March of this year Dr. Herb Haggler attended the Tenth Annual Meeting of Scanning Electron Microscopy Symposium held in Chicago and presented the paper: "Energy Dispersive X-Ray Spectroscopic (EDS) Analysis of Small Particulate Inclusions in Hypoxic and Ischemic Myocardium."

At the May meeting of the American Section of the International Society for Heart Research held in Pasadena, Calif. Dr. Max Buja presented the paper "Relationship Between Calcium Accumulation and Uptake of Technetium-99m Phosphorus Radiopharmaceuticals in Myocardial Infarcts."

Ms. Karen Burton also presented a paper at this meeting entitled, "Progression of Membrane Permability Alterations Induced by Hypoxia in Isolated Cardiac Muscle."

The laboratory of Dr. John Shadduck has just submitted two papers for publication. "A Canine Cutaneous Lymphoreticular Disease Resembling Human Mycosis Fungoides," Shadduck, J. A., Lawton, G., Freeman, R. and Reedy, L. to the American Journal of Pathology; and "Occurance of Canine Encephalitozoonosis in the United States with Isolation of the Causative Organism," Shadduck, J. A., Bendele, R. and Robinson, G. T. to Vet. Pathology.

The project currently underway in Dr. Shadduck's lab is an ultrastructural study of the effects of the antibiotic Fumagillin on **encephalitozoon cuniculi.**

New Staff Members: We wish to welcome Ms. Carol Grecio who joined Dr. Max Buja's staff on April 1.

General News: Congratulations are to be extended to George Lawton and Karen Neel for their May 7 wedding; and also to Mickey and Becky Glass on the birth of their sons, Jeffrey and Jeramy born May 5.

One final note, we are still patiently awaiting the arrival of our new JEOL 100S electron microscope.

HOUSTON: Baylor College of Medicine, Department of Medicine, Section of Cardiovascular Sciences.

Awards: Dr. Ann Goldstein, Assistant Professor of Medicine and Cell Biology is the recipient of a five year NIH Research and Career Development Award.

Presentations: Dr. Barry Van Winkle gave a seminar in July on ultrastructural and biochemical properties of fast and slow skeletal muscle at the University of Virginia Medical School, Department of Physiology.

News Briefs: Mr. David Murphy is a Research Assistant in Dr. Ann Goldstein's laboratory in the Fondren-Brown building and Ms. Cherie Gorman has joined the group as an electron microscope technician. Ms. Danna Bledsoe and Mr. David Wheadon have worked as summer students with Dr. Goldstein.

Publications: Goldstein, M. A., Thyrum, P. T., Murphy, D. L., Martin, J. H., and Schwartz, A.: Ultrastructural and contractile characteristics of isolated papillary muscle exposed to acute hypoxia. J. Mol. Cell. Cardiol, 9:285-295, 1977.

Entman, M. L., Bornet, E. P., Van Winkle, W. B., Goldstein, M. A. and Schwartz, A.: Association of glycogenolysis with cardiac sarcoplasmic reticulum: II. Effect of glycogen depletion, DOC solubilization and cardiac eschemia: Evidence for a specific phosphorylase kinase. J. Mol. Cell. Cardio., in press, 1976.

Entman, M. L., Goldstein, M. A. and Schwartz, A.: The cardiac sarcoplasmic reticulum: Glycogenolytic complex, an internal beta aderenergic receptor. Life Sciences (Mini-Review), 19:1623-1630, 1976.

Entman, M. L., Bornet, E. P., Van Winkle, W. B., Goldstein, M. A., Schwartz, A., Garber, A. J. and Levy, G. S.: The Sarcoplasmic Reticulum: A Potential Internal Cyclic AMP Effector Site and Beta-Adrenergic Receptor. Adv. in Cyclic Nucelo. Res., in press, 1977.

Bornet, E. P., Wood, J. M., Goldstein, M. A., Entman, M. L., Lewis, R. M. and Schwartz, A.: Physiological, biochemical and morphological characteristics of myocardial anoxia: The use of a semi-perfusion canine preparation. Cardiovascular Research, in press, 1977.

