TSM

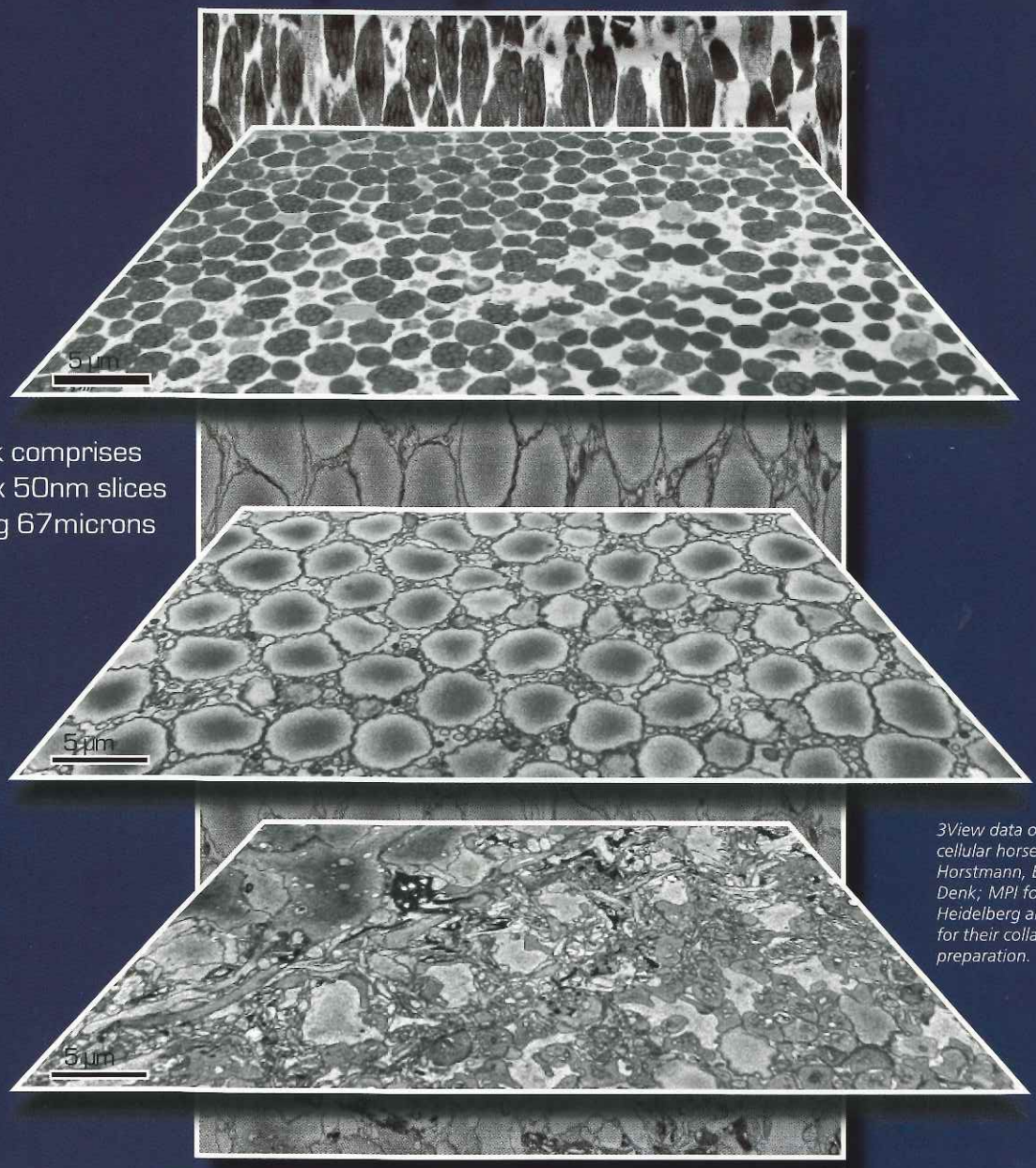
Texas Journal of Microscopy

Volume 38, Number 1, 2007 • ISSN 1554-0820 • Visit our web site at: www.texasmicroscopy.org

MULBERRY CYSTOLITH HISTOCHEMISTRY

3VIEW™

Perfectly aligned image stacks



Z stack comprises
1350 x 50nm slices
totalling 67microns

3View data of retina with extra-cellular horseradish peroxidase (HRP). Horstmann, Euler, Hausselt, Briggman, Denk; MPI for Medical Research Heidelberg are kindly acknowledged for their collaboration and specimen preparation.

Serial imaging in the SEM Resolution that approaches nanometer-scale, over a significant volume, can now be achieved by automated, *in-situ* microtomy and serial imaging of the block face. A highly stable stage and novel ultra-microtome design are used to achieve excellent registration between sequential 'slices'.

Cutting, retraction and specimen advance take only seconds, leaving valuable time for image acquisition. One software platform controls the automated cutting, SEM and image acquisition protocols such that a "Z-stack" of images is recorded at the end of the experiment.

gatan

www.gatan.com

A Revolution in 3D Microscopy

Contents

TEXAS JOURNAL OF MICROSCOPY
VOLUME 38, NUMBER 1, 2007
ISSN 1554-0820

Camelia G.-A. Maier, Editor
Department of Biology, Texas Woman's University, Denton, TX 76204

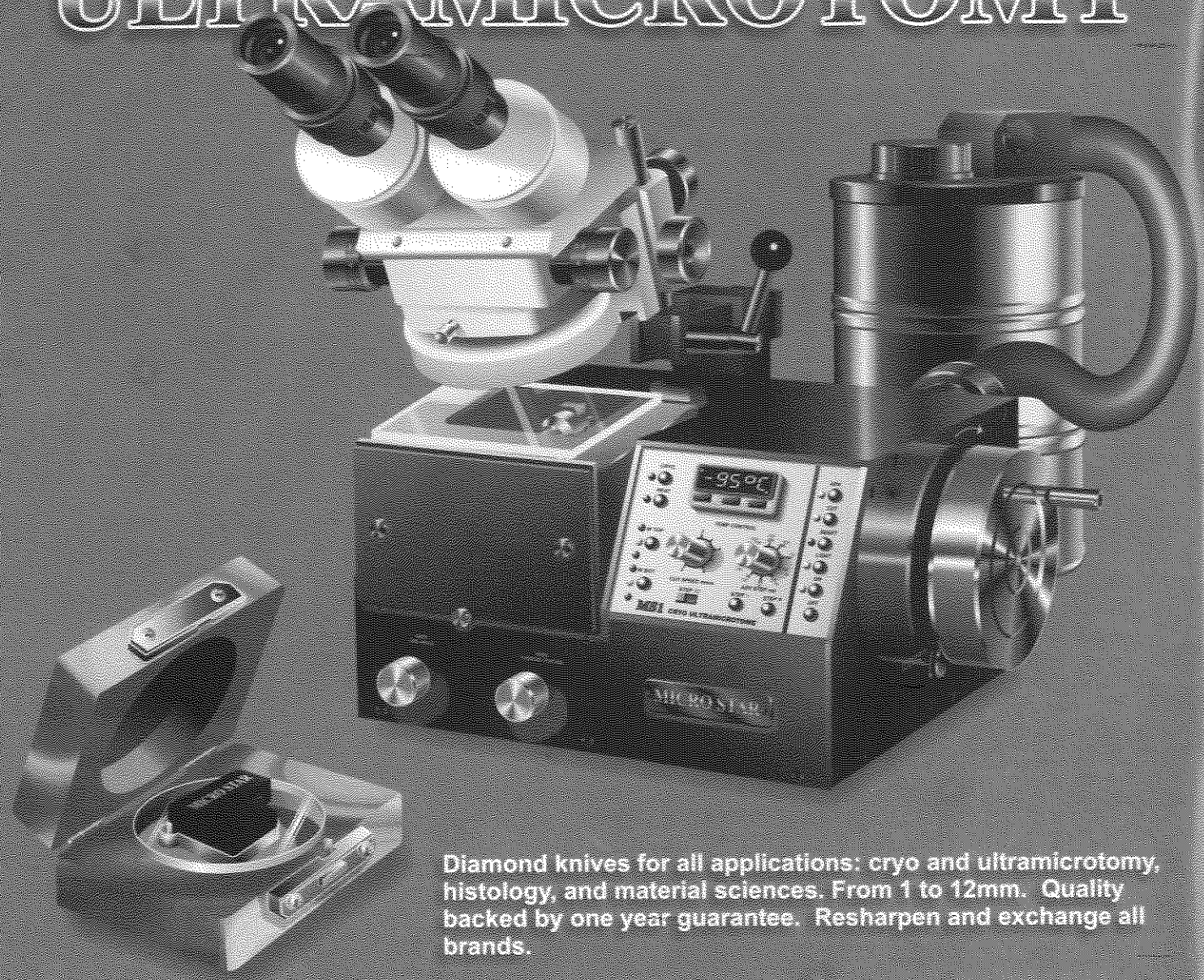
Official Journal of the Texas Society for Microscopy
"TSM - Embracing all forms of microscopy"
www.texasmicroscopy.org

ON THE COVER

This plate illustrates the utilization of histochemical extraction and Periodic acid-Schiff (PAS) staining to understand the nature of mulberry (*Morus rubra*) cystoliths. The histochemical techniques were adapted from W.A. Jensen 1962 (Botanical Histochemistry, 408 p. Freeman, San Francisco). This plate is organized into rows representing cystoliths treated in the same manner. The columns represent selected cystoliths, each being one replica of the corresponding treatment. ***Row One*** contains untreated cystoliths in absolute alcohol; note that the cystoliths vary in color. The cystoliths in ***Row Two*** have been demineralized. The $CaCO_3$ contained in their bodies was removed using a dilute HC1 solution. Without the Ca carbonate, the cystoliths still retain their shape. Cystoliths in ***Row Three*** have been stained by employing the PAS technique which stains carbohydrates. Note that the cystolith stalks do not stain because they are impregnated with silicon. ***Row Four*** contains cystoliths which have been treated with 0.5% ammonium oxalate which removes pectins; after this treatment, the cystolith stalks stain with PAS. After treatment with ammonium oxalate, the external (peripheral) part of the cystoliths swells, perhaps due the uncoupling of the cellulose fibers after the pectin is removed. ***Row Five*** shows PAS-stained cystoliths after treatment with 4% NaOH which removes hemicelluloses. ***Row Six*** shows PAS-stained cystoliths after treatment with 17.5% NaOH which removes non-cellulosic polysaccharides. The size of the cystoliths in rows five and six was reduced. Cystoliths in the ***Seventh Row*** have been treated with hydrofluoric acid which removes all traces of silicon and leaves only the cellulosic component. By this stage, there is considerable variation in the size of the individual cystoliths, however, they still show the characteristic body and stalk arrangement. Unpublished research of H.J. Arnott and M.A. Webb. Plate by H. J. Arnott.

MICRO STAR

ULTRAMICROTOMY



Diamond knives for all applications: cryo and ultramicrotomy, histology, and material sciences. From 1 to 12mm. Quality backed by one year guarantee. Resharpen and exchange all brands.

Cryo Ultramicrotome integrated in a single portable instrument. Designed for TEM and SPM sample preparation. Microprocessor controlled cryogenic system. Includes Dewar and complete set of attachments. Sections 25nm to 5µ, cryo temperatures to -130°C. Fully automatic or manual operation. High precision and stability at a fraction of the cost of other systems.

Request information, manuals and complete price list, or see them at the web.

800 533 2509
FAX 936 294 9861
MICROSTARTECH.COM

MICRO STAR
TECHNOLOGIES

President's Message

I wish to thank the Executive Council and the members of the Texas Society for Microscopy for support and advice during the past year. The leadership of Ernest Couch, President-Elect, and Pam Neill, Program-Chair, has resulted in our spring meeting to be held on another college campus in many years. A few years ago, Texas Woman's University was the host of a TSM spring meeting. This time, Texas Christian University is providing the impressive Alumni Center for our spring 2007 meeting. In order to encourage greater student participation, the members present at the fall 2006 TSM meeting in Allen voted to invite the TCU students to register for attending the spring meeting sessions without paying a registration fee. As further inducements for attending and presenting at our meetings, the number and amounts of awards for the "Howard J. Arnott" Best Student Competition have been increases. Tina and Amanda Halupnik prepared a flier to advertise this meeting and mailed the flier to our membership. Amanda Halupnik has also designed a new certificate for student presentation winners. Jodi Roepsch has continued to assist the Program-Chair and Webmaster, for which we are deeply thankful.

The dedicated supervision of our TSM website by Becky Holdford has resulted in recruitment of a diversity of biologists, materials engineers and bioengineers from academia and industry to join our Society and present at the meetings.

Camelia Maier, Editor of the *Texas Journal of Microscopy* has had a record number of requests for advertisements. With the support of the Executive Council she has increased the prices of advertisements in order to be able to increase the size of the journal. The spring issue of the journal, Vol. 38(1), will be the largest issue ever published! We greatly appreciate the wonderful support provided by our corporate members to TSM and its publication. Our editor has worked diligently to make arrangements for the *Texas Journal of Microscopy* to be listed in databases. This will substantially enhance the value of publishing in the Texas Journal of Microscopy.

We will have a change of officers at this spring meeting. I will become the Past-President with Ernest Couch the President, Ann Ellis the Treasurer and Phoebe Doss the Program Chair. Nabarun Ghosh will become President-Elect with Michael Pendleton the Secretary-Elect and Sandra Westmoreland the Program Chair-Elect. I greatly appreciate the very capable contributions of Bob Droleskey, Interim-Treasurer, and Pam Neill, Program Chairman. Tina Halupnik will continue as Secretary. With the approval of the By-Laws revisions, our officer-elects will have voting privileges on the Executive Council.

Thank you for the opportunity to provide leadership in the Texas Society for Microscopy. May the society continue to thrive!

Joanne (Judi) Tontz Ellzey
2006-2007 President,
Texas Society for Microscopy

Call For Papers

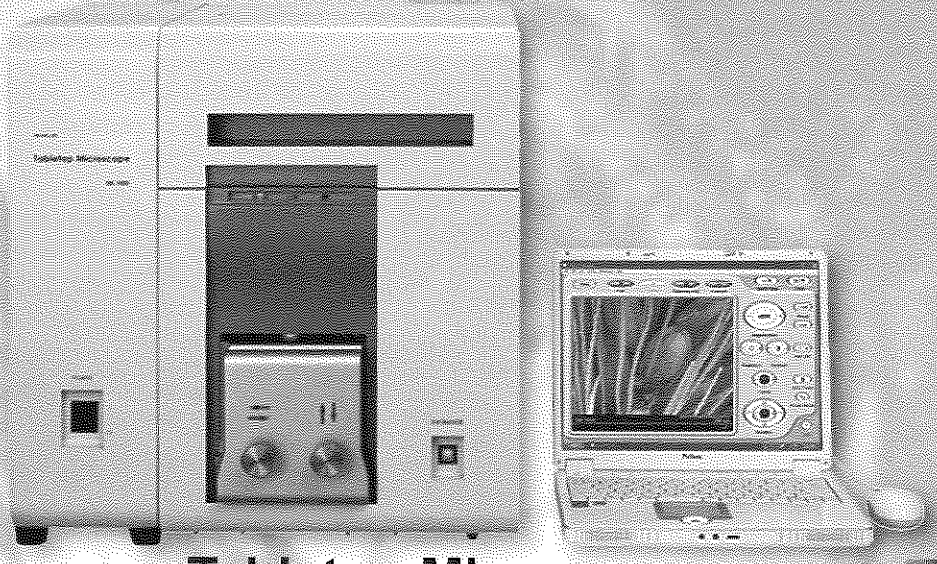
Manuscripts are needed for the next edition of the Texas Journal of Microscopy. Please send your work as short communications, full articles or review articles in biological sciences, material sciences or education to:

Camelia G.-A. Maier, TSM Journal Editor
Department of Biology, TWU, Denton, Texas 76204-5799
(940) 898-2358, cmaier@twu.edu

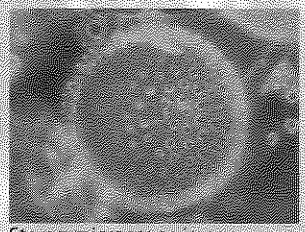
Manuscript deadline is July 15, 2007

Viewing the complex art of nature...

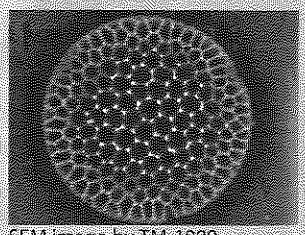
Simply.



Tabletop Microscope
TM-1000

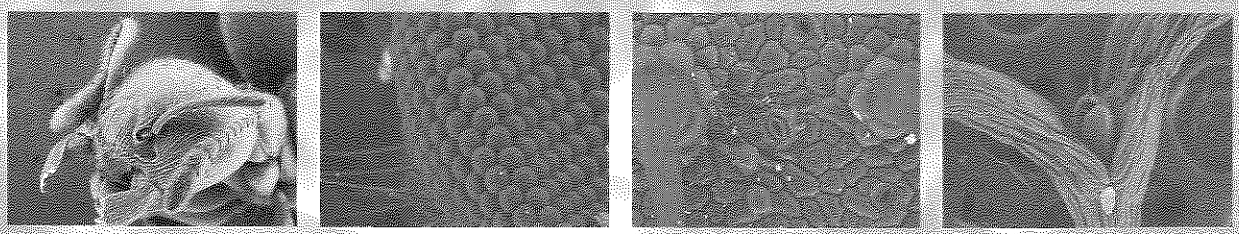


Stereo-microscope image



SEM image by TM-1000

No coating required.
As easy to use as a digital camera.
Stereoscopic observation with greater depth of focus.



Hitachi High Technologies America, Inc.
5100 Franklin Drive
Pleasanton, CA 94588
800.227.8877 / 925.218.3230 (F)
www.hitachi-hta.com

HITACHI
Inspire the Next

CORPORATE MEMBERS

Atomic Spectroscopy Instruments, Inc.
Graham R. Bird
1021 Yellow Rose Dr., PO Box 1035
Salado, TX 76571-1035
Phone/FAX (254) 947-8929
grbird@thegateway.net

Boeckeler/RMC Instruments, Inc.
Dave Roberts
4650 Butterfield Drive
Tucson, AZ 85714
(520) 745-0001 FAX (520) 745-0004
dave@boeckeler.com

Bruker AXS (PGT)
Alan Hollaar
5465 E. Cheryl Parkway
Madison, WI 53711-5373
(805) 523-1882 FAX (805) 523-1896
Alan_Hollaar@bruker-axs.com

CARL ZEISS SMT
German Neil
1 Zeiss Dr.
Thornwood, NY 10594
(914) 747-7700 FAX (914) 681-7443
neal@smt.zeiss.com

EDAX, Inc.
Tina Wolodkowich
Sales & Marketing Coordinator
(201) 529-6277 FAX (201) 529-3156
Tina.Wolodkowich@ametek.com

EMITECH
Linda Dailey
PO Box 680221
Houston, TX 77268
(281) 580-0568 FAX (281) 580-0593
emitech@earthlink.net

Electron Microscopy Sciences/Diatome
Richard Rebert/Stacie Kirsch
1560 Industry Road, PO Box 550
Hatfield, PA 19440
(800) 523-5874
sgkcck@aol.com
www.emsdiasum.com

FEI Company
Dennis Richards
8522 Old Quarry Drive
Sugar Land, TX 77479-1970
(281) 545-1353 FAX (281) 545-1393
drichards@feico.com
www.feicompany.com

Gatan, Inc.
Chad Tabatt
5933 Coronado Lane
Pleasanton, CA 94588
925-224-7318
ctabatt@gatan.com

Hamamatsu Photonic Systems
Butch Moomaw
360 Foothill Road
Bridgewater, NJ 08807-0910
(830) 885-2636 FAX (830) 885-7339
BMoomaw@hamamatsu.com

Hitachi High Technologies America
Kevin Cronyn
1375 N 28th Ave., PO Box 612208
Irving, TX 75261
(972) 615-9086 FAX (972) 615-9300
Kevin.Cronyn@Hitachi-hta.com

IXRF Systems
Travis W. Witherspoon
15715 Brookford Drive
Houston, TX 77059
(281) 286-6485
travisw@ixrfsystems.com
www.ixrfsystems.com

JEOL (U.S.A.), Inc.
Richard Lois
256 Green Cove Drive
Montgomery, TX 77356
(409) 449-4141 FAX (409) 597-6200
lois@jeol.com

Leeds Instruments, Inc.
Alex Butzer / Jeff Lovett
8150 Springwood Drive, Ste. 125
Irving, TX 75063
(972) 444-8333 FAX (972) 444-8435
abutzer@leedsmicro.com
jlovett@leedsmicro.com
www.leedsmicro.com

Leica Microsystems, Inc.
Robert Seilor
2345 Waukegan Road
Bannockburn, IL 60015
(847) 922-8902 FAX (847) 362-8873
robert.seiler@leica-mycrosystems.com
www.leica-microsystems.com

M.E. Taylor Engineering, Inc.
SEMicro Division
21604 Gentry Lane
Brookeville, MD 20833
(301) 774-6246
www.semsupplies.com

Meyer Instruments
Rob Meyer
1304 Langham Creek, Ste. 235
Houston, TX 77084
(281) 579-0342 FAX (281) 579-1551
ces@meyerinst.com
www.meyerinst.com

Micro Star Technologies, Inc.
Cathy Ryan
511 FM 3179
Huntsville, TX 77340
(936) 291-6891 FAX (936) 294-9861
mistar@msn.com

Nanotech America
Kim Kanggasniemi
313 S Jupiter Road, Suite 105
Allen, TX 75002
(972) 954-8014
Kim@nt-america.com

Smart Imaging Technologies
Ira Bleiweiss
1770 St. James Place, Suite 414
Houston, TX 77056
info@smartimtech.com

SPI SUPPLIES
Charles A. Garber, Ph.D.
Div. of Structure Probe, Inc.
569 East Gay St.
West Chester, PA 19381-0656
http://www.2spi.com/

Ted Pella, Inc.
James Long
1807 Slaughter Lane #200-487
Austin, TX 78748
(512) 657-0898 FAX (530) 243-3761
James_Long@TedPella.com

Thermo Electron Co.
David Leland
2551 W. Beltline Hwy
Middleton, WI 53562-2609
(970) 266-1164 FAX (408) 516-9882
david.leland@thermo.com
www.thermo.com

Tousimis Research Corporation
Callie Thomas
PO Box 2189, Rockville, MD 20847-2189
Yianni Tousimis
2211 Lewis Ave.
Rockville, MD 20851
(301) 881-2450 FAX (301) 881-5374

Abstracts

BIOLOGICAL SCIENCES
SPRING 2007

QUALITATIVE ASSAY OF INDOOR AIR USING A 3000 XTREME AIR PURIFIER WITH SPECIAL REFERENCE TO THE REDUCTION OF AERO ALLERGENS AND POLLUTANTS. RUPA PATEL[1], MANDY WHITESIDE[1], NABARUN GHOSH[1] and JEFF BENNERT[2], [1]Department of Life, Earth and Environmental Sciences, West Texas A&M University, Canyon, TX 79016, [2]Jeff Bennert, Air Oasis, 3401 Airway Blvd. Amarillo, Texas 79118.

Indoor aeroallergens are often the cause of serious allergic and asthmatic reactions, affecting millions of people each year. To aid to the diagnosis and treatment and reduce the ailments of individuals suffering due to indoor aeroallergens, a thorough understanding and control of the indoor aeroallergens and other airborne particles are necessary. Patients showed susceptibility to air spora, including *Alternaria*, *Cladosporium*, *Curvularia*, *Pithomyces* and many smut teliospores. Indoor Air Quality (IAQ) in offices is an important aspect. Indoor levels of air pollutants can be 2-5 times higher, and occasionally 100 times higher, than outdoor levels. We standardized the procedure to analyze and compare the mold spores, mycelia, hyphae or other fungal bodies and pollen present in the indoor air. We used different variables to assay the air purifier Xtreme 3000. Sets of the Petri plates with Brain Heart Infusion agar (VWR) and coated slides with Gelvatol (Burkard Co., UK) were placed within 1 foot, 2 feet, 4 feet, 6 feet, and 12 feet from the air purifier for various time intervals (24, 48, and 72 hours). All the experiments were carried out with High setting on the air purifier. The control sets of Petri plates and slides were placed in the room without using the air purification system. The prepared slides were examined, analyzed, and photographed using a BX-40 Olympus microscope attached to a DP-70 digital camera. Petri plates were examined with an SZ-40 stereoscope to observe and count the number of colonies. All control Petri plates showed vigorous growth of the microbial colonies after incubation at 37°C for 24 hours. The Petri plates placed closer to the air purifier (2 and 4 ft) produced least number of colonies after 24, 48, and 72 hours with the air purifier on. Minor trace of inoculum were observed in Petri plates at 2, 4, and 8 ft after 24 hours, indicating a definite reduction of the microbial aeroallergens in the room air. After 72 hours with the air purifier on, no microbial colony were observed on the Petri plates.

CYTOCHEMICAL LOCALIZATION OF REACTIVE OXYGEN SPECIES IN DIABETIC AND NON-DIABETIC KIDNEYS. E. ANN ELLIS[*], DENNIS L. GUBERSKI[**], and MARIA B. GRANT[***], [*]Microscopy and Imaging Center, Texas A&M University, College Station, TX 77843-2257, [**]Biomedical Research Models, Inc., Worcester, MA 01655, and [***]Department of Pharmacology and Therapeutics, College of Medicine, University of Florida, Gainesville, Florida 32610.

Superoxide and hydrogen peroxide are reactive oxygen species (ROS) with important roles in cell signaling and pathology. NAD(P)H oxidases are one of the major sources of ROS in biological systems. Sites of ROS generation in kidney have been localized with immunohistochemical procedures, which demonstrate areas where subunits of the enzymes are localized but not the actual enzyme activity and localization of ROS. Cerium based localization of NADH oxidase shows specifically the sites of superoxide and hydrogen peroxide localization and this method was used in age matched diabetic (BBZ/Wor rats) and non-diabetic (BB^{DR}/Wor rats) kidneys at the level of transmission electron microscopy. There was localization of ROS in the endothelial cells, lumens and red blood cells of the renal vasculature of both diabetic and non-diabetic rats. In the glomeruli there was localization in the plasma membranes of podocytes and fenestrated endothelial cells of the capillaries, basement membranes and red blood cells. Kidneys from diabetic rats had higher levels of ROS than those from non-diabetic rats. There were high levels of ROS in the apical microvilli and adjacent cytoplasmic vesicles as well as in the mitochondria of proximal convoluted tubules of both non-diabetic and diabetic rats. In addition, there was localization of ROS in the apical plasma membrane and cytoplasm of distal convoluted tubules of both non-diabetic and diabetic rats.

The kidney is a complex organ involved in filtration, selective reabsorption and secretion. Production of superoxide in the renal vasculature and capillaries of the glomeruli reduces the availability of nitric oxide by formation of peroxynitrite and contributes to the complications of diabetic nephropathy. Production of superoxide in the proximal convoluted tubules is thought to be involved in production and secretion of erythropoietin into the blood stream. Cerium based cytochemistry provides a direct way to investigate subcellular sites of ROS production in normal and diseased tissues.

ADVERTISER'S INDEX

EPICARDIAL FORMATION *IN SITU* AND IN CULTURE. JESSICA GREEN, RANDY CROSSLAND and J. KEVIN LANGFORD, Department of Biology, Stephen F. Austin State University, Nacogdoches, Texas 75962.

Once thought to be derived from the primitive myocardium, evidence demonstrates that the epicardial layer of the heart is derived from the proepicardium (PE), a cluster of mesothelial cells that form in the area of the dorsal mesocardium. During the early 80s, several SEM studies described the migration of these mesothelial cells from villous projections of the PE onto the myocardial surface of the heart, thus creating the epicardial layer and a matrix filled subepicardial space. Mesenchymal cells, present within the core of the PE populate this space and give rise to the coronary vasculature of the developing heart. To better understand these developmental processes, cells derived from the PE have been observed in tissue culture, including the growth and migration of these cells on and within type I collagen lattices. While this is a well accepted model for tissue culture, the current study compares the morphology of epicardial cells *in situ* with that observed in culture using SEM.

Cells present at the leading edge of the enlarging sheet of epicardium *in situ* display numerous filopodia as well as thin ruffling of the plasmalemma. On the surface of the most established area of the epicardium, the classic cobblestone morphology of mesothelial cells is evident. Small surface projections are common at the cellular junctions of these surface cells. In culture, the same cobblestone morphology and short projections are present throughout the epicardial cells. However, few filopodia were present at the edge of the mesothelial sheet in culture. This morphological difference may be due to the different substrates these cells are being challenged to spread upon, myocardial cells *in situ* and type I collagen in culture. Epicardial cells away from the leading edge likely are spreading on epicardially-derived matrix molecules, similar to that present in the subepicardial space. This study illustrates that while collagen type I serves as an adhesive and migratory substratum within which to study morphological processes, the interpretations must be tempered with the understanding that cells are continually modifying their substratum in culture, as they do *in situ*.

MICROSPOROGENISIS IN COTTON, *GOSSYPIUM HIRSUTUM* L. (*MALVACEAE*), STUDIED BY TEM. DAVID C. GARRETT[1], KENT D. CHAPMAN[2], and CAMELIA G.-A. MAIER[3*], [1]Department of Materials Science and Engineering, UNT, Denton, Texas 76201, [2]Department of Biological Sciences, UNT, Denton, Texas, 76201, [3]Department of Biology, TWU, Denton Texas 76204.

Breeding programs are aimed at developing improved cotton varieties, especially by employing genetically engineering technologies. Genetically transformation of cotton has been associated with poor pollination of flowers and increased boll (fruit) abortion. The development of cotton pollen was studied by TEM in developing floral buds and micrographs of tapetal cells, pollen wall, and pollen content were taken. Tapetum becomes amoeboid, very active, and fills the anther locule, depositing material (sporopollenin-covered white lamellae and lipids) on the surface of the exine and in the intercolumellar spaces (Fig. 1). Close to the maturation of pollen grains, tapetum degenerates. In early vacuolated stages, granular material is deposited in pericytoplasmic spaces, marking the intine development. In non-vacuolated stages, the intine appears to have a fibrillar structure. The protoplast shows an increase in ER vesicles, starch and lipid droplet abundance from the vacuolated stages to the non-vacuolated ones. Mature pollen grains have relatively thick lipid coats, which makes them stick together. The ontogenetic sequence of cotton pollen wall development follows the basic scheme in the angiosperms with an amoeboid/plasmodial tapetum type, forming a syncytium, with no orbicules or Ubisch bodies. In order to increase production of cotton lint and oil, understanding cotton reproduction is very important before employing genetically engineering methods for yield increase. This and further studies will provide insight into the microsporogenesis, pollination, and fertilization processes in cotton. The results presented here will encourage further assessment of the role of pollen coat in the reproduction of transgenic cotton.

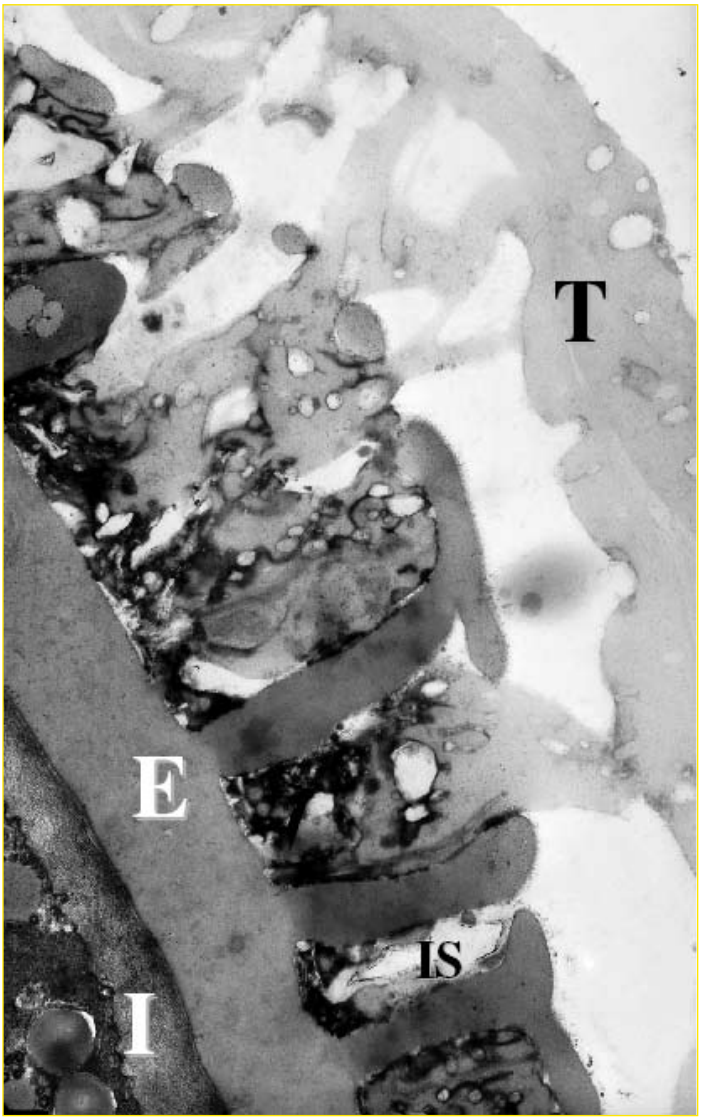


Fig. 1 – Representative thin section of cotton anther detailing the relationship between the tapetum and pollen wall. The amoeboid tapetum (T) fills the intercolumellar spaces (IS) of the exine (E) and deposits material on the surface of E. I, intine.

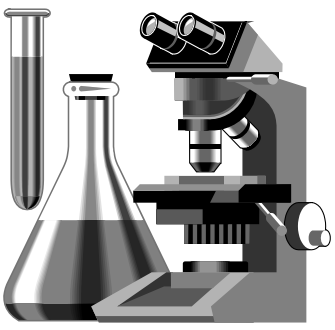
SEM/EDAXANALYSES OF MINERAL DEPOSITS IN PLANTS OF THE MULBERRY (*MORACEAE*) FAMILY. [1]BRANDON ROBETRS, [2]DAVID C. GARRETT and [3]CAMELIA G.-A. MAIER, [1]University of North Texas, Texas Academy of Mathematics and Sciences, [2]University of North Texas, Department of Materials Science and Engineering, and [3]Texas Woman's University, Department of Biology, Denton, Texas 76201.

Plants in the mulberry (*Moraceae*) family contain a variety of mineral deposits from the point of view of their morphology and chemical composition. We have previously reported on the morphology of mineral deposits in mulberry (*Morus alba*, *M. rubra*), Osage-orange (*Maclura pomifera*), edible fig (*Ficus carica*), fiddle-leaf ficus (*Ficus lyrata*), paper mulberry (*Broussonetia papyrifera*), all perennial species, and mulberry weed (*Fatoua vil-*

losa), a herbaceous exotic invader. All perennial species contained calcium carbonate deposits as primary and secondary cystoliths, and calcium oxalate prisms and druses. The herbaceous mulberry weed contained only druses. The leaves of fiddle-leaf ficus and paper mulberry possessed silicified trichomes, some of which with cystoliths inside their basal cells. EDAX analysis of the trichomes showed the presence of Ca, Si, and Mg. Calcium abundance increases towards the tip of the hair and Si abundance towards the base of the hair. Calcium decreases significantly at the base of the hair. The druses as well as the cystoliths extracted from the fiddle-leaf ficus leaves showed dominant peaks of Ca and traces of Si. In white mulberry, a small percentage of cystoliths have a dominant peak of Si than of Ca. Soil analyses will help explain the deposition of Si on cystoliths of mulberry. This study will help us better understand the genetics and environmental factors involved in mineral deposition in plants of the mulberry family.

A PRELIMINARY STUDY OF THE RELATIONSHIP BETWEEN HERBIVORE PREFERENCE AND THE ANATOMY AND MORPHOLOGY OF FORAGE SORGHUM LINES. M.W. PENDLETON[1], E. A. ELLIS[1], S. VITHA[1], F.R. MILLER[2], and B.B. PENDLETON[3], [1]Microscopy & Imaging Center, Texas A&M University, College Station, Texas 77843-2257, [2]MMR Genetics Ltd., Box 60, Vega, Texas 79092, [3]Department of Agricultural Sciences, West Texas A&M University, Canyon, Texas 79016-0001.

