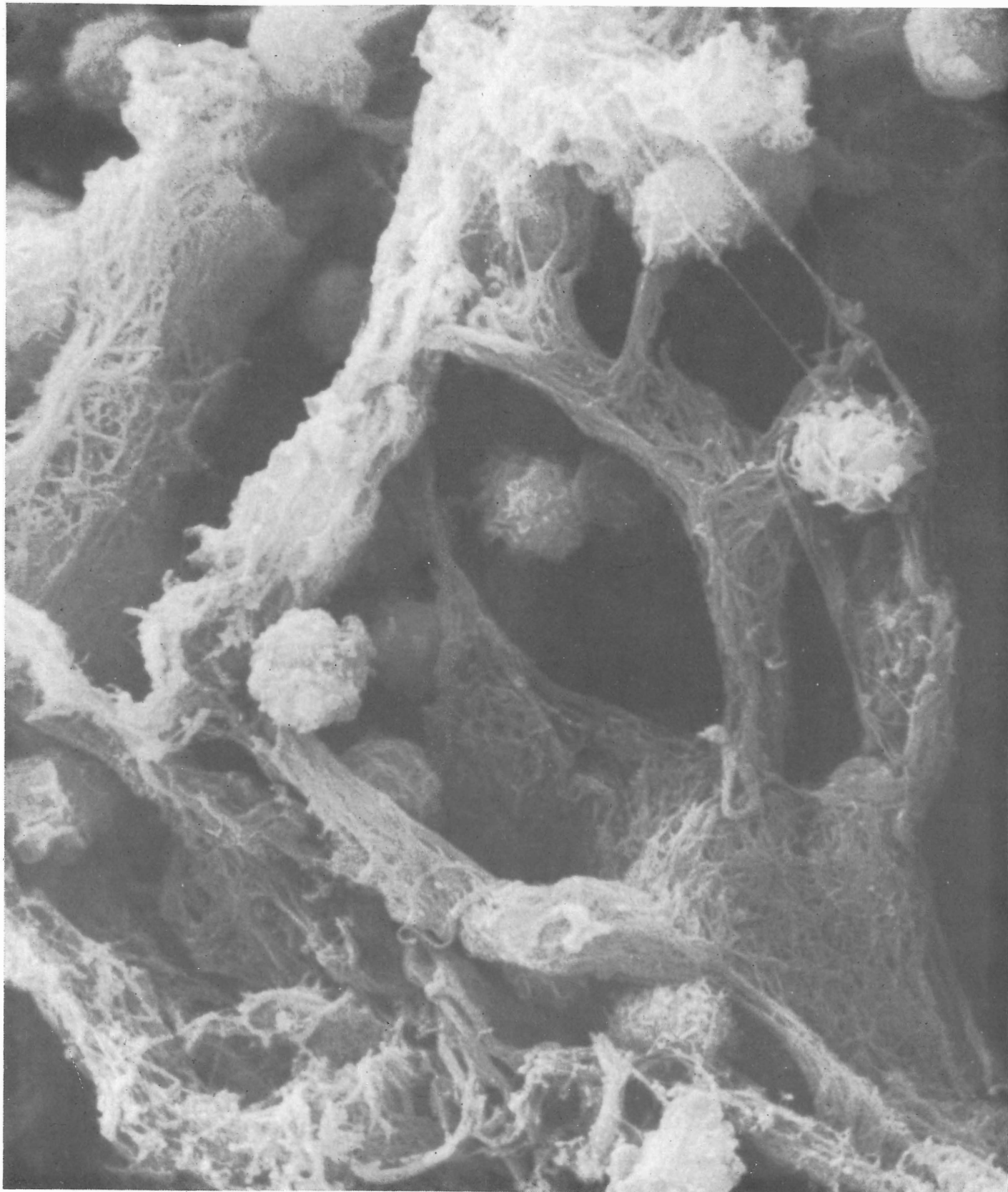
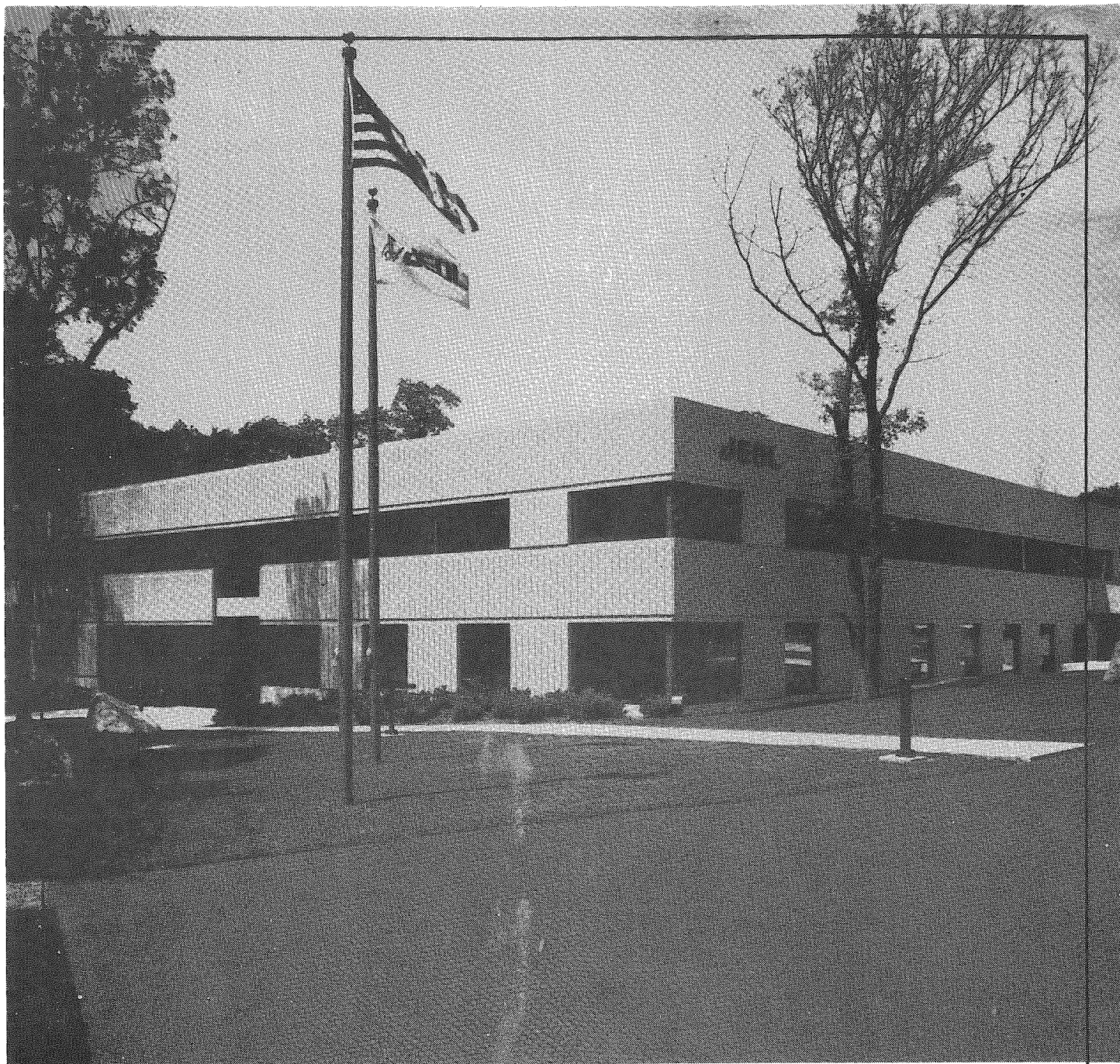




Texas Society for Electron Microscopy

JOURNAL
VOLUME 13, NUMBER 3
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EDITORIAL POLICY

LETTERS TO THE EDITOR

Letters to the editor are printed as they are received in the order of their arrival. These letters reflect the opinion of the individual TSEM member and do not necessarily reflect the opinions of the editor or the society. The content of the letters should be concerned with the philosophical or operational aspects of the TSEM, the Journal and its contents, academic or national policies as they apply to TSEM and/or its members and electron microscopy in general. Editorial privilege may be evoked to insure that the LETTERS SECTION will neither be used as a political forum nor violate the memberships' trust.

ELECTRON MICROGRAPHS AND COVER PHOTOS

Micrographs submitted for Cover Photos should be marked as such. The choice of photographs will be made by the editor. Photograph receipt and/or dispensation will not be acknowledged. Photographs will not be returned. Electron micrographs to be used for cover photos and text fillers (interesting micrographs) are welcome and should be selected with some attention to aesthetic appeal as well as excellence both in technique and in scientific information content.

(Continued On Page 22)

ON THE COVER

In this scanning electron micrograph, leukocytes are observed associated with fibrin strands forming the cell rich exudate material of a two day old full thickness (5 mm diameter) wound. Later, this fibrin matrix will become interlaced with collagen filaments and fibers (produced by fibroblasts) forming a tissue that will dehisce and become known as the scab. x4000.

Submitted by Paul S. Baur and Darrell Hudson, Division of Cell Biology, Shriners Burns Institute, Galveston, Texas.

President's Message

The Denton meeting was very successful, thanks in no small measure to the efforts of Marilyn Smith and her local arrangements committee. We enjoyed an attractive if at times somewhat blustery campus (I was glad I'd left my kilt in Houston), and met some very friendly natives. One of the most appealing aspects of T.S.E.M. is the opportunity it provides to visit academic centers in various parts of the State.

The news of Larry Thurston is of slow but encouraging progress, and it is good to know that he can now improve his motor skills and communication through use of a computer. Society members have been informed of this through a letter from A&M and invited to help, and the Society has, on the authority of the Executive Council, made a contribution. Our

thoughts are with Larry as he continues along the come-back trail.

The T.S.E.M. Journal is a credit to the Society, thanks to the efforts of Paul Baur and his predecessors, and we have the opportunity to make it even finer by expanding each issue with original contributions. The Journal is an ideal forum through which to share technical pearls, current research findings, and even to seek opinions on problems that come up in our work. I would encourage each of you to consider sending material to Paul for inclusion in the Journal.

Bruce Mackay
President TSEM
1982-1983

Editor's Message

I remember the TSEM newsletters of years past when the editors, officers or society members would fill more than a fractional portion of each issue with a variety of diatribes. I'll consciously try to refrain from such temptations.

This month three papers were submitted, three were reviewed and three were accepted. I didn't hear from anyone else! I don't know how to encourage your contributions other than these "pep-letters" and occasional admonitions. I do know that the Journal is expensive to publish and that the endeavor isn't going to be worth it unless the papers roll in! So please consider TSEMJ for one of your next offerings, be it an article, technical paper, review, announcement, comment, etc. Let's keep up the momentum.

The Galveston meeting seems to be arranged nicely. In view of that meeting I should tell you that Pat Davis and I have revamped the abstract form so that we can offset the abstracts directly into the Journal. This procedure will save us (TSEMJ) a lot of time and money in the future. Please examine the form

and note the new instructions. The bottom portion of the form that restates the title, author, and affiliation of the abstract will be used by the program chairperson to set up the program. Be sure you're accurate and neat with your abstracts. If you have comments or advice concerning the abstract forms please let me know.

By the way, did you note that I said program chairperson. A good policy to remember and an appropriate title change.

I left out Ann Goldstein's name as past-president in the list of officers. It was an accident or oversight pure and simple although I did get teased by Ann. (She left my name out of one issue). It's great to have friends such as we all have in the Texas Society for Electron Microscopy.

My best to everyone,

Paul S. Baur
Editor, TSEM Journal

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AN UNUSUAL CASE OF BACTERIAL CONTAMINATION IN TISSUE CULTURE: AN ELECTRON MICROSCOPIC STUDY

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INTRODUCTION

Omitting antibiotics from tissue culture media has the advantage, among others, of quickly revealing bacterial contamination. Contaminated cultures rapidly turn into turbid mixtures with rancid or unpleasant smell. However, the matter is not always so straightforward. From ultrastructural studies of many cell cultures grown in the absence of antibiotics we have learned that small numbers of bacteria can be present for long periods in some macroscopically, even microscopically normal cultures. These low-grade contaminations were only detected by electron microscopy (Fig. 1).