HOUSTON: Baylor College of Medicine, Department of Microbiology.

Presentations: Dr. Heather Mayor gave seminars on Parvo viruses at the University of California at San Francisco and at Stanford Medical School in April. Dr. Mayor also spoke on Parvo viruses at The First Cold Spring Harbor Symposium in May.

HOUSTON: Baylor College of Medicine, Departments of Obstetrics and Gynecology and Cell Biology.

News Brief: Dr. Russell Deter is now head of The ultrasound section of the prenatal diagnostic center being developed at Baylor. In his new position, he will be applying quantitative morphological methods in evaluating fetal development.

Application/Nomination For Membership

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

Name of institution applicant or corporation nominee person _____

P. O. Address _____

_____

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

_____

_____

_____

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

One year's dues in the form of a check or money order should be sent with the application for Membership form. (Regular $5.00, Student $1.00, Corporate $50.00)

Signature of one Member making the nomination:

_____

Dated _____ 19 _____

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

_____

Presented to the Council at _____ meeting. Date _____

Action _____

Remarks _____

Send Application to: Bill McCombs, Secretary
Department of Microbiology
Scott & White Clinic
Temple, Texas 76501

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Yesterday

When Coates & Welter was formed in 1970 to apply field emission technology to the scanning microscope, SEM performance was limited by the low brightness and energy spread of the thermionic electron gun. When our first CWIKSCAN® SEM's showed a 1000× gain in brightness and an order-of-magnitude reduction in energy spread, most other manufacturers grudgingly began field emission development programs of their own.

Coates & Welter's new Model 106A Field Emission Scanning Electron Microscope combines magnifications up to 200,000× (with depth of focus up to 20 times greater than other SEM's) with "matched" electron optics capable of 60Å resolution at 15kv, 300Å at 1kv and 50Å in STEM mode.

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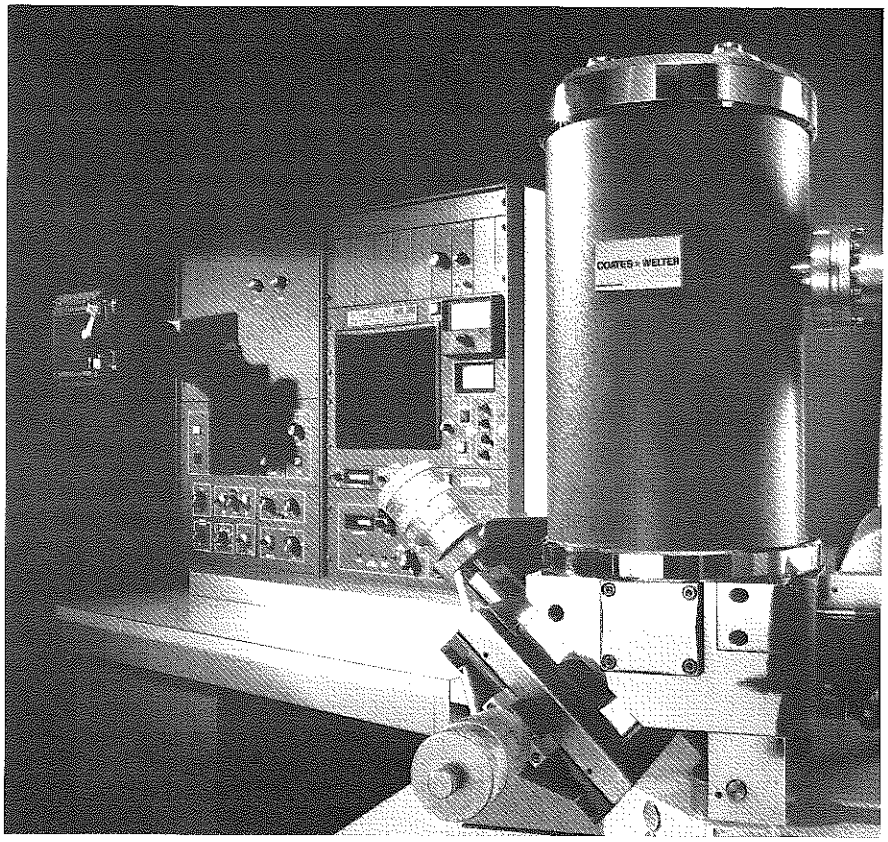
Today, you can buy a field emission SEM from Etec, Hitachi, JEOL and most other SEM manufacturers. Because of the substantial performance advantage of field emission over thermionic designs, they've introduced this new technology in "top of the line" premium-cost instruments. At Coates & Welter, we've built only field emission systems for six years. We offer a full range of models from $45,000 . . . and every one of the nearly 200 CWIKSCAN® SEM's in use worldwide incorporates a field emission electron gun.