Field trials have determined that cattle have a preference for a specific sorghum line. Anatomical and morphological characteristics of the preferred sorghum line are compared to those of seven other lines. Leaf blade cross sections were fixed in 2.5% glutaraldehyde-1.0% acrolein in 0.1 M HEPES buffer (pH 7.2), and post fixed in buffered 1% osmium tetroxide. Specimens were dehydrated in a graded methanol series and infiltrated and embedded in a low viscosity epoxy resin. Thick sections (0.5-1.0 µm) were examined with a JEOL JSM-6400 SEM to observe leaf epicuticular wax platelet pattern and size. Sections (5 mm thick) of the leaf blade at approximately 1 cm above the sheath were made. Each section was passed through an alcohol series and 2 washes of hexamethyldisilazane. Dry samples were mounted on aluminum stubs, coated with gold-palladium, and observed by SEM. The mean proportion of xylem vessel diameter in relation to the diameter of the leaf blade was very high for the preferred line. This proportion may be related to soluble carbohydrate and starch levels. The concentration and average size of epicuticular wax platelets on the adaxial leaf surface was highest for the preferred sorghum line. Wax platelet size and concentration influence water permeability through the leaf cuticle. Soluble carbohydrate levels, starch levels and water permeability may influence cattle preference for sorghum lines.



INVESTIGATNG THE DIFFERENT METHODS OF EGGSHELL THICKNESS MEASUREMENTS: A COMPARISON OF MICROCALIPER AND IMAGE ANALYSIS. H. POURARSALAN, M. GRACEY, and S.L. WESTMORELAND, Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, Texas 76019.

Eggshell thickness can be measured using a variety of methods. One of the instruments commonly used is the micrometer caliper. The Starrett micro caliper claims to allow measurements accurate to 2-3 micrometers. The shell of a single egg from Kroger supermarket was used for this experiment. Several 5 cm pieces were broken from the equator of the egg shell and each piece was treated with 6% sodium hypochlorite for 90 min and rinsed with deionized water. Ten pieces were selected and the thickness of each piece was measured three times using the Starrett micro caliper. The data were recorded in a Microsoft Excel Spreadsheet and the average of each measurement, as well as the overall averages, were calculated. For comparison, the same pieces were broken into 1.5 centimeter pieces and placed on stubs. Ten samples were analyzed per specimen. The samples were oriented for a radial (cross section) view and samples were sputter coated with gold and palladium. Micrographs of each sample were taken using the JOEL 35C SEM. The thickness of each sample was measured using the Image Pro Plus Image Analysis Software. Three measurements were obtained for each sample. The data were analyzed using the student's t-test. The average eggshell thickness of the two measurement methods were significantly different ($p = 0.03$, micro caliper mean = 333.0 ± 20.6 SD micrometers; Image Pro Plus mean = 352.6 ± 6.6 SD micrometers).

THE USE OF SCANNING ELECTRON MICROSCOPY AND IMAGE ANALYSIS TO DETERMINE CHANGES IN ZEBRA FINCH (*TAENIOPYGIA GUTATTA*) EGGSHELL MORPHOLOGY AFTER ORAL ESTROGEN EXPOSURE AS CHICKS. S. L. WESTMORELAND[1], H. POURSRSALAN[1], D. H. HAWKINS[2], J. R. ROCHESTER[3], J. R. MILLAM[3], The University of Texas at Arlington, Department of Biology and The Center for Electron Microscopy, Arlington, Texas[1], The University of Texas at Arlington, Department of Mathematics, Arlington, Texas[2], The University of California, Department of Animal Science, Davis, California[3]

Environmental estrogens have been implicated in changes in the oviduct histology, reproductive performance (egg production), shell breakage, and song systems in Zebra Finch birds (*Taeniopygia guttata*). We investigated the relationship of estradiol benzoate (EB) to eggshell thickness and morphology. Zebra Finch birds from a breeding colony at The University of California at Davis were fed a mixed seed diet and water *ad libitum* and kept on a 16L: 8D photoschedule. Female chicks were treated for 7 days, from post-hatch days 5-11, with either orally administered EB in canola oil (100 nmol/g body weight) or canola oil alone (control) at 1 microliter/g body weight. Males were treated only with canola oil. The 25 eggs for this study included 15 eggs from control females and 10 eggs from EB-treated females; all eggs were the second egg laid in the clutch. On the day of collection, eggs were split open at the equator, emptied, rinsed with deionized water, and air dried. Three samples were taken from each egg at the equator region and placed on aluminum stubs. The samples were oriented for a radial (cross section) view of the shell, coated with gold and palladium, and viewed using SEM. Digital micrographs were recorded for all shell samples at 500X. Micrographs of shell cross sections were analyzed for shell thickness using Image Pro Plus computer software. These data were statistically analyzed using SAS. The experimental shells were found to be significantly thinner than the control shells ($p = 0.02$, experimental mean - control mean = -6.45 micrometers, SE=2.58).

AN INVESTIGATION ON *IN VITRO* CULTURE OF SUGAR BEET (*BETA VULGARIS* L.) USING LIGHT AND FLUORESCENT MICROSCOPY. Mandy Whiteside[1], Esther Villanueva[1], Edward Caraway[1], Nabarun Ghosh[1] and Don W. Smith[2], [1]Department of Life, Earth and Environmental Sciences, West Texas A&M University, Canyon, TX 79016, [2]Department of Biological Sciences, University of North Texas, Denton, Texas 76203.

Sugar beet (*Beta vulgaris L.*) is a member of the family *Amaranthaceae*, subfamily *Chenopodiaceae*. Sugar beet roots contain 15-20% sucrose representing a major source for the sweetener industry. Rhizomania, the most devastating disease caused by BSBMV (*Beet Soil Borne Mosaic Virus*) and BNYVV (*Beet Necrotic Yellow Vein Virus*) resulted in vast decline of production in United States in the last decade. We established *in vitro* cultures of sugar beet for the regeneration of improved varieties and to study the pathogenesis from systemically infected tissue in culture. We excised the hypocotyl and cotyledon explants from the seedlings of *Beta-1395* germinated on ½ MS medium and implanted them into modified MS medium. After 21 days culture, callusing was observed from the cut ends of the explants. Development of shoot was achieved by the addition of various growth factors and coconut milk (5% v/v) to MS medium. Rhizogenesis was obtained using 2 mg/L of IAA to MS medium. After three weeks of transfer, the formation of roots at the bottom of the regenerated shootlets was recorded. Using callus we established cell suspension cultures to obtain protoplasts for further experimentation. The morphogenesis process was studied using light and fluorescent microscopy. Staining the cultured cells with vital stain Evan's Blue helped us screen the regenerative cells from suspension culture. We observed the torpedo shaped embryonic initial that exhibited characteristic fluorescence with FITC filter (Fig. 1).

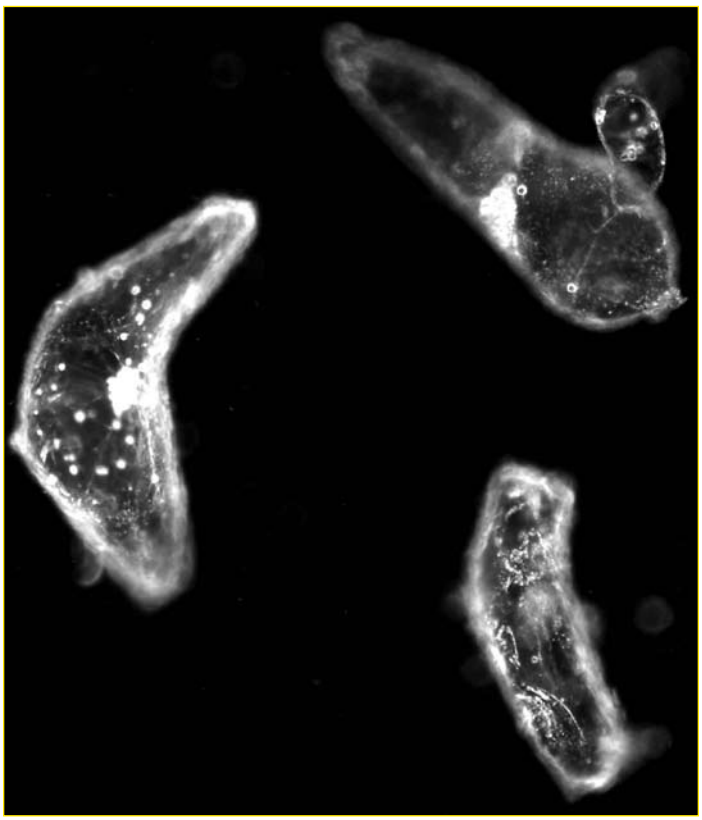


Fig. 1 – Viable cells from sugar beet suspension cultures stained with fluorescein under FITC filter.

COMPARING THE MELANIN-CONCENTRATING HORMONE-1 RECPTOR EXPRESSION IN THE BRAINS OF MICE AND RATS. S. CLAY WILLIAMS and HOWARD J. ARNOTT. The University of Texas at Arlington, Arlington, Texas 76019.

Melanin-concentrating hormone (MCH) is a cyclic 19 amino acid neuropeptide that was first isolated form the pituitary of salmon and was found to control the skin pigment melanin. Subsequently, MCH was isolated from the rat hypothalamus sparking intense research in mammals. Immunohistochemical studies revealed that MCH neurons reside in the perifornical region of the lateral hypothalamus as well as in the zona inserta and have axons that project throughout the rat brain including the olfactory bulb, cortex, hippocampus, amygdala and to hind brain structures like the pontine formation and medulla oblongata. It was discovered that direct intracerebroventricular injections of MCH into the brains of mice significantly increased food intake and fasting mice had greatly increased MCH mRNA levels in the brain. MCH null knockout mice are lean, hypophagic, and have higher metabolic rates. This suggests that MCH has an important function in the feeding behavior of rodents. Two G-protein coupled receptors specific to MCH have been identified, MCH-1R and MCH-2R and strangely, MCH-1R is the only MCH receptor expressed in rodents. Transgenic mice lacking the MCH-1R receptor (MCH-1R -/-) are lean, hyperphagic, and hyperactive. These mice are lean do to a decrease in fat pad volume and their hyperactivity is believed to offset their hyperphagia. These findings further support the idea of MCH controlling some aspect of feeding and also reveal that the MCH receptors could be a potential site for drug interaction to regulate food intake and possibly control obesity. Radioactive *in situ* hybridization studies have revealed a wide spread expression pattern of MCH-1R in the rat brain. There is strong MCH-1R mRNA expression in the olfactory nuclei, piriform complex, hippocampus, amygdala, locus coeruleus, and accumbens shell, as well as moderate expression in feeding centers including the ventromedial hypothalamic nucleus, arcuate, and the zona incerta. Currently, all studies that described the expression pattern of MCH-1R have been performed utilizing the rat brain. This presentation describes the MCH-1R radioactive *in situ* hybridization expression in the mouse brain and compares these results with the expression pattern found in the rat brain (Fig. 1).

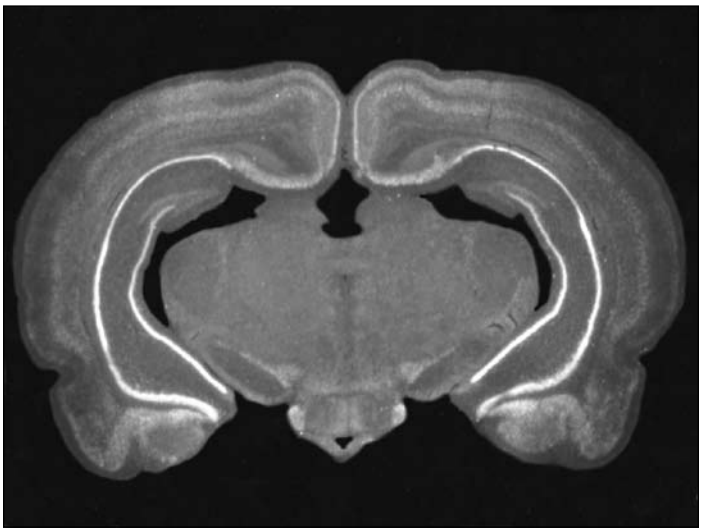


Fig. 1 – Cross section of a rat brain displaying MCH-1R mRNA expression pattern.

FROST RINGS IN TIMBERS FROM ROOM 43, SPRUCE TREE HOUSE, MESA VERDE, COLORADO

HOWARD J. ARNOTT

[1]Department of Biology and Center for Electron Microscopy, University of Texas at Arlington, Arlington, Texas, 76019

Spruce Tree House (Fig. 1) is one of the larger cliff dwellings in Mesa Verde National Park. Fewkes (1909) reported 114 rooms in Spruce Tree House and published photographs of Spruce Tree House as it was, before and after it was first "cleaned up." His photos clearly show both the breathtaking architectural characteristics of this place as well as the problems of maintaining it as a safe site for the public. Fewkes (1909) described many of the rooms in detail. Speaking of Room 43 he said, "Several rooms in this part of the ruins, especially rooms 43 (Pl 9) and 44 still have roofs and floors as well preserved as when they were built." Plate 9 contains photos of the inside of Room 43/1 (first floor) and "Main Street" where the entrance to Room 43/1 was located. The current nature of Room 43/1 and "Main Street" are shown in Figs. 2-4 taken in 2006.

Through the kindness of Professor Jeff Dean in the Laboratory for Tree-Ring Research (LTRR) I have been able to examine cores from both Spring House (Arnott and Adams, 2006) and from Spruce Tree House to determine whether they show frost rings. Frost rings are annual rings which show damage caused by freezing temperatures during the growing season. Many of the timber cores from Spring House and Spruce Tree House have frost rings. Almost all the freezing damage occurs in the early wood, hence they are called early frost rings. Because of rigor of dendrochronology, this ancient wood can often be dated to the exact year. Timbers from Spruce Tree House have frost rings from AD 906 to 1259. Because of the age of the trees used in the construction of this site, most of the frost rings date between 1150-1225 (Arnott, unpublished).

The cores from timbers of Room 43/1 are especially interesting because a comparison can be made between the timbers in a 2006 photos (Figs. 3-4) with those in Fewkes photo of 1909. Clearly, there were two primary timbers upon which 13 secondary timbers rested. The secondary timbers supported numerous tertiary members which supported fill and dirt forming the floor in Room 43/2 (Fig. 3-4). The timbers in the 2006 photos are the same as in Fewkes (1909) photo and thus, by extrapolation, the roof timbers are the same as the occupants left them in approximately 1300. Ten dated juniper wood cores from Room 43/1 were available in the LTRR's collection. Seven of the ten have the following numbers of frost rings per core: 1, 2, 3, 3, 5, 6 and 8 (Fig. 5); the other three had none. The timbers, dated by the LTRR, have beginning dates as early as 1143 and cutting dates from 1240 to 1250. Frost rings in 1149, 1154 and 1189 were each found in three cores. The 1179 frost ring is found in four cores; the earliest frost ring in Room 43/1 was in 1143, and the latest in 1209. All 29 frost rings found in the timbers of Room 43/1 are in the early wood. The individual frost rings are variable (Figs. 6-9) and each appears to chronicle a somewhat dissimilar incident. The 1179 frost ring is very narrow and is only approximately 15 cell layers thick with the frost damage extending throughout the radial dimension of the ring (Fig. 6). Other frost rings have only a limited number of aberrant cells, principally ray cells, demonstrating their frost damage. The 1162 frost ring shows that several rows of tracheids were already formed before the freezing episode occurred thus producing a "delayed early frost ring" (Fig. 8).

Frost rings were found in 70% of the cores available from Room 43/1. This percentage is substantially higher than in the overall samples from Spruce Tree House (Arnott, unpublished). Many frost rings occur in the early years of the trees that supplied the timbers for Room 43/1. However, frost rings also occur in much later annual rings, for example in the 25th, 36th, 42nd, 49th and 66th years. Fritts, et al (1965) using dendrochronology discovered a "prolonged dry period from 1276 to 1289" and suggests that it might be a factor in the abandonment. Salzer (2000) pointed to the cooling of the climate in the 12th and 13th centuries as important in considering the factors involved in abandonment. Obviously, the occurrence of many frost rings in these timbers merits further consideration regarding the climate at the time of abandonment. The frost rings are direct evidence of weather phenomenon at the Mesa Verde site.

REFERENCES

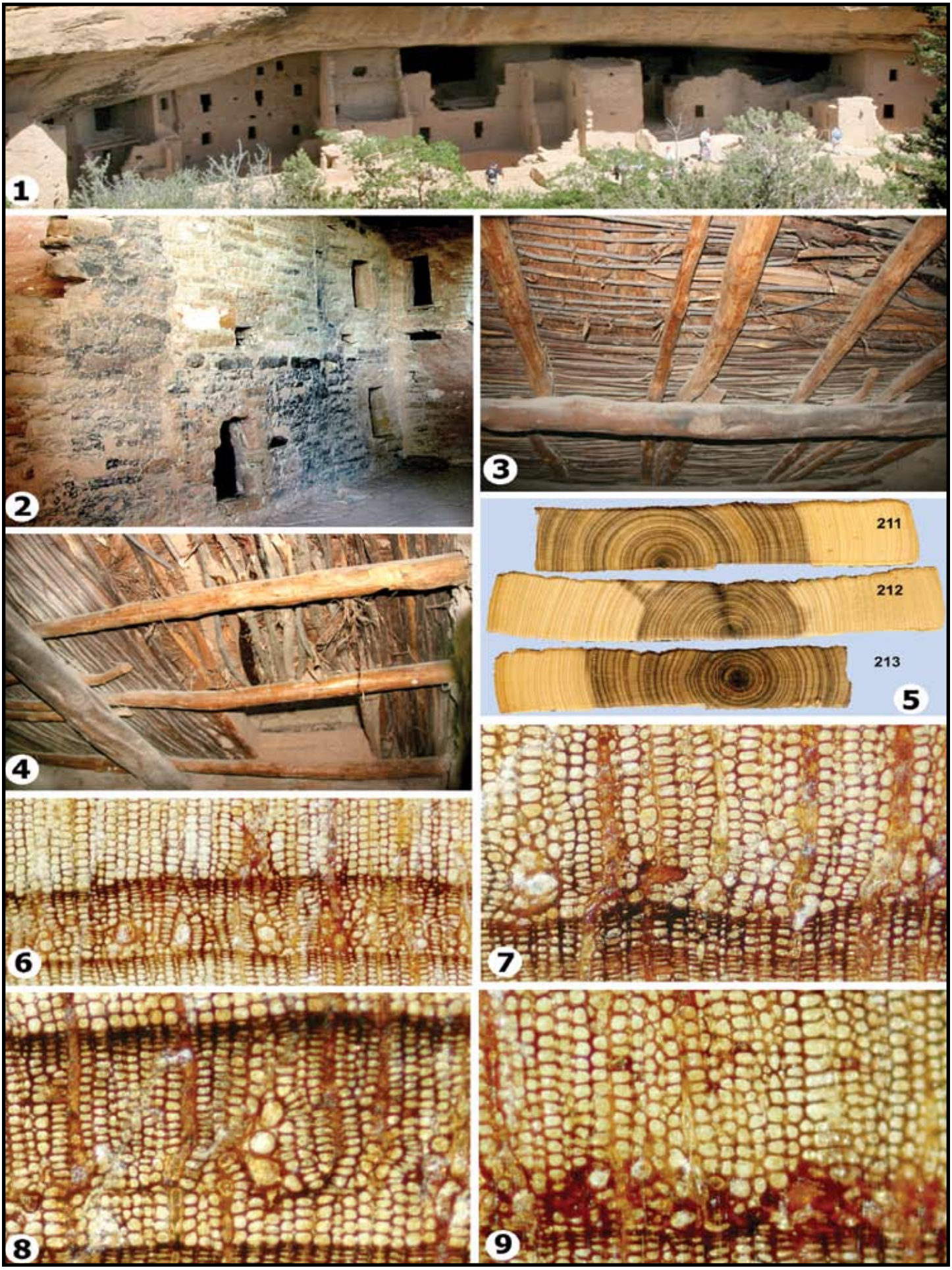
Arnott, H.J. and R. Adams. 2006. Frost Rings in Timber Cores from Spring House, Mesa Verde, Colorado. *Texas Journal of Microscopy* 37:56-57.

Fewkes, J. W. 1909. Antiquities of the Mesa Verde National Park. Spruce-Tree House. Bull. Bur. Amer. Ethnol. No. 41.

Fritts, H. C., D.C. Smith and M. A. Stokes. 1965. The Biological Model for Paleoclimatic Interpretation of Mesa Verde Tree-Ring Series. Am. Antiquity 31:101-121.

Salzer, M., 2000. Dendroclimatology in the San Francisco Peaks Region of Northern Arizona, USA. Dissertation University of Arizona, Tucson.

Figures 1-4 taken in 2006. **Figure 1.** Site view of Spruce Tree House, Mesa Verde, Colorado. **Figure 2.** View of "Main Street" in Spruce Tree House. The entrance to Room 43/1 is the first "door" on the left as you look down "Main Street." **Figure 3.** Roof of Room 43/1 showing one of the primaries and several secondaries. At right angles to the secondary many smaller "tertiaries" support the floor of the room above. **Figure 4.** Roof of Room 43/1 (the photo is almost perpendicular to Fig. 3). In this photo one can see the passageway that leads to the room above. Careful examination of the secondary beams reveals numbers and white core holes (filled). **Figure 5.** Examples of cores from the timbers in Room 43/1; number 213 contained 8 frost rings. ***Figures 6-9. Light micrographs of Core 211.*** **Figure 6.** The 1179 frost ring showing its scope. **Figure 7.** The 1154 frost ring showing typical rearrangement of the rays often seen in frost rings. **Figure 8.** The 1162 frost ring showing that several layers of tracheids were produced before the freeze occurred. **Figure 9.** The 1187 frost ring showing areas of cell damage which appear **red in this rendition.**


1
2
3
4
211
212
213
5
6
7
8
9

MATERIAL SCIENCES
SPRING 2007

INFLUENCE OF NITROGEN ON THE FORMATION OF NANOCRYSTALLINE COPPER THIN FILMS. R. CALINAS[1, 2], M. T. VIEIRA[1], and P.J. FERREIRA[21,] ICEMS, Departamento Engenharia Mecânica, Faculdade de Ciências e Tecnologia da Universidade de Coimbra, R. Luís Reis Santos, 3030-788 Coimbra, Portugal,[2] University of Texas at Austin, Materials Science and Engineering Program, Austin, Texas 78712.

The success of nanocrystalline materials is based on their unusual and promising properties compared to micro- and submicrograin size materials. However, maintaining the nanocrystalline character during intermediate and/or high temperature processes is not yet an easy task, due to the large driving force for grain growth exhibited by nanocrystalline materials. However, it is well known, that the addition of nonmetallic elements with a large affinity for grain boundary segregation can act as pinning centers and inhibit grain growth.

In this context, the aim of this work is to produce nanocrystalline Cu-based thin films through controlled additions of nitrogen, to inhibit grain growth. The concentrations of nitrogen are carefully controlled, such that the formation of copper nitrides is avoided.

Pure Cu and Cu-N thin films are deposited onto glass substrates by dc magnetron sputtering. In the particular case of the Cu-N thin films, the copper target is sputtered in reactive mode using different nitrogen/argon partial pressure ratios and a total deposition pressure of 0.3 Pa. In order to avoid accumulation of heat on the substrate, which is likely to lead to grain growth, the substrate was maintained at low temperatures by promoting heat flow through the substrate's holder. A detailed grain size analysis of the pure and N-doped copper thin films was conducted by TEM and HRTEM. Results indicate that the introduction of nitrogen, even in small amounts, is responsible for a significant decrease in grain size.

***IN-SITU* TEM NANO-INDENTATION OF INDIVIDUAL SINGLE-CRYSTAL NANOPARTICLES.** C.E. CARLTON[*], O. LOURIE[+], and P.J. FERREIRA, [**]Materials Science and Engineering Program, University of Texas at Austin, Austin, TX 78712, USA, [+]Nanofactory Instruments AB,Gothenburg, 412 58, Sweden.

Although it has long been noticed that dislocations are absent from individual nanocrystalline particles, a full understanding of the mechanisms associated with this behavior is still lacking. In this context, *in-situ* TEM nanoindentation experiments have been conducted on individual single-crystal Ag nanoparticles. Evidence for nucleation of dislocations and dislocation motion was observed during in-situ TEM nanoindentation, but upon unloading, dislocations were no longer visible. While the instability of dislocations in nanoparticles has been previously addressed for self-contained dislocation loops, the dislocations observed in the current experiments intersect the nanoparticles surface. Therefore, a new model for explaining dislocation instability is introduced.

***IN-SITU* TEM STUDY OF THERMALLY INDUCED VOIDS AND VOID FORMATION CHARACTERISTICS IN 180 NM CU INTERCONNECTS.** J. H. AN, and P. J. FERREIRA, Materials Science and Engineering Program, University of Texas at Austin.

Cu interconnects have decreased in width and are approximately 100 nm. A decrease in interconnect width has led to predominately bamboo structured Cu lines. In Cu interconnects, void formation during high temperature is a reliability issue, and this study looks at the void formation behavior in damascene Cu interconnects with a predominately bamboo microstructure. First, the crystal texture and grain morphology of the Cu interconnects was observed. Then to determine the void formation behavior, *in-situ* TEM was performed. Voids that formed as a result of *in-situ* heating were analyzed in terms of preferential void formation sites and crystal orientation. In bamboo-structured lines, voids formed at triple junctions where the grain boundary and the Cu/diffusion barrier (DB) interface meet. The relationship between crystal orientation and voids nucleation was studied to identify the paths for vacancy diffusion.

TEM MEASUREMENTS OF GRAIN ORIENTATION IN NANOSCALE CU INTERCONNECTS USING ACT SOFTWARE. J.H. AN AND P.J. FERREIRA, Materials Science and Engineering Program, University of Texas at Austin.

Void formation in Cu interconnects (CI) has been a concern for the microelectronics industry due to the fact that they affect the reliability of microelectronic devices. A major reason for this concern is that the mechanisms of void nucleation and growth, and related microstructure are not fully understood. While voids in the 180 nm CI used in this work formed at the Cu grain boundary/diffusion barrier interface, knowledge of the relationship between void formation and grain orientation in CI subjected to thermal stresses is currently lacking. While electron backscattered diffraction (EBSD) in a SEM has been instrumental in the past to determine grain orientation in CI, the continued downscaling of microelectronic devices has produced CI with nanoscale grain sizes, which makes the EBSD technique unreliable for grain orientation measurements. A possible alternative method is to use nanobeam diffraction (NBD) in a TEM. However, using NBD coupled with a conventional tilting technique in the TEM is very time consuming. To expedite grain orientation analysis in nanoscale CI by TEM, the ACT software was used to measure the crystallographic orientation of multiple grains. For the 180 nm CI, the results obtained by ACT show that while {111} grains are typically observed, {130}, {100}, and {112} grains have also been noted in other areas of the Cu interconnect. In addition, {011} annealing twins were also observed. The majority of grains in the {111} orientation seem to agree with previous XRD results. The results obtained by the ACT software can then be used as input data in a finite element method to calculate the distribution of local stresses across the microstructure. The use of this technique can be very helpful in understanding the mechanical behavior of various microelectronic devices.

NANO/SUB-MICRON GRAIN EVOLUTION IN AISI 301LN STAINLESS STEELS. S. RAJASEKHARA1, L. P. KARJALAINEN2, A. KYROLAINEN3, and P. J. FERREIRA1, 1Materials Science and Engineering, The University of Texas at Austin, Texas 78712, 2The Department of Mechanical Engineering, The University of Oulu, 90014 Oulu, Finland3, Outokumpu Stainless Oy, 95400 Tornio, Finland

Widespread acceptance of austenitic stainless steels (SS) for structural applications, particularly in the transportation sector, has been limited due to the low strength of SS in the as-annealed conditions. Since austenitic stainless steels possess a host of desirable characteristics, such as corrosion resistance, ductility, and formability, the objective of this work is to develop novel nano/sub-micron grained austenitic SS with high strength and high ductility for use in the above-mentioned industries. TEM has been used to characterize and analyze nano/sub-micron grain evolution in commercial AISI 301LN SS. This alloy has been 63% cold rolled to produce deformation-induced martensite (α'), followed by an annealing treatment at 600°C, 700°C, 800°C, 900°C and 1000°C, for periods of 1-100 seconds to produce nano/sub-micron austenite grains (γ). TEM studies revealed that cold rolled AISI 301LN SS have a microstructure containing dislocation cell-type martensite (α') and lath-type martensite, which might be ideal for the nucleation of nano/sub-micron grained austenite. Phase fraction analysis of the annealed samples reveal that at 600°C, the $\alpha' \rightarrow \gamma$ reversion is negligible. However, TEM images of samples annealed at 700°C reveal a mixture of unreverted martensite and newly nucleated nano/su-micron austenite, which may exhibit outstanding mechanical properties. At higher annealing temperatures, the $\alpha' \rightarrow \gamma$ reversion is almost complete, as well as rapid austenite grain growth is observed. Furthermore, secondary phase precipitates identified as CrN precipitates through nanobeam diffraction technique, were also present in these annealing regimes.

PERSISTENCE OF GUNSHOT RESIDUE CONSISTING OF LEAD, BARIUM, AND ANTIMONY PARTICLES AFTER TWO HUNDRED ROUNDS OF WINCHESTER WIN-CLEAN™ LEAD-FREE AMMUNITION. YVETTE WEBB[1], LAN LE[1], SANDRA WESTMORELAND[1], DAVID GARRETT[2], RON FAZIO[3], and MARTHA GRACEY[1], [1]Dept. of Biology, University of Texas at Arlington, Arlington, Texas, 760191, [2]Dept. of Material Science, University of North Texas, Denton, Texas 762033, and [3]Integrated Forensic Laboratories, Euless, Texas 760392.

Gunshot residue (GSR) is traditionally detected by the presence of lead (Pb), barium (Ba), and antimony (Sb) derived from lead styphnate, barium nitrate, and antimony sulfide, respectively. A common method used in GSR analysis is scanning electron microscope/energy dispersive X-ray detection (SEM/EDX). The removal of lead and other heavy metal particles from the primers, however, may make the recognition of GSR more difficult. Winchester WinClean™ is one brand of ammunition that has a heavy metal-free primer. The major element in the Winchester WinClean™ primer is potassium (K). There are also trace amounts of aluminum (Al), silicon (Si), calcium (Ca), sulfur (S), zinc (Zn), copper (Cu), nickel (Ni), chromium (Cr), magnesium (Mg), and iron (Fe). Gunshot testing was done at the Integrated Forensic Laboratories. Three shots of regular Precision Made Cartridges TM (PMC) 9mm Luger cartridges were shot to insure lead, barium and antimony particles were present in the Browning Hi Power 9mm Luger semi-automatic pistol. Two hundred rounds of the WinClean™ heavy metal-free ammunition were shot directly afterwards; three fabric samples were collected at 1, 50, 100, 150, and 200 shot intervals. Samples taken were analyzed to determine whether characteristic lead, barium, and antimony particles of gunshot residue would persist at the sample intervals. Samples were examined using SEM/EDX however, progress was limited due to the lack of an autosampler.

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 TSM 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 TSM, the Journal and its contents, academic or national policies as they apply to TSM and/or its members and 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.

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 are welcome and should be selected with some attention to aesthetic appeal as well as excellence both in technique and in scientific information content.

EMPLOYMENT OPPORTUNITIES

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 Editor's office.

TECHNICAL SECTION

The Technical Section will publish TECHNIQUES PAPERS, and HELPFUL HINTS. The TECHNIQUE PAPERS will describe new or improved methods for existing techniques and give examples of the results obtained with 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 Abstracts in the TEXAS JOURNAL OF MICROSCOPY is restricted to TSM members or to those whose membership is pending. A membership application form can usually be found in each issue of the TEXAS JOURNAL OF MICROSCOPY. Membership dues are as follows: student $10.00; regular members $30.00; corporate members $300.00 (corporate dues include all meeting registrations for the year, a link on the corporate sponsors' page, and other benefits. Contact secretary for more information). Research articles are accepted from both members and non-members. Individuals who belong to TSM 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.

BGA Solderability Issues Due to Nickel Carbonate Contamination

JODI A. ROEPSCH

Raytheon NCS Shared Services Failure Analysis Lab, McKinney, Texas 75071

Electrical failures of an assembled board led to an investigation into root failure cause. In-circuit testing identified electrical opens at Ball Grid Array (BGA) solder bumps to the Printed Wire Board (PWB) interface. Scanning Electron Microscopy (SEM), Energy Dispersive Spectroscopy (EDS), optical inspection and Fourier Transform Infrared were used to investigate this failure.

Failure was determined to be the result of poor solder connection of the BGA solder bumps to the gold plated PWB pads. Contamination was identified on the PWB pad surfaces causing the poor solderability. The cracked flaky appearance of the contaminant indicated the material was at one time in liquid form (Figure 1). A typical joint results in the SnPb solder bump wetting to the pad on the PWB by absorbing the gold plating and forming an intermetallic with the underlying nickel plating. In instances where the pads on the board contain contamination, the gold was unable to be absorbed by the solder and no solder joint was formed. Elemental analysis determined the contamination contains C, O and Ni (Figure 2). FTIR identified this material as nickel carbonate (Figure 3). The source of the nickel carbonate was isolated to the plating house but the exact cause could not be identified.

Considering the cost to manufacture this type of board, it was necessary to formulate a cleaning process in an attempt to salvage the populated boards. A significant concern with cleaning a populated board includes inducing damage to the board that could potentially go unnoticed resulting in a latent failure. This cleaning technique was deemed acceptable since the boards would only be used in test units and would not be placed in the field. Investigative studies into various acidic solutions led to success with a 10% Hydrochloric Acid solution. This solution was found to clean the pads in a reasonable amount of time. Damage was only identified from the 10% HCl cleaning process in instances when the gold plating was cracked or flaking. A microsyringe was used to isolate the acid to a contaminated pad thereby reducing the risk of damage to the board. Successful cleaning of pads allowed multiple boards to be cleaned and put back into process flow to later be installed in test units. This resulted in a significant cost savings to the program.

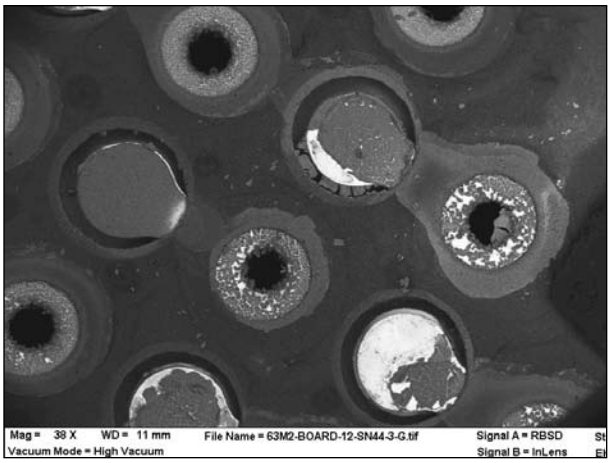


Figure 1: Low magnification image of contaminated pad on PWB. The bright areas contain gold and the dark areas on the pads contain nickel carbonate contamination. The contamination extends out onto the board surface.

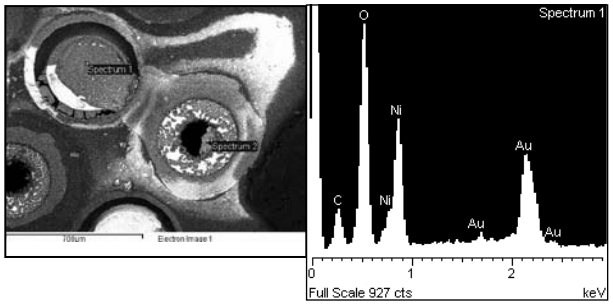


Figure 2: EDS data suggests the presence of C, O, and Ni on the gold plated surface.