During ongoing tissue culture studies of pleural effusion cells from metastatic cancers, we detected a peculiar case of bacterial contamination, to our knowledge without published precedent. A few weeks after initiation, primary cultures of cells derived from pleural effusion of a breast cancer patient underwent strange morphological changes. The cultures looked clear, with a normal pH. Only electron microscopy revealed what was wrong: large numbers of bacteria were replicating in cytoplasmic vacuoles of the cells. The bacteria were impossible to isolate on bacteriological media, but they did grow in HeLa cells. The

origin of these bacteria has not been established. Their characterization and sensitivity to antibiotics are currently under study. In this paper we report the major ultrastructural features of this very unusual bacteria-cell association.

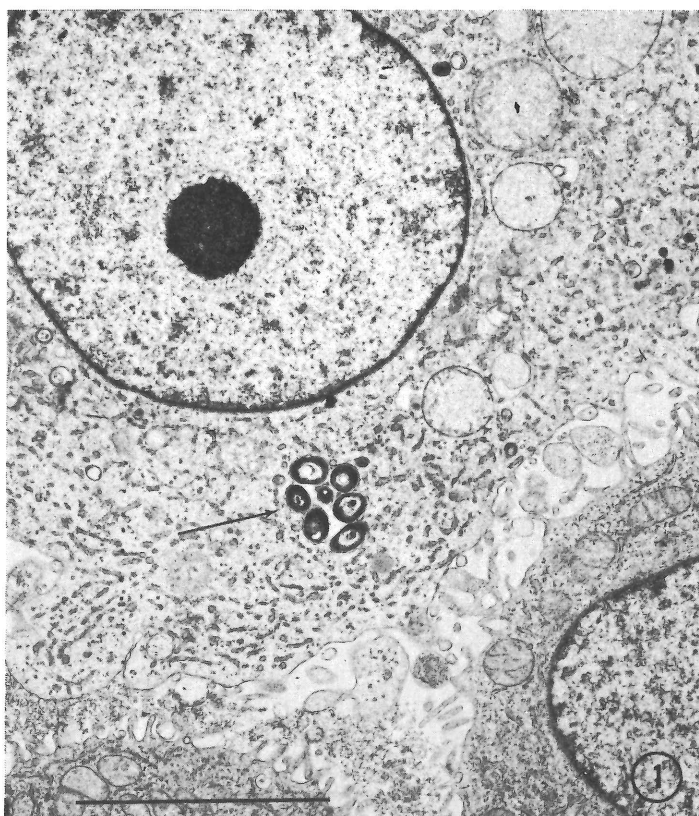


FIGURE 1: Part of a cell from established breast cancer cell line MDA-MB 157 showing a cluster of bacteria in the cytoplasm (arrow). Line = 5 μ m.

MATERIALS AND METHODS

Pleural effusion from a 48-year old patient with metastatic breast cancer was obtained on July 7, 1981. The patient had previously undergone mastectomy for infiltrating ductal carcinoma. She was treated with radiotherapy and chemo-therapy but did not receive adjuvant therapy with bacillus Calmette-Guerin (BCG) or *Corynebacterium parvum*. Tissue cultures were initiated by seeding, in each of two Corning T-75 flasks, cells from 50 ml aliquots of pleural fluid sedimented by low speed centrifugation. The cultures were grown in Leibovitz's L-15 medium with 10% fetal calf serum but no antibiotics. Flasks were kept at 37.5 C in a dry incubator flushed with air. Medium was changed about once a week. Subcultures were done by scraping half of a confluent monolayer and seeding the scraped cells in another T-75 flask, while the donor flasks were refed with fresh medium to allow regrowth of cells.

Electron microscopic examination was carried out on cells from the original pleural fluid and on cells in passage 3 from two different culture flasks. Cell pellets were fixed in 3% glutaraldehyde in Millonig's buffer and postfixed in 2% buffered osmium tetroxide. After dehydration in ethanols and propylene oxide, the blocks were embedded in epon-araldite. Thin sections were stained in uranyl acetate and lead citrate and then carbon-coated.

In attempts to characterize the bacteria, mixtures of scraped cells and culture medium from cultures in passage 3 and 5 were inoculated onto a series of 13 different isolation media in the Department of Laboratory Medicine of this institution. Two milliliters of clarified tissue culture medium (centrifuged at 5 min at 600 rpm) were added to HeLa cell cultures in attempts to propagate the bacteria.

RESULTS

Electron microscopic examination of cells from the original pleural fluid showed the presence of many cancer cells and of some cells of other types (mesothelial cells, macrophages and lymphocytes). However most cells in primary cultures appeared epithelial (Fig. 2). After about a month and a half, when subcultures had reached passage 2, cells in all the flasks, including those remaining from passages 0 and 1, progressively became abnormal (Fig. 3). The cells became larger and more frequently multinucleated, and increasing numbers of vacuoles, some very large, appeared in their cytoplasm. The larger vacuoles were filled with granules showing an intense Brownian movement. The medium remained neutral or slightly alkaline. At first, we assumed that the cultures were being overtaken by mucous-secreting cancer cells but no mucous was detected after using mucicarmine, alcian blue or PAS on fixed cells.

Electron microscopy gave the answer: the vacuoles were filled with bacteria. Some cells had one or several large vacuoles containing innumerable bacteria arranged at random (Fig. 4) or in near-geometric patterns (Figs. 5, 6). In many cells there were smaller vacuoles with fewer bacteria and very often single bacteria each contained in a

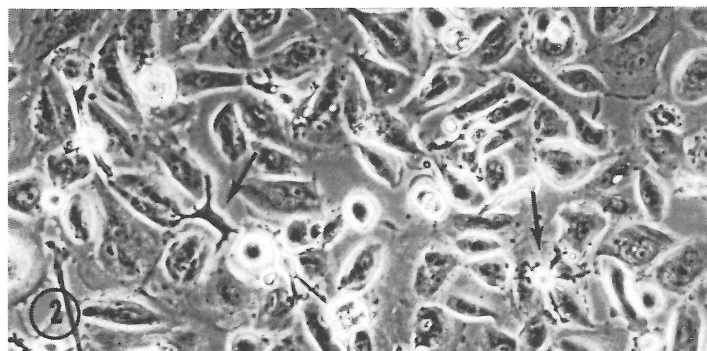


FIGURE 2: Phase contrast micrograph of pleural effusion cells in primary culture. Most cells are epithelial. The dendritic cells shown by arrows are histiocytes or macrophages. Magnification 160X.

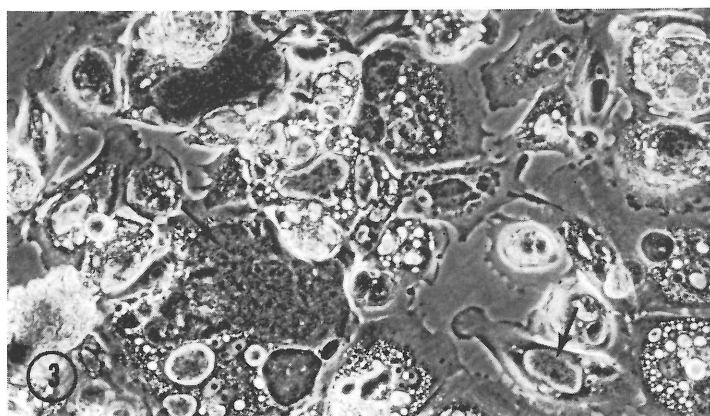


FIGURE 3: Infected pleural effusion cells in passage 3. Some cells contain large vacuoles filled with granules (arrows). Most cells are enlarged, often multinucleated with various inclusions in their cytoplasm. Magnification 160X.

vacuole. Bacteria-containing vacuoles always had a well-defined membrane: bacteria were never seen free within the cytoplasm. In some vacuoles the microorganisms were embedded in a finely granular matrix (Figs. 5, 7) while in other instances they were sequestered in typical lysosomes (Fig. 8). In Fig. 9 are shown bacteria imprisoned in one of the cytoplasmic balloons floating free in the medium. There were also numerous extracellular bacteria identical to those inside the cells.

The bacteria, up to 3 μ m long microns in length, were present in morphological forms varying from young, clear cells with well preserved ultrastructure (Figs. 4, 7) to condensed, osmiophilic elements probably undergoing degeneration (Figs. 4-6). All the forms could be seen both in cells and medium. Some intracellular bacterial bodies were abnormal in size and shape (Fig. 4). The bacteria were circular in cross section. Their outer walls were smooth and separated from the cytoplasmic membrane by a finely granular layer (Fig. 7). In the denser, osmiophilic elements, the outer wall and cytoplasmic membrane were often wide apart, with only empty spaces between them (Figs. 6, 8, 9).

The cultures died out slowly, ending in passage 5 after 7 months. Morphological alterations of the cells remained

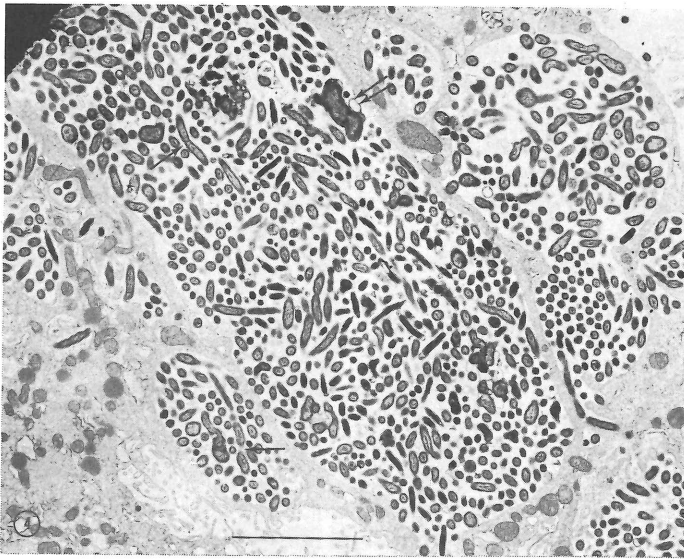


FIGURE 4: View of an infected cell with large vacuoles filled with bacteria. Besides well preserved, young bacterial forms, some seen dividing (arrows), there are denser and shorter osmiophilic forms. Some bacterial bodies are distorted, even gigantic (double arrows). Line = 5 μ .

the same throughout. The cultured cells became more and more sluggish, and at the end of the monolayers contained too few cells for successful subculture. The history of this peculiar case of bacterial contamination suggests that some state of equilibrium prevailed, at least for a while, between cells and bacteria. In effect, nearly all the cells were infected, yet they were able to proliferate. Even mitotic cells had bacteria in their cytoplasm. Bacteria also were dividing, either inside (Fig. 4) or outside the cells in plain medium. Thus, losses on both sides must have been balanced by replication. Cell killing was apparently very moderate. Infected cells, although altered in their ultrastructure, showed no damage and were simply shedding their bacteria-laden vacuoles (Fig. 9). Whether the cytoplasm was rebuilt after shedding could not be determined. Morphological evidence for bacterial destruction was also scant. Although bacteria could be seen sequestered into lysosomes (Fig. 8), their complete lysis was not evident. Like the cells, the bacteria probably slowly degenerated, littering the medium with dead, undigested organisms.

Bacteria must have penetrated into the cells by simple engulfment by cytoplasmic processes. No bacteria were seen adhering to plasma membranes. Whether the cells picked up from medium live and dead bacteria alike remains an unanswered question. It would appear, though,

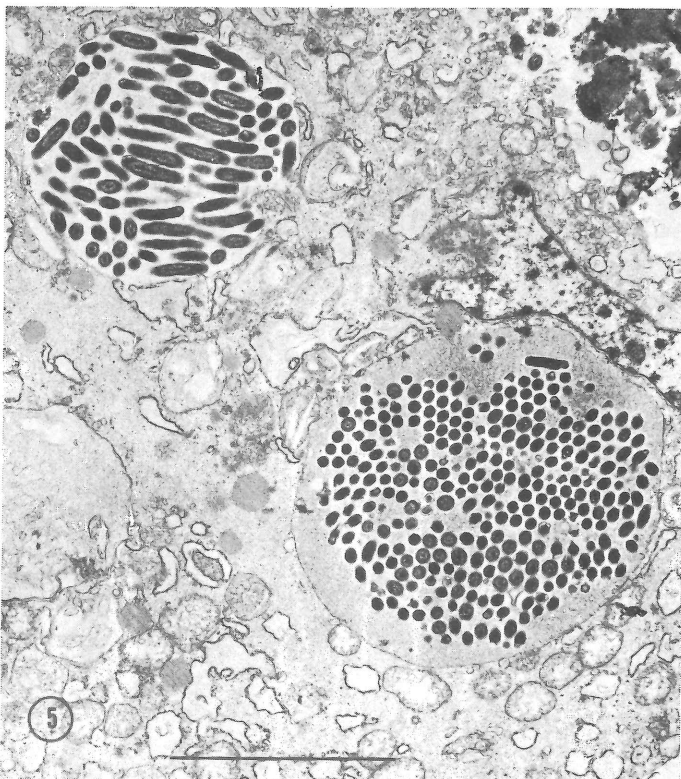


FIGURE 5: Two bacteria-filled inclusions in an infected cell. In one of the inclusions (bottom right), the bacteria, mostly osmiophilic, are seen mostly in cross section and are embedded in a finely granular matrix. No matrix embeds the bacteria present in the other inclusion (top left). Line = 5 μ m.