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As other manufacturers learn how to take full advantage of field emission technology, they'll discover exciting new SEM performance features. Eventually, they'll offer flicker-free tv-rate scanning at any magnification *(with a brighter beam, field emission SEM's can scan faster)*. High magnification micrographs of uncoated non-conducting samples with no damage to the sample *(tv scan rates minimize charge effects and sample damage)*. 30Å resolution at high voltage *(some thermionic systems may achieve 60Å)*. 300Å resolution at 1kv *(the best thermionics offer 1000Å at 1kv)*. Greatly reduced maintenance *(contamination-free ion vacuum pumps, no column cleaning, no liner or aperture replacement, for example)*. 20× depth-of-field gain at the push of a button *(like the CWIKSCAN® "lens-off" switch)*. Reduction of typical exposure times from 60 seconds to 16 seconds. Direct video-taping of microphotographic images. Ultra sharp micrographs *(with our unique 4200-line micrographic system)*. And constant, dependable resolution capability because there's no filament deterioration *(there's no filament)*.

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TSEM By-laws

Article I — NAME

The name of the Society shall be the **Texas Society for Electron Microscopy.**

Article II — PURPOSE

This Society is organized exclusively as a scientific and educational organization. The purpose of this Society shall be (a) to increase and disseminate knowledge concerning the biological and physical applications of electron microscopy and related instrumentation and (b) to promote free exchange of ideas and information among electron microscopists and interested participants. Not withstanding any other provision of these articles, this society shall not, except to an insubstantial degree, engage in any activities or exercise any powers that are not in furtherance of the purposes of this society. No substantial part of the activities of the Society shall be the carrying on of propaganda, or otherwise attempting to influence legislation, and the Society shall not participate in, or intervene in (including the publishing or distribution of statements) any political campaign on behalf of any candidate for public office.

Article III — MEMBERSHIP

Membership in the Society shall be open to individuals who share the stated purpose of the Society. The Society shall consist of regular members, student members, corporate members, and honorary members.

An applicant, other than a corporate organization, having an interest in electron microscopy may be considered for regular membership. An applicant enrolled in an academic undergraduate or graduate program will be considered for student membership. Students wishing to become more involved in the Society may elect to apply for regular membership. Any applying commercial organization having an interest in electron microscopy shall be considered for corporate membership. A corporate membership shall entitle that corporation to designate one representative who shall receive membership benefits as a regular member. Other representatives of the same organization may apply for regular membership to receive Society privileges. Honorary membership shall be restricted to either (a) distinguished scientists who are not members of the Society, but who have made significant contributions to this Society or (b) to Society members for extended and outstanding service to this Society.

Application for regular, student, and corporate membership shall require nomination by any regular member in good standing and shall be made to the Secretary, who, with the approval of the Executive Council, shall report same at the next business meeting of the Society. A two-thirds vote of the regular members present shall elect applicants to membership.

Nominations for honorary membership may be made by any member of the Society. Nominations shall be made in writing to any member of the Executive Council and must be accompanied by written evidence of the nominee's eligibility. The member of the Executive Council shall present the nomination at the next meeting of the Executive Council for consideration. The Executive Council shall act upon the nomination within one year of its presentation and shall notify the nominator of the final action taken on the nomination.