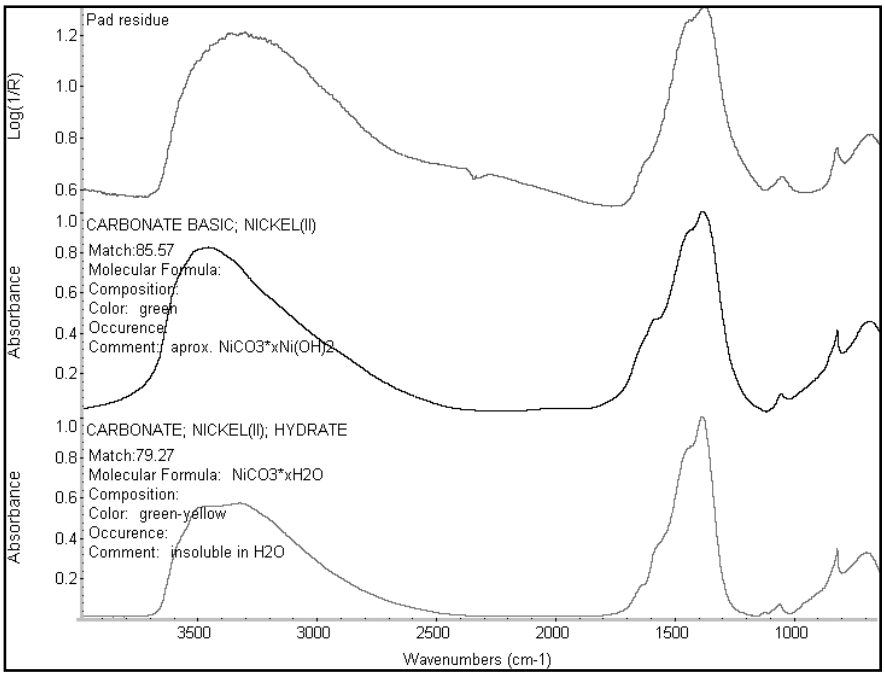


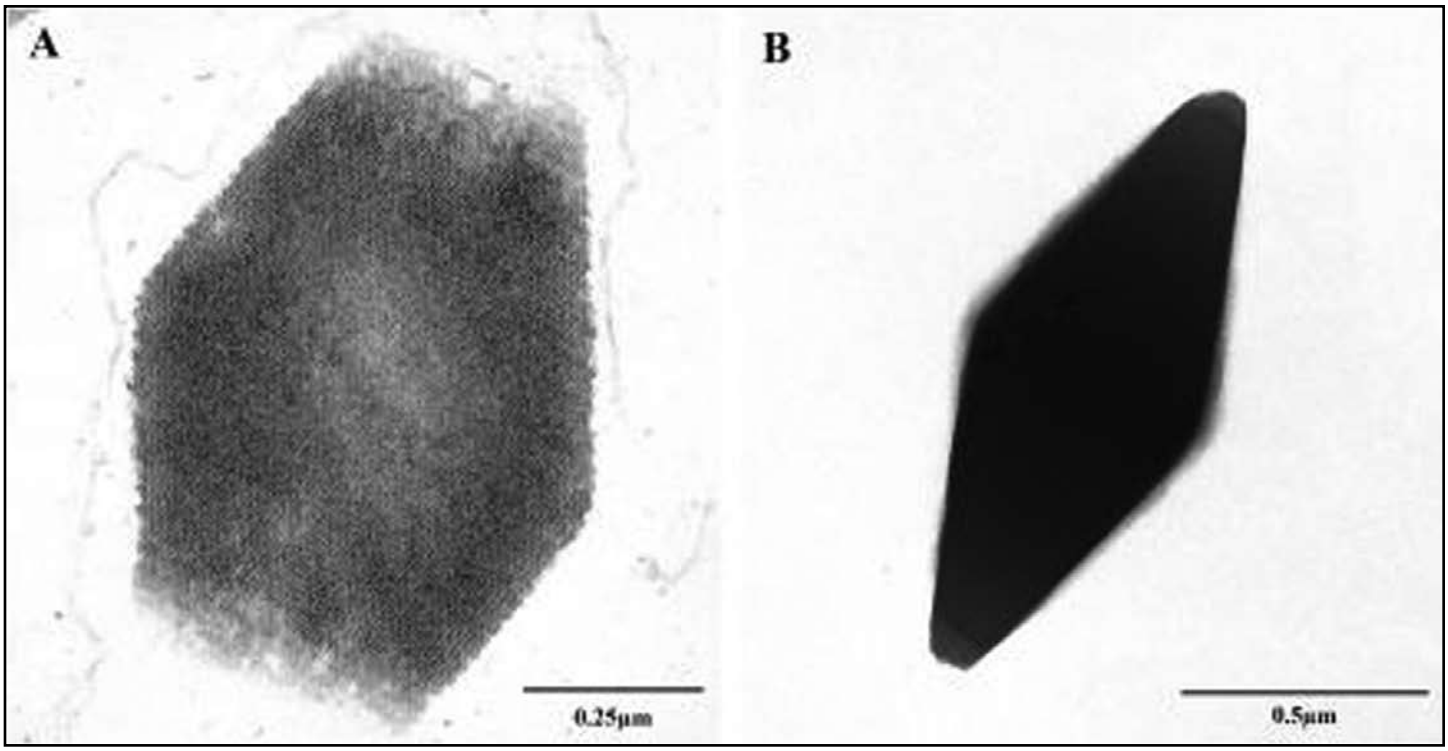
Figure 3: FTIR suggests the material is a nickel carbonate.

EDUCATIONAL SCIENCES
SPRING 2007

INSPIRING ENTHUSIASM FOR SCIENCE THROUGH ACCESS TO TECHNOLOGY: THE SCANNING ELECTRON MICROSCOPE AS A RECRUITING TOOL. JILL DEVITO (PRESENTER); CARL KNIGHT, CHERYL GREEN, MELANIE GILL-SHAW and DAYMI PARDO, Eastfield College, Dallas County Community College District.

The National Science Foundation Science Talent Expansion Program (NSF STEP) at Eastfield College is a multifaceted project designed to broaden access to the fields Science, Technology, Engineering and Mathematics (STEM). Our objectives include 1) educational outreach at the pre-college level, 2) fostering inquiry based learning through curriculum enhancements, 3) specialized recruitment of underrepresented students across STEM disciplines, and 4) providing early undergraduate research experiences to facilitate the transition to successful further study at four year universities and beyond. Our new Scanning Electron Microscopy facility centered around the Hitachi S-3400N VP SEM and the TM-1000 "tabletop" model plays a critical role in achieving each of these objectives. For example, we introduce students to SEM techniques at an early age by transporting our TM-1000 into the classroom for inquiry-based outreach activities in local public schools, and we utilize the same instrument to enrich the laboratory curriculum of science courses offered on campus. Both the S-3400N and the TM-1000 will be used extensively as research tools for our students, through collaboration with established research programs at four-year universities, for example, through our Biodiversity Summer Institute at the Big Thicket National Preserve (a joint venture with Texas A&M and several other institutions). These partnerships will create unique opportunities for community college students to engage in the scientific community as active participants, while they in turn will be valued for the use of their microscopy skills to enhance the work of researchers who would not ordinarily have access to SEM equipment.

"What Is It?" *Answer in Next Edition*



TEM micrographs provided by **Dr. Robert Droleskey**, USDA/ARS/SPARC, College Station, Texas 77845.

"The Crystal Trail." How one step leads to another!

Howard J. Arnott
Autobiography-Part Four

There seem to be distinct parallels between my scientific life on "The Crystal Trail" and the Victorian novel by H. Rider Haggard, "King Solomon's Mines" (Haggard, 1885, 1996). I thought that a comparison of the two could provide a framework for Part Four. Will such a comparison make an interesting narrative? You and I will have to decide that! Assuming that most of you have not read "King Solomon's Mines" recently, the following synopsis, adapted from the web*, will give you a basic understanding of the book. By the way, the two movies with the title "King Solomon's Mines" departed, big time, from the original plot as there is no female lead in the book.*

> **"King Solomon's Mines tells of the search by Sir Henry Curtis, Captain John Good and the narrator, Allan Quatermain, for Sir Henry's younger brother George. George has been lost in the interior of Africa for two years on a quest for King Solomon's Mines . . . The three companions encounter fearful hardships, fierce warriors, mortal danger, and the sinister and deadly witch named Gagool in one of the best adventure stories of its age. Quatermain, with touches of humor and great excitement, tells the tale of their struggle through unmapped Africa in pursuit of unimaginable wealth."**

A small section in Part III of my autobiography tells about the study that started me on the my crystal trail, namely, the examination of vertical files of calcium oxalate (CaOx) crystal cells in the root of *Yucca* (Fig. 1). For some time those crystal files remained absent from my mind, much as the map of King Solomon's Mines faded in the mind of Allan Quatermain. To start this study we need to look at the external forces that provide the impetus for these "travels." For Quatermain his recall of a map to King Solomon's Mines was merely a way to "help a fellow gentleman." The start of my crystal trail came from lessons in transmission electron microscopy (TEM). I wanted something to examine in the TEM. Like magic, the files of *Yucca* crystal cells popped to mind; yucca crystals cells seemed as good as anything. Not to draw too fine a point on the King Solomon's Mines comparison, but once begun, the search for crystals, whether diamonds (as in the novel) or biological crystals (as in my life), **crystals can quickly become an end in themselves.**

As a matter of convenience, to the reader and myself, I have decided to illustrate this paper as follows: text figures will relate to the current dialogue; plates will illustrate the characteristics of a variety of crystal systems (summarized in Plate 1); calcium oxalate in higher plants, calcium oxalate in fungi, calcium oxalate in insects, protein crystals in insect viruses, etc. The plate legends are important as some micrographs found in the plates will not be discussed in the text. I hope that if you are interested in a specific point or micrograph you will seek the original publications listed in the citations. Most of the micrographs used here are my own or those of a student of mine. Some unpublished micrographs of mine are included and are cited as such. I will respond to questions about them or any other part the "autobiography."

*The complete text of *King Solomon's Mines* is available free on the internet at: www.pagebypagebooks.com/H_Rider_Haggard/King_Solomon's_Mines/

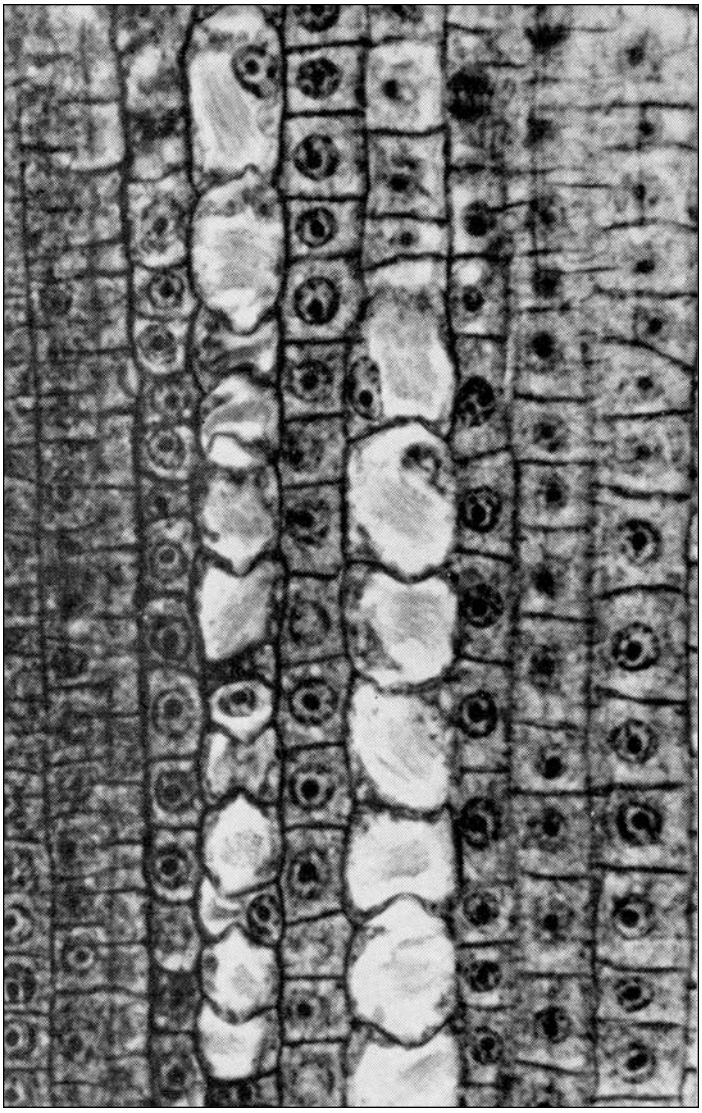


Figure 1. The trailhead incarnate. Two files of raphide idioblasts in the root of *Yucca whipplei.* Note the development pattern is from the root tip upward, Adapted from Arnott, 1962.

Figure 2. Prince Valiant fights for his life and possibly King Solomon's gold. Adapted from Hal Foster's Prince Valiant by Gianni and Schultz, 2006.

I am a long time devotee of the comic strip* Prince Valiant. *First thing Sunday morning I look for it in the paper. Other comic strips do not interest me, but somehow, when I was young "Prince Valiant" captured my imagination. All of the panels are extremely well done, in fact, each is like a piece of fine art. The colors are rich and the characters are robust. The artist(s) utilize different size panels rather than the traditional four used in many comic strips. Sometimes they use only a single very large panel. These panel size changes add character to the strip. The drawings always exhibit a great feeling for perspective and a fantastic sense of reality. The story line is continuous and moves slowly; one "episode" may take several weeks. Prince Valiant in the days of King Arthur, ©King Features Syndicate, was originally written by Hal Foster (no relation to Adriance Foster) starting in 1937 and over the years was illustrated by a number of artists. It is now written and drawn by Gary Gianni and Mark Schultz as Hal Foster's Prince Valiant. Some of you will say this paragraph is interesting but completely immaterial. However, there is a connection! On Sunday three weeks after starting Part IV, and tentatively deciding that I could relate "my crystal trail" to the events in the novel King Solomon's Mines, I found Prince Valiant enmeshed in a fight for King Solomon's Treasure (Fig. 2). Whether it was a fluke, a twist of fate, or coincidence I took it as a sign! It gave me confidence that I was on the right trail. In November and December of 2006 "the coin of Solomon" and King Solomon himself, returned to the plot of Prince Valiant.

THE CRYSTAL TRAIL

Crystals of CaOx in plants will be the first stop on the trail. From there the trail goes in diverse directions (Plates 2, 3). Perhaps the crystal trail is more like a web since it led to the study of algae, plants, fungi, vertebrates, plant and insect viruses and finally, believe it or not, to wood. The crystal research described here involves my own work and joint studies with students, seasoned investigators, a housewife and even a detective. In many ways the "webbiness" of this trail came from the influence of Dr. Frederick G. E. Pautard, the friendly Englishman (Fig 3). Soon after I met him in the middle 60's, we became both friends and research associates. Fred was trained in biophysics in the Astbury Lab at Leeds University. He was a persistent fisherman at home or abroad; a bon vivant, and an original "multitasker." But above all he was always a "ruthless intellectual." In many ways, the "webbiness" also derives from Gordon Whaley, who, I believe, moved in the background in many ways to expand my research life. As you will see later, Fred Pautard, Kenneth Smith and Colin Nicol were all "pawns" in Whaley's pastime of local edification. I will recount these stories in a chronological manner, with occasional flashbacks, as in King Solomon's Mines. The chief members of my crystal trail safari were: Adriance Foster (UC Berkeley); Fred Pautard, Kenneth Smith, Colin Nicol (UT Austin and U of South Florida); Mary Alice Webb (UT Arlington and Purdue U). There were others but these five "carried the load" just as the porters did for Quatermain. I will introduce each of them as we go along the trail.

Figure 3. F.G.E. Pautard in the snow at Davos, Switzerland, 1965.

To embark on "The Crystal Trail," first I want to recall an incident in the life of Adriance S. Foster. In 1955 he gave the retiring president's address at the annual banquet of the American Botanical Society held at Michigan State University. His address was entitled, "Plant Idioblasts: Remarkable Examples of Cell Specialization" (Foster, 1956). A note at the beginning of the published version of his address says, "Dr. Foster's address was illustrated with a series of excellent slides of mixed botanical and psycho-entomological nature." His address included pictures of sclereids and crystal idioblasts much like those we studied in his plant anatomy class but he also showed cartoons of grasshoppers with sclereids stuck in their "teeth" hence the editor's comment. In 1968, well along on my crystal trail, I had the pleasure of addressing the American Botanical Society in a symposium on plant idioblasts. My lecture ended with a photograph of Adriance S. Foster, and naming him the ***"greatest idioblast of all."*** See Part III for pictures of Foster.

While Foster's course in plant anatomy was near the beginning of my crystal trail, the actual start was a study of longitudinal sections of yucca roots, wherein I found that raphide crystal idioblasts arise in files (Fig. 1). In my dissertation I wrote: "Large numbers of raphide-containing idioblastic cells occur in the cortex of the roots of many *Yucca* species, especially *Y. whipplei*. They are found most often in vertical files. Idioblasts develop acropetally in a zone of cortical cells not far removed from the root apex. As far as could be ascertained, raphide crystals develop inside the vacuole. The early development of these crystals occurs in areas where cell division is still common". Later, I published a picture and a short paragraph describing the situation (Arnott, 1962) and set the whole thing aside (*"out of sight, out of mind"*).

Before leaving UC Berkeley and Adriance Foster's influence, here is another anecdote that is pertinent to the crystal trail. As his Teaching Assistant, he and I often visited the Coke machine

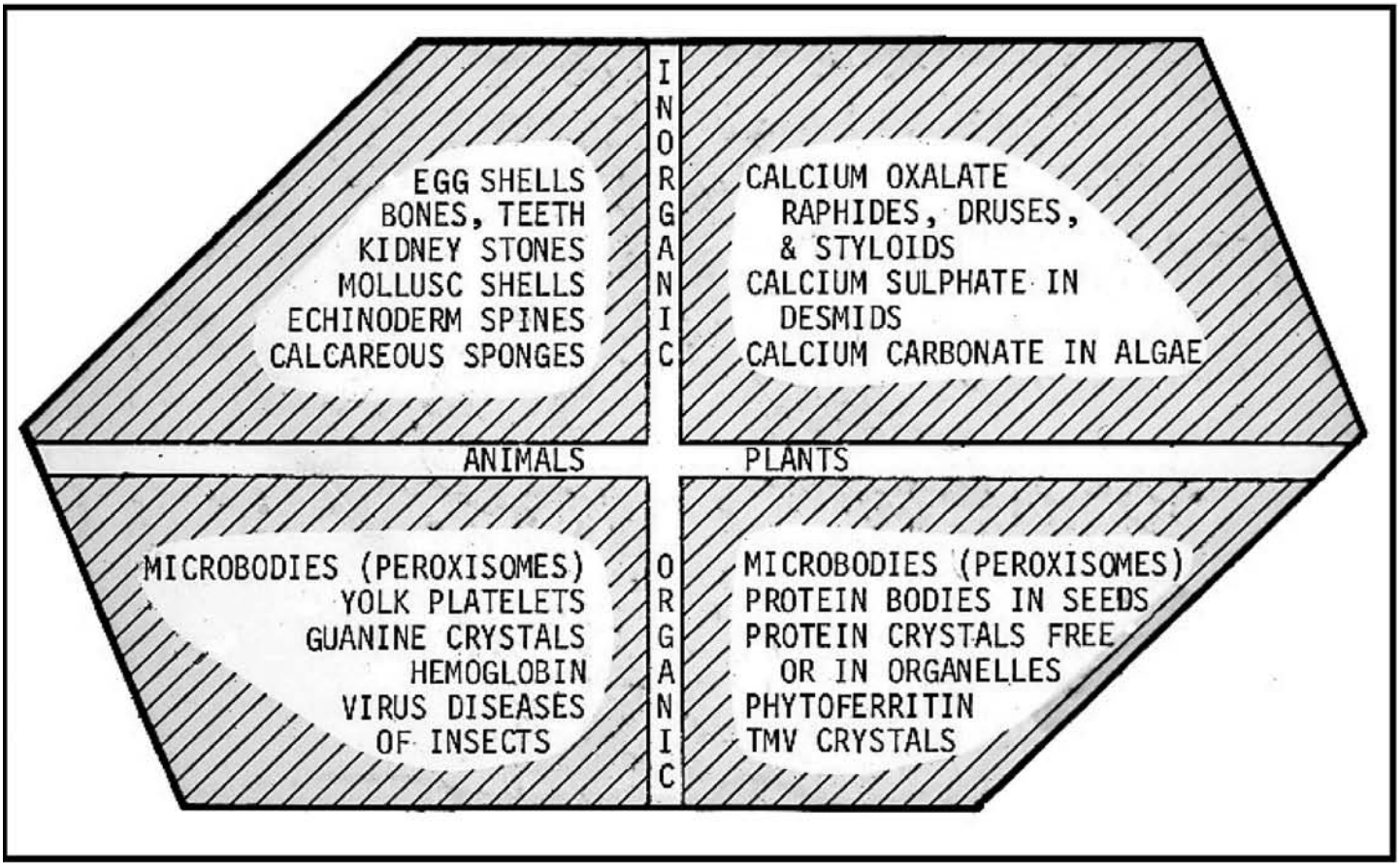


Plate 1. Biological crystals. This chart is a virtual map to the crystal trail; like the map to King Solomon's Mines, it can guide you to many of the traditional "crystal systems" found in biological tissues. The figure was made to call attention to crystal development in both animals and plants. Despite the importance of some of these systems, in 1970 there was little broad interest in crystals, possibly because of the general thought that crystals are "inert" and not involved in the metabolism of organisms. The figure is divided both horizontally and vertically with inorganic crystals above the median line and organic crystals below it. On the upper left various animal systems are listed; on the upper right crystals of a number of plant systems are found. The figure is not meant to be inclusive but rather to deal with many of the systems that have been a part of my "crystal trail." Egg shells (currently the end of my crystal trail) are made up of a layer of large radially oriented calcite crystals of calcium carbonate grown together and somewhat supported by a protein matrix. The regulation of egg shell thickness is a major problem for the 10 billion dollar poultry industry. Bones and teeth are formed by crystals of calcium phosphate (hydroxyapatite crystals) imbedded in a protein matrix. The extremely small crystals (crystallites) of bone are produced by osteoblasts associated with collagen fibrils. Specialized cells called ameloblasts in teeth produce long thin ribbon-like hydroxyapatite crystals in enamel prisms also embedded in protein matrix. Kidney stones represent a third important mineral system of humans and other mammals; the most common are crystals of calcium oxalate dihydrate but many are also associated with colestreol. In the ocean and in some marine deposits, the shells of molluscs are made up of calcium carbonate (both calcite and aragonite) also embedded in a protein organic matrix. Echinoderms and sponges use calcium carbonate crystals to construct parts of the their bodies; these creatures also have a protein matrix that is associated with their crystals. In plants, crystals of calcium oxalate are extrememely common. Over three fourths of plant groups produce CaOx crystals. The CaOx crystals are produced always in the vacuole, and may be found one per cell or one cell may have more that 2000 crystals. The shape of the crystals in plants seems to be under genetic control since individual species always produce the same kind of crystals. However, soil conditions can also regulate the presence or absence of crytals in certain species. Calcium sulfate crystals are found in desmids but is rare or unknown in other groups of plants. Crystals of calcium carbonate are sometimes found in the walls of various seed plants. Amorphus calcium carbonate deposits are common in approximately ten families of flowering plants. These specialized cells, called cystoliths, each have a deposite of calcium carbonate in an internal structure called a lithocyst. Occasionally, the lithocysts become crystalized through the formation of calcite crystals. Organic crystals, usually protein crystals are common in many organisms. In animals, yoke platelets and peroxisomes have organic crystal. In these crystals, the lattice plane of the protein can often be resolved. The viral diseases of insects are also associated with protein crystals which often surround one or more virus particles. The guanine crystals found in various tissues of fish (eyes and skin) are common examples of an organic crystal serving an important structural role. Protein bodies are common in the seeds of many plants; they are also found in microbodies (peroxisomes) or free in the cytoplasm. TMV and other viruses may crystalize in plant cells. One can not help but notice the routine association of proteins with crystals in many of the the systems discussed. The role of the proteins is of universal interest. Adapted from Arnott and Pautard, 1970.

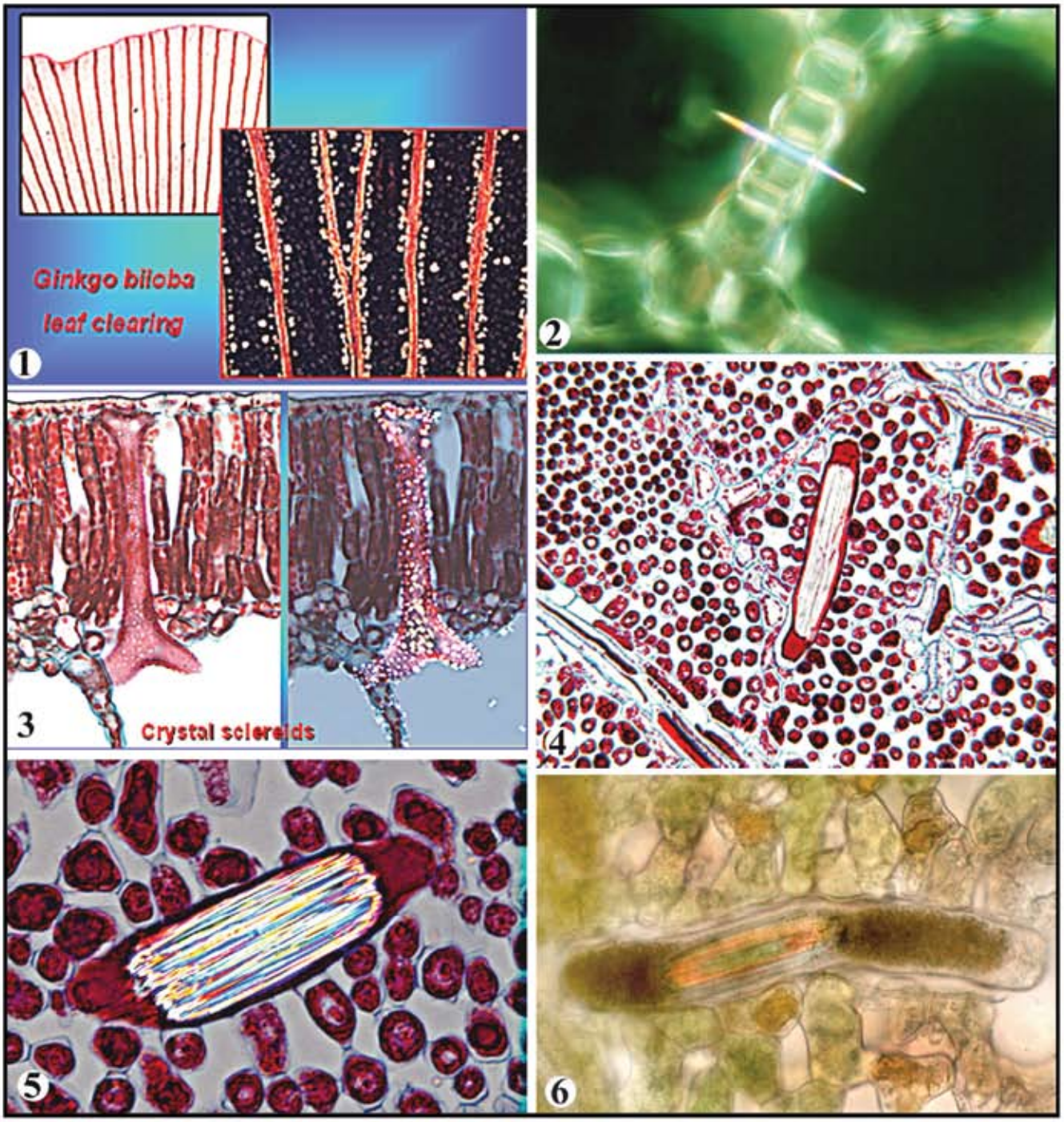


Plate 2. A series of light micrographs which show the graphic colors often seen when observing plant crystal cells either directly or under polarized light. 1. Micrographs showing a cleared leaf of *Ginkgo biloba*. Upper figure clearing as seen in normal light, lower right figure as seen in polarized light. Note the numerous druses which occur along each vein. 2. A single styloid crystal in *Eichhornia crassipies* (the water hyacinth) as seen under polarized light. The styloid is contained within a cell but under these conditions the cell walls are not seen. 3. Crystal sclereids of *Nymphaea* as seen in normal light (left) and polarized light (right). The lower part of the sclereids has grown into the airspace of the leaf. 4. Paradermal section of a *Vitis vinifera* leaf showing large raphide idioblast. 5. Paradermal section of *V. vinifera* showing a raphide idioblast under polarized light. 6. Living section of *V. vulpine* showing large raphide cell with small group of crystals in its center.

during the class break between lecture and lab. We generally talked about students because then, as today, they are always of interest to instructors. However, on the day in question, we discussed the use of electron microscopy in the study of plant cells. Specifically, we spoke about a recent paper by Dr. Flora Murray Scott, a distinguished plant anatomist at UCLA, and her colleagues. Her paper presented electron micrographs of onion epidermal cells (Scott, *et al*., 1956). However, we could not relate her micrographs to plant cells as seen in the light microscope. In fact, "we" dubbed her micrographs "**rug patterns**." We had a **great laugh** about these "useless pictures!" (I think that "Rug patterns" was probably Foster's idiom, in fact sometimes I used "**Wallpaper patterns**" when relating this incident). Obviously, we did not have much regard for the use of electron microscopy in the study of plant cells. That "contempt" especially on my part, is incredible, when you realize that in just few years, I was fully engaged in studying plant cells with the electron microscope. By the early 1960's Dr. Scott published electron micrographs that contained obvious plant cells components. Her 1956 paper was apparently near the beginning of her use of electron microscopy to study plant cells. Foster stuck with the tried and true and never moved on to electron microscopy.

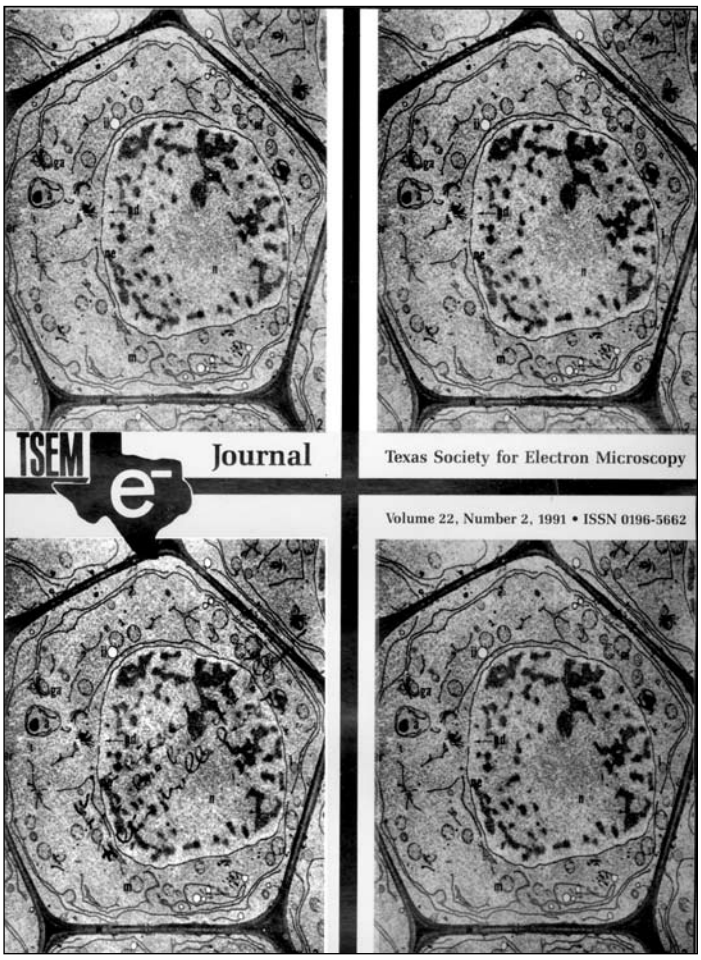


Figure 4. "Mollenhauer Cover" appeared on TSEMJ 22(2), 1991. The cover was created by H .J. Arnott by adapting a micrograph from Whaley, *et al.*; 1960.

In 1960 I was back in Berkeley teaching Botany in summer school. That summer, Foster and I discussed a recent paper by W. Gordon Whaley, H. H. Mollenhauer and J. H. Leech, "The ultrastructure of the meristematic cell" (Whaley, *et al*., 1960). It got our attention "big time." In fact, "**That paper changed everything!**" Here was electron microscopy that showed real plant cells, not "rug patterns." Now we could actually see plant cells in transmission electron micrograph, images that corresponded to what we could see in the light microscope. However, there was a **critical difference**, now we could see the **internal details** of plant cells that microscopists struggled to make out since the time of Leeuwenhoek and Hooke. There were "the granules," that E. B. Wilson spoke of in his book on cells (Wilson, 1928). Unknown to us, in the previous year, Hilton H. Mollenhauer reported on the $KMnO_4$ fixation of plant cells (Mollenhauer, 1959). That paper was the real breakthrough and it gives Mollenhauer (alone) the priority for the introduction of permanganate fixation of plant cells. Dr. Hilton H. Mollenhauer was president of the Texas Society of Microscopy in 1985-86 and was honored by a festschrift given by the Society in 1996 and a cover showing one of Mollenhauer's classic micrographs of permanganate fixed plant cells (Fig. 4).

Now, let's go back to the crystal trail. In 1963-64 Jack Horner, a graduate student of mine at that time (see Part III), and I began to study transmission electron microscopy in the lab of Professor James C. Hampton, Chair of the Department of Anatomy, Northwestern University Medical and Dental Schools, Chicago, Illinois. We received excellent direct instruction from both Hampton and Benjamin (Ben) Rosario. However, preparation of thin sections was difficult, as glass knives didn't work well with plant material. In any event, at that time I began looking for a plant system to study and my thoughts brought back the raphide crystal cells of yucca. Like Quatermain remembering the map. I promptly fixed some yucca root tips using $KMnO_4$ and embedded them in Epon. Later, I purchased a diamond knife and sectioning became much easier. In 1964, at the American Botanical Society meeting in Denver, I gave a paper entitled "The Ultrastructure of the *Yucca* Root" in which I spoke about the electron microscopy of raphide idioblasts for the first time (Arnott, 1964). It is interesting to note that Dr. George R. Johnstone was in the audience at my presentation. That was the last time I saw my USC mentor; he died in 1971.

I spoke of my "stroke of luck" in being accepted in the Graduate School at Berkeley (see Part III). By another similar "stroke of luck" I arrived at The Cell Research Institute, UT Austin, late in 1964. I became an NIH postdoc with Gordon Whaley and began a critical training program in electron microscopy. I transferred my NSF grant on "The Anatomy of *Yucca*," to the University of Texas and soon graduate students (not mine) were in the field (in Mexico and South Texas) collecting and fixing yucca material. The students somehow got the "idea" that it would be good to freeze the fixed specimens – of course, nothing could have been worse. All their collecting was for naught, since the freezing destroyed the integrity of the specimens and made them useless to me.

That "setback" ended a research avenue that I hoped to follow, namely, to study the anatomy and ultrastructure of yucca. However, I was already examining the ultrastructure of several kinds of plant idioblastic CaOx crystal cells, and with the fixation setback, they became my chief area of study (plan B). In retrospect, "the crystal trail" was an outstanding choice, even if it was forced by circumstances. Some of my first crystal cell samples, in addition to *Yucca*, were duckweed, *Lemna minor*, the water hyacinth, *Eichhornia crassipes* and the castor bean, *Ricinus communis* (Plate 3). They were all fixed with $KMnO_4$.