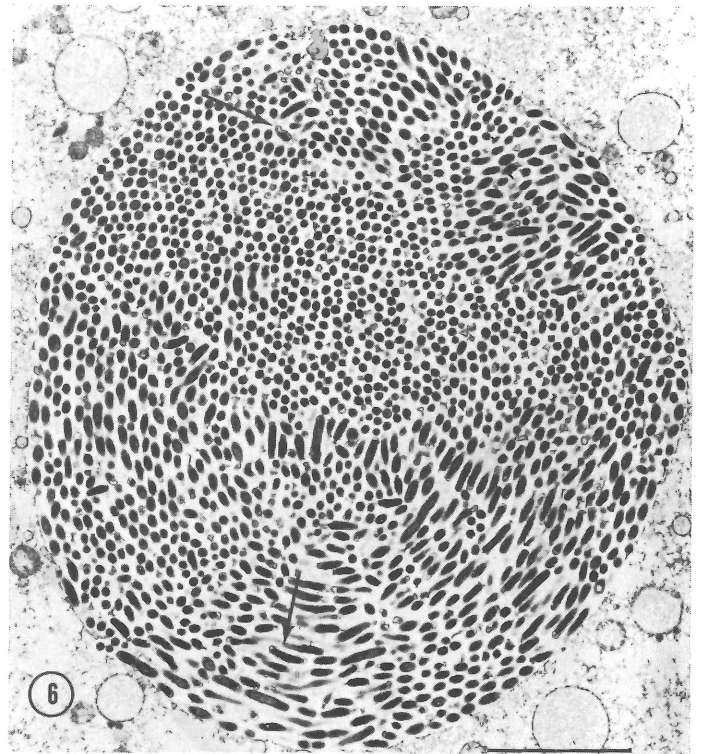


FIGURE 6: Huge inclusion containing more or less geometrically arranged bacteria. The distribution of bacteria suggests that they form a colony derived from one or only a few of the microorganisms. All the bacteria are dense and osmiophilic. Many of them show a dehiscence of the cytoplasmic membrane from the outer wall (arrows). Line = 5 μ m.

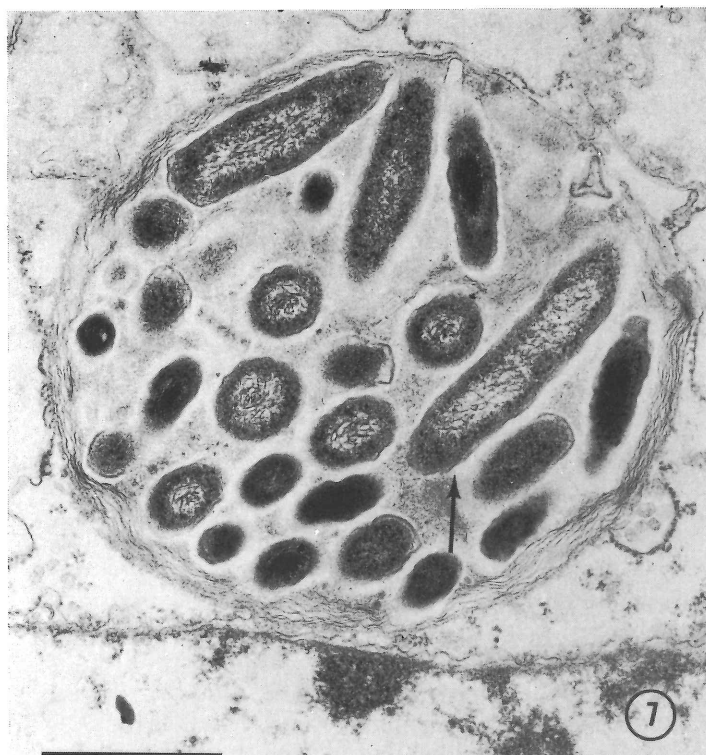


FIGURE 7: Inclusion containing mostly young, well preserved bacteria, embedded in a finely granular matrix. This inclusion probably represents a colony. The onion-ring disposition of the vacuole. The cell wall is separated from cytoplasmic membrane, in the young bacteria, by a grayish layer (arrows) a disposition characteristic of gram negative bacteria. Line = 1 μ m.

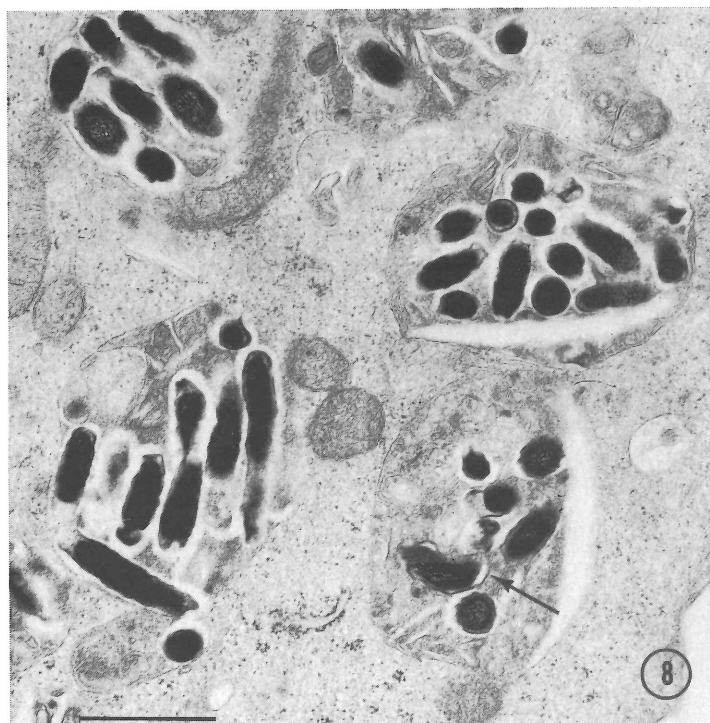


FIGURE 8: Micrograph showing bacteria sequestered in typical lysosomes. The bacteria are dense and shortened. Cell wall and cytoplasmic membrane are often wide apart (arrows). Line = 1 μ m.

that a number of bacteria were able to form intracytoplasmic colonies on their own, which is the only way to explain the formation of inclusions such as those shown in Figures 5 and 6. Therefore, it is possible that the bacteria-laden vacuoles were the result of two distinct phenomena: one of indiscriminate phagocytosis of extracellular bacteria, whether dead or alive, and the other of colony formation by intracellular bacteria.

Infected cultures retained an epithelial morphology. The finding of typical desmosomes in cells of passage 3 confirmed the epithelial nature of the cells. Part of a cell in passage 3 is shown in Fig. 10. The cells had ragged outlines, convoluted nuclei, dilated rough endoplasmic reticulum, and many pleomorphic mitochondria. The last two features were undoubtedly caused by bacterial infection.

As expected from the ultrastructure of their cell walls (1), the bacteria were gram negative. No growth was observed, even after a month, after inoculation of clarified medium into plain L 15 with 10% fetal calf serum, or of cell-medium mixtures into 13 different bacteriological media (thioglycollate; trypticase soy broth at 37° or 22° C; trypticase soy broth with sheep blood; chopped meat carbohydrate broth; chocolate agar; Schadler agar; egg yolk agar; Schadler agar with gentamycin and vancomycin; Lowenstein-Jensen slant; Lowenstein-Jensen slant with

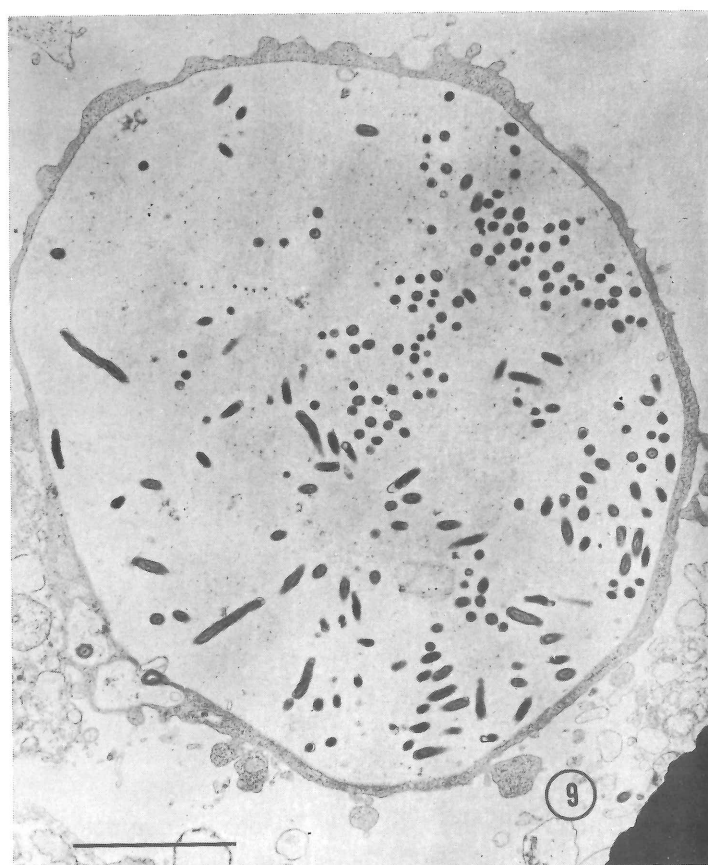


FIGURE 9: Cross section of a cytoplasmic balloon, filled with osmiophilic bacteria and floating free in medium. Besides bacteria, this formation is practically empty. Line = 5 μ m.

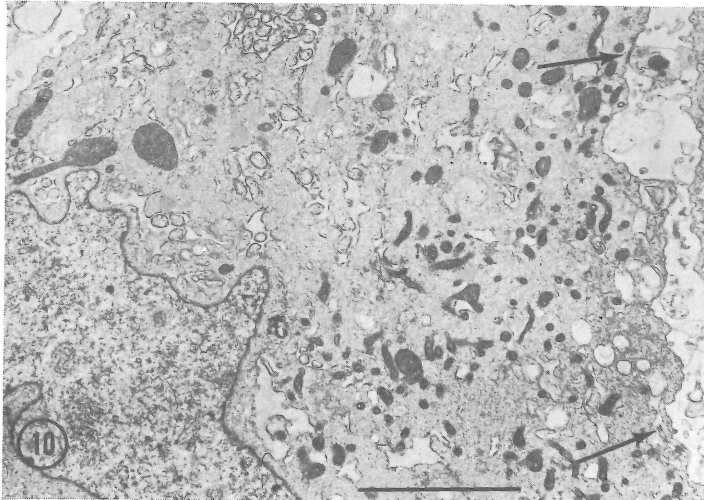


FIGURE 10: Part of the bacteria-free cytoplasm of a cell in passage 3. The cellular contour is ragged. Endoplasmic reticulum is highly developed; mitochondria are numerous and pleomorphic. Two rather inconspicuous desmosomes are indicated by arrows. Line = 5 μ m.

cycloheximide, lincomycin and naladixic acid; Middlebrook 7 H-10 broth; Middlebrook 7 H-10 agar). However, after addition of cell-free medium from an infected culture in passage 5 to a culture of HeLa cells in L 15 medium, HeLa cells showed (5 weeks later) signs of infection. Increasing numbers of HeLa cells with large vacuoles containing motile granules (Fig. 11) appeared in the original cells as well as in two subcultures. The mechanism of this intracellular infection is now under study.