Only regular members shall have the right to vote, to nominate new members, to hold office, or to serve on committees. Corporate members may exhibit at the Society's meetings (additional exhibition charges may be levied by the Executive Council). An honorary member shall be exempt from dues and shall be entitled to all privileges of regular membership. All members shall receive Society mailouts except for ballots which will be mailed only to regular members.

The amount of dues shall be set by the Executive Council. Dues shall become payable on January 1 of each year. Members unpaid by the Spring meeting shall be notified and if still unpaid will be dropped from membership after the Fall meeting.

Article IV — OFFICERS

A. Elected Officers

The elected officers of the Society shall be President, President-Elect, Immediate Past President, Secretary, Treasurer, Program Chairman, and Program Chairman-Elect. The President-Elect shall serve one year as such, one year as President, and one year as Immediate Past President. The Secretary shall be elected in even-numbered years and serve for a two year term. The Treasurer shall be elected in odd-numbered years and serve for a two year term. The Program Chairman-Elect shall serve one year as such, followed by one year as Program Chairman. The installation of incoming officers shall be at the Spring meeting. All officers shall arrange for the orderly and timely transition of their offices within 30 days after the installation of officers. However, all officers shall continue until relieved by their successors. The duties of the officers shall include:

1. **President**: shall preside at all business meetings of the Society and at meetings of the Executive Council. The President shall represent the Society at the annual meeting of the Electron Microscopy Society of America. The President shall conduct the business of the Society between Executive Council meetings.
2. **President-Elect**: shall assist the President and substitute for him in his absence and perform such duties as assigned by the President.
3. **Immediate Past President**: shall assist the President and Executive Council.
4. **Secretary**: shall maintain the records of the Society other than financial, and distribute announcements to the membership.
5. **Treasurer**: shall be custodian of the Society funds and shall account for them in accordance with accepted business practice. The Treasurer shall be bonded and the cost of such shall be borne by the Society. The Treasurer shall have his records examined annually by an internal audit committee chosen by the Executive Council at the Winter meeting. A written report of the internal audit shall be presented to the Executive Council and the membership at the Spring meeting.
6. **Program Chairman**: shall be responsible for organizing the various scientific activities of the Society. The Program Chairman shall not commit any funds of the Society unless authorized by the Executive Council via an approved budget or as authorized by the President and Treasurer under conditions of exigency.
7. **Program Chairman-Elect**: shall assist the Program Chairman and substitute for him in his absence and, additionally, extend the planning of programs into his own term of office as Program Chairman.

B. Appointed Officers

The appointed officers of the Society shall be the Newsletter Editor and the Student Representative who shall be appointed by the Executive Council.

1. **Newsletter Editor**: shall publish a Newsletter three times a year promoting the purpose of the Society, unless otherwise ordered by the Executive Council. The term of appointment shall be for two years and may be renewed.
2. **Student Representative**: shall represent the student membership of the Society on the Executive Council. The term of appointment shall be for one year.

Additionally, the officers of the Society shall perform the duties prescribed by the Bylaws and, as appropriate, by the parliamentary authority adopted by the Society. No part of the net earnings of the Society shall inure to the benefit of, or be distributable to its members, trustees, officers, or other private persons, except that the Society shall be authorized and empowered to pay reasonable compensation for services rendered and to make payments and distributions in furtherance of the purposes set forth in Article Two hereof.

Article V — MEETINGS

There shall be three scientific meetings per year: fall, winter, and spring, unless otherwise ordered by the Society or by the Executive Council. Exact times and places of these meetings shall be designated by the Executive Council. A business meeting will be held at each scientific meeting of the Society. Parliamentary procedures to be followed in the business meeting shall be those specified in the current edition of **Robert's Rules of Order Newly Revised**. Ten percent of the regular members, or 35 regular members, whichever is smaller, shall constitute a

quorum at a business meeting.