In each of these species the formation of CaOx crystals, i.e., "crystal production system," was different in specific ways; however, they had many common characteristics. All produced the CaOx crystals in the vacuole, **a truly significant point** since the plant vacuole at that time was characterized as a simple water sac. Even though I suggested that the crystals of yucca appeared to be in the vacuole (Arnott, 1962), electron microscopy made it "crystal clear." In *Eichhornia* I found over 2000 needle-shaped

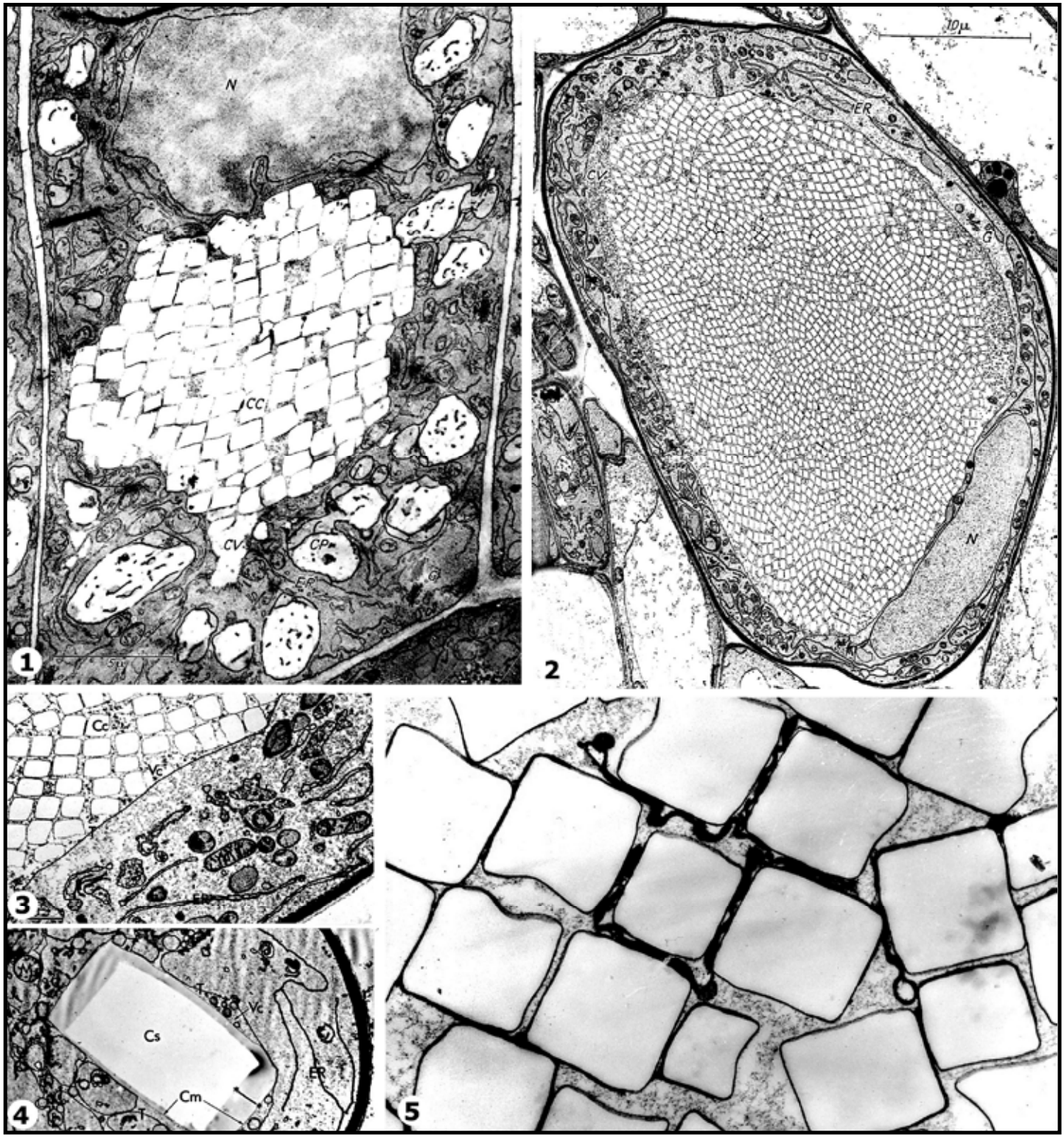


Plate 3. Early 1960's transmission electron micrographs of crystal idioblasts. All samples were fixed with $KMnO_4$ and stained with urinal acetate 1.My first published micrographs of a Yucca root crystal idioblast showing many empty crystal chambers in the vacuole, the nucleus, endoplasmic reticulum and "crystaloplastids," a type of plastid characteristic for certain spices of Yucca, each having only a few thylakoids. 2. Large crystal cell of water hyacinth, *Eichhornia crassipes*. This raphide cell has over 2000 crystal chambers, each representing a single needle-like crystal of CaOx monohydrate. Note the prominent ER, many mitochondria, and several dictyosomes in the cytoplasm. A large nucleus is seen in the lower part of this micrograph. 3. Magnified detail of a water hyacinth crystal idioblast showing the individual crystal chambers (Cc), the cytoplasm with Golgi bodies, mitochondria and ER; the cell wall is lower right. 4. A crystal idioblast of *Eichhornia crassipes* with one large styloid crystal of CaOx in the vacuole; Cs = styloid crystal, Cm = crystal chamber membrane. 5. Portion of an *Eichhornia crassipes* central vacuole with many crystal chambers. Note the membranes that define each crystal chamber which with higher magnification show a "unit membrane" structure. Membranes and tubules are seen between the crystal chambers. Adapted from Arnott, 1965.

raphide crystals in the vacuole of a single cell (Plate 3:2-5); each crystal being formed in a unique membrane bound chamber associated with tubules (Plate 3). In *Eichhornia* there were also cells in which a large solitary styloid crystals of CaOx were also produced their vacuoles. In *Lemna,* I found the crystal chambers were formed between the points of a complicated membrane system, still all within the vacuole (Arnott and Pautard, 1970).

$KMnO_4$ fixation made the electron microscopic observation of the membranes easy. Unfortunately, as Mollenhauer pointed out, some cellular components were lost; but then, in the 60's that point was overlooked as researchers were happy just to see something of the ultrastructure of plant cells. Bulk staining with uranyl acetate extracted most of the calcium oxalate from the crystal chambers where it was replaced with plastic making the material easier to section (Plate 3:1-5).

F. G. R. PAUTARD BIOLOGIST-BIOPHYSICIST

In 1965, at the insistence of Fred Pautard (Fig. 3 and Part I), I attended the Third European Symposium on Calcified Tissues held in Davos, Switzerland (Arnott, 1965). Fred was a visiting scientist from the Astbury Biophysics Laboratory at Leeds University. He was completely at home in meetings about mineralization (or meetings about anything else for that matter) as he was well versed in the structure and biophysics of bones, teeth and kidney stones. However, despite Pautard's endless chatter about animal mineralization I was still almost completely in the dark about it. Pautard was well traveled in Europe but it was only my second time there and first time in Switzerland. I flew from the US to Leeds where I met Pautard and visited with his family and he showed me his favorite fishing places. We took a train from Leeds to Heathrow and flew to Zurich. From there we took a train through the mountains to Davos. The symposium was held in the Hotel Schatzalp, located some 300 meters above the town level, and the view of the Davos countryside was spectacular. At the time, the Hotel was an ageing monarch; currently it is still an attractive hotel. We were greeted at the symposium check-in by H. Fleish, our Swiss host. While at the check-in table, Dr. Fleish was talking to several people, more or less at the same time, but what was amazing to me was that he was talking in four different languages (you may remember that I am language challenged, see part III). While there, we were able to go to the top of the ski lift where I saw people of all ages (small children 4-5 years old, to old men and women 80's+) heading down what looked like an unending path to destruction. Luckily there was a coffee shop with hot chocolate at the summit as I had only a suit coat and sweater on.

The meeting was as advertised; many important people in mineralization were there. Most papers focused on either medical or scientific calcification in animals and humans. As it turned out I was the only one at the Davos meeting that spoke about plants. In my paper I clearly demonstrated that in plants, CaOx crystals develop inside plant cells, to be precise, they are produced in the vacuole. With regard to my paper the view of the medical/animal oriented delegates ranged from those who thought internal calcification was unlikely (the kind ones) to those that thought it was completely out of the question (the majority). I left thinking that both groups felt my work had no relationship to animals at all. In their minds, internal crystal fabrication was not possible since the mineral crystals of bones and teeth always appear to arise outside the cells. I was blissful that the delegates and symposium officials allowed me to appear in the delegate photo (See Part I). Later, because of the open minded and far-sighted urologist, Dr. Birdwell Finlayson, this attitude changed.

In 1970, after over two years in press, Fred Pautard and I published "Calcification in Plants" (Arnott and Pautard, 1970). That paper helped inform people about how extensive calcification is in plants. As time went on, a number of my students, or former students, including Harry T. (Jack) Horner, Frances Fletcher, Ezequiel Riviera, Mary Alice Webb, Mark Grimson, Genie Brackenridge and Jackie Van de Veire took up the study of CaOx crystals in plants. Early on, Diane Teigler investigated CaOx crystal formation in the Malpigian tubules of the silkworm (*Bombyx mori*). Ten years after the review by Arnott and Pautard, Vincent Franceschi and Jack Horner published another review of calcium oxalate in plants (Franceschi and Horner, 1980; Fig. 19). Vincent who earned a masters degree under Horner went on to establish an animated research program on calcium oxalate in plants as a professor at Washington State University. Vincent Franceschi passed away in 2005, however, his program continues with the research of his former students. Some early transmission electron micrographs of calcium oxalate in various plants are presented in Plate 3. Later, some modern views of this interesting system are shown. The reader should not assume that the study of plant crystals began with my work. Nothing could be more wrong. There is substantial literature about plant crystals beginning in the 19th century. Most of the literature was in German and the illustrations were drawings made from the light microscope. See the work of Albert Frey-Wyssling for example (Frey-Wyssling, 1981).

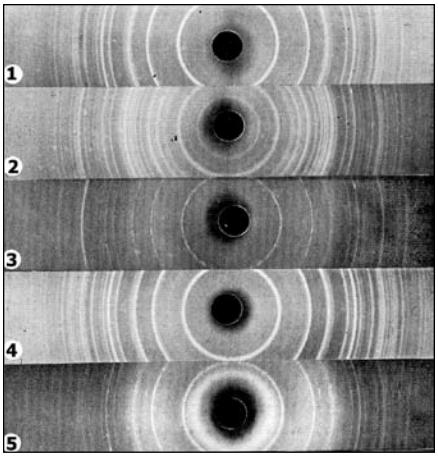


Figure 5. Powder diffraction patterns comparing known calcium oxalate monohydrate (1) with crystals isolated from several other plants (2-5). Adapted from Arnott and Pautard, 1970.

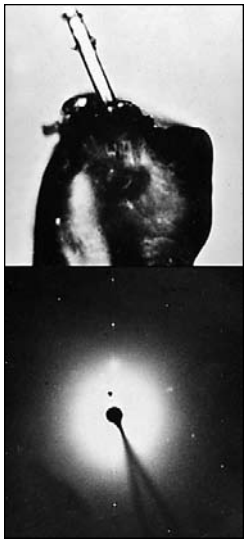


Figure 6. Single crystal isolated from *Yucca rupicola* used for x-ray diffraction (upper panel). Single crystal x-ray diffraction pattern for calcium oxalate monohydrate (lower panel). Adapted from Arnott and Pautard, 1970.

Over the years we learned to isolate the CaOx crystals from the plant tissues. At the Cell Research Institute, under my direction, my assistant, Virginia Stork, was the first of my associates to experiment with isolation of oxalate crystals. Using a blender and centrifugation she was able to compact the number of crystals but unfortunately they were always contaminated with cellular debris. These preparations were quite satisfactory for powder x-ray studies (Fig. 5) but they were not useful for LM or SEM studies. Later at UTA, Mary Alice Webb and I worked out a protocol for the isolation of CaOx crystals; a protocol which provided large number of crystals that were completely clean and free of other cell debris. An outline of the procedure was recently published (Webb and Arnott, 1983; Maier and Arnott, 2002).

It was Pautard that showed me the **power** of x-ray diffraction and the difference between powder and single crystal diffraction. It was, of course, as a biophysicist second nature to him. When we studied the crystallography of calcium oxalate monohydrate, I isolated the very large styloids from the leaves of *Yucca rupicola* by macerating the tissue and separating the large crystals with

dissecting needles. Then Fred mounted single crystals on a thin glass thread. These preparations were used for x-ray diffraction studies in which the crystal structure of calcium oxalate monohydrate was worked out using the large CaOx crystals of *Yucca* (Arnott, Steinfink and Pautard, 1965) (Figs. 5, 6).

"COMMENTARY ON THE CELL RESEARCH INSTITUTE"

During my residency in the Cell Research Institute I was fortunate to meet many of the noteworthy cell biologists of the time. This came about though a lecture program Gordon Whaley initiated which brought world famous cell biologists to Austin. I mention a few such as Keith Porter, Christian de Duve, Jean Brachet, Myron Ledbetter, George Palade, Philip Siekevitz, J. G. Gall, F. G. E. Pautard, Francis O. Schmitt, and Kenneth Smith. This was a great program as these lectures and visits helped bring Texas into the main line of cell biology, which was developing mostly in the East. For a few of us, the lucky ones, Whaley hosted us to lunch with some of these "famous" individuals. These lunches were always at the same place, Mi Casa Es Su Casa, a Mexican restaurant on the 6th street in downtown Austin. Despite the fact that the food was unusually good and presented in a superb manner, not every guest loved Mexican food as much as Whaley did! *C'est la vie.*

There are a number of steps along my crystal trail; however, space, time and my memory will not let me deal with all of them. In fact, some of the research chronology has already grown dim in my mind (please bear this in mind as you read on). That being said, I will explore my research using several examples. I have chosen the examples I am presenting here because of their contribution to the understanding of crystals in biological systems, i.e., their contribution to my crystal trail (autobiography). Unlike some of my previous work (see part III), most of the crystal associated material found its way into publications. In some cases, as an aid to understanding, the material is not presented chronologically.

"PUBLICATIONS ARE THE ABSOLUTE ANALOG OF GOLD AND DIAMONDS"

In keeping with my comparative line of thought, it should come as no surprise to hear me say that, "**Publications are the absolute analog of the gold and diamonds found in King Solomon's Mines**." Scientists of previous centuries understood that publications along with ethics are two essential parts of "a complete scientific life." In publications *Priority* is all, and *priority* comes from timely publications because research is never complete until it is published. In fact, it may just as well not have been done, if it is not published. There is no gold; there are no diamonds, until your paper is "***in press***." Without being pretentious, it is my intention to display some of my gold and even a diamond or two in the coming paragraphs. I also want to present some of the "travels" and "travails" of life on the crystal trail.

KENNETH SMITH AND INSECT VIRUS CRYSTALS

My association with insect virus crystals chiefly involves work with Dr. Kenneth Manely Smith, CBE, FRS (Fig. 7) (Kassanis, 1982). Dr. Smith served as Director of the Virus Research Unit, Cambridge University for twenty years. While there, he supervised and interacted with some of most noteworthy virologists of the era. Following his retirement, he spent four years as a Visiting Professor in the Cell Research Institute at U.T. Texas where it was "my luck" to come to know him. Smith was very well recognized for his work on both insect and plant viruses. He was elected F.R.S. in 1938. When we first met he was 73 and I was 37. This is an interesting turnaround of numbers for you that are concerned with numerology. At Cambridge he was interested in insect viruses, especially those that had crystals at a certain stage in their life history (he was already on the crystal trail). I am not certain about the first time I met him, it was probably in one of Gordon Whaley's receptions or after one of his lectures at the Cell Research Institute.

Figure 7. Kenneth Smith CBE, FRS. Biographical Memoirs of the Royal Society, Vol. 28-451-477, 1982

I do remember my first visit with Kenneth Smith in his office. During that visit, he broached the question of whether I would be interested in looking at the ultrastructure of an insect virus that produces protein crystals around the virus particles. Since I was already hooked on crystals I promptly accepted his offer and for the next few years we had a cooperative research program (Fig. 8). It only ended when Kenneth was forced by bad health to return to England.

One of the first projects we investigated was the virus that infects the Indian Meal Moth, *Plodia interpunctella*. As the name suggests, this insect was (and still is) an economically important pest. The moth is susceptible to a virus with a protein crystal around it, the whole unit being called a capsule (Fig. 8). The disease caused by this virus is termed a **granulosis**, a term derived from the many capsules, "granules," seen in the infected larval cells. The protection of the protein "coat" allows the virus to maintain its infective potential for many years. Kenneth believed they would survive for more than 20 years outside of the host, in the dust or dirt where they were released at the death of the insect. When the capsules are ingested by a larva, the virus undergoes reproduction in the cells of the fat body, typically causing the death of the moth. The moths were easily raised by placing a few moths in 10 lb stone jars with nylon covers. The moths laid eggs and the larvae developed on a mixture of bran, glycerin and baker's yeast. Both control and virus infected colonies could be easily propagated. This part of the research was handled by Kenneth Smith.

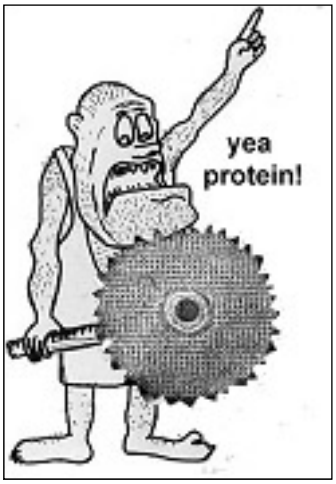


Figure 8 The crystal Gagool got the message; he is shown with a protein virus club.

The cylindrical DNA virus reproduces within the cells of the fat body in the infected larvae of *Plodia* (Fig. 9; Arnott and Smith, 1968a). During the reproductive phase, the virus obtains a membrane from the endoplasmic reticulum. The membrane surface is used to nucleate the development of a protein crystal, which ultimately grows to completely envelop the virus particle and form the mature capsule. Numerous capsules are formed in a single cell. The growth of the protein capsule may begin at the end or the side of the virus particle or occasionally even from two ends (Plates 4, 5). The complicated reproduction of this virus occurs within

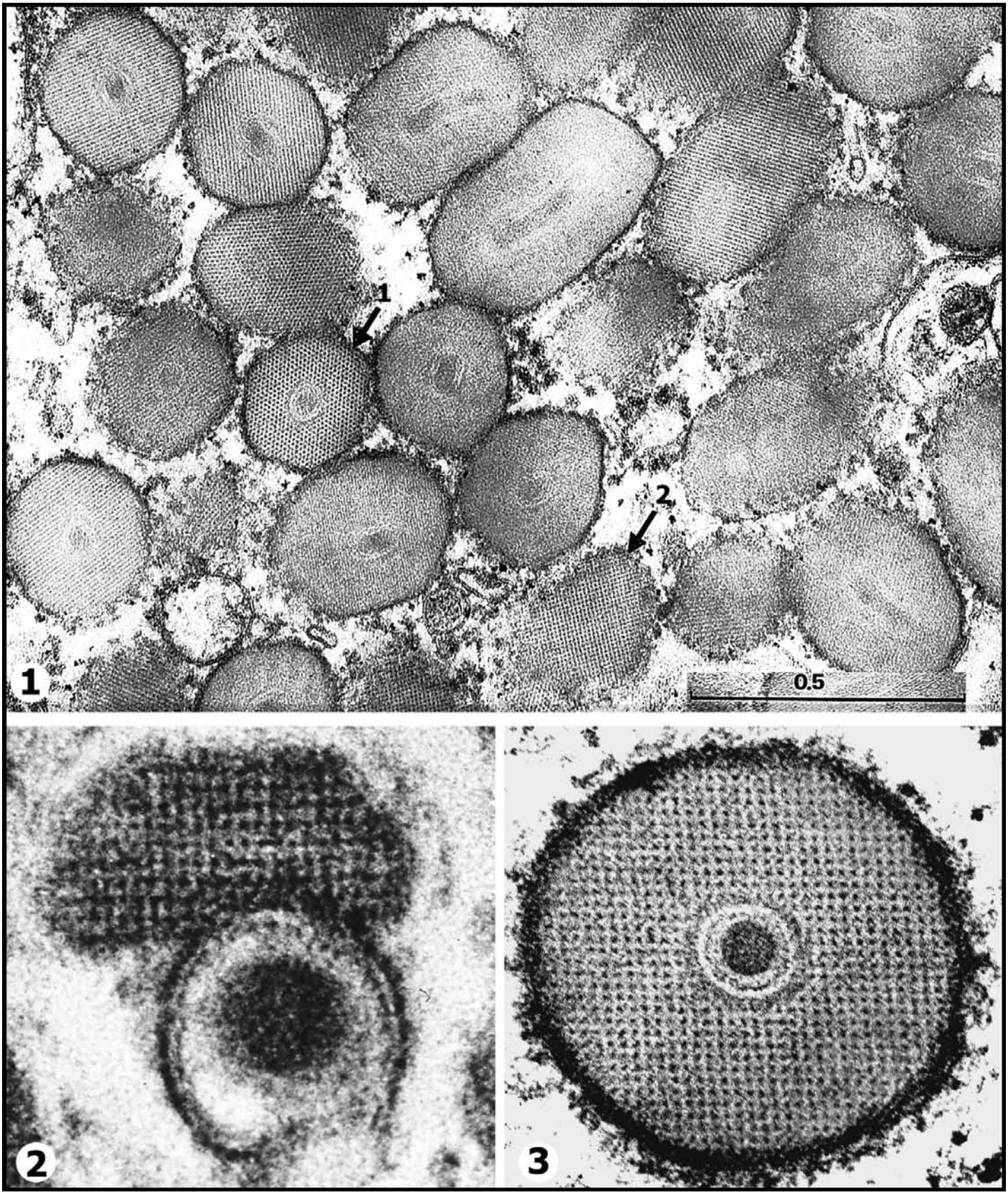


Plate 4. ***Plodia interpunctella*** **viral capsules as seen in TEM. 1. Sections of several capsules showing lattice planes. Note that the lattice planes form at least two patterns, which are presumed to be the crystal lattice shown in planes that are perpendicular to one another. Also note that some capsules (protein crystals) have facets, a general characteristic of crystals. 2. Cross section of a virus particle surrounded by a membrane on which a small part of the protein capsule is growing. Note that the membrane and the crystal lattice are integrated at the point of contact. 3. Cross section of a capsule showing the virus particle, membrane and mature capsule with very clear lattice planes. Periphery of the capsule has a boundary consisting of dense material. Adapted from Arnott and Smith, 1968.**

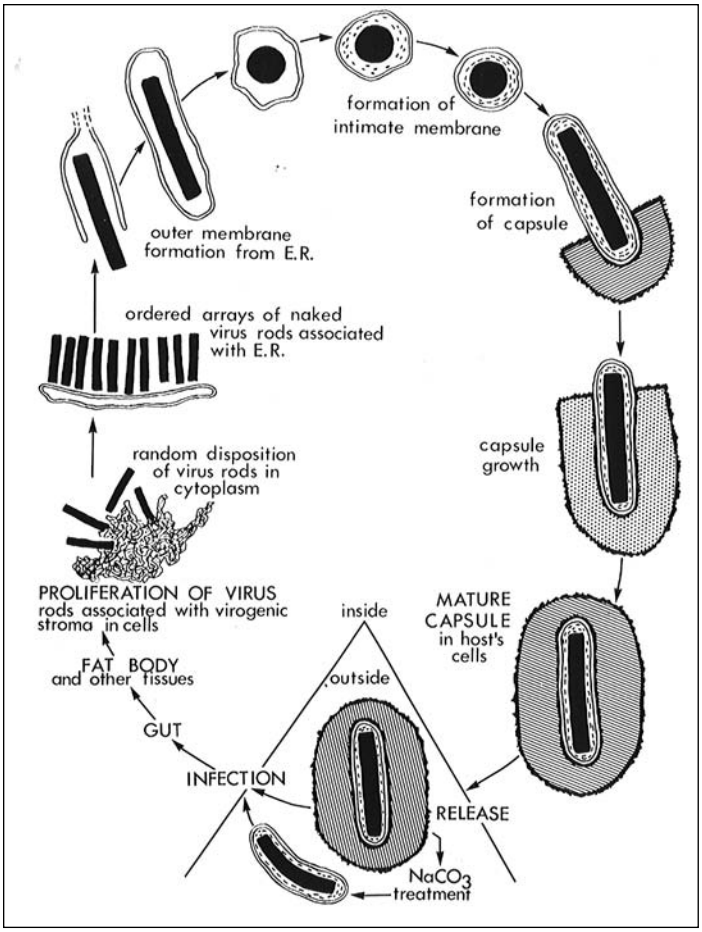


Figure 9. "Life History" of the *Plodia* virus. Adapted from Arnott and Smith, 1968.

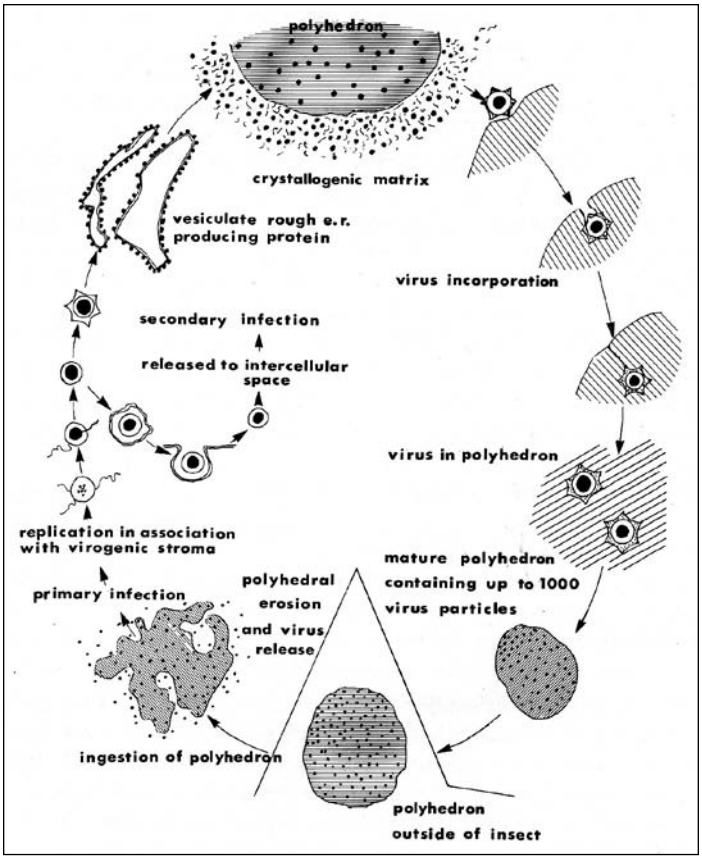


Figure 10. Cytoplasmic polyhedrosis virus of the Monarch butterfly. Adapted from Arnott, Smith, and Fullilove, 1968.

the cytoplasm, never in the nucleus. When we started working on this project, I knew little about insect viruses, or for that matter much about other viruses. I remember my introduction to plant virus work when Kenneth took me to the greenhouse and showed me how easy it was to transfer tobacco mosaic virus from an infected tomato plant to one that was uninfected. Even I could do it.

The *modus operandi* of our working arrangement was as follows: Smith would raise the insects, infect them with the virus, and then, at the proper time, he would dissect the appropriate tissues and place them in glutaraldehyde (the primary fixative). Then I would take the tissues, post fix them with osmium tetroxide and then run them through remaining procedures to embed them in plastic. Much of the actual manipulation was carried out by assistants, Virginia Stork being the most prominent. After the plastic was hardened, I mounted appropriate pieces and made thin sections using the hand driven Porter/Blum MT1 microtome; we had more advanced microtomes but I preferred the MT/1. Then the sections were stained with lead or uranium. At that time, the Cell Research Institute had several transmission electron microscopes. The best one was the Siemens Elmiskop IA, which was used for the above study. Earlier I was trained on the RCA 3D and 3F by Hilton Mollenhauer and Marianne Dauwalder.

I was trained on the Siemens microscope by Henry Threm. Henry was a German Tank Commander from WW II who was hired by Whaley as a full time microscope service engineer in the Cell Research Institute. Being German, Henry had a real affinity for the Siemens microscope and although likable he had somewhat of an autocratic personality. After training on the Siemens microscope, I could produce 30-40 micrographs per day. We used glass plates (instead of film) and they made extremely fine micrographs. I could get the best micrographs by slightly adjusting the position of objective aperture to do the final stigmation. But of course I did not tell Henry. *Es ist nicht in der Handbuch!!* After the micrographs were printed I would put them together and then discuss the results with Kenneth. With regard to writing, he generally did the literature survey (often it was mainly his work) and introduction and I wrote the observations based on a set of micrographs. Both of us would write an integrated discussion and then we would come to an understanding on the complete text. I was always in charge of putting the plates together, a job I enjoyed. That work is now made simple by the use of computer programs such as Word, Power Point or Photoshop. When there were diagrams to be made, I drew them by hand using the skills I learned at USC and Berkeley (Figs. 8, 10).

After we studied and published the normal reproduction for the *Plodia* virus (Plate 4; Arnott and Smith, 1968a) we were then able to study some "strains" in which aberrant "capsules" were frequently found. The development of aberrant capsules made a second interesting paper on the *Plodia* virus (Plate 5; Arnott and Smith, 1968b).

Another investigation which contrasts with the *Plodia* studies was a virus disease of the Monarch butterfly, *Danaus plexippus*. In the latter disease, the virus consists of small icosahedra particles. During the course of the disease, many virus particles would be enveloped in a large protein crystal, called a polyhedron. The virus and their protein crystals were assembled in the cytoplasm, thus the disease was called a **Cytoplasmic Polyhedrosis Virus** (Fig. 10, Plate 6). This was quite different from the case in *Plodia*. It was fascinating to see the stages of virus incorporation into the polyhedrons as well as the effect of the virus particles on the protein crystal lattice; by studying these effects you could "see" how the polyhedron developed. Smith and I published several additional papers that had some relationship to crystals or crystal-like inclusions as well as several on plant viruses. The work on the monarch butterfly was done together with Dr. Susan Fullilove

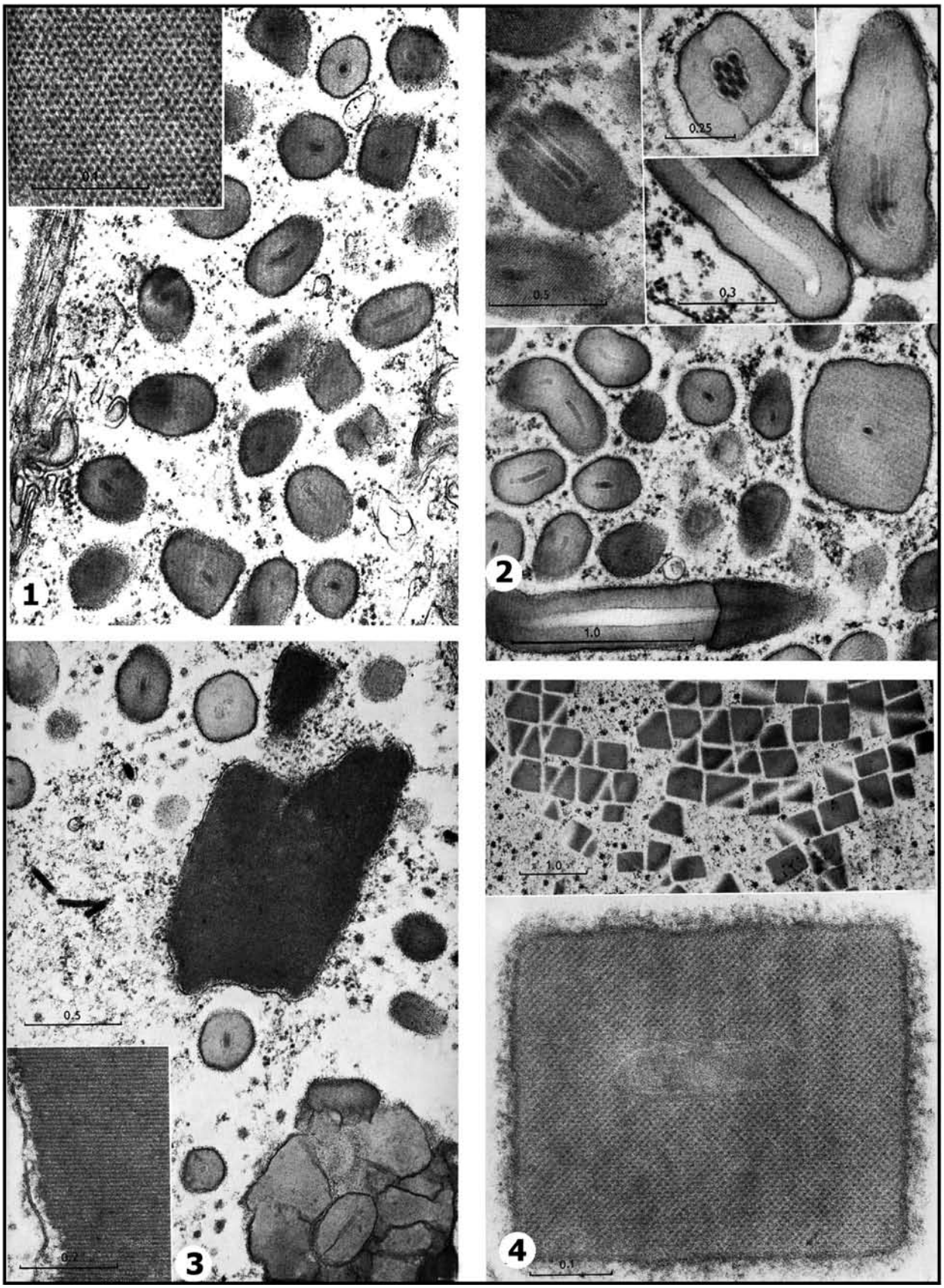


Plate 5. Aberrant capsules and irregular masses of capsules in abberent strain of the *Plodia* virus. 1. Mis-developed capsules among some slightly aberrant or normal capsules. 2. A variety of aberrant capsules including: jointed capsules;capsules with extra virus particles or with no virus particles; enlarged capsules. 3. A large protein crystal (inset shows the lattice of the large crystal which is contained in a membrane sac). Note the mass of capsules in the lower right. 4. Upper panel shows a cell with only square capsules; triangles represent squares cut obliquely. Lower panal shows lattice planes of a square capsule. Note the virus particle included in the squre capsule. Adapted from Arnott and Smith, 1968.

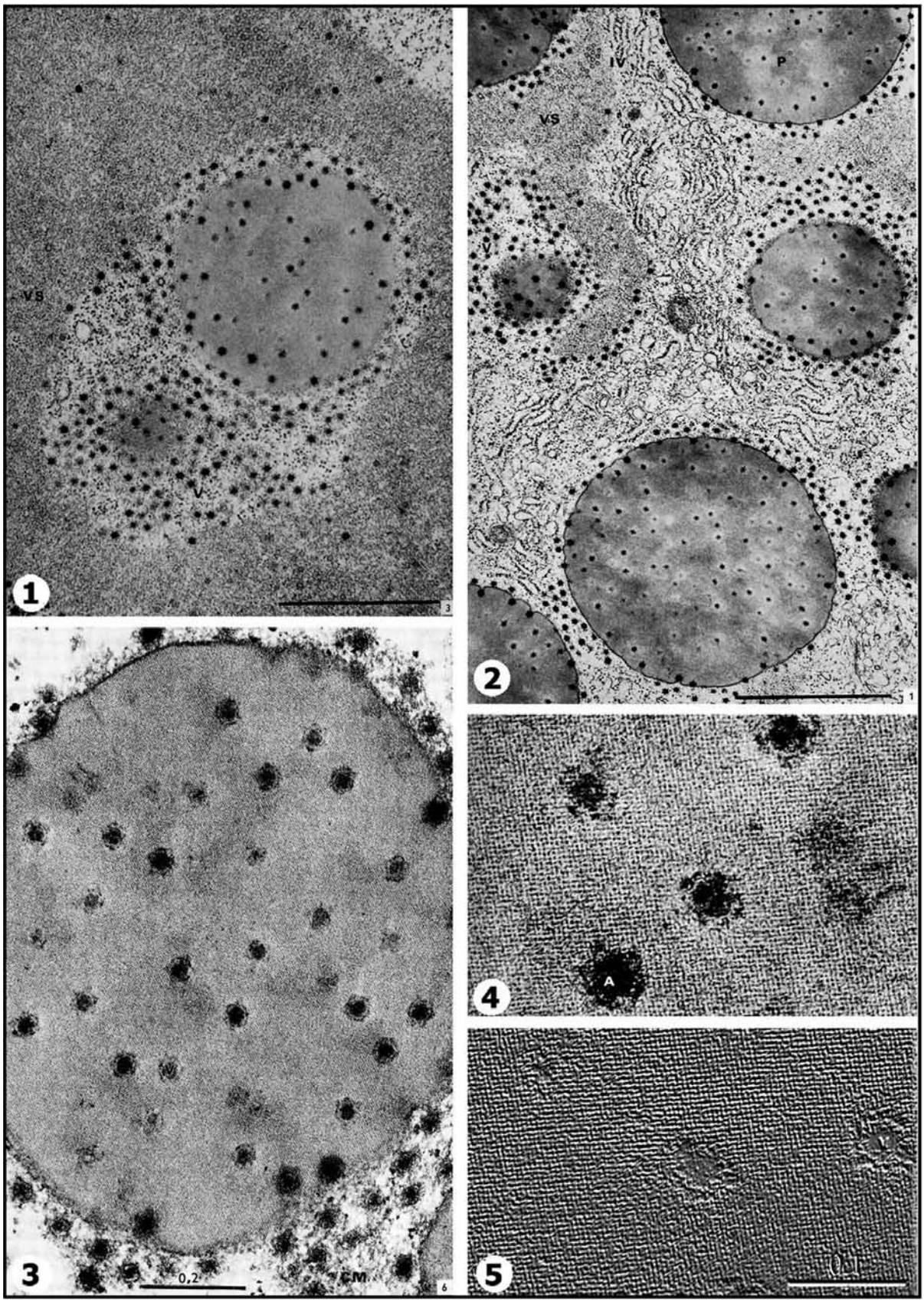


Plate 6. Cytoplasmic polyhedrosis in the Monarch butterfly. 1. Early stage in polyhedron formation. Note many free virus particles. 2. Later stage in polyhedron formation. Note massive amounts of endoplasmic reticulum. 3. Mature polyhedron showing many included star-shaped virus particles within the polyhedron which is a single protein crystal. 4. Crystal lattice in a polyhedron with included virus particles.Note that you can distinguish the coat and a central core in the virus particles. 5. Crystal lattice that has been embossed to further distinguish the lattice planes. Line equals 10 µ or a portion of 10 microns as indicated. Figs. 4 and 5 are the same magnification. Arnott, Smith, and Fullilove, 1968.