DISCUSSION

To our knowledge, no study in the literature describes the ultrastructural aspects of chronic bacterial contamination of tissue cultures.

The origin and species of bacteria found in this particular culture of breast cancer cells remain a mystery. No bacteria were seen in thin sections of the original pleural cell preparation, but small numbers of bacteria possibly picked up from the patient's skin during thoracentesis could easily have been missed by electron microscopy. No contamination of the same kind occurred in other cell lines fed in July, 1981, with the same stock of L-15 medium. The simultaneous occurrence, in passages 0 to 2, of cellular changes in cultures derived from this particular pleural effusion suggests that contamination was present very early. Thus there are no definite clues as to whether the origin of the bacteria was endogenous or exogenous.

The bacteria were gram negative, but more work will be necessary to establish their species, using different bacteriological media and other target cells. The apparent dependence of these bacteria on nonmacrophage cells, such as breast cancer or HeLa cells, is intriguing and point to some close relationship between cellular and bacterial metabolisms.

Following the discovery of this peculiar case of bacterial contamination, we have not destroyed the infected

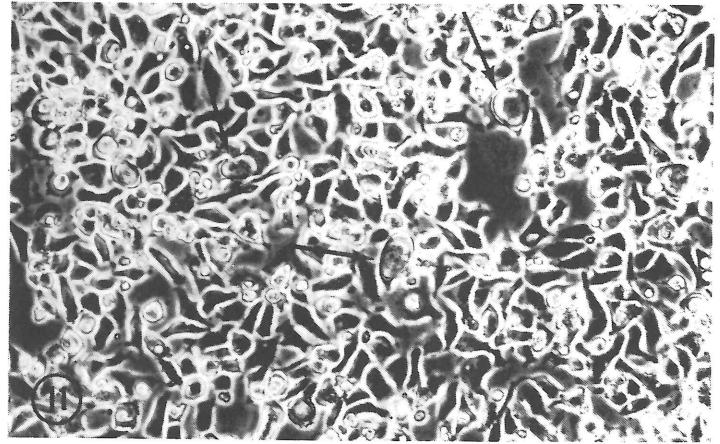


FIGURE 11: Phase contrast micrograph showing a culture of HeLa cells one month after infection with cell-free medium from a pleural effusion cell culture in passage 5. The HeLa cells are from the second subculture after infection. A number of cells show large granule-laden vacuoles identical to those that first appeared in pleural effusion cells. Magnification 160X.

cultures or attempted to eliminate the bacteria with antibiotics. We will do so only once the bacteria are fully characterized. The bacteria may belong to a new strain with metabolic requirements of interest to bacteriologists.

Our immediate aim is to examine at the ultrastructural level the mechanism by which the bacteria penetrate nonmacrophage, epithelial cancer cells.

REFERENCES

1. M.T. Silva, and J. C.F. Sousa. Ultrastructure of the cell wall and cytoplasmic membrane of gram negative bacteria with different fixation techniques. *J. Bact.* 113 (1973) 953-962.

ACKNOWLEDGEMENT

We thank Dr. Roy L. Hopfer (Department of Laboratory Medicine, The University of Texas M.D. Anderson Hospital and Tumor Institute) for his assistance in bacteriological procedures.

ABSTRACT

An unusual case of chronic bacterial contamination of tissue cultures derived from pleural effusion of a breast cancer patient has been studied by electron microscopy. Despite large numbers of bacteria inside and outside the cells, the cultures appeared macroscopically normal. Bacteria invaded and replicated in cytoplasmic vacuoles, which ultimately were shed into medium. Mutual destruction of cells and bacteria was apparently moderate. The cultures slowly died out after 7 months, without any flask's contents being lost to acute infection. The contaminating bacteria have not been characterized so far, because they failed to grow on any of thirteen bacteriological media used. However, chronic contamination of HeLa cells by these bacteria has been achieved experimentally, reproducing the same morphological pattern as in cancer cells derived from pleural effusion.

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. Notwithstanding 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 Chair-

man, 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

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 com-

pleted 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.

TISSUE REPLICA PREPARATION FOR SCANNING ELECTRON MICROSCOPY

By
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Galveston, Texas 77550

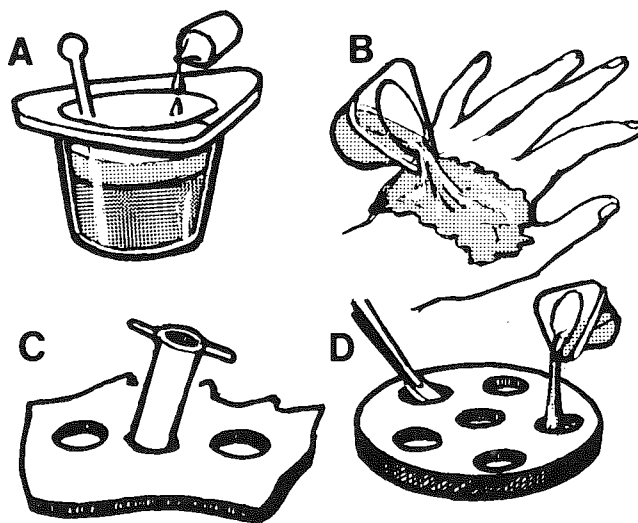
INTRODUCTION

Impressions (casts or negative replicas) and the positive replicas made from them are useful tools by which to examine the surface details of a tissue by scanning electron microscopy (SEM) without necessitating the material's excision and subsequent destruction. The replica technique can also be employed in SEM to study materials that would be distorted or destroyed by fixation or drying procedures. It is also useful for studying delicate organic materials which would be altered by the electron beam and/or large objects which could not be accurately subdivided (1). The replicas are especially beneficial in SEM studies of the integument (wrinkle patterns, keratinization, scar formation, dermatology studies, etc.) (2). Impressions and replicas can be made from a wide variety of materials (3). However, silicone rubber impressions tend to retain greater detail and are easiest to use (4).

METHODS AND MATERIALS

Impressions in this study were made using Dow Corning Silastic 382 Medical Grade Elastomer (80%), Dow Corning 200 CS Fluid (20%), and Dow Corning Catalyst M (<1%). Ingredients were measured on a volume basis following the manufacturer's recipe (Fig. 1A). The mixture was stirred thoroughly, applied quickly to the desired area of study, and allowed to harden (< 10 minutes) (Fig. 1B). Longer or shorter working and/or vulcanization times could be achieved by changing the amount of catalyst added to the silastic mixture. In order to accurately replicate the surface detail and to avoid bubble or void artifacts, care was taken to minimize the air stirred into the mixture and to insure total penetration or coverage of the area by the elastomer. Although biological tissues seldom require a mold release agent, when one was needed a thin film of petroleum jelly was often sufficient. After the impression or cast was peeled from the specimen surface, a 5% solution of sodium hypochlorite was used to dissolve away adherent biological material from the cast surface. The

silastic cast was then rinsed several times with distilled water and air dried. The sites on the silastic impression that were selected for study were punched out using a 15 mm diameter cork borer (Fig. 1C). The silastic discs were then transferred (detail side up) to a silastic mold with



1

FIGURE 1: The components used to formulate the silastic casting material were: Dow Corning 382 Medical Grade Elastomer (80%), Dow Corning 200 CS Fluid (20%), and Dow Corning Catalyst M (<1%) (A). After careful mixing, the casting material (approximately 25 mls) was applied to the area of interest (B). After vulcanization, the cast or impression was removed from the subject and cleaned. Regions on the impression surface chosen for study were then punched out with a cork borer (C). The impression discs were placed in a silastic mold (detail side up), an Epon mixture was added to each well, and the resin polymerized at 60°C for 36 hours (D).

cylindrical depressions 15 mm in diameter and 8 mm deep. An Epon mixture measured on a volume basis (Epon 812-44%, NMA-42%, DDSA-14%, and benzyldimethylamine- <1%) was then layered over the impression discs and the mold placed in a 60°C oven for 36 hours (Fig. 1D). After polymerization, the positive Epon replicas were removed, mounted on stubs, coated with 300-500 Å of gold, and examined by SEM.

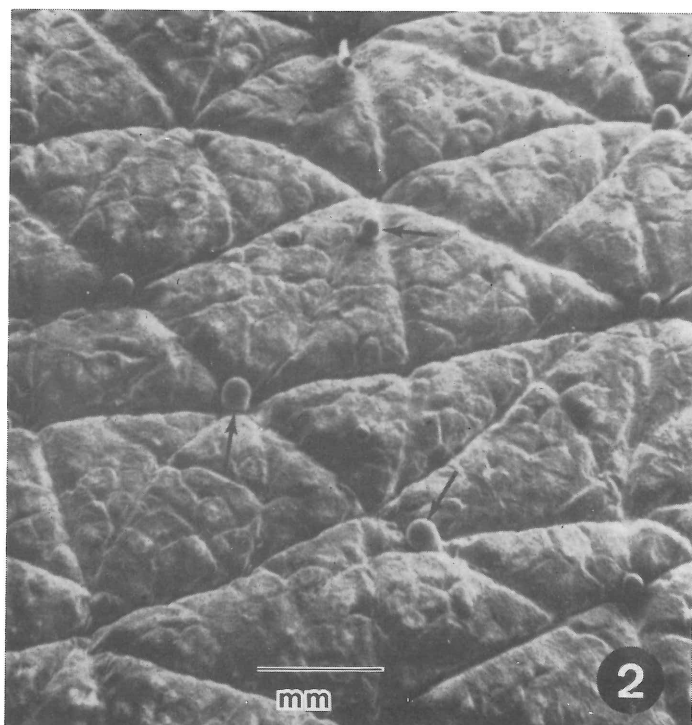


FIGURE 2: An Epon replica of normal skin showed distinct wrinkle or crease patterns and demonstrate the air bubbles (arrows) that were occasionally incorporated in the impression surface. x60.

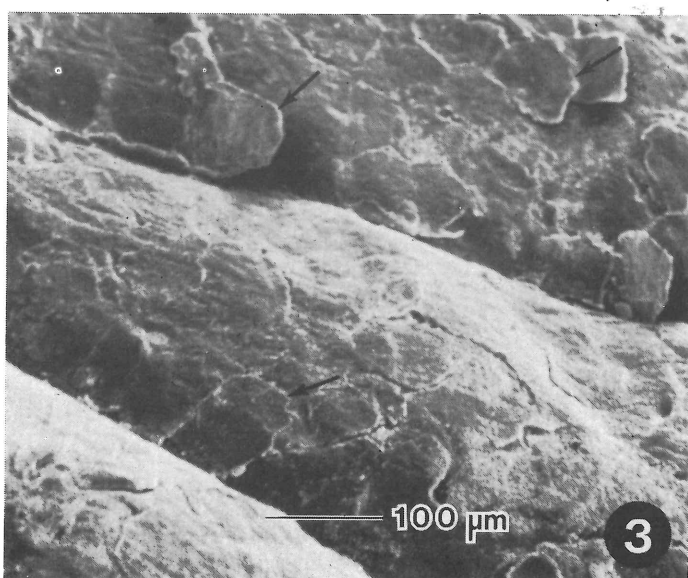


FIGURE 3: An Epon replica of normal human integument demonstrating the placque-like arrays (arrows) of epidermal cells, a characteristic feature of keratinizing skin. x570.