Article VI — EXECUTIVE COUNCIL

The Executive Council shall be responsible for the scientific and administrative obligations of the Society. It shall determine policies for the good of the Society in accordance with these By-laws; it shall plan scientific and business meetings, it shall authorize the expenditure of Society funds, and it shall conduct other duties as required for the benefit of the Society. The Executive Council shall meet prior to the business meeting at each scientific meeting of the Society. Special meetings of the Executive Council can be called by the President and shall be called upon the written request of three elected members of the Executive Council.

At each spring meeting the Executive Council shall appoint a Student Representative who shall represent the student membership of the Society on the Executive Council the following year as a voting member. The Executive Council shall also appoint Local Arrangements Chairmen for each of various meetings and in so doing shall duly consider the recommendations of the Program Chairman and the President. Local Arrangements Chairmen are **ad-hoc**, non-voting members of the Executive Council.

Executive Council meetings are open to the membership.

The elected and appointed officers shall constitute the Executive Council. The President and four other elected officers or the President-Elect and four other elected officers shall constitute a quorum.

Article VII — COMMITTEES

Standing or special committees shall be appointed by the President as directed by these By-laws or as the Society, or the Executive Council, shall from time to time deem necessary to carry on the work of the Society. The President may appoint advisory committees at any time without prior consultation with the Executive Council. The President shall be **ex officio** a member of all committees except the Nominating Committee.

Article VIII — ELECTIONS AND INTERIM VACANCIES

In February of each year the Executive Council shall appoint three regular members to serve on the Nominating Committee with the President-Elect, and the Secretary. The Secretary shall serve as chairman of the Nominating Committee. The Nominating Committee shall nominate two candidates for each officer position becoming vacant that year. In preparing the slate of nominees, due consideration shall be given to the geographical area and fields of interest represented by the membership of the Society and to the nominees previous participation in the Society's affairs. The Nominating Committee shall also ascertain the willingness of each nominee to serve if elected. The report of the Nominating Committee shall be announced to the regular membership by March 1.

Additional nominations may be initiated by the membership by a petition to the Secretary signed by a minimum of ten of the regular members. Such petitions must be received by the Secretary by March 15.

Ballots shall be mailed to the regular members in March and completed ballots shall be accepted by the Secretary until April 15. The Secretary shall count the ballots on the next appropriate day and announce the results of the election at the spring business meeting and by mailout to the regular membership. Any regular member may examine the ballots at the spring business meeting.

The candidate receiving the largest number of votes shall be the winner. In the event of a tie vote, the Executive Council shall decide the winner. The ballots shall be examined by the Executive Council at the spring meeting.

A two-thirds vote of the entire membership of the Executive Council shall remove any officer or appointee derelict in their duties. The Executive Council shall accept resignations in good faith.

An interim vacancy in the presidency shall be filled by advancement of the President-Elect, who will go on to serve his anticipated term as President and Immediate Past President. In the event there is no President-Elect to advance, the Executive Council shall elect one of its members as acting President to serve until the completion of the next regular election. An interim vacancy in the office of Program Chairman shall be filled by the Program Chairman-Elect, who will go on to serve his anticipated term as Program Chairman. If there is no Program Chairman-Elect to advance, the Executive Council shall appoint a Program Chairman to serve until the completion of the next regular election. Interim vacancies in the offices of Secretary or Treasurer shall be filled by appointment by the Executive Council until the completion of the next regular election. Interim vacancies in the offices of Newsletter Editor or Student Representative shall be filled by an appointment made by the Executive Council.

Article IX — DISSOLUTION

Upon the dissolution of the Society, the Executive Council shall, after paying or making provision for the payment of all the liabilities of the Society, dispose of all of the assets of the Society exclusively for the purposes of the Society in such manner, or to the Electron Microscopy Society of America. Any such assets not so disposed of shall be disposed of by the Court of Common Pleas of the county in which the principal office of the Society is then located, exclusively for such purposes or to such organization or organizations, as said Court shall determine, which are organized and operated exclusively for such purposes.