(Arnott, Smith, and Fullilove, 1968).

Kenneth Smith and his wife lived a short distance from the UT Austin campus in a multi-story apartment building between the Texas Capitol Building and the Biological Science Building. Smith did not drive, he walked to work as the distance was something less than a mile. On these "excursions" he passed through a "redevelopment" area, and being a consummate collector looked at all the plants on the way, even those arising out of sidewalk cracks. One day he came into my "office" with a couple of leaves of a sunflower plant. He pointed out that the leaves had some "funny" regions, which might well be the result of a virus. That was the discovery of the Sunflower Virus, another "notch on Kenneth's belt." The sunflower virus is of considerable interest since sunflowers are a major crop in the U. S. and other countries. The next morning I met him and he showed me some of the plants with the purported virus. I photographed the plants and took some of the leaves back to the lab. We fixed and processed them and soon we could confirm by electron microscopy that the sunflower had a virus, and a few months later we submitted a paper on the sunflower virus (Arnott and Smith, 1967).

Nothing beats **keen eyes**, whether in the lab or in the field, or on a walk to school. In closing, I must tell you that Kenneth later discovered another virus in *Lantana* plants growing in that same area. Because of his return to Britain we have never finished that work. When I looked for the *Lantana* plants at a later time, the area had been rebuilt and the plants were gone.

I am now close to Kenneth's age (when we worked together), and presumably have a different and more mature perspective; however, I still continue to wonder what he thought of his zealous (American) companion in research (apologies to Sigma Xi). In Biological Memoirs of Fellows of the Royal Society (Kassanis, 1982) it is written, "He later wrote 'The four and half years I spent at Austin were among the most fruitful of my career; there was available an almost virgin field of virus research'. His association with Professor Howard Arnott was indeed a very successful and productive one." Among **all** the investigators I worked with, Kenneth was unsurpassed in the ease with which we cooperated. With every other "**research partner**" I had moments of doubt and/or differences of opinion, but nothing like that ever happened with Kenneth Smith. I am still looking for a 37 year old researcher to "mellow out with" in my dotage; someone to bring back to the "**good old days**."

COLIN NICOL AND REFLECTION IN ANIMAL TISSUES

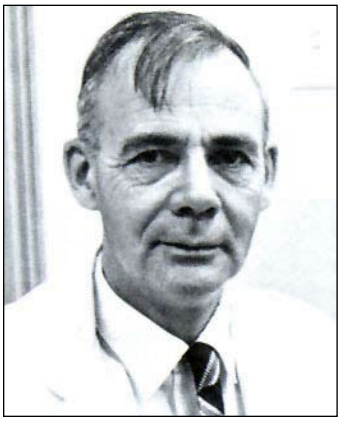


Figure 11. J. A. Colin Nicol FRS. Adapted from UT Obituary by Lee A. Fuiman, 2005.

Joseph Arthur Colin Nicol FRS was born in Canada (Fig. 11). He published his first scientific paper at 13 and continued to publish through his 80's; a 70-year span of time! The research papers of Colin Nicol are a treasure trove. To say he was creative is really a gross understatement. His papers are especially notable because they deal with problems that are inherently difficult. While many researchers turn away from thorny problems, Colin was more or less oblivious to thorns and prickles. Although Colin Nicol was important to the research of my crystal trail, in the reverse, I was just one of many collaborators he worked with. At least four others, E. J. Denton, A. G. C. Best, E. L. Thurston (a former TSEM President), and B. A. Fineran co-

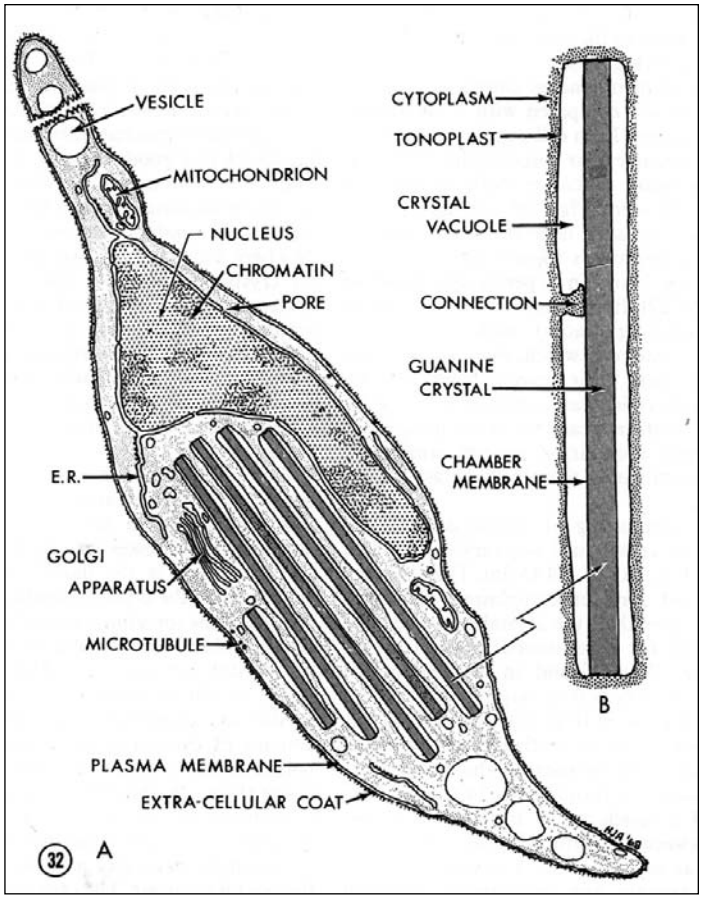


Figure 12. Diagram of a ratfish skin guanophore. Guanine crystals are formed in chambers as seen on the right. This is quite similar to crystal formation in plants. Adapted from Arnott and Nicol, 1970.

operated with him in various electron microscopic investigations.

During the time Colin and I worked together I was located at UT Austin and he was at the UT Marine Science Institute in Port Aransas; the two institutions are about 200 miles apart. As result, cooperation with Colin was inherently more difficult than with Kenneth Smith or Fred Pautard. Working with Nicol was something like playing tennis! He would send materials, usually fixed, to me, I would send the resulting micrographs to him; he would send them back to me along with many comments. And then a new set and so on. He would write manuscripts and I would rewrite them. Eventually we would agree on the general text but in the end he often changed or added something before it went to the editors. We investigated reflecting systems in both fish skin (Fig. 12; Plates 7:2, 8) and in fish eyes (Fig. 13; Plates 7:1, 9). On occasion we would travel to the other's institution, that way making more rapid progress.

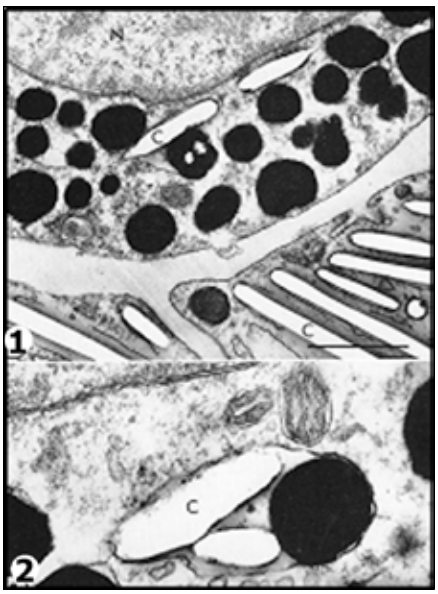


Figure 13. TEM views of the tapetum lucidum of the stingray. From Arnott, Best, and Nicol, 1970.

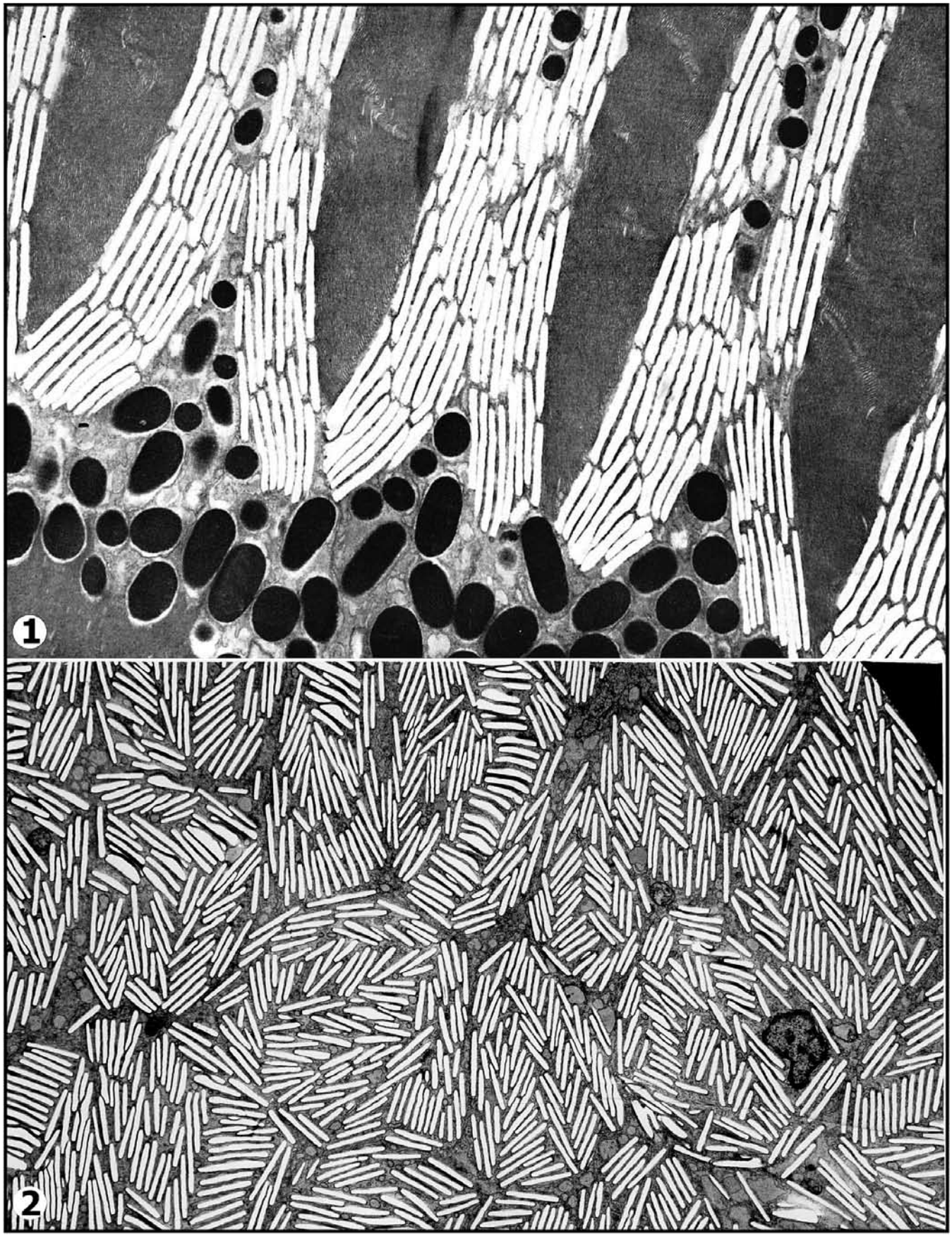


Plate 7. Tapetum lucidum and iridophores in fish. 1. Tapetum lucidum of the bay anchovy showing the interrelationship between the rods and the guanophores of the tapetum lucidum. The large black ellipsoidal objects are melanosomes. Individual guanophores contain both guanine crystals and melanosomes. 2. Iridophores of the stingray skin. The general outline of the individual iridophores is seen by the position of the guanine crystals; individual iridiophores have almost 75 guanine crystals per cell. Note the nucleus in the complete cell at the lower right.

Colin Nicol was always keen to get on with his work; in all probability, his colleagues would describe him as focused, or single-minded, or driven, or even obsessed by his research. I would probably choose the latter description. If it meant working all night, working in the dark, or working in an inconvenient place (boat) that was just part of research, his drive certainly had a great deal to do with the fact that Colin Nicol was "a very good scientist." On one occasion when I was visiting him at Port Aransas, he was in the process of fixing the eyes of some stingrays. He wanted the eyes to be dark adapted so the stingrays were in buckets in a "very dark room;" perhaps there was a tiny red light in the room. In any event, I was glad to learn that he cut the stingers off the fish before putting them in the dark. Colin used a rather large machete to deal the fatal blow, all done in "that very dark room," while I remained outside fighting off the famous bird-like mosquitoes of Port Aransas. Colin had **hyperopia** (farsightedness) and used two pairs of glasses to deal with that situation. Depending on the circumstances he used one set for normal viewing and a second for close-up vision. I am very familiar with that situation since I too have **hyperopia**. Benjamin Franklin invented bifocals to solve this problem. However, for me (and I guess Colin) reading glasses are better for reading and an absolute requirement for using the SEM. The remarkable thing about Colin was that he could switch from one pair to the other incredibly fast and did so often. The only comparison I can make is with the "fast guns" of the old west.

Colin Nicol involved me deeply in the study of animals' eyes; like the viruses in the Kenneth Smith saga, this was another subject about which I knew almost nothing at the outset. You might infer, correctly I think, that I did a great deal of "on the job training." However, I believe that in both cases I contributed more than might be imagined at the outset. The interaction with Nicol was important to my development in that it taught me to get on with things quickly and to sort out what was important from the rest; working with Colin there was no time for "diddling around." Observers, noting his level of activity might have called it the "Nicol syndrome."

I have just written a paragraph telling you about a "whirling dervish" but now, to set things straight, I must tell you about Colin Nicol the host, family man and friend. On a couple of occasions Jean and I spent an evening with Colin and his wife in their home at Aransas Pass; they shared that home with a very large, black dog (I think it was a Mastiff). As a host, Colin was very gracious, considerate, completely relaxed. Like night and day, the drive associated with his research activities was absent and he was the most affable host you can imagine, in fact, he was always a big hit with Jean.

Colin and I published 16 papers together, most dealing with eye structure (Fig. 13). In eyes we studied the following: the tapetum lucidum of the stingray (Arnott, Best and Nicol, 1970); reflecting spheres in the eyes of weakfish (Arnott, Nicol and Querfeld, 1971); the tapetum lucidum in the eyes of seatrout (Arnott, Nicol, and Querfeld, 1972); riboflavin containing spheres in the eyes of gars (Nicol and Arnott 1972; Fig. 14); the tapetum lucidum in the eyes of gar (Nicol and Arnott, 1973); the tapetum lucidum in the eyes of catfish (Arnott, *et al*., 1974); tapetum lucidum in the eyes of goatsuckers [birds] (Nicol and Arnott, 1974); and the diffuse reflectance of retinal tapetum lucidum in drum (Nicol, Best and Arnott, 1973). We also studied the reflection of the ratfish (*Hydrolagus colliei*) skin (Arnott and Nicol, 1970). I also started a study on the guppy skin (Plate 8) but it has never been published. A few of these studies deal directly with crystals. Many of these studies were reminiscent of the swamps, sand dunes, and mountains of Africa that sidetracked the Quatermain party from their travels on the trail to King Solomon's Mines. While traveling on my crystal trail I was often diverted, even the smallest dune (interesting subject or student complaint) could provide a distraction. After I moved to South Florida, Nicol and I tried to continue joint research but the distance and the complexities were too much. That pleasant and effective cooperative relationship was killed by my "urge to administrate."

Which of my studies with Nicol contributed to the crystal trail? First let's reflect on the silver color seen in the skin of many fish, for example the skin of a guppy (Plate 8), sardine or king salmon. Where does that silver color come from? The short answer is that silver color is caused by the reflection of certain wavelengths of light (silver light) by special layers of cells in the skin. The cells in these special layers contain guanine crystals. Each cell, termed a guanophore, or sometimes an iridiophore, may contain ten or more large flat guanine crystals arranged in an oriented array. For example, consider the well known neon tetra. Guanophores are involved in the bright blue lateral stripe and the red posterior. In the neon tetra the red and blue colors come from cells exterior to a guanophore layer. Light from outside passes though these pigments, which act like filters, and are reflected back by the guanine layer, hence the colors seem to glow. When there is very low room light the fish are almost transparent. Likewise the angle of view can cause the blue line to change to green.

We studied the origin of the silver/gold color(s) in the ratfish skin (Arnott and Nicol, 1970). The general structure of the reflecting cells (guanophores) is presented in a line drawing (Fig. 12). In that case we see an elongate cell section containing: five guanine crystals, a nucleus, mitochondria, Golgi body, rough endoplasmic reticulum and a series of vesicles. In actuality the cells are flattened perpendicular to the plane of cut seen in the diagram. Each plate-like guanine crystal was produced and is contained in a chamber. The organization seen in the ratfish skin and guppy are very much like that seen in plants where the CaOx crystals, regardless of their shape, develop and are contained in crystal chambers. One interesting question regarding this system is what physical of biochemical forces are involved in the precise arrangement and orientation of the crystals. In some fish there is clear evidence that the distance between the guanine crystals can be "manipulated" by the guanophores. A change in the distance between the members of a guanine stack can change the wavelength of the reflected light because the individual crystals work as a quarter wave reflecting system. Thus, when the distance between guanine crystals changes, the light reflected from skin can vary in color.

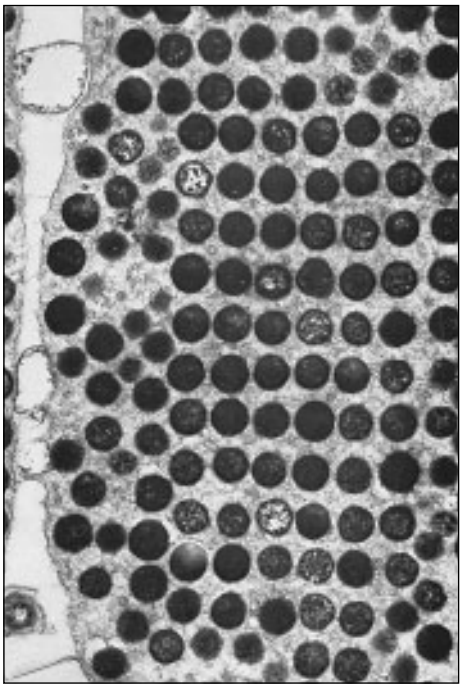


Figure 14. Ordered arrangement of spheres which form the reflecting layer in the eyes of the alligator gar. Adapted from Nicol and Arnott, 1973.

Colin Nicol also introduced me to eye-shine in fish. We investigated the ultrastructure in several examples of fish with eye-shine. The cause of eye-shine in these fish is a layer called the tapetum lucidum (reflecting layer). The tapetum lucidum is situated behind (outside) the retina. The two are formed as concentric zones in the eye. Various substances are involved in fish eye-shine. For example spheres are used by

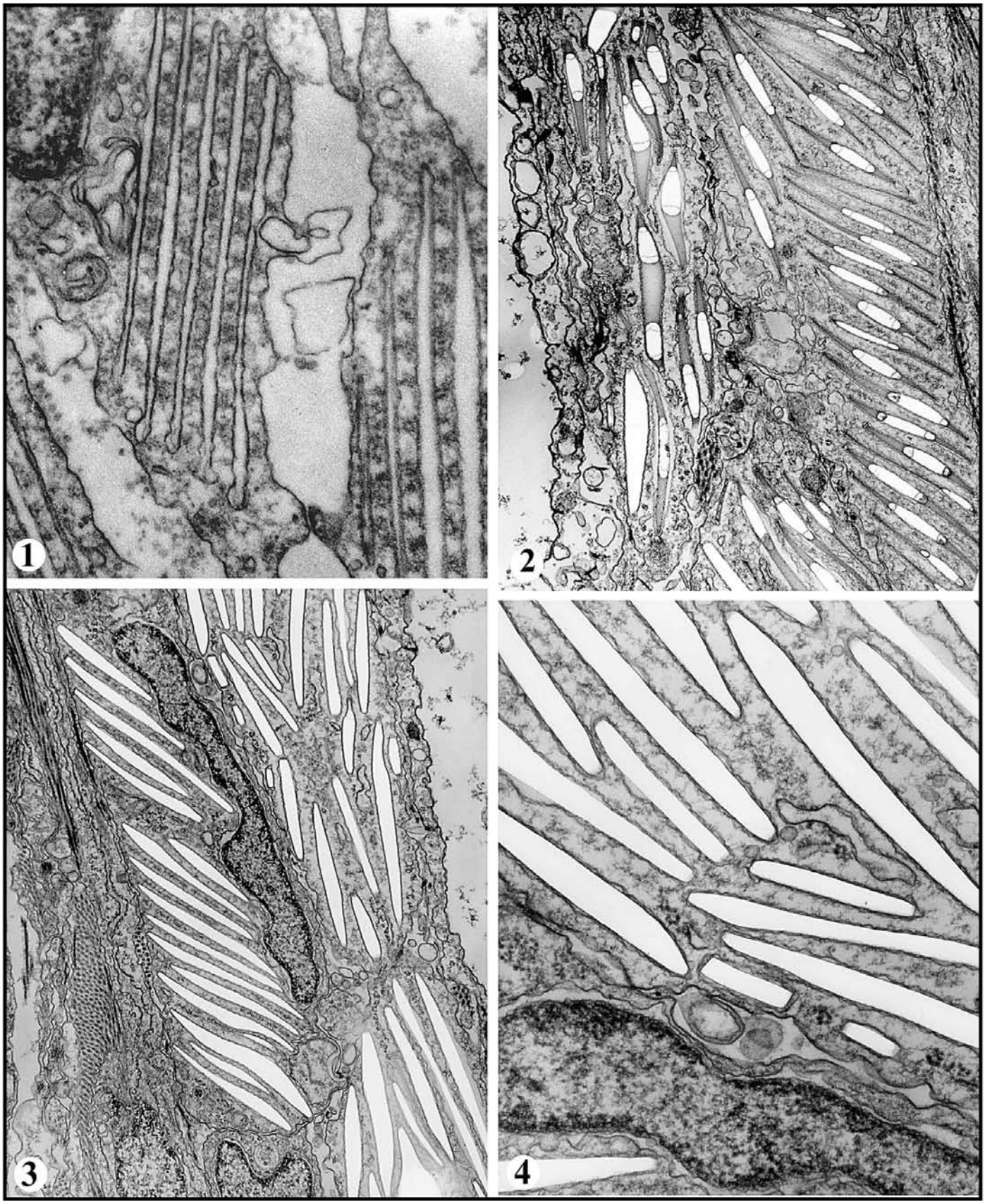


Plate 8. Iridophores of the guppy (*Poecilia reticulata*) as seen with the TEM. 1. A series of crystal chambers before the formation of the guanine crystals. Note that crystal chambers are all orientated in parallel. 2. Early stages in the formation of the guanine crystals within the individual chambers. At least three different iridophores are shown each with the guanine crystals oriented differently. 3. Good example of the structure of an iridophores with parallel oriented crystals and an elongated nucleus. 4. Two cells with guanine crystals in each cell. Processing of the tissue has removed the guanine crystals, which are now seen as white area surrounded with the chamber membranes. This is similar to what happens with CaOx crystals in plants when treated with uranyl acetate stains. Unpublished micrographs by HJA.

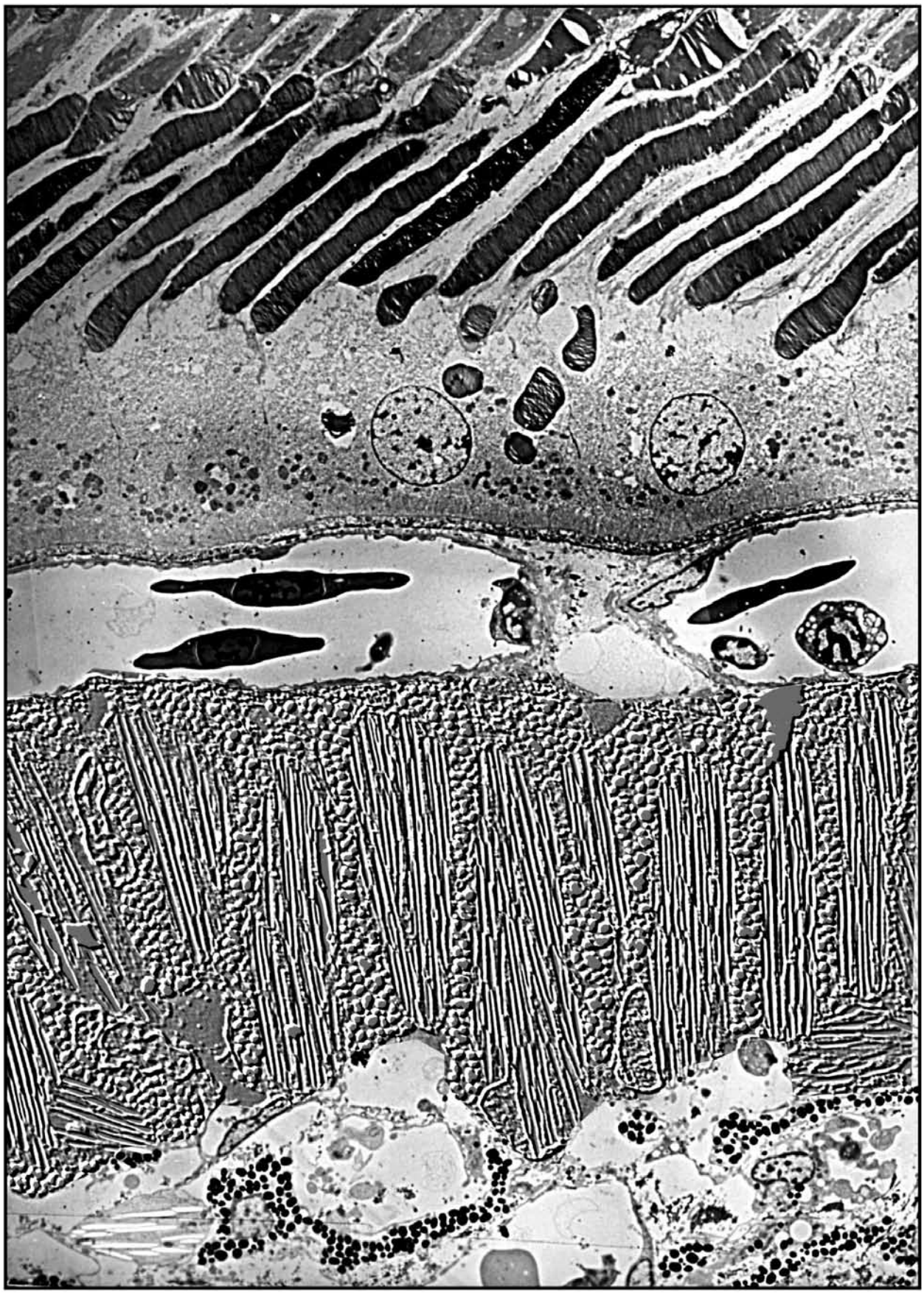


Plate 9. Tapetum lucidum in the eye of the sting ray. The upper layer shows the outer segments of rods and cones underlined by the epidermis which is associated with the retinal vascularization (two capiliaries containing blood cells). The bottom layer is the tapetum lucidum consisting of gaunophore and pigment epithelial cells with many melanosomes. The gaunophore pigment cells have been treated with an embossing filter to emphasize the orientation of the guanine crystals. Unpublished work by C. Nicol and H. J. Arnott.

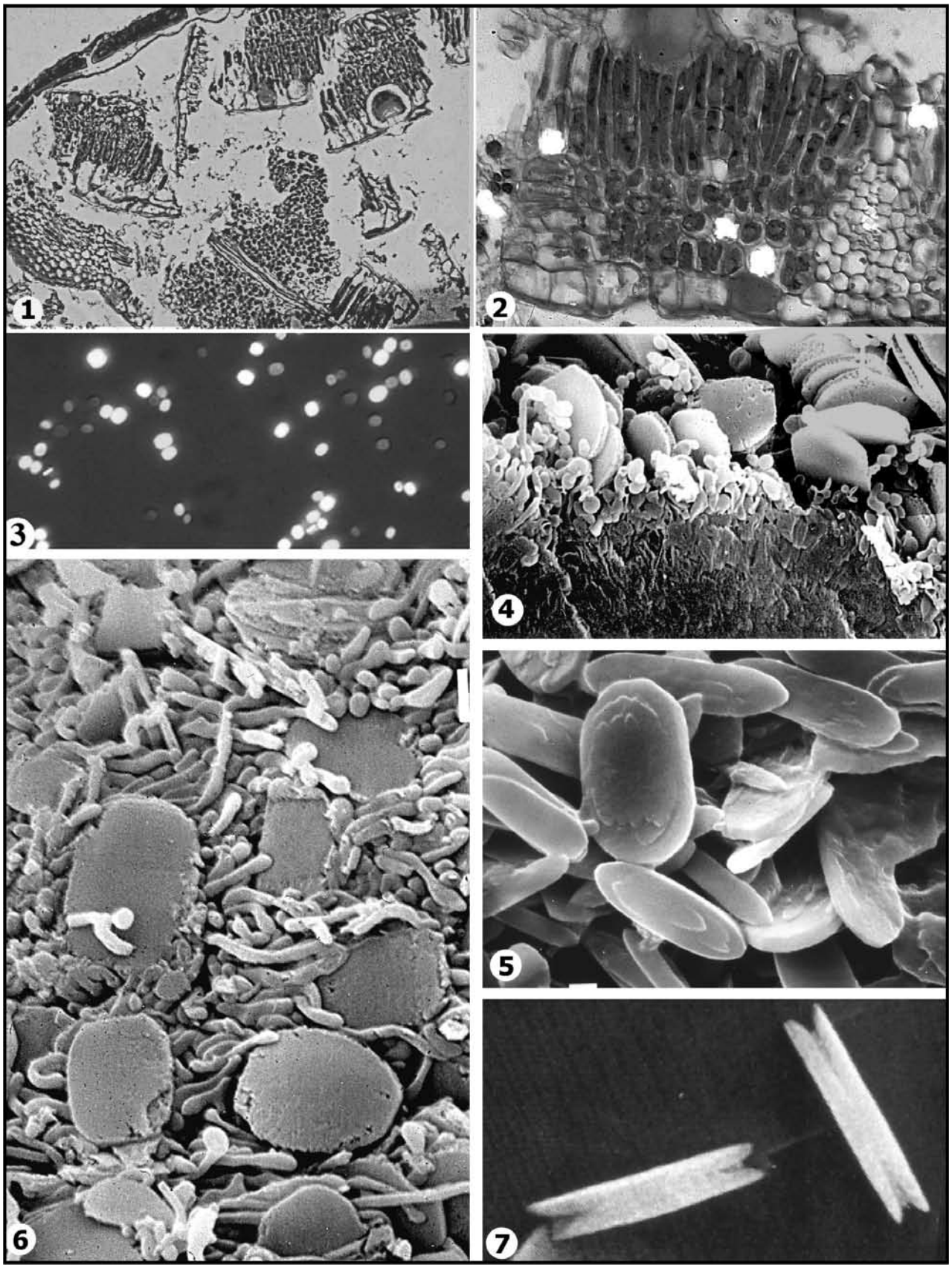


Plate 10. Calcium oxalate crystals associated with the silk moth, *Bombyx mori*. 1. Section of the silkworm's gut containing pieces of mulberry (*Morus alba*) leaves. Note various anatomical features of the leaf that are still well preserved, including a cystolith. 2. Single piece of mulberry leaf in the caterpillar's gut showing druses of calcium oxalate under polarized light. 3. Isolated crystals from the silkworms' Malpigian tubules viewed under polarized light. 4. A fractured Malpigian tubule cell with attached CaOx twin crystals. 5. Twin crystals of CaOx produced on the surface of the Malpigian tubule. 6. Twin crystals of CaOx associated with a lawn of elongate microvilli. 7. Side view of two twin crystals of CaOx produced in the Malpigian tubules.

gars to form their tapetum lucidum (Fig. 14). Within the cells of the tapetum lucidum in gars, the spheres are organized in psudocrystaline arrays. Guanine crystals are found in cells associated with the production of eye-shine in other fish. In the eyes of the bay anchovy organized arrays of guanine crystals and melanosomes are involved in their tapetum lucidum (Plate 7: 1). Depending on the position of the melanosomes, the reflection can be greater or less. If the melanosomes are in a position to shield the guanine crystal reflectors, the eye-shine will be less and the fish will be light adapted. In dark adaptation (at the time of maximum eye-shine), the crystals will be uncovered by the melanosomes.

The subject of the evolution of guanophores seems first to be tied directly to the ability to form guanine crystals at all. In most guanophores the crystals are clearly formed in "vacuoles" or vacuole-like chambers. Examination shows that each guanine crystal is surrounded by a crystal chamber membrane (similar to what we found in plants). When we examined the skin of the stingray we found guanine crystals and melanosomes in the same sacks. Extrapolating from that, perhaps the origin of guanine crystals is related to the origin of melanosomes. Estimates that the tapetum lucidum originated in at least three groups over 350 million years ago suggest that the guanine crystal system is very old.

During the time I worked with Nicol, Diane Teigler was working on her Ph.D. with me studying the crystals found in the Malpigian tubules of the silkworm moth, *Bombyx mori*. These were first identified by us as calcium oxalate monohydrate using powder x-ray diffraction (Teigler and Arnott, 1972a). Massive numbers of CaOx crystals are produced in the lumen of the Malpigian tubules. Zoologists believe the Malpigian tubules represent a primitive (kidney-like) excretory organ. Silkworms feed entirely on mulberry leaves. Mulberry leaves produce numerous cystoliths (see later) but have numerous crystals of CaOx as druses in most mesophyll cells. Both CaOx crystals and cystoliths pass through the gut of the silkworm and can be found in their fecal pellets (Plate 10). During instar stages, the moths eliminate (flush out) the CaOx crystals contained in the Malpigian tubules (Plate 10). Some of these crystals are deposited in the new larval skin and are responsible for the iridescence seen in various larval stages and even on the mature silkworm moth (Teigler and Arnott, 1972b).

The Malpigian tubules are long tubes closed at one end and attached to and emptying into the gut at the end. When the tubules are cross-sectioned, they generally consist of three cells surrounding a central tube. These tubule cells have numerous elongated microvilli (Plate 10:4, 5), which are unique in that each contains an elongated mitochondrion which almost completely fills the internal space of the microvillus. The CaOx crystals are produced in extensions of the endothelial cell membranes in between the microvilli. In the membrane extension, a dense material precedes and is replaced by the development of CaOx crystals. Each tabular crystal is actually a twin. Twinning is common in both the monohydrate and the dihydrated crystals of CaOx. In fact, many CaOx crystals in plants are twins or multiple interpenetrant twins (see twins later). In this case the twin plane is associated with the z axis making flattened twin crystals (Plate 10:4-7). However, sometimes multiple interpenetrant twins are produced; the later may be similar to the druses so common in many plant species. Please note that this represents still another crystal system in which the crystals are produced in sacs bounded by membranes.

Jumping ahead, in the 1980's at UTA, I began once again to study CaOx crystals in the Malpigian tubules of the silkworm. With absolutely no concern we raised the silkworms in open cardboard cartons on the center laboratory bench. Mulberry leaves were easy to find, I had a tree in my yard and there were several in the vicinity of our laboratory. As the result we had a bumper crop of silkworms to use in our Malpigian tubules studies. The larvae just eat and eat and eat; they remind me of Wimpy in the Popeye comic strip. As most of you will know, as the silkworms go through their instars, they get bigger and bigger. One week they are mere specks or short green rods with a diameter of a match stick; seemingly in the next week they are two or three inches long and twice the diameter of a pencil; during this time, they never left the open boxes. Following about 4 weeks of growth and constant feeding, our silkworms answered the call of nature and began forming cocoons. Initially this was fine, as they were carefully spinning their cocoons in the confines of their boxes. I left for a weekend, and on returning, a new fact of nature was apparent. Silkworms **migrate**; in fact, they can migrate quite some distance in order to find just exactly the right place to spin their cocoons. As the result of this migration, rogue silkworms had spun their cocoons all over the laboratory; on the ceiling, on walls and doors, in cabinets, behind the microtome, in other words, just about everywhere. There it was several hundred cocoons in the nooks and crannies of the laboratory. Of course, at the proper time, the moths disembarked from their silky domiciles looking for mates. Thus we had moths trying to fly throughout the lab in an effort to perpetuate their kind. Some colleagues thought this was very funny, however, they did not have to clean up the mess. An eager forensic scientist might still find a cocoon here and there in the lab; in fact, there are probably mummified silkworm eggs waiting to be uncovered in the 2200's. That is the way I remember things, however, they may have been even worse. Talk about your "red face!"