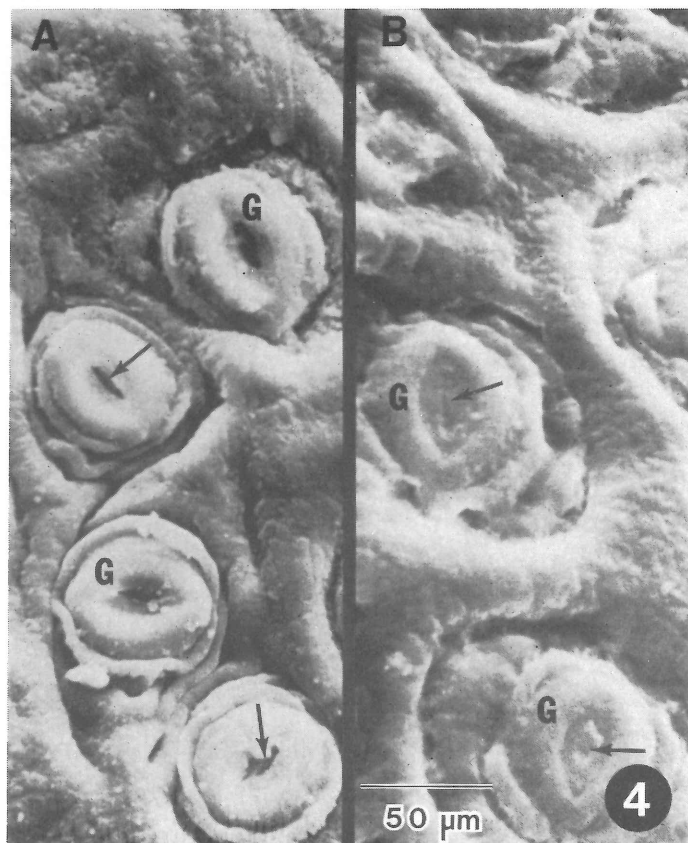


FIGURE 4: The stomatal surface of a leaf prepared by standard scanning electron microscopy procedures (A) is compared to an Epon replica of a similar surface (B). Note the guard cells (G) and stomata (arrows). x1120.

RESULTS AND DISCUSSION

Replicated surface details of the integument of the hairless mouse, human skin, burn scars, and the stomatal surface of a leaf, when examined by SEM, compared favorably to those of the actual biological material (Figs. 2, 3, and 4). Epon resins, epoxy glues, and methacrylate esters were used to produce the best positive replicas. However, the Epon castings provided the best retention of detail. While, in several instances, the replicas proved useful up to 2000 magnifications the technique was most beneficial in studies of surface topography requiring a lower range of magnifications (20 x - 500 x).

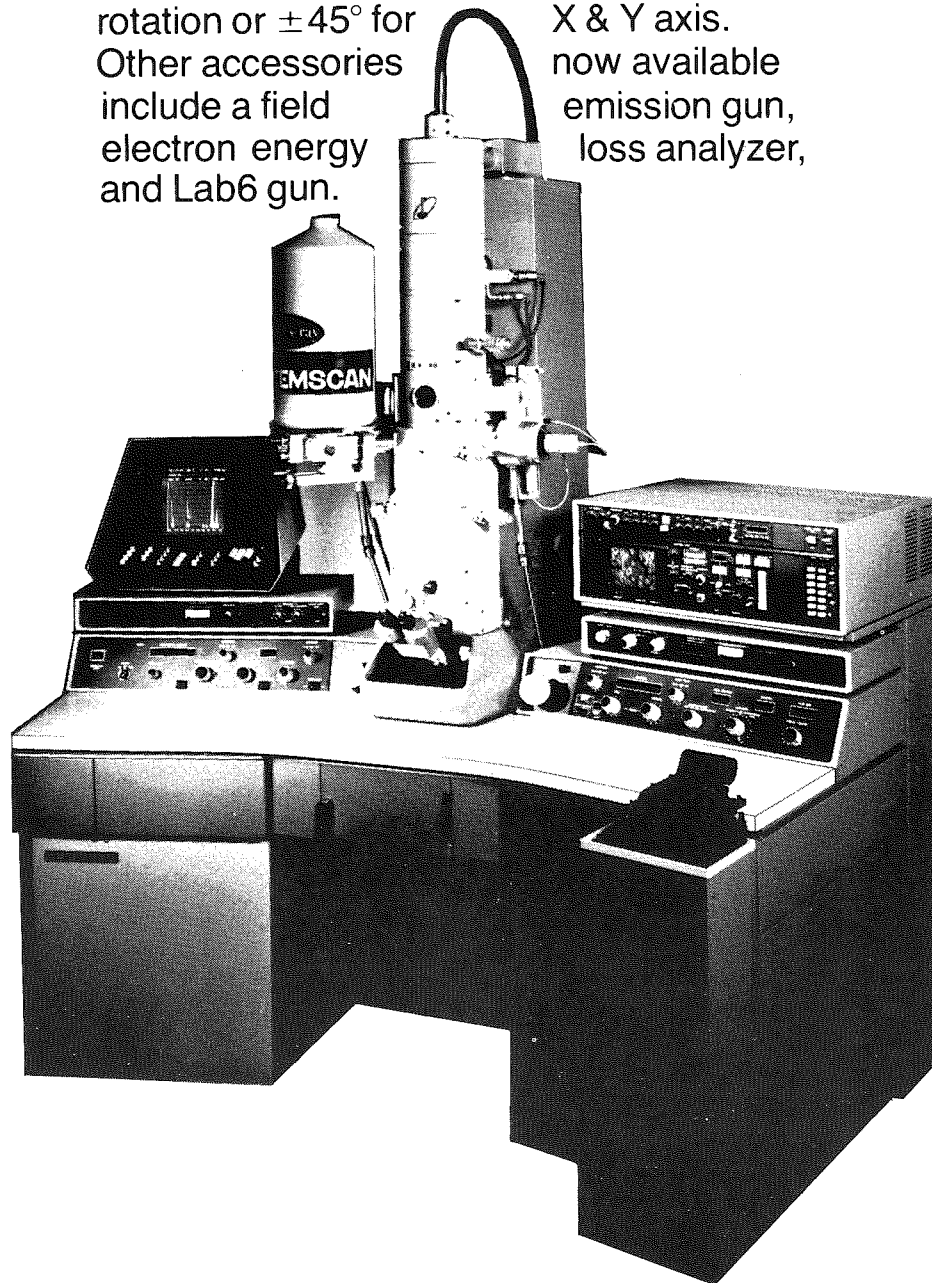
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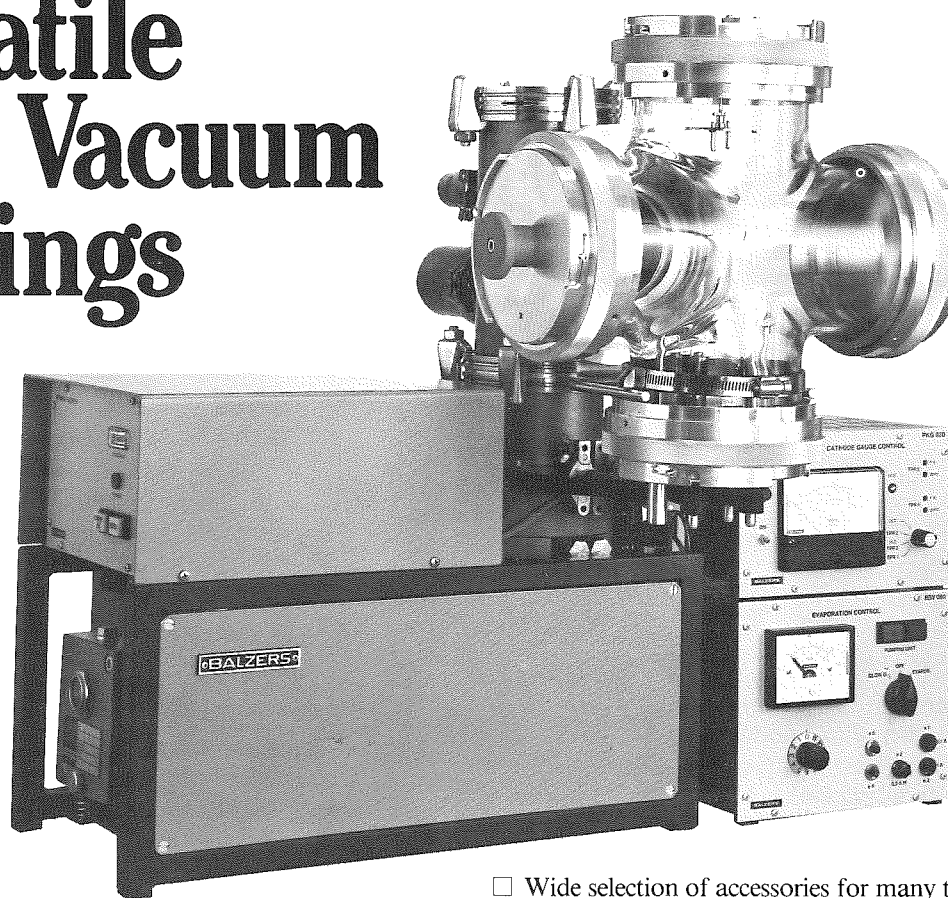


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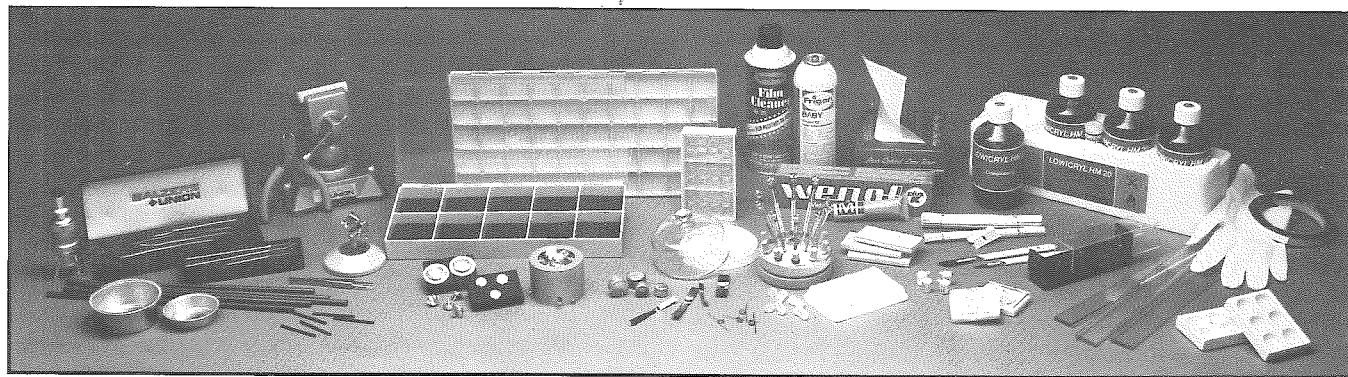
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THE DISAPPEARING SUBSTRATE PROCEDURE — ELECTRON MICROSCOPY OF TISSUE CULTURE CELLS

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This work was supported by
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SUMMARY

In the disappearing substrate procedure, tissue culture cells are fixed and dehydrated *in situ*, then are separated from the surface of the glass slides upon which they were grown by dissolving away an intermediary parlodian substrate. The cells are then pelleted, embedded in plastic, and thin sectioned. This technique allows the microscopist to section and examine large numbers of tissue culture cells while maintaining their normal morphology and fine structure.

INTRODUCTION

Ultrastructural studies of tissue culture cells involve the use of flat embedded cells or cell pellets (1, 2). The former process allows the sectioning of one or two specific cells of interest, while the latter technique permits the investigator to simultaneously section and examine many randomly arranged cells. Flat embedding is ideally suited to pharmacological, histochemical, cytochemical, and morphological studies. However, drawbacks to this procedure can include the following: the limited number of cells that can be sectioned at one time, the uniform orientation of the cells in the plastic, and the difficulty in sectioning the thin monolayers (1, 2).

Sectioning cell pellets allows the microscopist to examine a large number of cells, statistically improving the chances for accurate biological characterization. However, the formation of pellets normally requires the separation of the cells from the substrate prior to fixation. This is usually accomplished by trypsinization, mechanical means (scraping), and/or the use of various chelating agents (EGTA or EDTA) (3). Undoubtedly, these detachment pro-

cedures induce various ultrastructural alterations (removal of the glycocalyx, configurational changes, membrane disruptions, organelle displacement, etc.) that are undesirable.

The disappearing substrate procedure was developed as an alternative technique to the above processes. This method allows fixation, post-fixation, and dehydration of the cells *in situ* which, unlike other methods involving cells detached from their substrate, preserves each cell's *in situ* gross morphology as well as the spatial relationships of its ultrastructural components (1, 2).