Article X — AMENDMENTS

Amendments to these By-laws may be initiated by individual members of the Executive Council or by petition to the Secretary signed by ten regular members of the Society. Amendments must be approved by a two-thirds majority of the Executive Council, the proposed amendment shall then be promptly submitted by mail to the regular membership by the Secretary with statements of support and/or opposition by the Executive Council. The ballots shall be accepted by the Executive Council for one month after the date of mailing. The Executive Council shall count the ballots; the amendment(s) shall be ratified if it receives a favorable two-thirds majority of the votes cast. Any regular member can, if he so desires, be present at the counting of the ballots.

Editor's Comments

By now, after the Fourth issue of the TSEM Newsletter, I feel like an old seasoned editor. Believe me, I'm only kidding It's true I've learned alot and have received a lot of callaces and bruises on my one synapse but still have lots to learn. It's a continuous pleasure working with the advertisers and members getting this newsletter out. Of course, without them we just simply would not have a newsletter. Everyone has been extremely cooperative; especially the corporate people when I was forced to increase the rates on advertising.

The winter and spring issues of 1978 will be my last issues as your editor; therefore, if any of you are interested in being my replacement either contact myself or Ivan Cameron, who will be president at that time.

Again I would like to thank everyone out there in the E. M. world for sending in all the items for this issue of the newsletter. You have all made my job alot easier and pleasurable through your generous cooperation.

BOB TURNER
TSEM Newsletter Editor

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Materials Analysis Division

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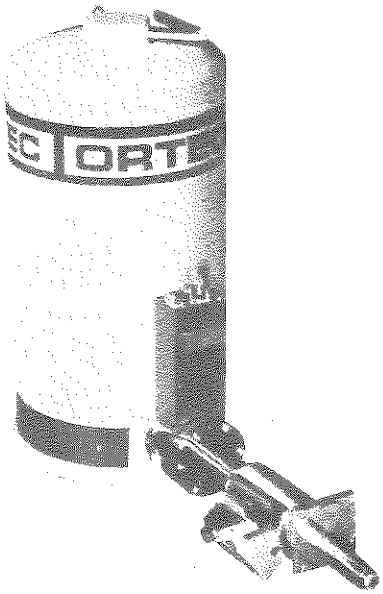

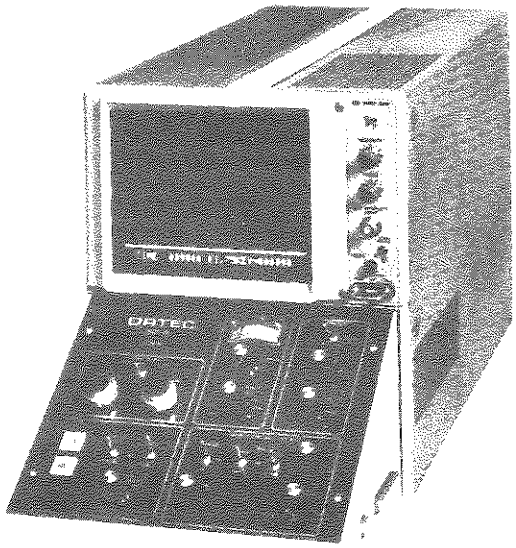

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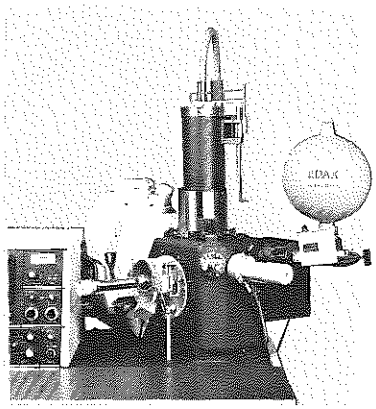

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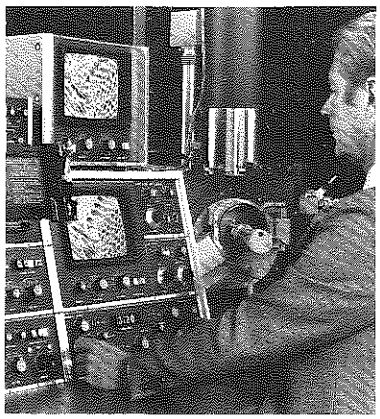