Dr. Zek Rivera was among my last students at UT Austin. He worked on cacti and produced some of the first SEM micrographs of CaOx crystals. I was amazed when I saw his pictures and realized that the SEM could provide a great deal of information about calcium oxalate in plants in a much shorter time than with TEM. Before you could finish processing material for TEM, a sharp investigator could complete an SEM study. Zek is currently a Professor of biology at the University of Massachusetts. Dr. Rivera is an amazing painter, perhaps as good, although not as well known as his namesake, Diego Rivera. He is also a master woodworker and genuine all around "artist." His pioneering effort on the SEM of cactus druses was certainly an inspiration for me to begin SEM studies (Rivera, 1973).

Dr. Genie Brackenridge Eliert was my last student at UT Austin (Fig. 19). She chose to work on the development of CaOx crystals in the root tips of *Yucca torreyi* (Eilert, 1974). That species of yucca is found in West Texas and produces very large seeds. When germinated, these seeds produce relatively thick roots and like *Y. whipplei* develop crystal idioblasts in files. The larger roots of *Y. torreyi* were ideal for investigating the crystal idioblasts. Taking advantage of this, she studied the complicated system of membranes involved in the production of the six-sided single crystals which arise in these idioblasts. Later, I sent seeds of *Y. torreyi* to Jack Horner, for use by his student Dr. Albert Kausch (Fig. 19; Kausch and Horner, 1984).

John Gottsch finished his M. S. with me during my two year stay at the University of South Florida. I guess it was natural for John to study eye-shine since it followed directly after my studies of eye-shine with Colin Nicol. He investigated the bright eye-shine found in the eyes of the opossum, *Didelphis virginiana*, which is caused by small spheres in the pigment epithelium. His experience led him to study ophthalmology and he now teaches ophthalmology at the Medical School of John's Hopkins University, where he is an Associate Professor.

Heather Koch finished her Master's Degree at UT Arlington in 1979 (H. M. Koch, 1979). The subject of her thesis was eye-shine in *Dorosoma cepedianum*, the gizzard shad. She was able, with the help of Dr. Tom Hellier (UTA, Biology), to catch this small fish in Lake Arlington, Arlington, Texas. *D. cepedianum* was a

convenient subject since Zyznar and Nicol (1973) had recently determined that the reflecting material in that fish was made up of pteridine crystals, 7, 8-dihydro-xanthopterin. The crystals were in the pigment epithelial cells and responsible for the reflectance seen as eye-shine. Her study was initiated as a natural evolution of my interest in animal reflecting systems. Heather was my first graduate student to receive a degree at UT Arlington.

In an elegant light and transmission electron microscope investigation, Heather worked out the development of these reflecting crystals (Plate 11). She found that the crystals originate in chambers which are ultimately formed by endoplasmic reticulum. Each crystal, or multiples of up to four crystals is/are formed in a crystal chamber which is clearly contained in a membrane bounded vacuole-like sac. Many of the crystals have grooves. Masses of these crystals are found in the pigment epithelium which was seen with SEM (Koch and Arnott, 1979). The crystals are intermixed with melanosomes, which are also contained in chambers and migrate during light adaptation. Note, once again, we find that the reflecting crystals are produced and contained in membrane sacs (Plate 11: 1-4).

THREE SYSTEMS OF BIOMINERALIZATION IN PLANTS

Another "stage on my trail" (NPI) involves looking at calcium carbonate in plants. The start of this research coincides with moving to UT Arlington, writing an article on "The Three Systems of Mineralization in Plants" (Arnott, 1982) and finally (perhaps most importantly) obtaining access to a scanning electron microscope. Previously, I mentioned the table top ISI scanning electron microscope I purchased with my UTA set up money (Autobiography Part II). I used it to do my first studies on cystoliths. Some micrographs taken with that microscope were published. At that time, I knew that crystals of $CaCO_3$ are formed in the cell walls of plants, especially in the walls of some red algae. In higher plants, deposits of $CaCO_3$, called cystoliths, are found in a few plant families. Many important examples occur in the fig family, e.g. the cystoliths of *Ficus elastica*, the cultivated rubber plant.

The carbonate deposits are formed in large adaxial epidermal cells called lithocysts. Within the lithocyst cell, a cystolith is formed (Fig. 15). It forms inside the cell wall but external to the cytoplasm thus is still intracellular mineralization (Plate 12). Recently, the site of cystolith formation has been called "a cell wall sac." However, unlike CaOx crystal formation, cystoliths are formed between the plasma membrane and the cell wall that is outside the cytoplasm (Plate 12). The body of the cystolith forms around a stalk that grows inward from a thickened cell wall. Generally, the stalk develops from the outer (adaxial) cell wall. In many cases, the stalk is also impregnated with silicon (Fig. 16). Incidentally, lithocysts and cystoliths have been used as a diagnostic characteristic for the drug plant *Cannabis sativa* or Marijuana.

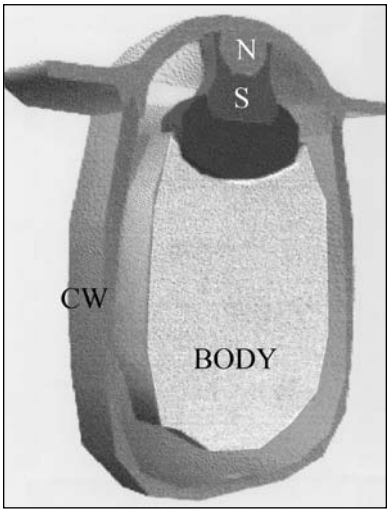


Figure 15. A three-dimensional computer model of a lithocyst containing a mature cystolith (body). Arnott and Webb, unpublished.

It is relatively easy to isolate the cystoliths from plants that have them. The technique is similar to that used to isolate CaOx crystals. Mostly leaves but also flowers, stems, and roots are collected and carefully washed to remove dirt particles. The leaves are blended for 10 to 20 seconds in 100% ETOH (use care) in a blender. Absolute alcohol is used because the amorphous calcium carbonate will quickly form crystals if water is present. The blended material is then filtered though several layers of cheese cloth. Finally, a spot plate is used to isolate the crystals in absolute alcohol. A pipette is used to generate centrifugal force which causes the heavy cystoliths to collect in the center of the spot plate depression. The cell debris can be removed from the cystoliths using suction with a pipette, and in two or three cycles very clean cystoliths can be isolated (Plates 13-15). These can be used for microscopy and/or chemical studies (Maier and Arnott, 2006).

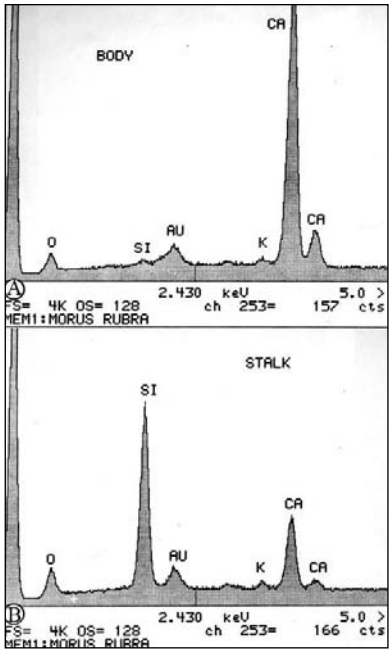


Figure 16. Energy dispersive x-ray analysis of the body and the stalk of *Morus* cystoliths. Note the Si peak in the stalk that is absent in the body. Adapted from Arnott and Webb.

In some plants, for example in the leaves of hackberry (*Celtis occidentalis*), cystoliths have an array of crystalline components but in most species the $CaCO_3$ deposits are non crystalline. I isolated cystoliths from several pants and showed their variation using SEM (Arnott 1982). In the light microscope you could see that there was some sort of structure inside the cystoliths so I tried slow demineralization using EDTA. When the carbonate mineral was removed, the hydrated cystoliths still maintained their general "football-like" structure (a prolate spheroid) as viewed with the light microscope. After drying the demineralized cystoliths changed and their internal structure was not maintained. However, they did still demonstrate the integrity of the matrix upon which the amorphous calcium carbonate was deposited. Later, in 1990, at Purdue, Mary Alice Webb and I worked out the developmental sequence of cystoliths in *Morus alba*. We also used histochemistry coupled with differential extraction to show which parts of the cystoliths were made of various carbohydrates (cellulose, hemicelluloses, etc.; see cover).

For some time, it has been suggested that cystoliths may be organs for the storage of CO_2. According to this hypothesis, the CO_2 from respiration would be stored during the night and released in the day time when a lower concentration of CO_2 is likely to be a limiting factor for photosynthesis. Reacting to this possibility, we began an experimental study to show the relationship between cystoliths and the CO_2 used in photosynthesis; unfortunately, after 15 years, the experimental materials are still in their fixation jars. Another potential breakthrough opportunity lost! Carbonate deposits in plants like *Morus* turned out to be tangential to the crystal trail but they are of significant interest. In a recent presentation on *Morus* (Arnott and Maier, 2006) the audience expressed considerable interest in the structure and function of cystoliths. In a recently published paper we began to explore the nature of the elusive nebencystolithen (secondary cystoliths) found in the mulberry (Arnott and Maier, 2006).

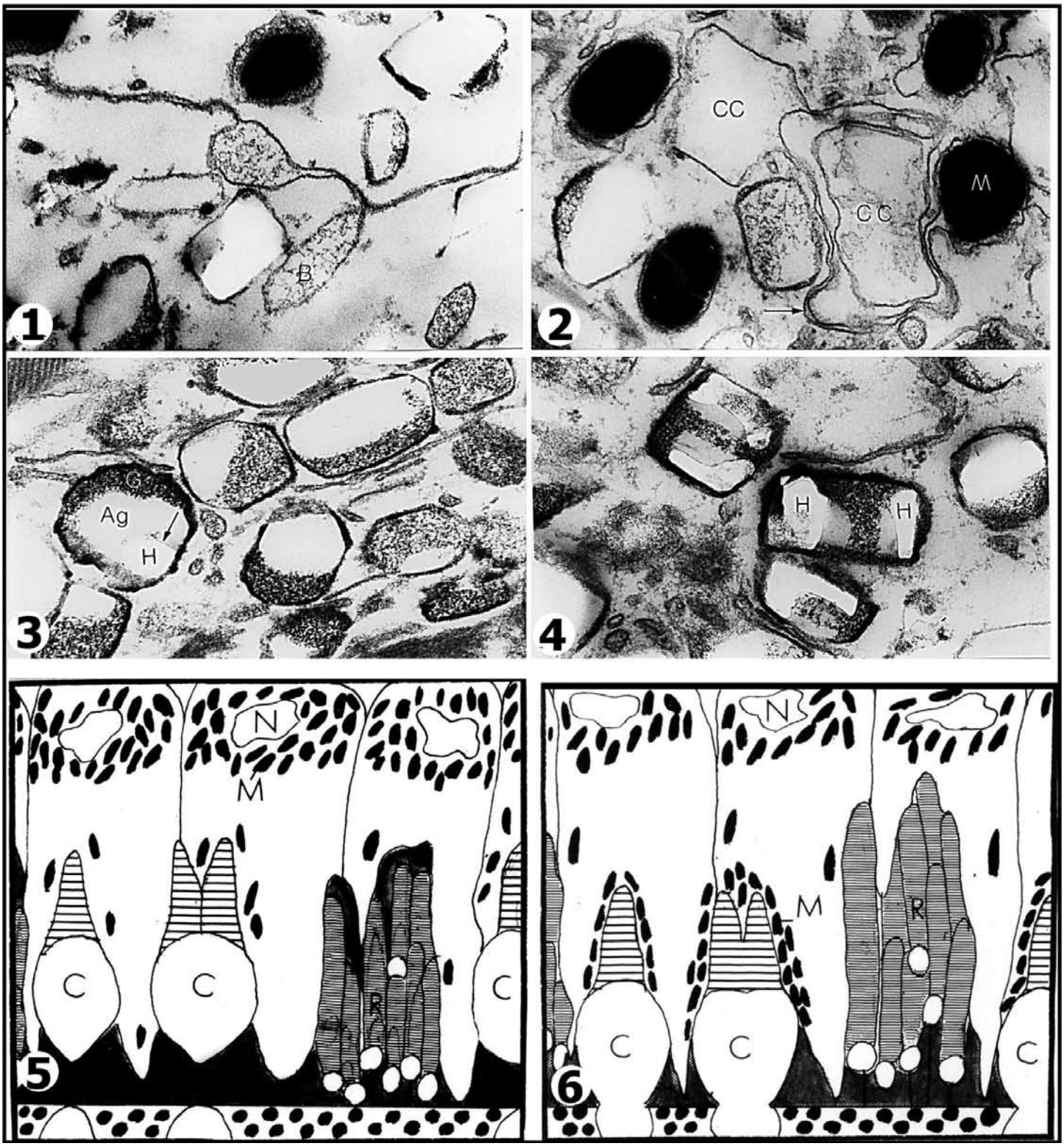


Plate 11. Tapetum lucidum cells of *Dorsoma cepedianum*. 1-4. Stages in the formation of reflecting crystals. 1. Expanding endoplasmic reticulum (B) prior to the formation of a crystal chamber. 2. Early stages in the shaping of the crystal chambers (CC) (M = melanosome, arrow = ER membrane). 3. Crystal chambers containing granular material before the formation of the reflecting crystals (G = granular area, Ag = agranular area). 4. Early stage in the formation of multiple crystals in a crystal chamber (H = crystal hole). 5. Diagram showing pigment epithelium in the light-adapted state: C = cone, N = nucleus, dark bodies are melanosomes, and the remainder of area within the tapetal cells is occupied with a large number of reflecting crystals. 6. Diagram showing the pigment epithelium in the dark-adapted state. A, B = 17300X; C, D = 27900X.Adapted from Koch, 1974.

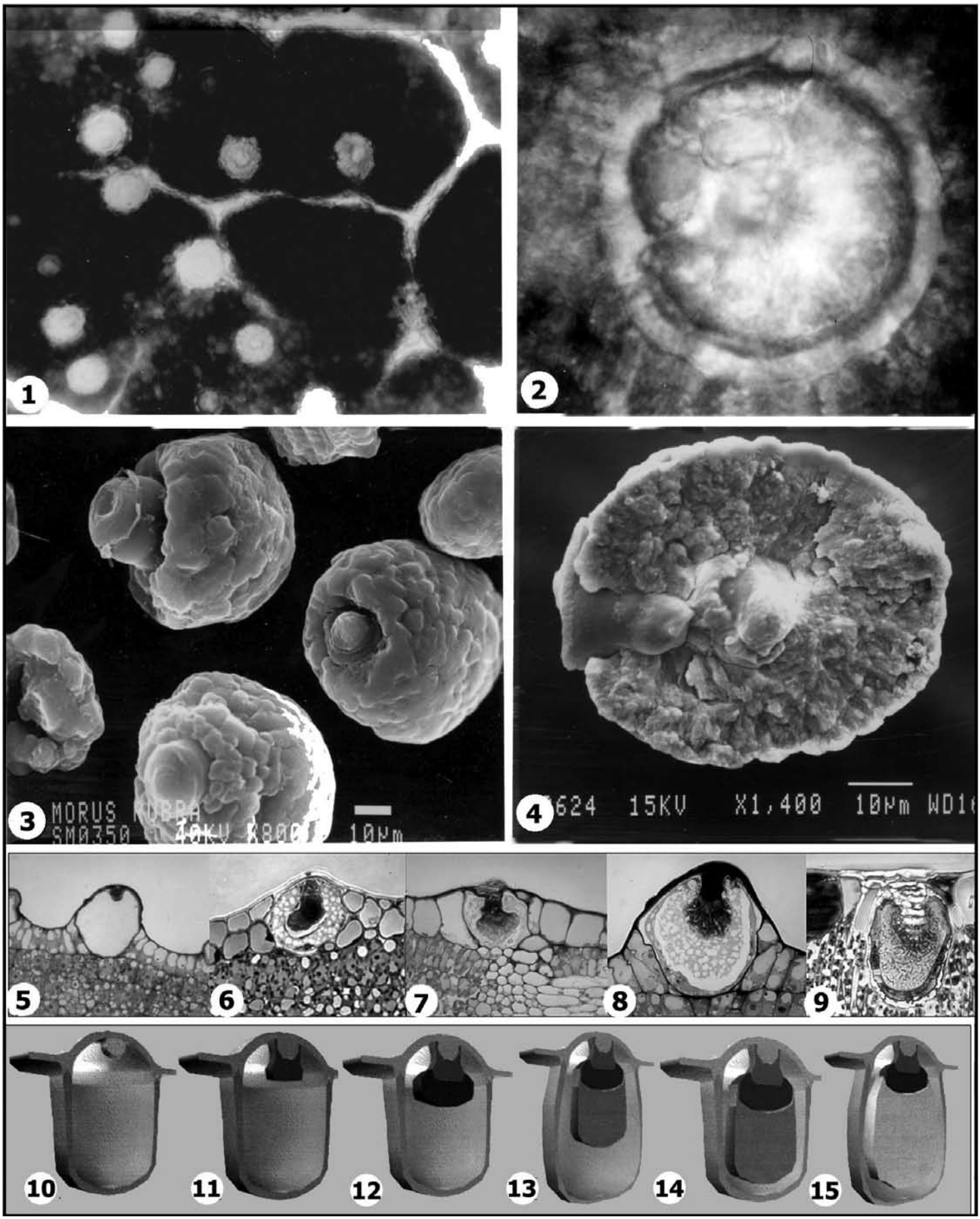


Plate 12. Cystoliths of *Morus rubra*. 1. Paradermal view of the leaf showing the distribution of cystoliths. 2. Paradermal view of cystolith in situ in living leaf. 3. SEM view of isolated cystoliths. 4. SEM view of cystolith fractured in the middle. Note how the stalk relates to the cystolith body. 5-9. Thin sections of leaf showing various stages in the development of cystoliths. 10-15. Computer models of the development of cystoliths with their parent lithocyst (cell). Adapted from Arnott and Webb, unpublished.

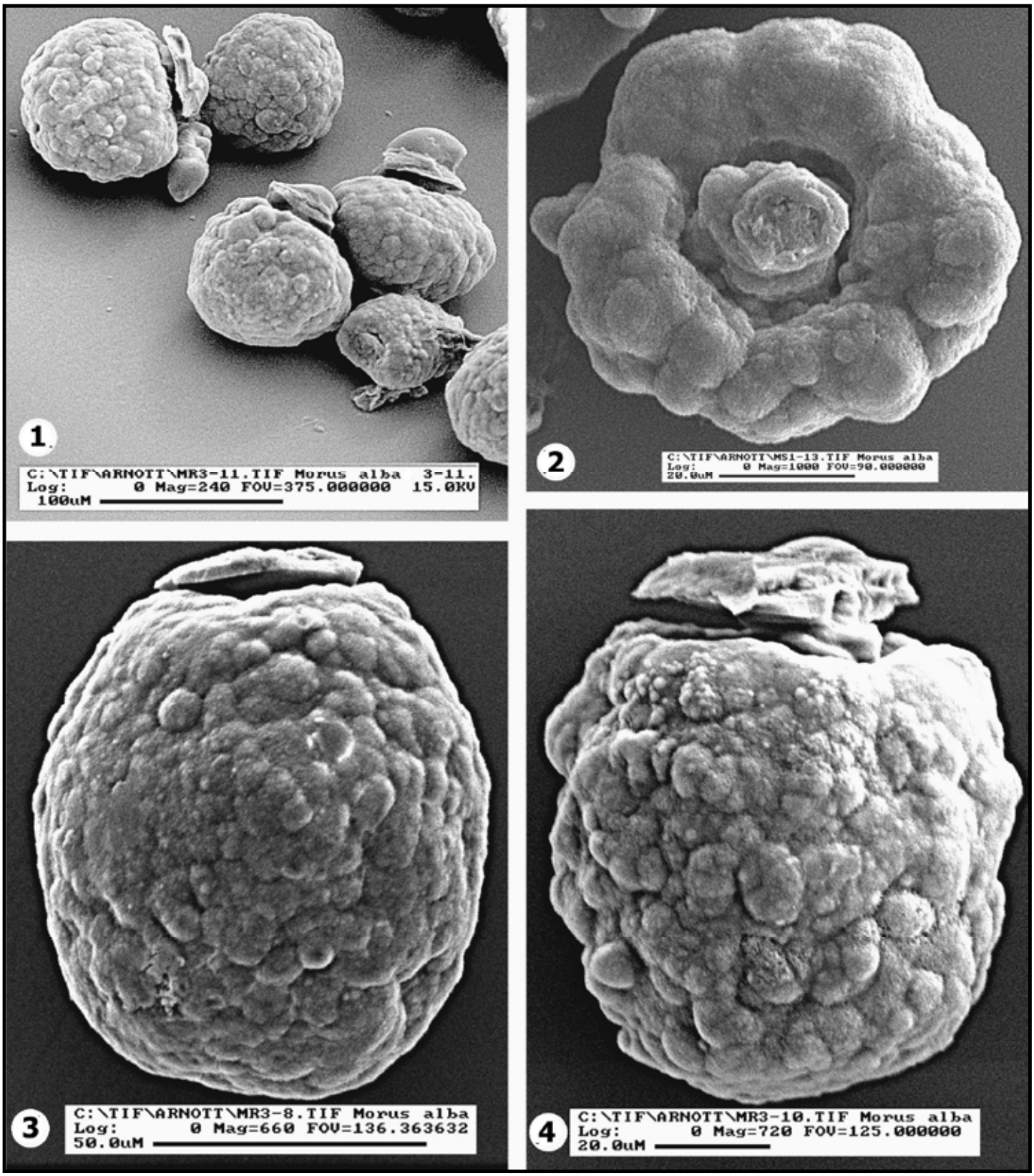


Plate 13. Cystoliths of *Morus alba* isolated from the leaves of a female tree from the Denton campus of Texas Women's University. 1. Isolated cystoliths showing stalks and bodies; a nebencystolithen is in upper left. 2. Cystolith showing the stalk and body from an "adaxial" viewpoint. 3-4. Lateral views of cystoliths showing stalks broken away from the upper wall of the lithocyst. Note the "bumpy" surface of the cystoliths. Adapted from Arnott and Maier, 2006.

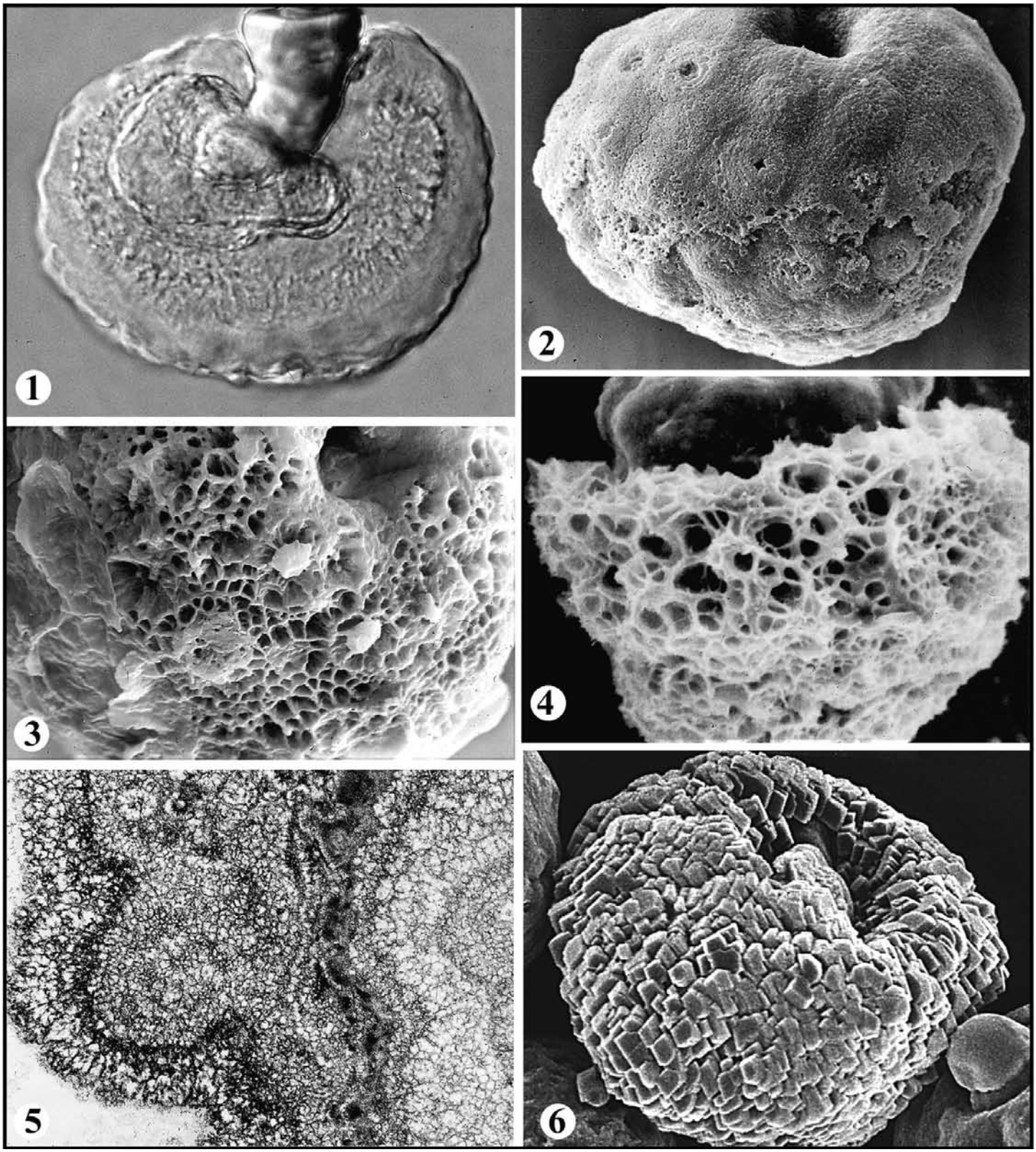


Plate 14. Cystoliths of *Morus alba*. 1. Light micrograph showing the internal details of a cystolith. 2. SEM of partly eroded surface of a cystolith. 3. Partly demineralized cystolith showing "honeycomb-like" array left after the crystallites of the cystolith wall have been removed. From the structural appearance is seems that the material surrounding the crystallites may be a polysaccharide since it is not destroyed by demineralization. 4. Demineralized cystolith showing non-carbonate material. 5. TEM showing several structural layers of a cystolith from which the mineral has been extracted. 6. Rare cystolith with its surface formed by hundreds of calcium carbonate crystals. 4-6 adapted from Arnott, 1983; 1-3 previously unpublished.

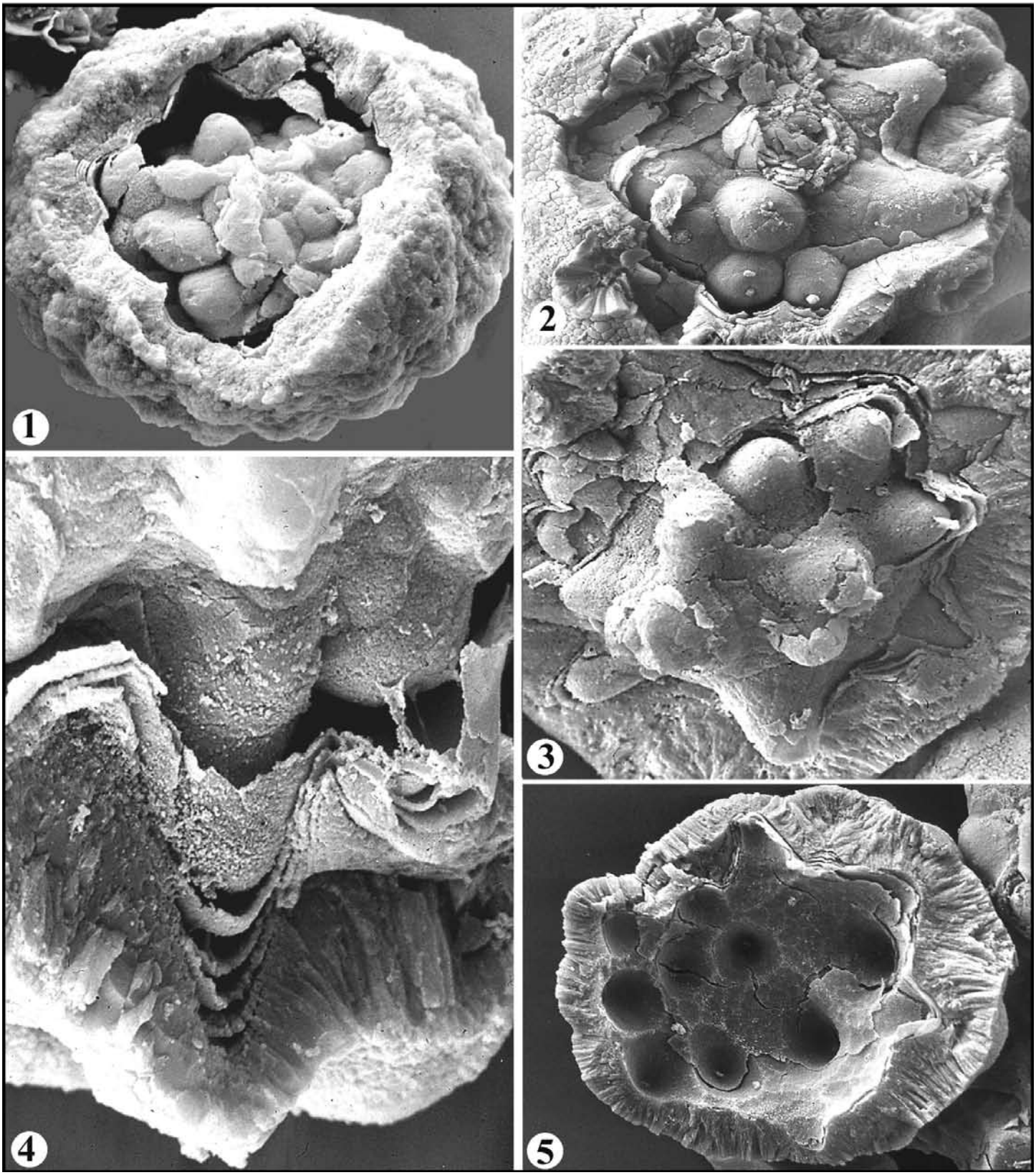


Plate 15. Fractured cystoliths of *Morus alba*. 1. Cystolith with part of its external wall layer shown. Internal "nipple-like" protrusions are clearly seen. 2. Fracture showing parts of the "nipple-like" protrusions and their relationship to the central cystolith body. 3. Fracture showing the independence of the central body of the cystolith. Note also the organized array of crystallites forming the cystolith wall. 4. Fracture showing a series of layers involved in the formation of the "nipple-like" protrusions and the crystallites of the wall are also seen. 5. Outer most portion of the wall separated from the central body showing how the protrusions extend into the outer wall of crystallites. Image 5 is essentially a negative of image 3. Previously unpublished.

Autobiography continued on page 44

Soft Imaging System

YOUR SPECIFICATIONS – OUR SOLUTION
VELETA – 2k x 2k SIDE-MOUNTED TEM CAMERA SOLUTION

Meeting the demands on today's TEM camera market – our new side-mounted TEM camera has the capabilities that customers are looking for in a 2k x 2k camera. The new Veleta fills this description perfectly. If you're familiar with our MegaView III and Morada cameras, the Veleta's capabilities position it right between these two.

This new side-mounted TEM camera is our answer to the most current expectations on the TEM market. The Veleta is a Peltier-cooled camera featuring up to 2048 x 2048 highly sensitive pixels and has a 14-bit dynamic range. Veleta offers more than 10 fps at full view and about 20 fps at binning mode 2.

Veleta is optimized for today's latest applications – such as tomography or immunogold labeling. Naturally the camera is applicable for all standard TEM imaging tasks.

TEM – Cameras

For detailed information please contact:

Olympus Soft Imaging Solutions
info.osis@olympus-sis.com
www.soft-imaging.net

OLYMPUS SOFT IMAGING SOLUTIONS

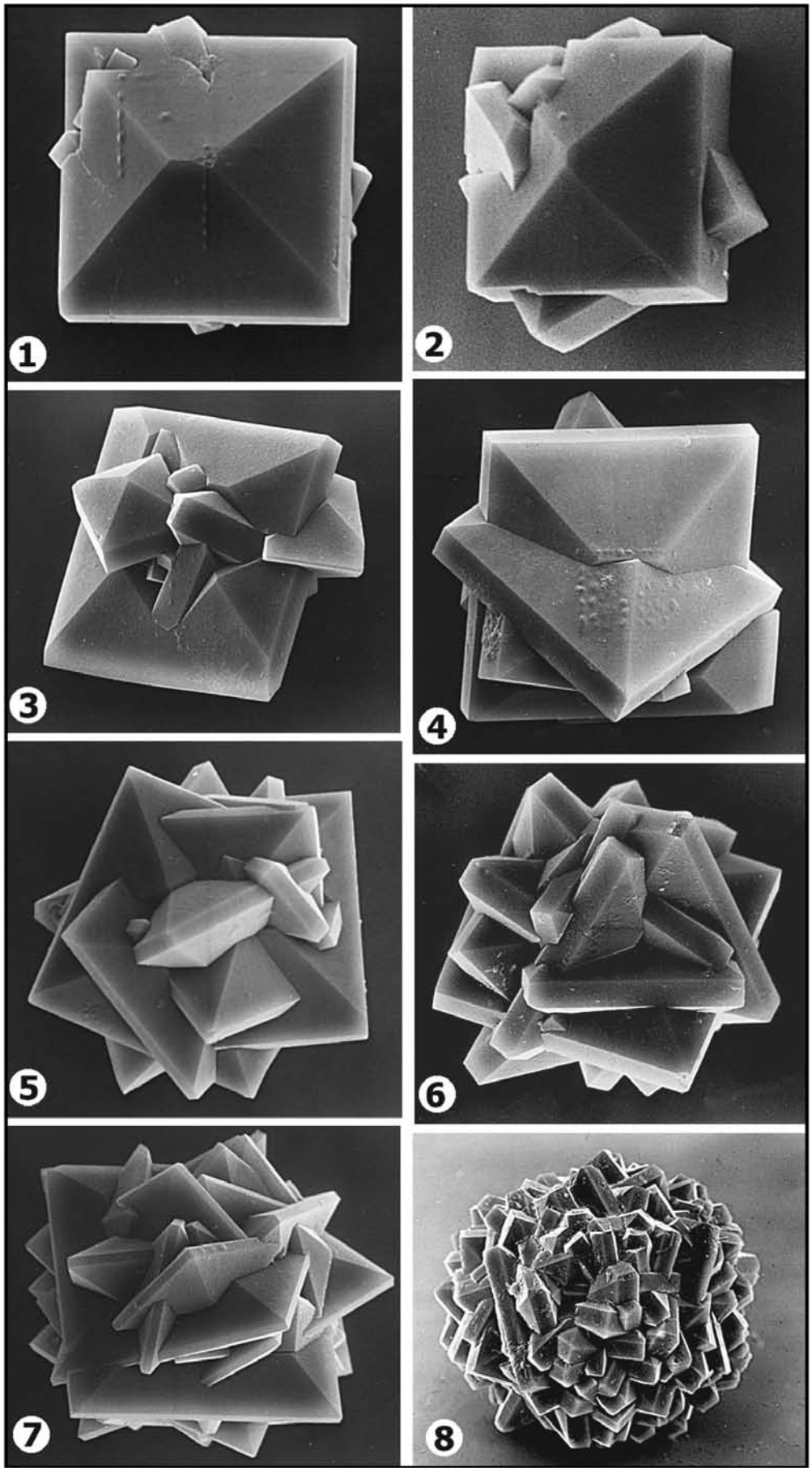


Plate 16. Isolated CaOx dehydrate druse crystals of *Begonia* sp. 1-8 Increasing complexity of the druse crystals, which are all interpenetrant twins. The nature of the twinning can be seen in these individual druses. Original micrographs by HJA.

Plate 17. Participants in various meetings associated with biomineralization and CaOx. 1. Gordon conference on Calcium oxalates, June 9, 1986, organized by Birdwell Finlayson (8th from the left, front row). Howard Arnott upper row left, Mary Alice Webb 2nd row extreme right. 2. 2005 Calcium oxalates meeting sponsored by FASEB and organized by Mary Alice Webb (1st row 6th from the left. Howard Arnott upper row left). 3. Fifth international Biomineralization Conference, May 1986. Meeting held at the University of Texas at Arlington, Organized by Rex Crick (not identified) and Howard Arnott(last row middle).

Autobiography continued on page 47

Beating the Competition is Easy, When You've Got Products They Don't Offer!