MATERIALS AND METHODS

Cleaned glass slides 25 x 75 mm, completely frosted on one side (Esco, Buffalo, N.Y.), were totally immersed in a 3% solution of parlodian in amyl acetate. The slides were left in the parlodian solution for 10 minutes to insure sterilization. They were then withdrawn, tilted vertically to allow the excess solution to drain off, and dried in a dessicator to prevent moisture accumulation which often occurred as a result of rapid solvent evaporation. The coated slides were then placed in petri plates (frosted side up) to which media and cells (human fibroblasts) were added. After a period of 1/2 to 2 hours, the cells were firmly attached to the parlodian substrate. At 24, 48, and 72 hours post-subculture, the slides were withdrawn from the medium and gently rinsed by dipping several times in a serum-free medium solution or phosphate buffered saline. The slides were then processed through a series of coplin jars containing the various electron microscopy (EM) preparative solutions. The primary fixative consisted of a 0.1M PIPES (piperazine N-N'bis (2 ethanol sulfonic acid))buffered (pH 7.4) 3% glutaraldehyde solution (1 hr.

at 24°C). After several buffer rinses of PIPES, a buffered 1% osmium tetroxide solution was used as a secondary fixative (30 minutes at 24°C). Several of the samples were prepared with ruthenium red (500 ppm) in the fixative, buffer rinses, and post-fixation solutions in order to demonstrate acid mucopolysaccharides (AMPs) (4). A brief water rinse preceded dehydration through a graded series of ethyl alcohol solutions (75, 95, 100%).

Following dehydration, the slides were immersed in propylene oxide which dissolved the parlodian substrate, releasing the cells. The propylene oxide solution containing the cells was transferred to a glass centrifuge tube and centrifuged at a low speed for several minutes. Routine infiltration of the resultant pellet with resin continued in the same centrifuge tube using a modified Luft (5) epoxy mixture. After embedment, the samples were thin-sectioned on a DuPont Sorvall MT-2B ultramicrotome using a diamond knife. The thin sections were stained with uranyl acetate and lead citrate, and examined on a Phillips 300 transmission electron microscope.

RESULTS

The parlodian membrane remained intact and tenaciously attached to the frosted surface of the slide throughout the course of the experiment. Smooth surfaced glass slides proved unsatisfactory because of frequent failure of the membrane to remain adherent to the glass. The cultured cells remained firmly attached to the parlodian membrane throughout processing for electron microscopy. When the slides were immersed in the propylene oxide, the parlodian dissolved, separating the cells from the substrate. Additional propylene oxide rinses appeared to remove all traces of the parlodian from the sample which was then pelleted and embedded. Light microscopic surveys of thick sections (0.5 to 1.0 μm) allowed selection of cells of interest for subsequent thin sectioning and electron microscopic survey (Fig. 1).

Since the parlodian membranes did not withstand autoclaving, occasional culture contamination was noted. However, several repetitions of each sample usually provide an adequate number of uncontaminated petri plates, slides, and cells. Parlodian coated slides can be gas sterilized with ethylene oxide if sterilization is required.

The morphology of the fibroblasts and their inherent ultrastructural detail was excellent (Fig. 2) and compared favorably to that observed in flat embedded cells. Concomitantly, the ability to section a large number of cells without using a procedure that required initial trypsinization and repeated centrifugation at every step was a time saving feature.

Visible structural details included bundles of cytoplasmic 60-80 Å diameter microfilaments and 100 Å intermediate filaments frequently oriented parallel to the long axis of the cell (Figs. 2 and 3). These filaments are often not observed in cells subjected to trypsinization. Such procedures tend to cause the flattened cells to "round up" (become spherical) with corresponding changes noted in fine structure. The configuration of the nucleus (Fig. 4) in the disappearing substrate procedure was similar to that

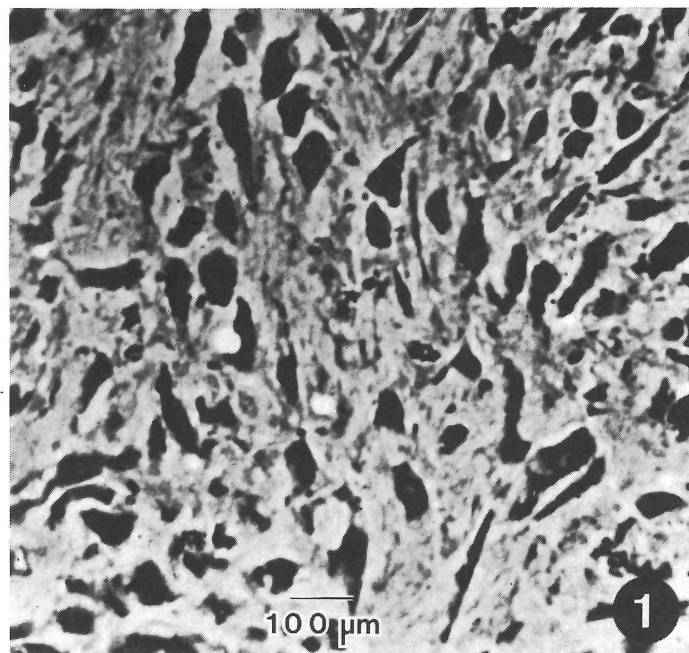


FIGURE 1: Light micrograph of randomly oriented cells observed in a thick section (0.5 to 1.0 μm) of a pellet produced using the disappearing substrate procedure. A total of 270 cells were noted in this particular section, which was stained with methylene blue-azure II. X125.

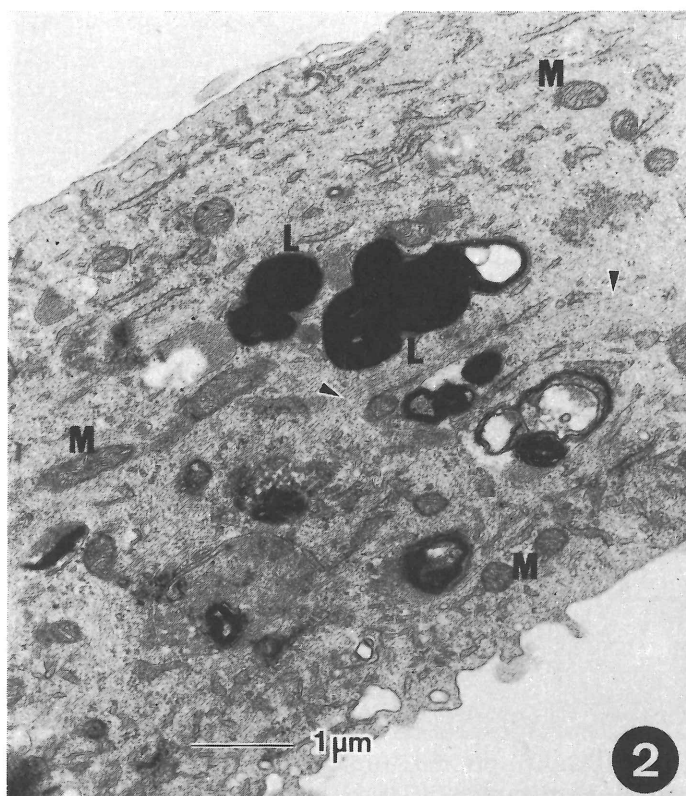


FIGURE 2: Cultured fibroblast demonstrating excellent preservation of ultrastructural detail using the disappearing substrate procedure. The cytoplasm contained the usual organelles including lysosomes (L), mitochondria (M), and longitudinal arrays of intermediate sized filaments (arrows). X18,240.

of living cells examined by phase microscopy and differs from the spherical or incised shapes observed in trypsinized cells (Fig. 5). Also, the reaction products formed by the ruthenium red and AMPs in the cell coat were apparent. The glycocalyx was clearly visible and could be observed uniformly coating the upper and outer portions of the various cells (Figs. 3, 4 and 6). The cell surface facing the substrate was more or less devoid of reaction products (Figs. 3 and 4). All of the structural differences characteristic of the free and attached surfaces of cells *in situ* were observed in these pelleted cells. Pinocytotic vesicles facing the upper and outer surfaces of the cell were observed to contain reaction products (Figs. 3, 4 and 6), demonstrating the cell's ability to re-incorporate the AMP material.

DISCUSSION

The disappearing substrate procedure proves useful in cytological investigations of animal tissue culture cells. A large number of cells can be simultaneously sectioned,

each providing a random sectional view. The retention of cellular detail using the disappearing substrate procedure is similar to that observed by light or TEM studies of viable or flat embedded cells. Also, appropriately modified preparative treatment preserves the detailed structure of the glycocalyx. The processing steps for EM, utilizing coplin jars and slides, is both rapid and efficient. The several low speed centrifugation steps that are required can be carried out on the fixed cells without concern for induced cellular disruptions or distortions. Although variations of the procedure have been previously reported, this particular technique appears to be the most useful and reliable method for the TEM study in *in vitro* cells (Figs. 6, 7 and 8).

Modifications of this procedure may prove useful when samples of suspended cells are required. For example, this process may be helpful in flow-microfluorometer (FMF) studies and would permit the survey of fixed and treated cells without requiring initial cell release by enzymatic, mechanical, or chemical means. Similarly, this procedure would be applicable to scanning electron microscopical studies of the adherent (substrate facing) surfaces of tissue culture cells.

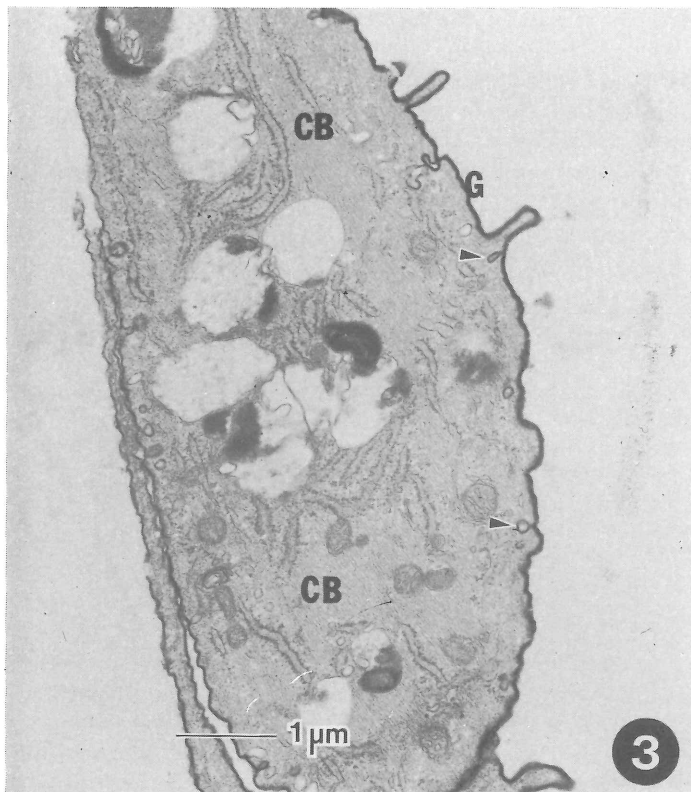


FIGURE 3: The presence of the acid mucopolysaccharide containing glycocalyx (G) on the upper surface of the plasma membrane was quite apparent in ruthenium red treated cells. Contractile bundles (CB) of microfilaments and intermediate sized filaments predominated in the cytoplasm while pinocytotic vesicles (arrows) were observed to incorporate ruthenium red on their inner surfaces. X18,760

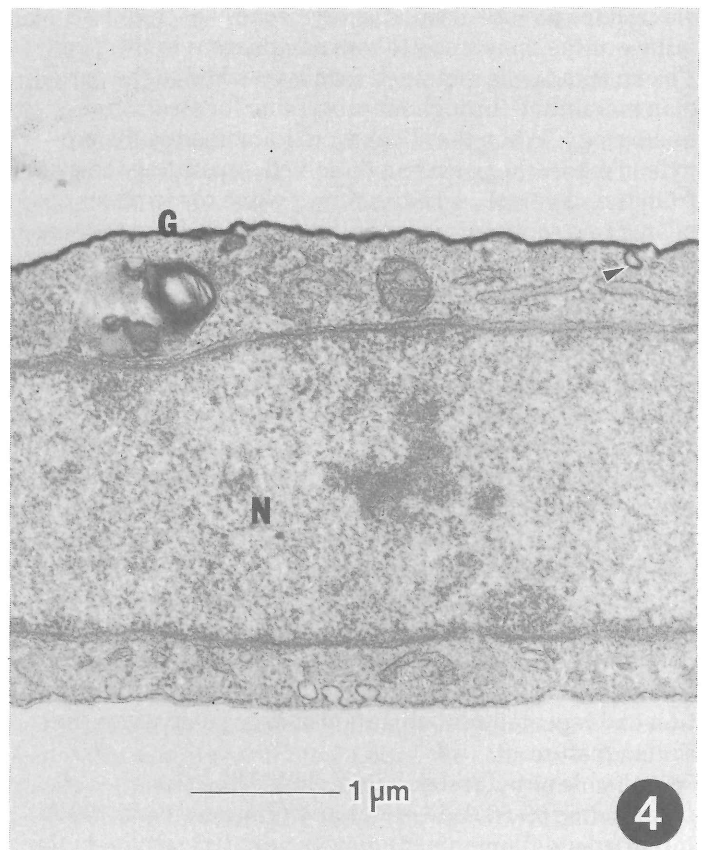


FIGURE 4: Nuclear (N) morphology, consistent with that observed by phase light microscopy, was noted in this transmission electron microscopy section of a cultured fibroblast. The glycocalyx (G) found on the upper cell surface and the lining of the pinocytotic vesicle (arrow), demonstrated an affinity to the ruthenium red. X27,700