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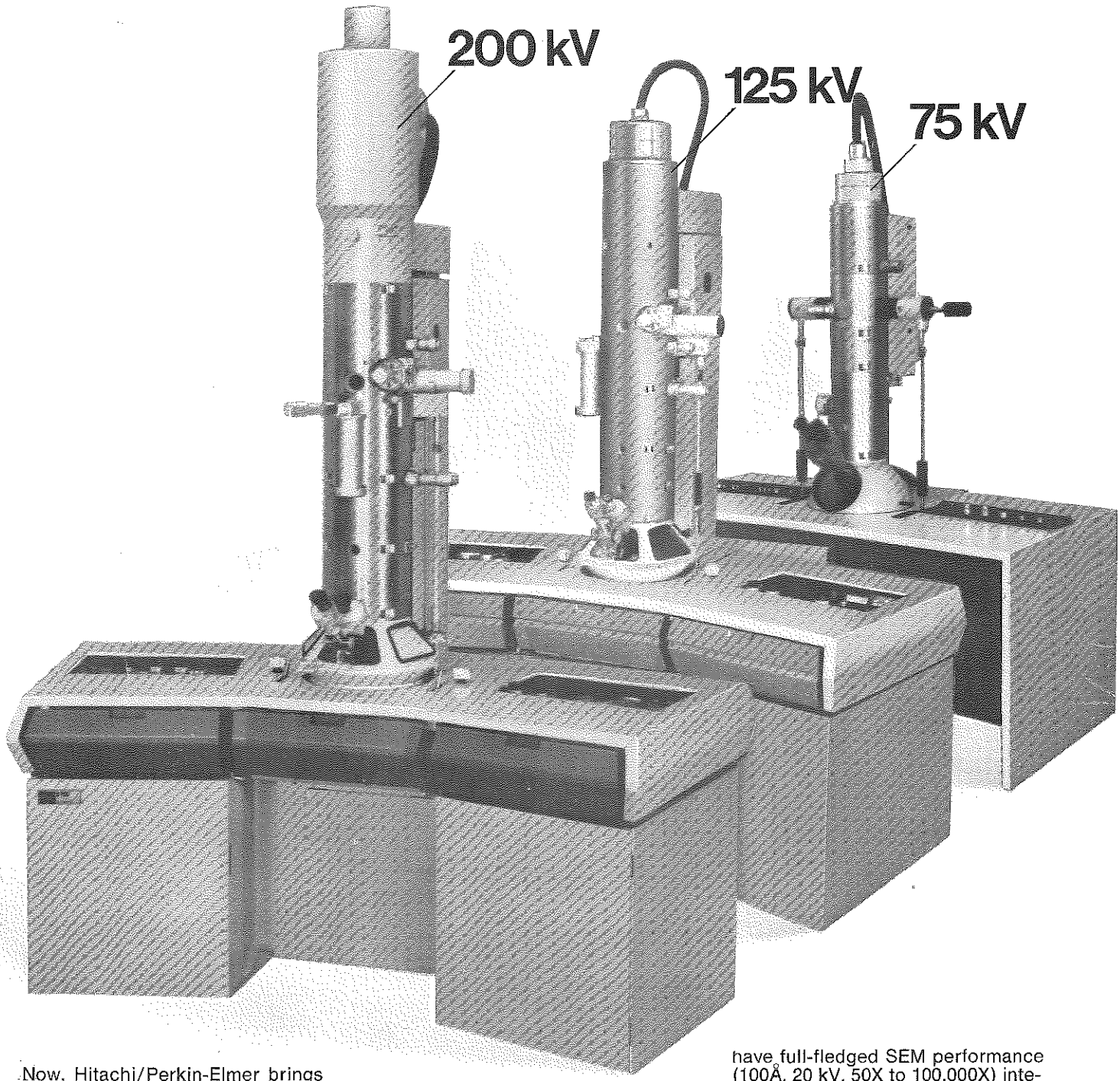

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