Si-Li EDS Detectors,
IXRF offers guaranteed, premium ≤ 130eV, resolution SiLi detectors. *(*industry standard detector resolution 133-138eV)* which carry a three year warranty.

New 30mm's at 133eV, three year warranty available for cost effective fast X-ray Mapping.

X-Beam, Micro XRF,
40-60 micron spot. 10,000 times more analytically sensitive than EDS. Out performs WDX for less than half the cost.

fX SEM XRF,
400-1000 micron spot. 10,000 times more analytically sensitive than EDS.
Perfect for environmental applications as well as non-conductive samples.

50mm Silicon Drift Detector, the largest Active Area in the industry (50mm). 133eV resolution, with optimum peak stability, and high input count rates. Who says "Bigger isn't better"?

IXRF does not stratify their software suite into low, medium, and high-end levels; there's only one high-end package that includes a myriad of Spectra Analysis, Image Analysis, X-ray Mapping, and unsurpassed SEM/EDS Automation.

Free Software Upgrades for Life...no compromises.

IXRF SYSTEMS

IXRF Systems, Inc. 15715 Brookford Dr, Houston, Texas, 77059 (Tel): 281/286-6485 (Fax): 281/286-2660
www.ixrfsystems.com

TWINNING

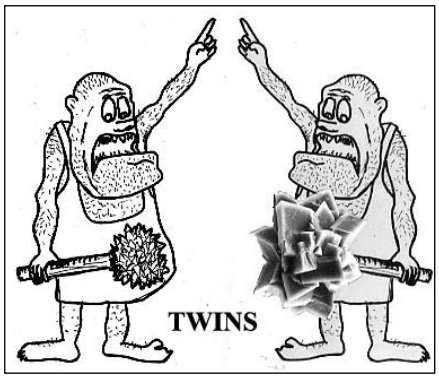


Figure 17. Twin crystal monsters! That's one to many!

After examining CaOx crystals extracted from a variety of plants, it became clear that twinning was a very common characteristic of both calcium oxalate monohydrate and dihydrate in plants (Fig. 17). These observations resulted in a paper entitled, "An SEM study of twinning in calcium oxalate crystals of plants" (Arnott, 1981) which deals with the process of twinning in plant crystals. Recognition of twinning in plant crystals helps to explain the shapes and forms that are found. Raphides and styloids can be either single or twin crystals. For example, the raphides of *Yucca* are single crystals, while those of *Vitis* are twined. Druses are always twins, and they are best described as multiple interpenetrant twins. Some excellent examples of twinning are seen in crystals isolated from *Begonia* (Plate 16). In *Begonia,* different "degrees" of twinning are present in the dihydrate crystals of calcium oxalate. Interestingly, sometimes monohydrate crystals appear to "twin" on the surface of the dihydrate crystals. Without careful examination of this occurrence, it is probably not twinning but rather just nucleation on the dihydrate crystal surface. The monohydrate crystals of CaOx are highly birefringent, whereas the dihydrate crystals are much less birefringent under polarized light. Later I will discuss the complications that arose around the determination of the exact nature of the twin plane in the raphide crystals of *Vitis*. Regarding twinning, an interesting situation was found in okra (*Hibiscus esculentus*). Two types of druses are produced in the leaves of okra, the first are normal in size and morphology. The second type is termed planar druses, which are very large and arise in the palisade layer where their long axis is often oriented vertically (Arnott and Workman, 1981). They were termed planar because all the twin crystals seem to be produced (more or less) in a single plane. The significance of this kind of CaOx crystal system is not understood.

THE ORGANIZATION OF THE GORDON CONFERENCES ON CALCIUM OXALATES BY BIRDWELL FINLAYSON

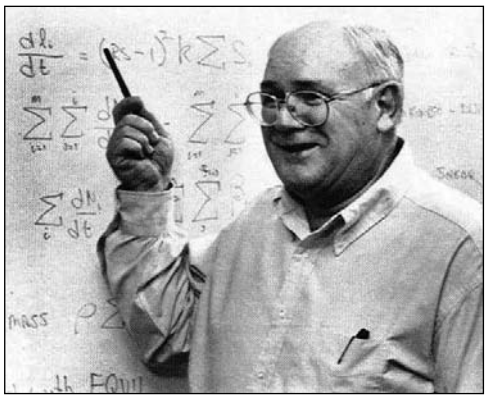


Figure 18. Dr. Birdwell Finlayson. Photo courtesy Saeed Kahn.

My relationship with Birdwell Finlayson, M.D., Ph.D., and subsequently the Gordon Research Conferences on Calcium Oxalates began in 1980 (Fig. 18). At that time I was invited by Finlayson to give a talk on "biological crystals" at the University of Florida, Department of Urology. Seems like a **strange** place for a botanist to lecture? Perhaps not since calcium oxalate is of interest to both groups. This lecture came about through

Figure 19. Students of calcium oxalate in plants at 1989 Gordon Research Conference at Plymouth State College, New Hampshire. Left to right, Xingxiang Li, Kenneth Whitney, Mary Alice Webb, Albert Kausch, Vincent Franceschi, Genie Brackenridge, and Harry (Jack) Horner. Photo by HJA.

Figure 20. Delegates at the 5th International Biomineralzation Conference in Arlington, Texas in 1986. Photographer unrecorded. HJA in the center.

Saeed Kahn who I met as a student in the laboratory of Henry Aldridge at U. of Florida. After graduation Kahn began working with the kidney stone group in the University of Florida Medical School. Finlayson specialized in urology in his medical practice, but he was also a prominent kidney stone researcher associated with the Medical School. The obvious connection between my studies on calcium oxalate in plants was the fact that a large proportion of kidney stones are in part or wholly calcium oxalate.

After my seminar, Finlayson told me that he was organizing a Gordon Conference on calcium oxalate to be held in the near future. He asked me to help him by organizing plant calcium oxalate workers to be part of this conference. I agreed and thus, when the conference met at Kimball Union Academy, there were a number of speakers on calcium oxalate in plants (Plate 17). It would not surprise me to learn that Finlayson argued vigorously with some of his colleagues to make this bilateral conference happen. Not everyone had the insight to reason that this was a natural combination of interests. In the end, Finlayson's groundwork led to not only "his" 1986 meeting, but six additional meetings with still another scheduled in 2008. Finlayson convinced others to name me as the chairman of the second conference. Sadly, Birdwell Finlayson died in 1988 at the age of 55; at that time he was still a young and vigorous person.

Table 1. Gordon and FASEB Calcium Oxalate Conferences, 1986 to 2005.

First Gordon Conference on Calcium Oxalates; Birdwell Finlayson, Chair; Kimball Union Academy, Meriden, New Hampshire, 1986
Second Gordon Conference on Calcium Oxalates; Howard Arnott, Chair; Plymouth State College, Plymouth, New Hampshire, 1989
Third Gordon Conference on Calcium Oxalates; Lynwood Smith, Chair; Plymouth State College, Plymouth, New Hampshire, 1991
Fourth Gordon Conference on Calcium Oxalates; Harry Horner, Chair; Plymouth State College, Plymouth, New Hampshire, 1993
First FASEB Conference on Calcium Oxalates; Cheryl Scheid, Ross Holmes and Marguerite Hatch, Co chairs; Copper Mountain, Colorado, 1999
Second FASEB Conference on Calcium Oxalates, Marguerite Hatch, Chair; The Vermont Academy, Saxons River, Vermont, 2002
Third FASEB Conference on Calcium Oxalates, Mary Alice Webb, Chair; Tucson, Arizona, 2005

My role in organizing the first Gordon conference was relatively easy. However, the organization of the second Gordon Conference was very different. For one thing, I was counting on Finlayson's help. I soon found out that the Chairman is responsible for a number of things. First, I had to convince the organizers of the Gordon Conferences that there was a need for another conference on calcium oxalates; it was not automatic, nor was it taken for granted by the Gordon Conference Organizers that there would be any more calcium oxalate meetings. I made a special trip to Texas A&M University to visit the Chairman of the Gordon Conferences specifically to convince him that our intentions were serious. Once I convinced the powers that another conference was in order, we had to raise the money to support travel and attendance. The obvious place to seek support was the Urology Section of NIH, an agency that I had never been associated with. In any event, money was raised and the conference took place at Plymouth State College in 1989. Since that time, many of the same researchers have continued to attend the conferences under the auspices of Gordon Conferences and FASEB (Table 1; Plate 17). The unique interaction between botanists and urologists has been helpful to both groups. Throughout this series of conferences, Saeed Kahn has been a driving force, following in the footsteps of his mentor, Birdwell Finlayson.

Figure 21. Mary Alice Webb, Purdue University, at the 1989 Gordon Conference on Calcium Oxalates. Photo by HJA.

In May, 1986, I was a host to the Fifth International Conference on Biomineralization which was held in the Geological Sciences Building on the UTA campus. The conference was a part of a continuing series of meetings devoted to biomineralization. Jean and I attended the Third International Conference in Japan. Along with Rex Crick, I helped organize the meeting, obtaining funding, and sending invitations to speakers. The meeting in Arlington was very successful. I remember a sunny afternoon under the trees of Doug Russell Park where the conference members enjoyed their fill of Texas barbeque. Members of the conference came from all over the world and there was a substantial contingent from Japan. I remember visiting the Shogun Japanese Restaurant with several of the Japanese delegation; they just had to have their tempura, etc. (Fig. 20). Dr. Rex Crick, UTA Geology, did a great job of editing the volume that originated from the conference (Crick, 1989). I gave a paper at the Conference; however, I did not write a paper for the volume, largely because of lack of time.

The next year, I organized a Symposium on Calcium Oxalate for the International Botanical Congress in Berlin, Germany. It was a very good opportunity to discuss this important component of plant systems in a major venue. My recollection, which is feeble on this point, is that very little was accomplished in the symposium. In the end, the participants agreed to disagree with regard to the function and many other details of calcium oxalate in plants. All in all, it was not the finest hour for calcium oxalate in plants, or for me.

MARY ALICE WEBB AND PLANT CRYSTALS REVISITED, BUT WITH PANACHE

Figure 22. Left to right, Mary Lou Kelly, Randy Allen, Mary Alice Webb, and Mark Grimson at Disneyland for Scanning Electron Meetings in 1982. Photo by HJA.

During the early 80's I had a very fruitful association with Dr. Mary Alice Webb (Figs. 19, 21, 22). Mary Alice was born in Falfurrias, Texas (81 miles SW of Corpus Christi and 73 miles N of Mexico). She is the daughter of a Presbyterian Minister and grew up in the quiet environment of rural South Texas. She has three brothers; one is a lawyer, one is a student of Japanese Philosophy, and the third is an MD. From what I understand, all the Webb children were musically inclined; Mary Alice played the flute and piano and her three brothers the piano. Rumor has it that, when young, they all dreamed of being conductors when they grew up. Dr. Rob Webb is an emergency room physician in Odessa, Texas. In 2005, influenced by his sister, Rob commuted from his home in Midland to Arlington (340 miles) to take my course in scanning electron microscopy. His SEM class project was on frost rings in a ninth century burial wood from Mongolia. On several occasions I have listened to David Webb play jazz piano. He is an intellectual property lawyer in Washington DC.

At UTA, Mary Alice learned transmission electron microscopy from Dr. James Butler (see part II) and scanning electron microscopy from me. In addition we both received instruction in TEM and SEM from Linda Lopez who, at that time, was supervising my laboratory in the Center for Electron Microscopy at UTA (See Part II). Mary Alice became proficient in both TEM and SEM and soon was able to make very high quality micrographs. However, many of her micrographs were not just high quality but both idiosyncratic and artistically imaginative. The "artistic eye" is still a characteristic of her current work.

Mary Alice completed a B.A. degree in botany at UT Austin before moving to Arlington in 1978. As a master's degree student,

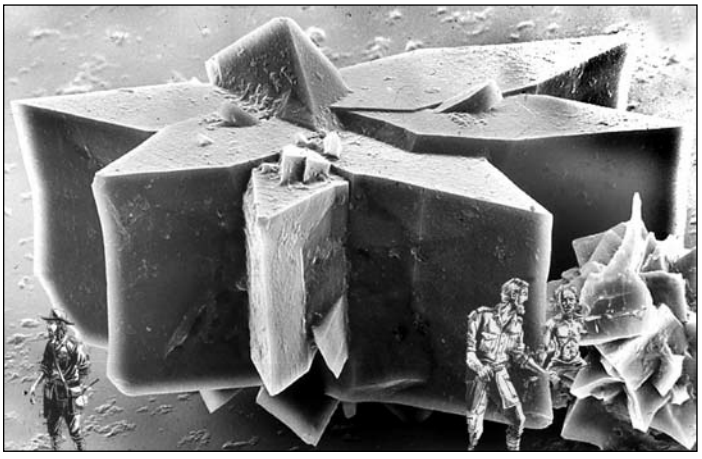


Figure 23. Isolated planar druse of okra. Adapted from Arnott and Workman, 1981.

Autobiography continued on page 51

Next Generation Silicon Nitride Support Films for TEM

Advantages:

- 3.0 mm circular frame for standard TEM holders
- EasyGrip™ edge for ease of handling
- Resilient and chemically inert planar substrate
- Usable across a range of microscopy platforms (SEM, AFM, TEM through to optical)
- Free from debris or broken edges

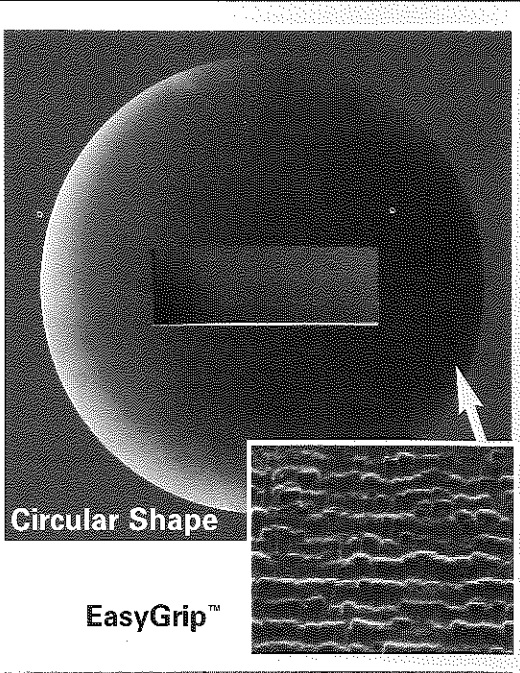
Applications:

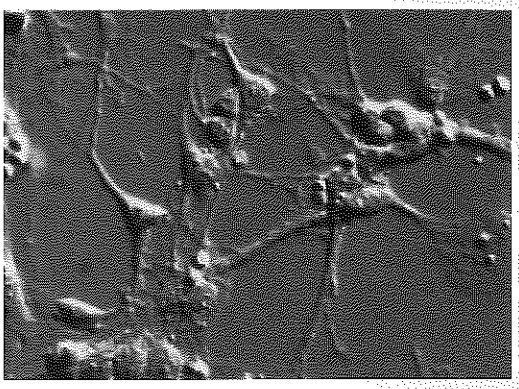
- High temperature experiments
- Observation of deposited thin films
- Direct cell growth including electron tomography
- Imaging and analysis of nanoparticles, nanofibers and nanotubes

Specifications:

- Window sizes: 0.5 x 0.5; 1.0 x 1.0 and 1.5 x 0.5mm
- Special size for electron tomography, 0.5mm x 1.5mm, allowing extended area imaging at high tilt
- 200 micrometer silicon substrate with 50nm ultra-low stress silicon nitride

21500-10 Silicon Nitride Membrane, 0.5 x 0.5mm, 50nm thickness, pkg/10
21500-100 Silicon Nitride Membrane, 0.5 x 0.5mm, 50nm thickness, pkg/100
21502-10 Silicon Nitride Membrane, 1.0 x 1.0mm, 50nm thickness, pkg/10
21502-100 Silicon Nitride Membrane, 1.0 x 1.0mm, 50nm thickness, pkg/100
21504-10 Silicon Nitride Membrane, 1.5 x 0.5mm, 50nm thickness, pkg/10
21504-100 Silicon Nitride Membrane, 1.5 x 0.5mm, 50nm thickness, pkg/100





DIC image of hippocampus neurons grown on a silicon nitride substrate by Prof. M. Stowell, et. al., MCDB, CU-Boulder, Colorado

PELCO® Silicon Aperture Frames (without support film)

The PELCO® Silicon Aperture Frames are 3mm disk type frames with a thickness of 200µm and square or rectangular apertures have found a variety of applications:

- Support frame to attach TEM lamellas made with FIB
- Support frame for thin films, foils, wires and fibers
- Mask for thin film research (deposition mask)

www.tedpella.com/grids_html/silicon-nitride.htm

21540-10 PELCO® Silicon Aperture Frame (no support film) 0.5 x 0.5mm, pkg/10
21541-10 PELCO® Silicon Aperture Frame (no support film) 0.5 x 0.5mm, pkg/10
21542-10 PELCO® Silicon Aperture Frame (no support film) 0.5 x 0.5mm, pkg/10

Silicon Nitride Coated 3mm Disks (blanks)

These 3mm silicon disks have a 50nm ultra low stress silicon nitride layer (Si_3N_4) on both sides and can be used for a number of applications:

- Specimen mounts for SEM and FESEM applications
- Specimen disks for AFM applications which need a Si_3N_4 background
- Blanks to built the PELCO® Liquid Cell™ together with the PELCO® Silicon Nitride Membrane

21555-10 Silicon Nitride 3mm Disks, pkg/10

TED PELLA, INC.
Microscopy Products for Science and Industry

4595 Mountain Lakes Blvd., Redding, CA 96003
Phone: 530-243-2200 or 800-237-3526 (USA)
FAX: 530-243-3761
Email: sales@tedpella.com
Web Site: www.tedpella.com

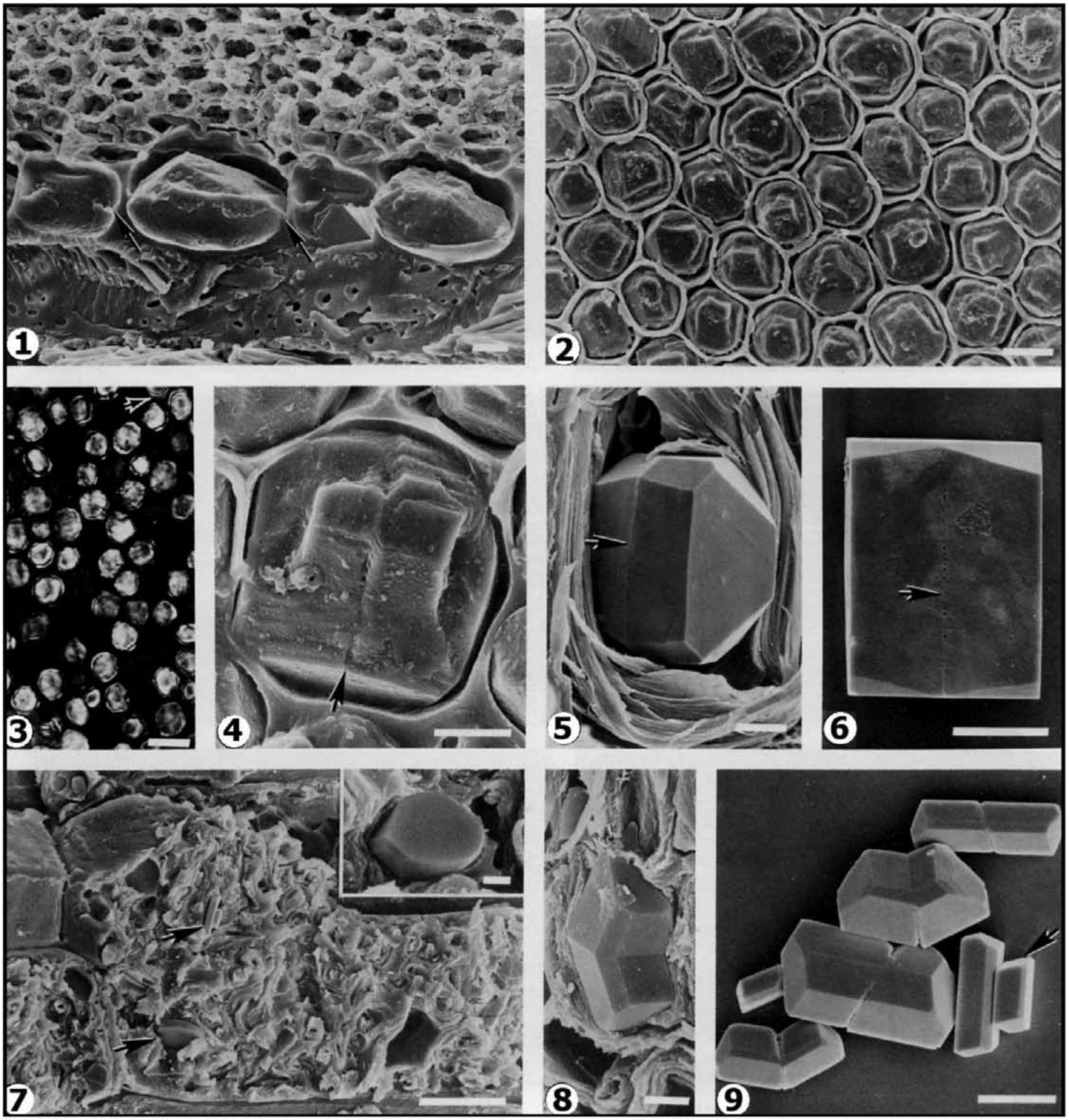


Plate 18. Micrographs of CaOx crystals in various seeds. 1-4. *Carica papaya* seeds. 1. Fractured view of seed coat showing crystals. 2. Face view of crystals in the seed coat. Note that ach cell has an individual crystal; at this time in growth you can no longer see the vacuole within which the crystals develop. 3. Polarized light view of seed coat; showing bright CaOx crystals. 4. Twin crystals of seed coat. 5-6. Prismatic CaOx crystals in the seed of *Juniperus virginiana*. 6. Isolated crystal from the seed of *Juniperus virginiana*. 7. Crystals in the endosperm cells of *Magnolia grandiflora* seed. 8. Kinked twin crystal of CaOx in the seed coat of *Phaseolus vulgaris* (common bean). 9. Isolated kinked and straight twin crystals of CaOx from the seed coat of common bean, *Phaseolus vulgaris*. Adapted from the work of Webb and Arnott, 1980.

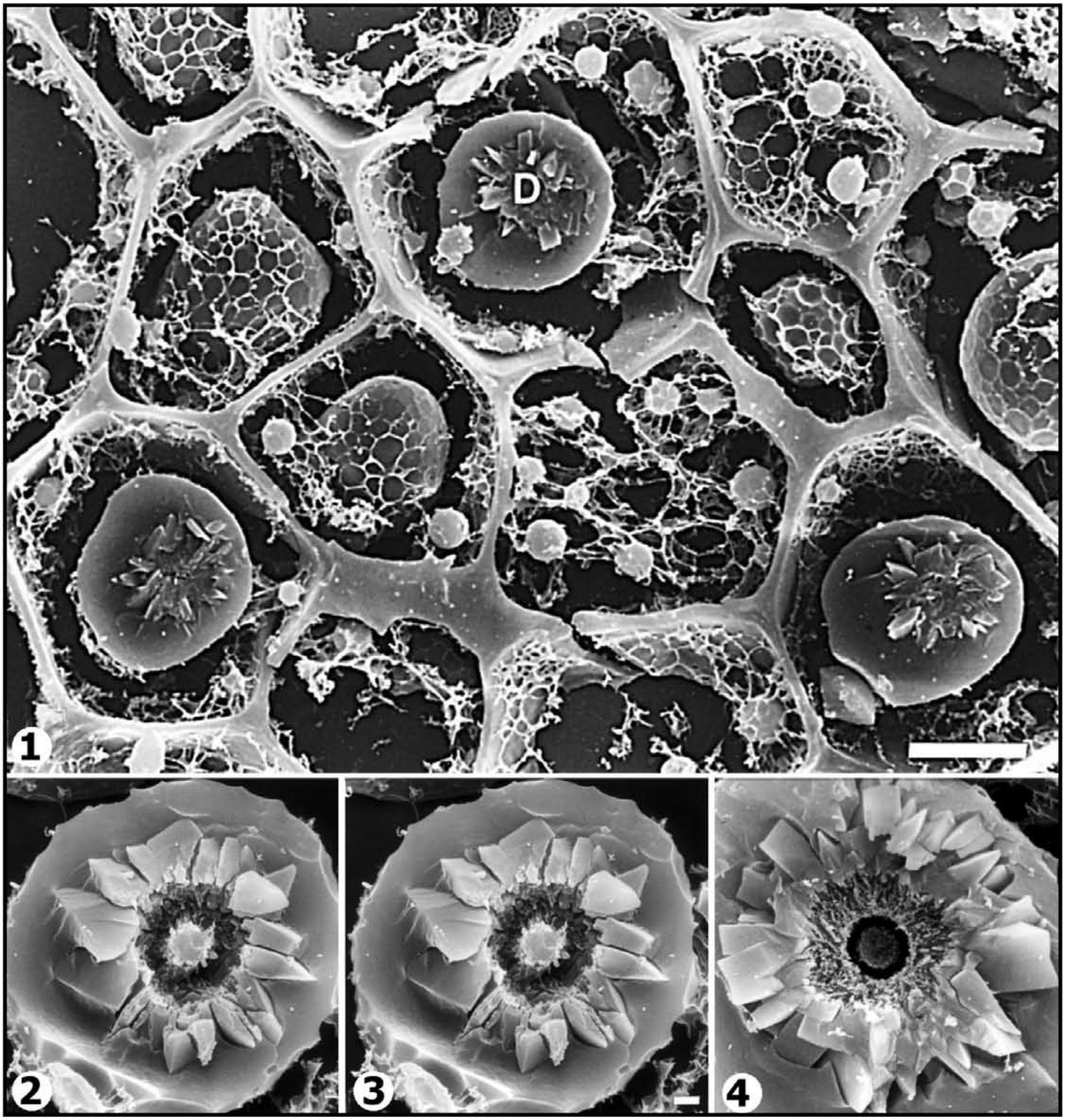


Plate 19. Representative SEM showing endosperm cells of *Vitis*. 1. Freeze fracture showing several endosperm cells with the remainder of the cytoplasm. Some cells contain large protein bodies or globoids. Within three of the globoids a large calcium oxalate druse (D) can be seen. Holes in the cytoplasmic matrix represent the position of lipid bodies, common in endosperms cells. 2-3. Stereo pair showing a fractured druse inside a globoid. Note the individual CaOx crystals and the central "nucleating" area around which the druse develops. 4. Fractured globoid druse treated with the protein-dissolving enzyme, pronase. Note that many areas within the nucleating area are now missing. Adapted from Webb and Arnott, 1982.

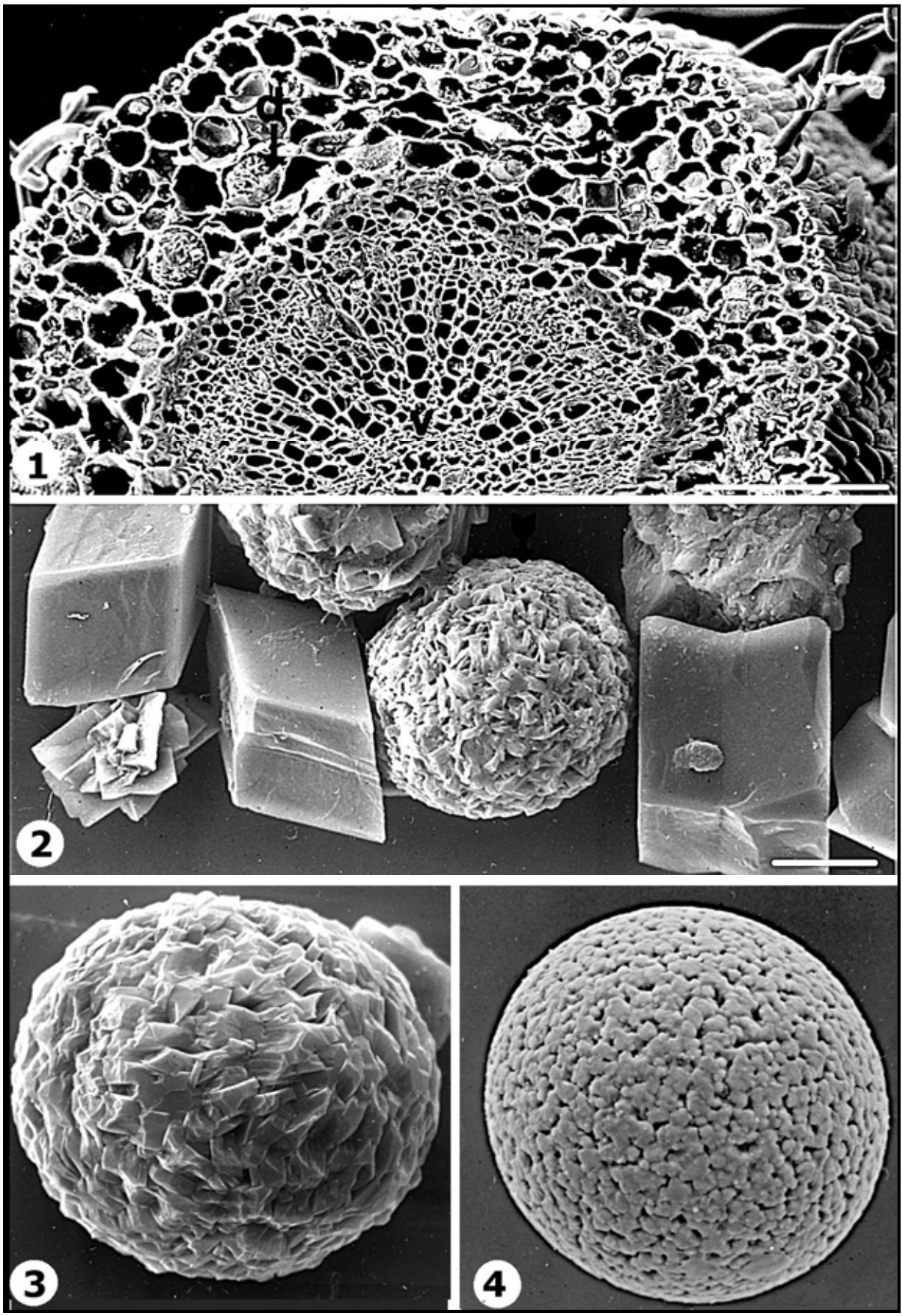


Plate 20. SEM of calcium oxalate crystals in *Rosa multiflora*. 1. Cross section of the leaf midrib showing many crystal deposits in place. 2. Isolated crystals from the leaves of Rosa multiflora. Note a single crystal (upper left) and a twin (lower right) associated with four multi interpenetrant twins, or druses. 3. Druse or multi interpenetrant twin where in individual twin crystals are still visible. 4. Almost smooth druse from *Rosa*. The origin of this type of druse is unknown. From Kelly and Arnott (1981) *Texas Soc. Electron Microscopy J.* 12:23.

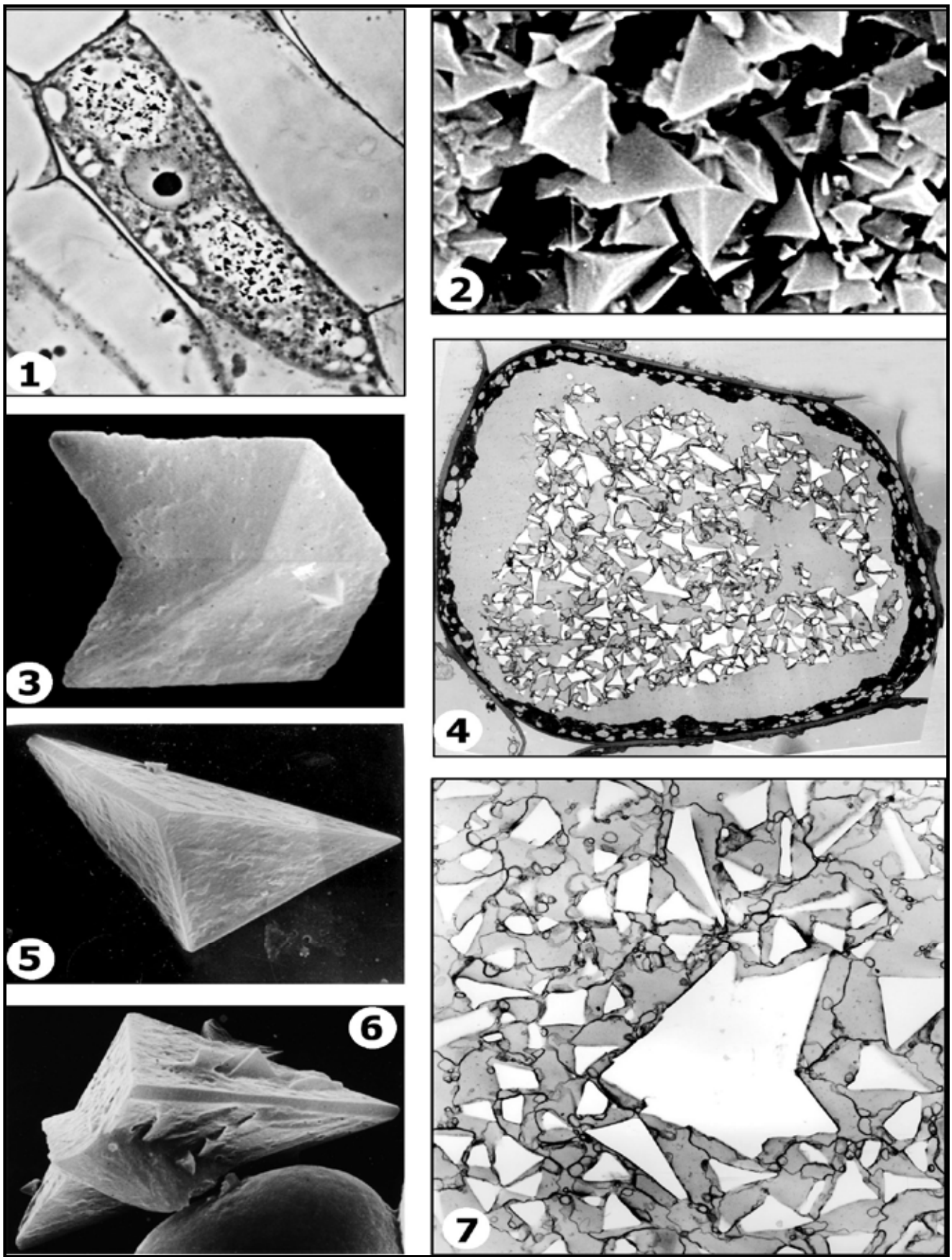


Plate 21. Crystal sand from the stem of the potato, *Solanum tuberosum*. 1. Light microscopic view of crystal sand cell with central nucleus and two large vacuoles with calcium oxalate crystals, seen as dark spots. 2. SEM of crystal sand. 3-6. Characteristics of isolated crystals from crystal sand. 4. TEM of a crystal sand idioblast. Note the dark cytoplasm and wall surrounding the crystals in a large central vacuole. 7. Micrograph showing crystal chambers associated with many interconnecting membranes. Adapted from the thesis of Mark Grimson (1984).

she worked on the seeds of zucchini (*Curcubita pepo*) finishing her thesis in 1980. Her study of the dormant (completely dry) seeds of zucchini is unique. Most ultrastructural research on seeds was (is) done with seeds that had been hydrated. A set of surprising results came from this research in which the fixation was by osmium vapor. For example, the cell walls of the dormant cells are folded in regular arrays that help maintain wall structure during the dormant period and allow for the imbibition of water and expansion during germination. A uniquely interesting finding from this research was a material that filled the intracellular spaces in dry seeds. This material "falls out" of its position in specimens when they are fractured. The material is flexible and apparently gel-like in consistency. Through a series of excellent micrographs, Mary Alice also showed exactly how the protein bodies of the dry seed become protein vacuoles and then develop into vacuoles during the germination process. Like me, Mary Alice's first research was on seeds. I have examined and re-examined her thesis recently, and without doubt, it is equivalent of most Ph.D. dissertations at UTA or in the other four institutions where I taught. Unfortunately, at the time, because of pointless state government interference, we were not authorized to offer a Ph.D. degree (See Part II). Working out the methods with which to examine seeds in their dormant condition was a *bona fide* forward step in the study of seeds that can be attributed to Dr. Webb.