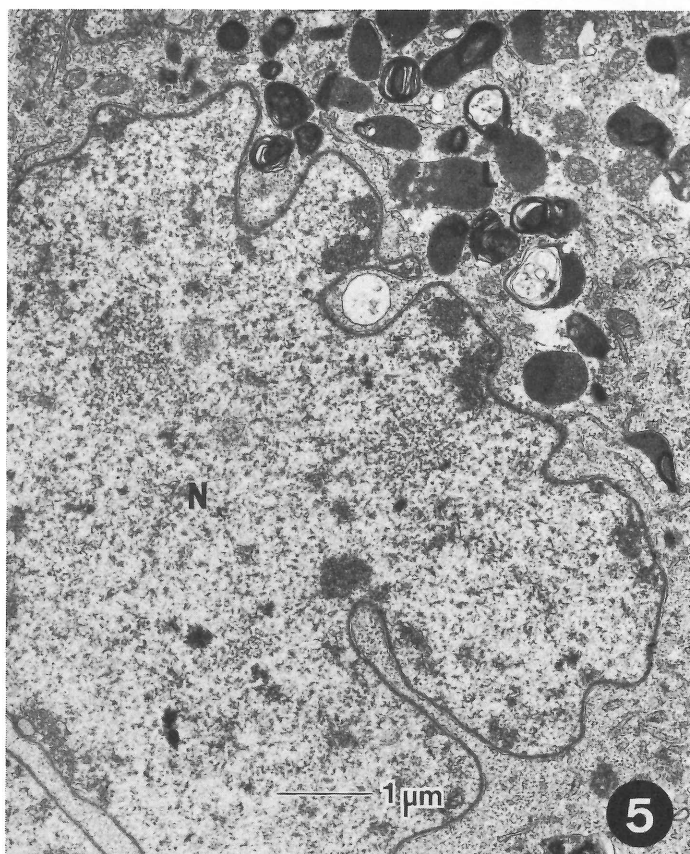


FIGURE 5: The ultrastructural details of a human fibroblast tissue culture cell trypsinized from the substrate and pelleted by centrifugation prior to fixation. The irregular configuration of the nucleus was in contrast to that observed in cells prepared using the disappearing substrate procedure. The heavy concentration of lysosomes (L) were characteristic features of plateau phase cells from scene-scent non-transformed cultures. X18,000

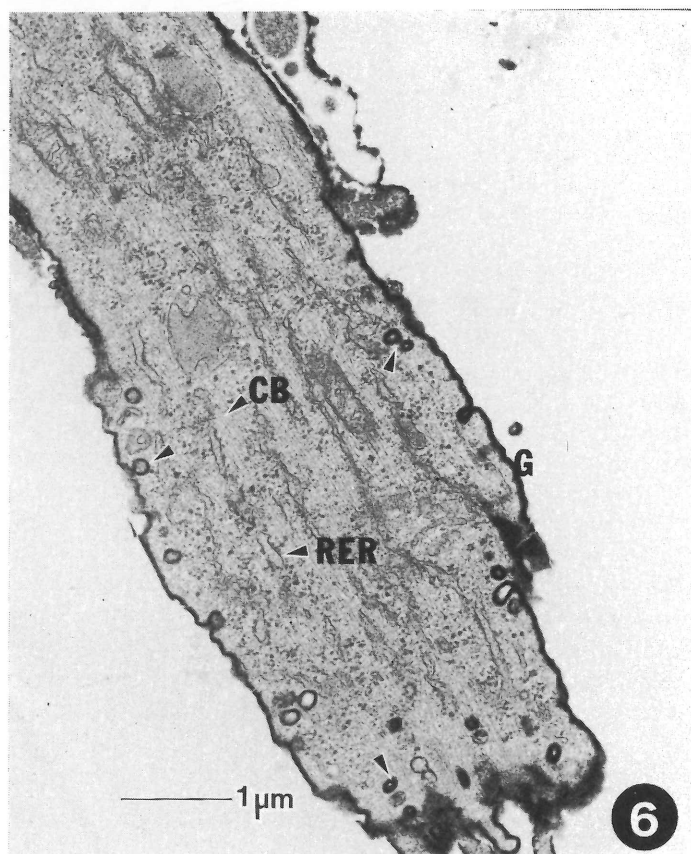


FIGURE 6: Contractile bundles (CB) of intermediate sized filaments and rough endoplasmic reticulum (RER) were prominent features of this tissue culture cell. The glycocalyx (G) and the lining of the pinocytotic vesicles (arrows) demonstrated an affinity to the ruthenium red stain. X20,500

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Secretary's Message

TSEM now has 447 members, including 423 regular and student members and 24 representatives of corporations which are our friends. TSEM Journal is currently being sent to 80 libraries within the state of Texas and to 55 libraries at major universities and institutions elsewhere in the United States and in Canada. These numbers give you some idea of the exposure your material receives when published in the Journal.

Not all of our readers live in Texas. The membership list shows addresses in California, Maryland, Minnesota, New York, Illinois, Louisiana, Oregon, Arizona, Utah, Oklahoma, Massachusetts, Kansas, Ohio, Arkansas, Florida and Mexico. Not all of us consider ourselves primarily electron microscopists, but we all appreciate electron microscopy and value the unique insights

it can bring into problems in our chosen disciplines. There is a valuable opportunity here for interaction between disciplines, for the sort of cross-fertilization that can produce highly original research. Interaction can take place both in person and through the Journal. To take full advantage of this we all need to publish more in our journal. Most of us are going to think first about writing for a journal in our area of specialty — but how about writing the material up twice? Once for your specialty and once, from a different point of view and stressing the electron microscopy, for TSEM? In addition to the benefits from expanded interaction — those extra publications never hurt anyone!

Elizabeth Root

TSEM Minutes

The TSEM Business Meeting was called to order by President Goldstein at 1:15 p.m., April 3, 1982, in Hubbard Hall on the TWU Campus in Denton, Texas.

1. The minutes were read and approved.
2. The treasurer's report was presented and approved.
3. Future meetings were announced by Pat Davis: The Fall meeting at Hotel Galvez at Galveston, October 7-9, 1982. The Spring 1983 meeting in Austin or San Marcus or vicinity.
4. New officers were presented: Charles Mims, President-Elect; Elizabeth Root, Secretary; Ernest Couch, Program Chairman-Elect.
5. Paul Baur reported on the TSEM Journal: Hope to have 5 to 6 articles per issue. The cost is about \$3 per issue. Want active regional editors. Randy Moore is the Editor in charge of

Regional News. Hilton Mollenhauer is the new Technical Editor. Robert Blystone is the Editor in charge of Advertising.

6. Awards were presented to past editors: Bob Turner, Ann Goldstein and Elaine McCoy.

7. Thirty-six new members were accepted into the society.

8. Dr. Goldstein thanked TWU and the local arrangements committee for the meeting.

9. Dr. Goldstein handed the gavel to the new President, Bruce Mackay, who acknowledged the contributions of the outgoing President.

10. The meeting was adjourned at 1:37 p.m.

Respectfully submitted,
Marilyn N. Smith
Secretary, TSEM

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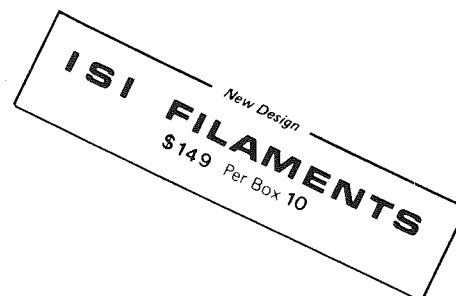
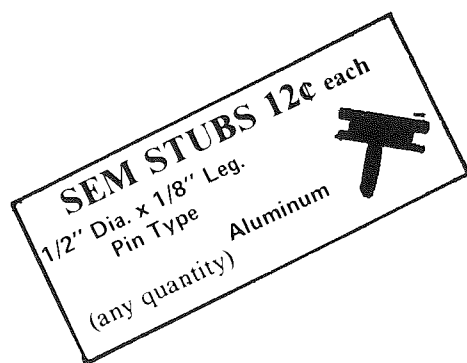
SEM	Diameter	Order Part No.
ISI-mini	7.7mm	ISI-D2
ISI	13.5mm	ISI-D1
AMR 1200	3/8"	AMR-1200
AMR	3/4"	AMR-D19
Cambridge—600	1/2"	S-600D
Cambridge other	3/8"	use AMR-1200
Etec	0.365"	ET-1
CamScan III	18mm	CS-3
Hitachi	20mm	H-20
* (1) Hitachi	19.8mm/ metal ring	H-198-M
* (2) ARL-Probe	3/4" x 0.285"	ARL-D
JEOL	20mm	H-20
SEMCO/NOVASCAN	16.6mm	NS-1
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Job Opportunities

**POSITION AVAILABLE
POSTDOCTORAL APPOINTMENT OR RESEARCH ASSISTANTSHIP
OPPORTUNITY TO TRAIN IN ELECTRON PROBE X-RAY
MICROANALYSIS OF BIOLOGICAL TISSUE**

Individual will be responsible for laboratory work and collection of data for one-year grant project involving x-ray microanalysis of diffusable elements in biological tissue. July 1, 1982 to June 30, 1983. Duties will include handling of animals; sacrificing animals and removing tissue; freezing tissue; cryoultramicrotomy and freeze-drying of tissue; electron probe x-ray microanalysis of tissue, which involves operation of scanning electron microscope and computer-based x-ray analysis system; compilation and statistical analysis of data; routine laboratory procedures and cleanup; keeping laboratory records; and library research. Training will be provided.

Cryoultramicrotomy requires high degree of manual dexterity, concentration, and patience. Previous experience with ultramicrotomy, electron microscopy, and ultrastructure desirable. Some previous familiarity with computers required. Must demonstrate quantitative aptitude. Prefer course in quantitative chemical analysis and instrumental analysis.

Contact: Dr. Nancy Smith
Department of Anatomy
University of Texas Health
Science Center
7703 Floyd Curl Drive
San Antonio, TX 78284
512-691-6983

**ELECTRON MICROSCOPE TECHNOLOGIST II
(Full Time Permanent)**

Must be proficient in the following: prep of biological tissues for TEM and SEM, ultramicrotomy with diamond knives, EM photo procedures, WORKING knowledge of TEM and SEM, thin film prep and ability to instruct persons in routine procedures of EM. Experience in freeze etching desirable. Minimum requirements include 2 yrs University training in biological or physical sciences and at least 1 year full time TEM work experience in EM lab, AFTER training. MORE EXPERIENCE DESIRABLE. Send vita and references to:

Dr. Judy Murphy
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Salary Range: \$1200-\$1350/month + civil service benefits.

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Editorial Policy Continued . . .

REGIONAL NEWS

News items should be submitted through the regional editor in your area and made to conform to the standard format used by the regional news section. Regional contributions should be sent to the Regional News Editor. Editorial privilege may be executed for the sake of brevity or to preserve the philosophical nature of the TSEM Journal.

The JOB OPPORTUNITIES section will be comprised of a "Jobs Available" and a "Jobs Wanted" sub-section. Anonymity of individuals listing in the Jobs Wanted or Jobs Available sub-sections may be maintained by correspondence routed through the Regional News Editor's office.

TECHNICAL SECTION

The Technical Section will publish TECHNIQUES PAPERS, HELPFUL HINTS, and JOB OPPORTUNITIES. The TECHNIQUES PAPERS will describe new or improved methods for existing techniques and give examples of the results obtained

with these methods. The format of the Technique Papers will be the same as that used for regular research reports. HELPFUL HINTS will be in the form of a brief report with an accompanying illustration, if required for clarity. Helpful Hints should embody techniques which will improve or expedite processes and/or procedures used in EM.

PUBLICATION PRIVILEGES

The right to publish in the TSEMJ is restricted to TSEM members or to those whose membership is pending. A membership application form can usually be found in each issue of the TSEMJ. Membership dues are as follows: students \$2.00; regular members \$10.00; and Corporate members \$75.00. Individuals who belong to TSEM by virtue of a corporate membership are invited to participate in Journal submissions as are our regular or student members. However papers of a commercial nature, either stated or implied, will not be accepted for publication as a Research Report or Techniques Paper. Such papers may be acceptable as advertising copy.

Meeting Announcements

(1) Texas Society for Electron Microscopy's Annual Fall Meeting — 1982

October 7-9

Marriott's Hotel Galvez in Galveston

Tentative Agenda

Thursday: Registration; Demonstration sessions on microprocessors, statistics and digitization of images

Thursday evening: Social

Friday and Saturday mornings: Platform and Poster sessions

Friday: Featured Speaker — Lee D. Peachey, President, EMSA

Friday Night: Banquet

Saturday: TSEM Business Meeting

Room Rates: \$40 S/D

Registration: \$25/R; \$15/S; \$35/NM

Contact: C.P. Davis, Program Chairman, Dept. of Microbiology, University of Texas Medical Branch, Galveston, Texas 77550.

(2) Texas Society for Electron Microscopy's Annual Spring Meeting — 1983

April 7-9

Quality Inn in Austin

Tentative Agenda

Thursday afternoon: Registration; Tour of U.T. Campus

Thursday evening: Social

Friday and Saturday mornings: Platform and Poster sessions

Friday evening: Invited Speaker

Saturday Noon: TSEM Banquet

Room Rates: \$40/S; \$45/D

Contact: C.P. Davis, Program Chairman, Dept. of Microbiology, University of Texas Medical Branch, Galveston, Texas 77550

(3) Louisiana Society for Electron Microscopy's Annual Fall Meeting — Nov. 5, 1982

Held at the University of Southwest Louisiana, Lafayette, Louisiana

An invited speaker program — Registration begins at 10:00 a.m.

Sessions start at 1:45 p.m.

Contact: Roy Brown, Dept. of Biology, University of Southwest Louisiana, Lafayette, Louisiana 70504

(4) American Society for Cell Biology's Annual Meeting — 1982

Nov. 30-Dec. 4

Baltimore Convention Center, Baltimore, Maryland

Contact: Professional Associates, 2012 Big Bend Boulevard, St. Louis, Missouri 63117, (314) 781-9192

APPLICATION FORM FOR TSEM MEMBERSHIP

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

Name of nominee _____

P.O. Address _____

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

Date 19

Signature of TSEM Regular Member making the Nomination

This application for Membership in the Society or this application for transfer from the grade of Student to Regular or Regular to Student Member should be sent to the TSEM Secretary. The form will be presented at the next meeting of the Executive Council for their approval (majority vote). The nominees will then be presented by the council to the membership at the next general business meeting for their approval (majority vote). Nominees will be added to the membership rolls at that time.

Presented to the Council at _____ meeting. Date _____

Action _____

Send Application to: Elizabeth Root
GEA 115
The University of Texas at Austin
Austin, Texas 78712

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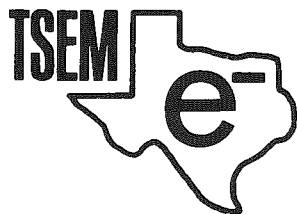
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Regional News

TEXAS TECH UNIVERSITY HEALTH SCIENCES CENTER

NEW FACULTY

The Department is pleased to announce the arrival of our new department chairman, Dr. Harry Weitlauf, who comes to us from the University of Oregon. We also welcome Dr. Fred Jackson who will be joining the faculty this year as a visiting assistant professor. We are sorry to lose Dr. Kenneth Karkos who will be leaving Texas Tech to return to school at the University of Wyoming. Dr. Karkos desires to obtain a Ph.D. in Clinical Psychology.

POSITIONS AVAILABLE

The Department of Anatomy will have four positions available to be filled over the next two years. We are interested in individuals with established research programs or individuals who show promising research potential. They should be willing to contribute to the teaching of Anatomy disciplines to medical and graduate students.

AWARDS

Drs. Roger Markwald and Ken Karkos were presented awards for excellence in teaching by the graduating class of 1982.

GRANTS

Coates, Penelope W., "Comparison of the Lateral Antebrachial Cutaneous Nerve to the Digital Nerve for Consideration as a Desirable Autograft", American Society for Surgery of the Hand, April 1, 1981 - March 31, 1982, \$2,000.

Hutson, James C., "Characterization of Sertoli Cells in Diabetic Animals", National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, April 1, 1982 - March 31, 1983, \$30,167.

Hutson, James C., "Effects of TSH on Sertoli Cells", National Institute of Child Health and Human Development, September 1, 1981 - August 31, 1982, \$31,263.

Markwald, Roger R., "Hyaluronate Binding Sites on Migrating Embryonic Cardiac Mesenchymal Cells", Southern Medical Association, July 1, 1981 - June 30, 1982, \$1,000.

Markwald, Roger R., "Mucopolysaccharide Metabolism in Cardiac Anomalies", National Heart, Lung and Blood Institute, April 1, 1982 - March 31, 1983, \$42,195.

Roberts, Lou A., "Atrial Pacemakers Characteristics and

Dominance", National Heart, Lung and Blood Institute, April 1, 1982 - March 31, 1983, \$46,286.

Seliger, William G., "The Study of Macrophages in the Diagnosis and Prognosis of Breast Cancer", Audrey B. Jones for Cancer Research and Treatment, September 1, 1980 - Indefinite, \$3,500.

Seliger, William G., "Reaction of Bone and Fibrous Connective Tissue to Selenium Deficiency in the White Rat", Institute for Nutritional Sciences, January 1, 1982 - April 31, 1983, \$5,000.

Yee, John A., "Effect of Insulin on Osteoblasts In Vitro", American Diabetes Association, North Texas Affiliate, July 1, 1981 - June 30, 1982, \$9,500.

Yee, John A., "In Vitro Studies on Isolated Endosteal Bone Cells", National Institute on Aging, March 1, 1982 - February 28, 1983, \$47,774.

PUBLICATIONS

Hay, D.A. and R.R. Markwald. 1982. Cardiac Development: Role of the Extracellular Matrix. *Anat. Rec.* 202:77A.

*Johnson, R.C. 1982. Origin of Mesenchyme in Mural Endocardium in Trabecular Regions of Chick Ventricle. *Anat. Rec.* 202:91A.

*Markwald, R.R., D.A. Hay and G.T. Kitten. 1982. Modifications of Extracellular Glycosaminoglycans (GAG) and Glycoprotein (GP) Ordering During the Migration of Embryonic Chick Cardiac Cushion Mesenchyme. *Anat. Rec.* 202: 120A.

Markwald, R.R. and T.P. Fitzharris. In Press. Cellular Migration Through the Cardiac Jelly Matrix: A Stereo-Analysis by High Voltage Electron Microscopy. Running Title: HVEM Stereo-Analysis of Cardiac Matrix.

*Runyan, R.B. and R.R. Markwald. 1982. Extracellular Components Trigger Endocardial Cell Seeding into Three Dimensional Collagen Cells. *Anat. Rec.* 202:163A.

Shew, R.L. and P.K.T. Pang. 1981. "Parathyroid Hormone and Uterine Contraction", presented at the Satellite Symposium on Comparative Endocrinology of Calcium Regulation, Urawa, Japan.

Shew, R.L. and P.K.T. Pang. 1981. "Effect of Parathyroid Hormone on Uterine Contraction in vivo", presented at the Ninth International Symposium on Comparative Endocrinology, Hong Kong.

Yee, J.A. and G.L. Stallings. 1982. Characterization of Bone Cells Isolated from the Cortical Endosteum of Adult Rabbits. *Calcified Tiss. Int.* 34:S16.

*Papers presented at the American Association of Anatomists Meeting.

Information for Authors

PURPOSE: The goal of the TSEM Journal is to inform members of the society and the Journal's readers of significant advances in electron microscopy, research, education, and technology. Original articles on any aspect of electron microscopy are invited for publication. However, the TSEM Journal is biologically oriented and articles along those lines will be preferred. Guidelines for submission of articles are given below. The views expressed in the articles, editorials and letters represent the opinions of the author(s) and do not reflect the OFFICIAL POLICY OF THE INSTITUTION with which the author is affiliated or the Texas Society for Electron Microscopy. Acceptance by this Journal of advertisements for products or services does not imply endorsement. Manuscripts and related correspondence should be addressed to Paul S. Baur, Jr., Ph.D., Editor, TEXAS SOCIETY FOR ELECTRON MICROSCOPY JOURNAL, Division of Cell Biology, Shriners Burns Institute, 610 Texas Avenue, Galveston, Texas 77550.

GUIDELINES: Manuscripts written in English will be considered for publication in the form of original articles, historical and current reviews, case reports and descriptions of new and innovative EM techniques. It is understood that the submitted papers will not have been previously published. Accepted manuscripts become the full property of the TEXAS SOCIETY FOR ELECTRON MICROSCOPY JOURNAL and may not be published elsewhere without written consent of the Editor. The author should retain one complete copy of the manuscript. The JOURNAL is not responsible for loss of the manuscript in the mail.

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MANUSCRIPT PREPARATION. Manuscripts should be submitted in conformance with the following guidelines:

FORMAT: Submit an original and two copies of the entire manuscript, typed, double-spaced, on 8-1/2 x 11 white paper, leaving ample margins. Number each page and identify the article by placing, at the top left of the page, a shortened form of the title, followed by the last name of the first author.

TITLE PAGE. Include:

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SECTIONS. The text of each original article and technical

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Historical and current reviews and case reports do not need to be divided into the aforementioned sections.

ABSTRACT. Summarize the article in no more than 150 words. This takes the place of a final summary paragraph.

REFERENCES to other work should be consecutively numbered in the text using parentheses and listed at the end, as in the following examples:

(1) A. Glauert, Practical Methods in Electron Microscopy, Vol. 2 (North-Holland, Amsterdam, 1974) 82-88

(2) P.S. Baur, Jr., G.F. Barratt, G.M. Brown and D.H. Parks, Ultrastructural Evidence for the Presence of "Fibroclasts" and "Myofibroclasts" in Wound Healing Tissues. J. of Trauma. 19 (1979) 744-756

(3) D. Gabor, Information Theory in Electron Microscopy, in: Quantitative Electron Microscopy, Eds. G.F. Bahr and E. Zeitler (Williams and Wilkins, Baltimore, 1965) 63-68

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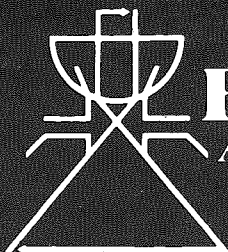
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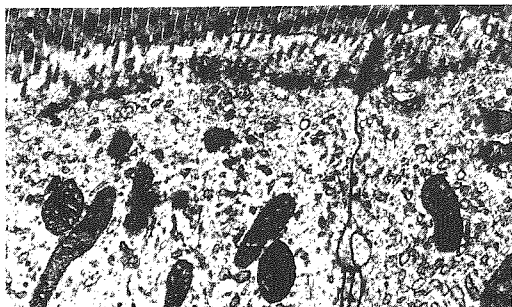
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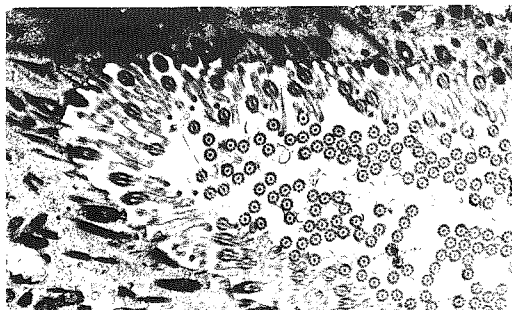
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