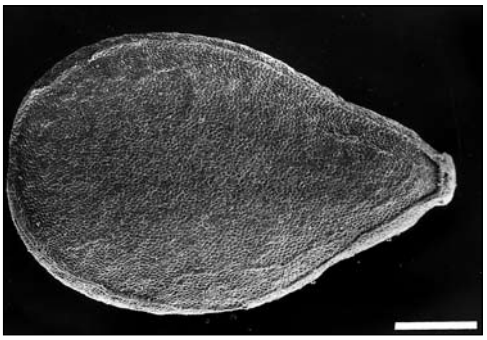


Figure 24. Sesame (*Sesamum indicum*) seed. The surface of the seed is covered with cells which produce crystals of CaOx. Adapted from Webb and Arnott, 1982.

Following her graduation, Mary Alice moved to North Carolina where she worked for Dr. J. David Robertson at Duke Medical Center. Dr. Robertson, well known for his studies of membranes, is perhaps generally remembered for developing the concept of the "unit membrane." Through that and other research he was responsible for the stimulating interest in membrane structure. After spending a year in North Carolina Mary Alice returned to Arlington.

During the next few years Mary Alice worked as my research associate as we began to study calcium oxalate in plants once again. By this time many technical changes in the fixation of specimens for the TEM had occurred and the SEM was providing views of plant cells unattainable a few years earlier. Most of our joint research was on crystals of CaOx and the cells that produce them. Our publication record indicates that we were most active in 1980-1983 and again in 1990-1994 producing 38 joint citations. Between those periods of activity, Mary Alice finished her Ph.D. degree in Botany at the University of Wisconsin and became an Assistant and then Associate Professor at Purdue University.

As my research associate, Mary Alice worked mostly on projects related to calcium oxalate. At the time, I was an administrator and had limited time for research (See Part II), so she did a lot of the literature research. However, when it came to microscope work, our working arrangement was, more or less, that of a friendly contest; i.e. we each tried "to out do the other" with better and better micrographs. Actually this was "good practice" since the competition produced many excellent micrographs. By the 90's it was sometimes difficult to know who took which picture and we still have "friendly arguments" about that topic. On her return to Texas, Mary Alice and I began to study CaOx crystals in various

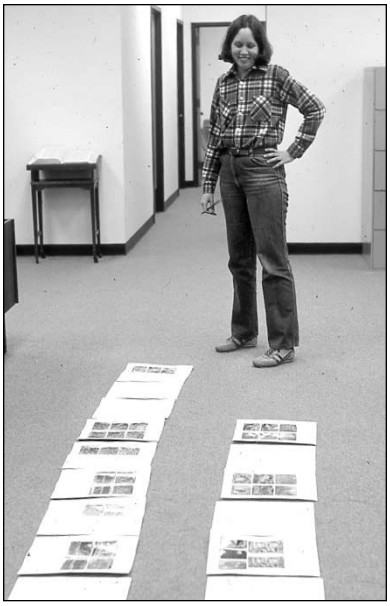


Figure 25. Mary Alice Webb standing in the U. T. Arlington Dean of Science office reviewing a paper destined for the journal "Scanning Electron Microscopy." Typing of the manuscript was and had to be letter perfect. Photo by H. J. Arnott, about 1980.

subjects but especially in seeds. Seeds were one of my special interests from the "get-go" (See Part III). The seed research soon produced a substantial paper dealing with crystals in a variety of seeds (Plates 18, 19). Indirectly, this line of research resulted in the long term investigation of raphides in the leaves of *Vitis*. From that work, the raphides of grape leaf became a model for calcium oxalate production in plants. See the "adventures in the vineyards" later.

When Mary Alice returned to UTA, my lab had several students working on biocrystal systems. In addition to running the EM lab, Linda Lopez was working on the air space system and calcium oxalate in *Eichhornia crassipes*. Mary Lou Kelly, an interdisciplinary M.S. student, was studying the CaOx druse development in the leaves of *Rosa multiflora* (Plate 20). Mark Grimson (Fig. 22), a Master's student, was working on the twin CaOx crystals of *Phaesolus vulgaris* (bean) and on the development of CaOx crystal-sand idioblasts in the tuber of *Solanum tuberosum* (potato) (Plate 21). Chris Workman, an undergraduate, was helping me investigate the planar druses of CaOx in the leaves of okra (Fig. 23).

In 1982, I had three papers in succession in *Scanning Electron Microscopy*. The first, "A survey of CaOx crystals and other mineral inclusions in seeds" (Webb and Arnott, 1982), provided a review and added new information about crystal in seeds. That paper produced a set of principles for understanding problems of minerals (crystals) in seeds. It provided references to crystals in 81 families of seed plants and new information on 18 species. It included many transmission and scanning electron micrographs. It included the first micrographs of *Vitis* seed coat raphide idioblasts and of grape endosperm crystals. *Sesamum indicum* seeds remind me of the following anecdote. One night in the 70's, I happened to run into Dr. Larry Thurston (see part I) at Los Angeles Airport. We decided to have dinner in the Theme Building Restaurant (that's the large octopus-like building shown in advertisements about LAX). As a part of dinner we were served some small sesame seed crackers. A discussion came up as to where you could find subject matter for SEM research. My position was that you could find it anywhere. As a joke, I bet Larry that I could publish a picture of a sesame seed from one of the crackers in a scientific journal. I wrapped up a cracker in a paper napkin and took it home and placed it in a small specimen box until work on this seed paper came up. I kept the box with the sesame seed cracker for some years (Fig. 24).

The second of the three papers (Grimson, Arnott and Webb, 1982) deals with winged crystals in bean. The third paper, "Calcium oxalate (weddellite) crystals in forest litter" (Arnott, 1982)

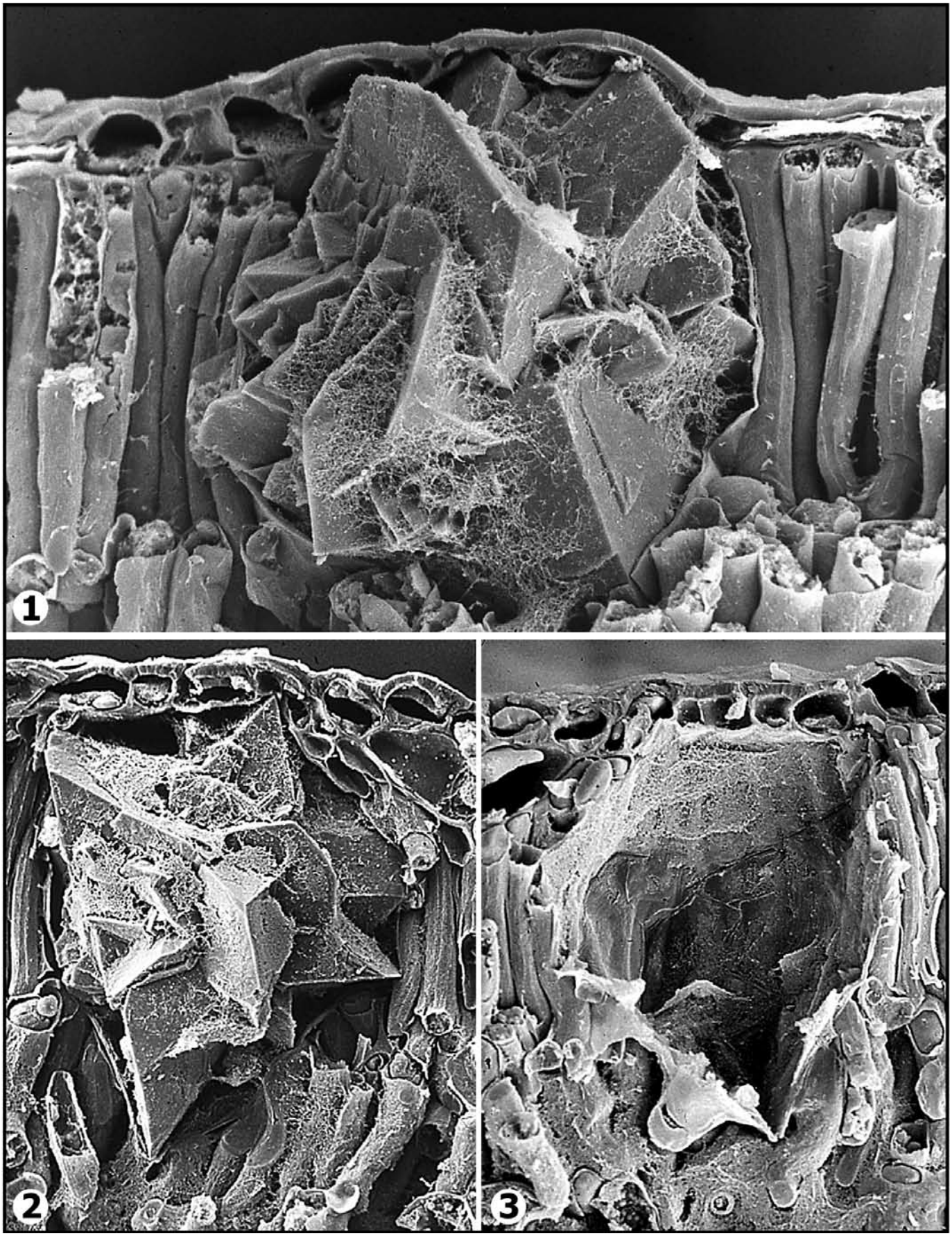


Plate 22. SEM of crystal idioblasts in the leaf of pecan, *Carya illinoinensis*. 1. A large multifaceted crystal of CaOx covered with cellular remnants. The crystal idioblast, originally an epidermal cell, expands within the palisade parenchyma when mature. 2-3. A pair of representative micrographs showing a crystal idioblast from both sides of a fracture. The empty space in 3 was filled with the crystal shown in 2. Adapted from Arnott, 1980.

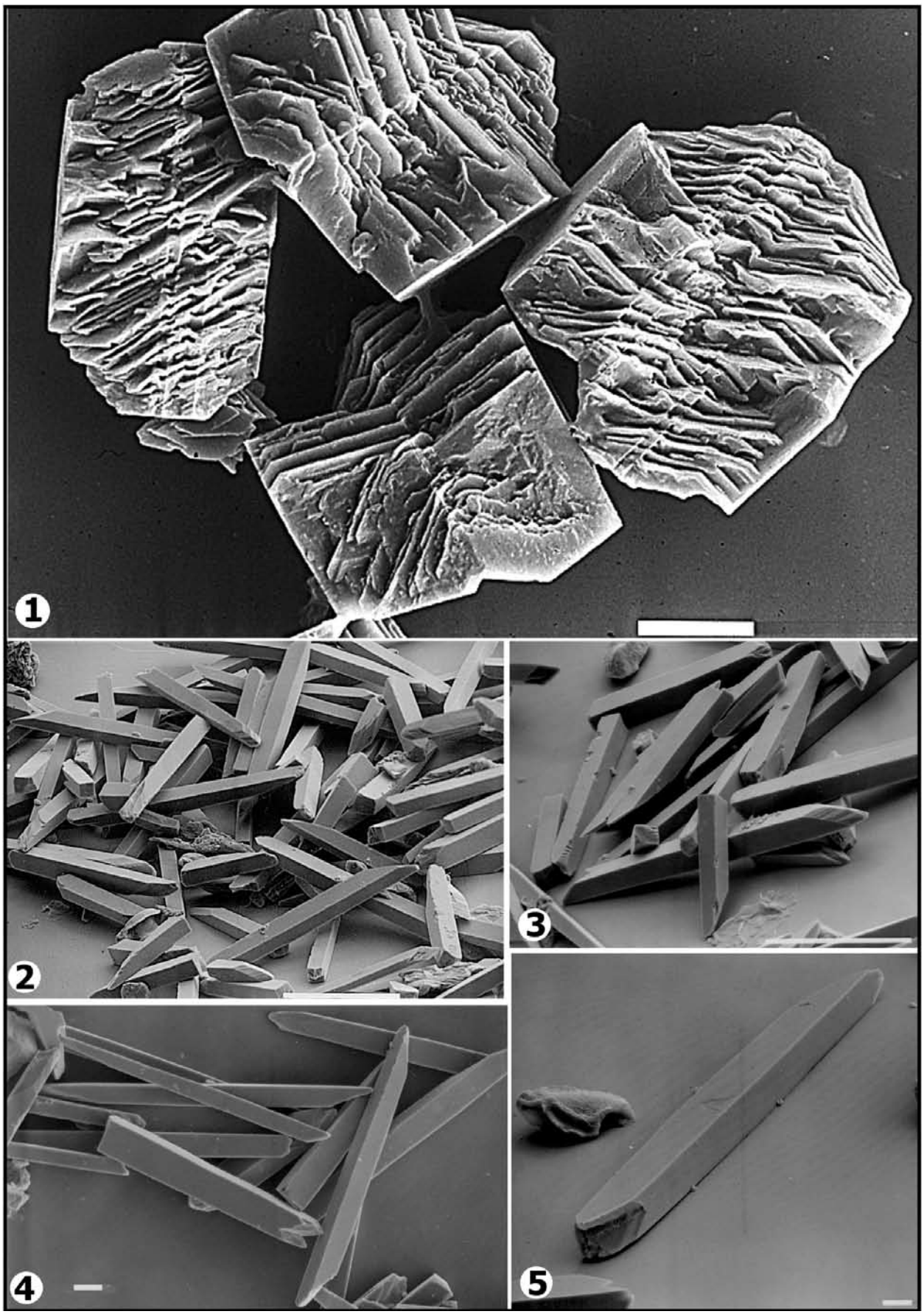


Plate 23. Crystals of *Oxalis dillenii* and *Iris* sp. 1. Isolated twin crystals from *Oxalis dillenii* leaves. These crystals appear to be eroded, although they were not treated for demineralization, bit rather isolated with the usual techniques mentioned in the text. They definitely are bizarre. 2-5. Isolated styloids from the leaf of *Iris*. Each styloid crystal is a twin. Note the reentrant angles more visible in 4 and 5. Bar equals 1 micron.

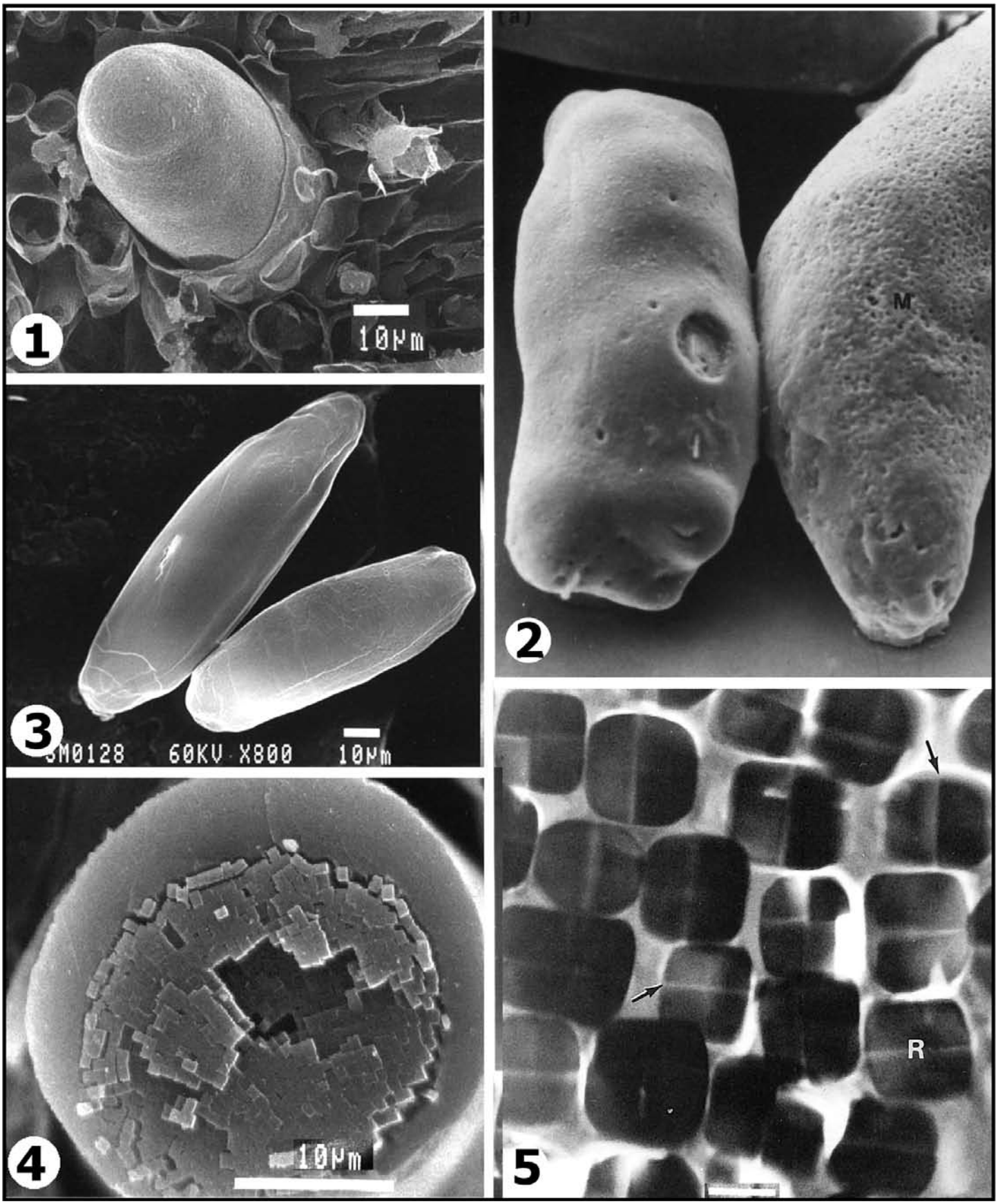


Plate 24. Structural features of the raphide system in *Vitis vulpina*. 1. Raphide packet *in situ* in a leaf. Note the elongate nature of the packet and the cell wall surrounding it. 2. Two isolated packets showing their elongate nature and a large "hole" in one of them. 3. Two isolated packets showing narrow ends and wide centers. 4. Fractured packet showing the disposition of the raphide crystals contained within. 5. TEM showing the twin nature of the calcium oxalate raphides. Adapted from Arnott and Webb, 2000; Webb and Arnott, 1995.

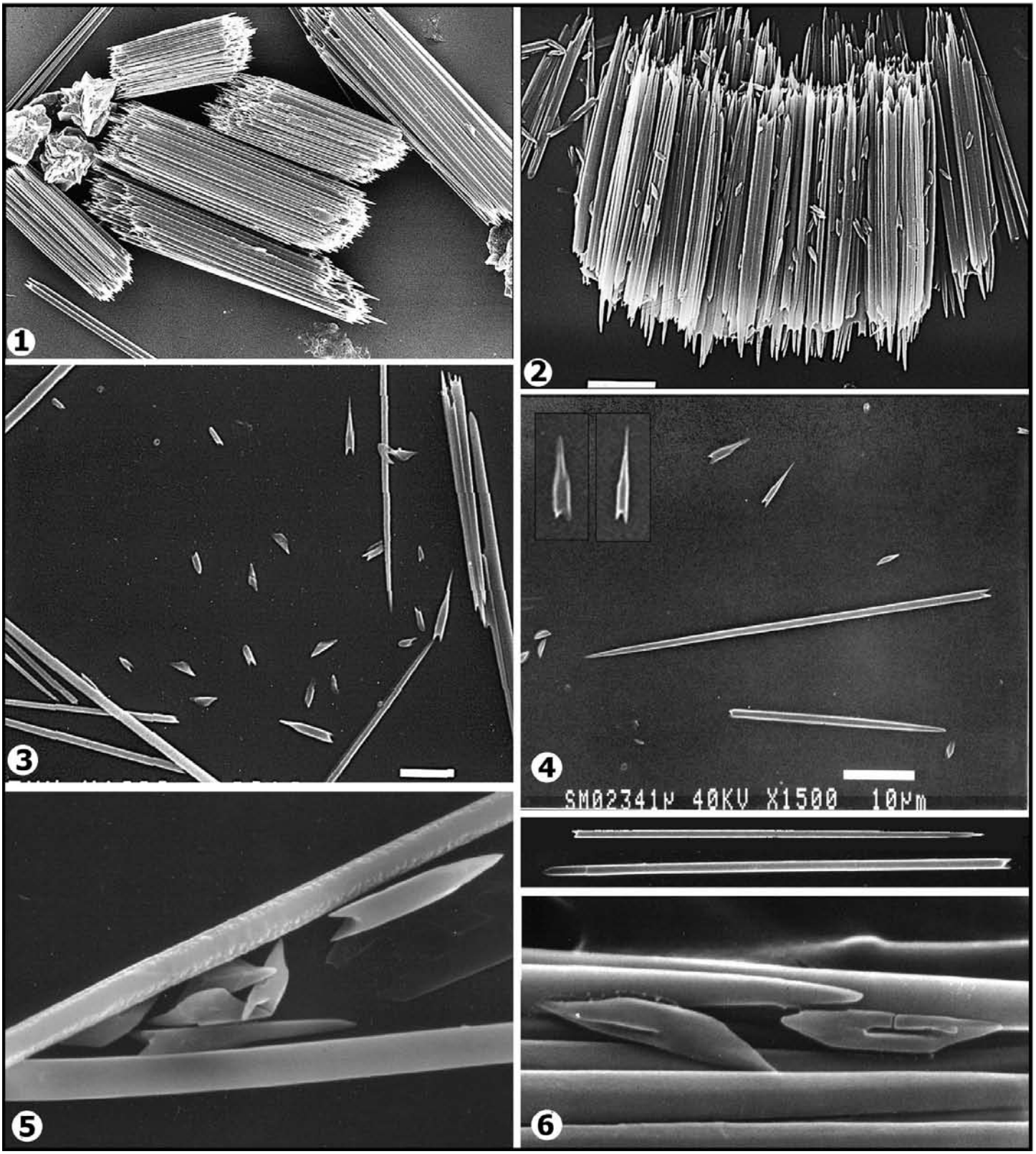


Plate 25. Calcium oxalate crystal twins isolated from *Vitis vulpina*. 1. Isolated raphide crystals without their crystal matrix. Note also the three isolated druses. Each group of raphides contains about 120-200 twin crystals. 2. Raphide crystals spread out by the addition of water to several packets. Note that there are several small initials seen on the surface of the group of raphides. 3-4. Isolated mature raphides and raphide initials. Several of the initials are intermediate in size. 4. Inset shows two intermediate-sized initials. Two isolated mature raphides show the narrow and the sharp pointed ends of the twins. 5. Enlarged view of several raphide initials. 6. Two raphide initials with holes between the individual twins. Adapted from Arnott and Webb, 2000.

has another out of the ordinary story in its background; a story that includes another small specimen box, but that anecdote fits better in the section of "my trail" which covers fungal crystals. At that time I also worked on some other types of plants crystals, e.g. pecan leaf crystals (Plate 22) in which we showed for the first time both, the A or "positive", and the B or "negative" faces from fractured leaves. I also worked on the isolated crystals from *Iris* and *Oxalis* leaves (Plate 23).

Many of the papers we produced in the 80's era were published in the Journal *Scanning Electron Microscopy*. In the light of modern electronic submission of manuscripts it is interesting to review the process of publication in that journal. The traditional publication protocol usually involved the following steps: submission of a manuscript; peer review process; and acceptance or rejection. If the manuscript was accepted, there were always changes suggested by reviewers and the editor, followed by the submission of a revised (final) manuscript. Then you waited for its ultimate acceptance by the editor, the printing process, review of the final proof, and finally publication. In my experience, these steps represent the mode of scientific publication in use throughout the last half of the 20th century. The process could be quite lengthy, I mean protracted "big time." A period of six to ten months (or more) would be considered normal for the process to run its course. Of course *Science* and *Nature* had their "fast track rubrics," namely, Letters to the Editor.

Scanning Electron Microscopy was run by Dr. Om Johari who was Editor and Chief; to say the least, Dr. Om Johari was an interesting and enterprising publishing entrepreneur. *Scanning Electron Microscopy* had a good reputation mostly because of Johari's leadership and attention to details. However, there were several things that were different in his publication scheme than in the traditional process outlined above. The first step was the submission of a complete manuscript which would be appropriately reviewed. If accepted, the manuscript would be returned and the authors became responsible for preparing a **camera ready** manuscript for publication. Publication in *Scanning Electron Microscopy* was my first experience with the concept of the "camera ready process."

"Camera ready" manuscript for *Scanning Electron Microscopy* involved the laborious typing on a wide carriage typewriter using a specific font and font size on special large stock paper sent by the publisher. The final paper had to have the illustrations (micrographs) attached in the appropriate place between the typed parts of the manuscript; obviously, considerable planning had to accompany the typing. Many of our papers were typed by Mary Jane Goad or the staff of the Dean's office (See part II). The preparation of such a manuscript obviated the type setting, proof reading and other workings usually done by a printer. It was a good scheme and with Johari's supervision it produced excellent papers. Figure 25 shows Mary Alice Webb viewing a "camera ready" manuscript (laid out on the floor for illustration purposes) about to be returned to the editor. Looking at Johari's method of publication from today's perspective, it is obviously an intermediate step between the traditional and the modern electronic publication process.

Figure 26. Grape plants in an ancient vinyard by the Mathematics Institute, just outside of Trento, Italy. I collected some of the grape leaves for later studies. Photo by HJA.

Scanning Electron Microscopy had another innovative component. It was entitled "Discussion with Reviewers" and accompanied each paper. "Discussion with reviewers" was added to the end of the paper and could be quite lengthy. In that section reviewers asked questions, sometimes hard questions, about the research content. Authors were obligated to supply answers to the questions in print. As you might guess, this section sometimes initiated vigorous discussions, which did not always end with the printed arguments.

ON THE BIOMINERALIZATION (CRYSTAL) TRAIL IN EUORPE

I was invited in 1981 to attend the Dahlem Konferenzen (Conference) on "Biological Mineralization and Demineralization" which met in West Berlin, (this was briefly mentioned in Part II). This was my first time to visit Berlin, a city that is familiar to anyone who lived in the 20th century, as it played a central role in WWI, WWII and again in the Cold War. We were accommodated in an excellent hotel where I learned about the German penchant for cold cuts at breakfast; quite a shock when compared to the traditional "coffee, eggs and grits" of Texas. The hotel was a short walking distance from the meeting rooms in a famous DBAG Building, the building with a colossal Mercedes Star on its roof. We met on an upper floor and had incredible views of the Kaiser Wilhelm Memorial Church, the Kurfurstendamm and other parts of 1980's West Berlin. Further out you could see East Berlin. With a short ride you could get to the Berlin Wall (See part II).

There were approximately 50 scientists attending this meeting, and with German efficiency we were divided up into groups by interest and background (See Part II, Fig. 32). In fact, organization was the "name of the game" with the Silke Bernhard and other Dahlem officials. I arrived at the meeting place a little early and, after checking in, I found I had some free time. Immediately, so that no time was wasted, the wife of the organizer, Mrs. George Nancollas, and I were sent off to the Dahlem Museum, that was Dahlem organization in action. On our tour of the Museum we saw some of the world's most famous paintings; including Rembrandt's 'Self-Portrait' signed and dated 1634; Rembrandt's 'Study for the Head of Christ' painted about 1650. However, the most extraordinary painting we saw was Pieter Bruegel the 'Elder's Netherlandish Proverbs', painted in 1559, a bizarre tour de force of the 16th century country life. The Dahlem Museum was a delight with glorious paintings one after another, until your senses were on overload.

On another day, the entire conference group traveled *via* bus into East Berlin through Check Point Charlie. From there we went on into East Germany where we visited a huge Soviet war memorial and had lunch at a small "local" restaurant. On our way through East Berlin we saw the "other" (east) side of the Brandenburg Gate. After lunch, we visited the Pergamon Museum, where we saw another famous gate from a much earlier time. The Ishtar Gate was built by Nebuchadnezzar II around 575 BC. It is interesting to note that King Solomon's Temple (sometimes called The Temple) was destroyed by Nebuchadnezzar II in 586 BC. We also saw the Pergamon Altar originally built in the 2nd century BC in the Greek City of Pergamon (now in Turkey) and rebuilt in the Pergamon Museum. However, none of this was as remarkable as the difference between East and West Berlin. Only 35 years after WWII, West Berlin was already a gleaming city of beauty. I learned a lot of biomineralization science at that meeting; however, the real take home lesson was that plant scientists were always second class to animal scientists, and third class to medical scientists.

Regrettably, one is obliged to live within the pecking order one finds himself pecking in!

Over the years, I lectured on biological crystals and calcium oxalate in some outstanding places, including Germany, Switzerland, England, Ireland, Japan, Australia, and Canada, as well as many places in the U.S.A. After the Dahlem Konferenzen in 1981, I gave lectures in several European universities. The first lecture was at the University of Fribourg, Switzerland, where my host was J. Wattendorf, an investigator of calcium oxalate in plants. He discovered the six-sided raphide crystals of *Agave* (Wattendorf, 1969). J. Wattendorf was a robust Swiss man about my age. He met me at the train station where the trains are punctual to the minute. He and his is family lived in the second story of an urban house built in the 1500's. The family hosted me to an excellent lunch. Most of lunch conversation was carried by Mrs. Wattendorf and centered on women's rights; at that time women's suffrage had not yet come to the Wattendorf's Canton (state) although it had arrived in the majority of Switzerland by 1971. Mrs. Wattendorf was very curious about American women's lives, what kind of homes and appliances they had, and how they felt about voting.

Following my lecture, Wattendorf's only remark was to tell me that I had "fractured" the pronunciation of Latin plant names. I was disheartened by his forceful comment which dashed any hope that we would become friends. Apparently, I am easily put off; however, I did not take it as an example of European snobbery. Wattendorf and others apparently think that Latin pronunciation in America and Europe are the same, but from my experience that is not true. While I am not perfect, I learned to pronounce scientific names from some of the best botanist in the world. So it seems, at least for me, that the "invariance of dead languages concept" is deceased.

Later on that trip, I traveled through Lichtenstein and then through a snowy Brenner Pass into northern Italy. Finally, I reached the beautiful mountainous locale of the ancient walled city of Trento in northern Italy. Beauty aside, it was the strangest lecture circumstances I have known. Dr. Luigi Salvatore, a mathematician at UTA, made the local Italian arrangements. I thought the lectures would be given at the main university where biologists would be in attendance; however, that was not the case. My lecture was given at the Mathematics Institute of the Free University of Trento. The Mathematics Institute was far removed from the main campus in a gorgeous area of ancient vineyards (Fig. 26). Most would agree that a Mathematics Institute is probably not the place for a lecture on "Biological Crystals" or "The Importance of Electron Microcopy in Biology." However, a group of about 25 mathematicians, including my host, Dr. Salvatore, endured my two lectures. Here, I must emphasize my hosts were indeed very gracious. Except for my misgivings about the lectures, that stop was the best on "my European Crystal Trail." Among other things the food was excellent; I believe it was the best I have ever tasted.

Luigi drove me from Trento to Venice in his Alfa Romero, he went fast big time. I had one day on my own in that fascinating environment of Venice. While walking around, I came upon an amazing Pescara (fish market) and Erbaria (vegetable market), in an active open-air bazaar. The skin and eyes of the fish in that market just begged to be studied. Likewise the vegetables required scrutiny and I am sure that many of them there contained CaOx crystals. After a while, as I wandered about the city, I began to feel uneasy. All of a sudden, some of the busiest streets I ever been on were becoming empty, the people just seemed to disappear. Finally, I realized that it was "lunch" time. **When it's lunch time in Venice, it's really lunch time.** While in Venice, I rode in a gondola, walked in the Piazza San Marco (St. Marks Square), well known for its many pigeons, had a tour of St Mark's Basilica, climbed the Campanile and visited the Doge's Palace. What a day! Visiting Venice means lots of walking. Tired, I went back to Trento by train.

On that tour I also lectured at the University of Heidelberg in Germany where my host was Dr. H. Dietmar Behnke. I met Behnke during the time I was a professor at UT Austin and he had no comments on my Latin. Behnke used data from his ultrastructural studies to help him understand plant systematics. He and his wife toured the western U.S. and they became fascinated by **kachina** dolls and began to collect them. Jean and I saw some of their collection in their home on a different trip. Heidelberg is on both sides of the beautiful Neckar River. The University is one of the oldest in Europe. The campus is divided in two parts, the old buildings of the original university near town and the new campus, which was ultramodern, in the suburbs. The Hauptstrasse is the center of Old Town. The whole area was striking because of its narrow streets and ancient buildings. In one ancient building I found a McDonald's with a diminutive golden arch. Luckily, while there, I was able to visit a wonderful exhibit of Salvador Dali paintings in the Heidelberg Castle. In addition to the instantly recognizable surreal paintings that Dali is famous for, the exhibit had examples of Dali's botanicals, the so called "FlorDali" which, as a botanist, fascinated me. Dali was an excellent botanical illustrator, something I had never read about.

THE TRAIL MEANDERS THROUGH THE "VINEYARDS"

The work on CaOx crystals in *Vitis* started with a study of the endosperm of the cultivated grape seeds (*Vitis vinifera*). (Please note, almost all the research on *Vitis* was carried out jointly with Mary Alice Webb). The endosperm cells contained crystals of CaOx (see below). That study also showed that grape seeds have a number of raphide idioblasts in the seed coat tissues. A look at a wonderful slide of a *Vitis vinifera* leaf, given to me by Dr. George Denel, made it clear that grape leaves contained many CaOx crystals in raphide and druse containing cells. Through these investigations it became obvious that the leaves of *Vitis* were a potential source of large quantities of CaOx crystals. At the time, two species of grape were available on the UTA campus, *V. mustangensis* and *V. vulpina*, both growing along the Trader Horse Creek on the south side of the campus. The mustang grape leaves are very hairy and difficult to work with; of the two species, *V. vulpina* was a better specimen source for crystal studies and received the most attention. For reasons I do not comprehend or appreciate, the grape plants have been systematically eradicated from the banks of Trader Horse Creek. *C'est La Vie*.

The "vineyard trail" is a major part of my crystal trail. It began with a TEM investigation of the endosperm in the seeds of *Vitis vinifera*; the original seeds came from my bag lunch. Endosperm cells contain abundant protein and lipid reserves in the form of relatively large protein bodies associated with smaller lipid spheres. CaOx druses were found in the center of many protein bodies (Plate 19). Using a Zeiss EM9 TEM and thin plastic sections, we found that CaOx druse crystals were entirely enclosed

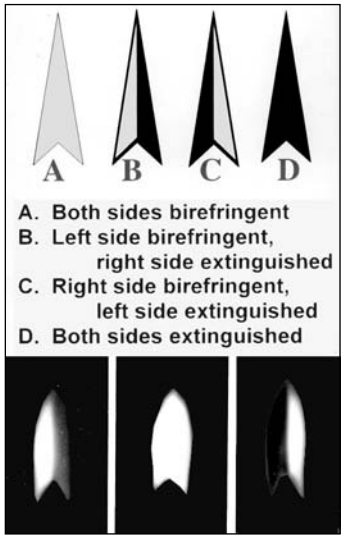


Figure 27. Diagrammatic and optical views of twins in *Vitis* initials. Adapted from Arnott and Webb.

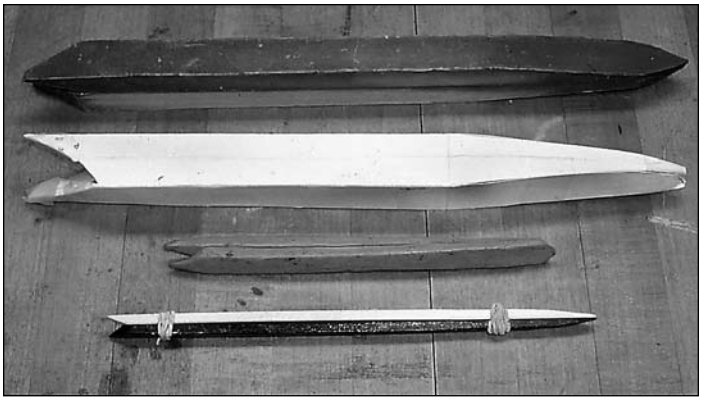


Figure 28. Models of raphide crystals of *Vitis*. From top to bottom, ceramics, paper, clay and chop sticks were used to make the models. Models made by Arnott and Webb.

within the protein bodies. Each druse crystal had an organic central "sphere." The nature of that central sphere was investigated using digestive enzymes. Digestion with pronase (a protein digestive enzyme) demonstrated convincingly that the center sphere contained mostly proteins. The druse crystals in grape seed endosperm probably form in a vacuole and the central core of proteins seems to represent the nucleating agent where calcium oxalate crystallization begins (Webb and Arnott, 1983).

From almost our first encounter with the raphide crystals of *Vitis* we understood that they were twins. Under polarized light you could demonstrate that one twin extinguished while the other was birefringent. By rotating the specimen stage, and thus the specimen, you could then extinguish the opposite twin while the original extinguished twin became bright (Fig. 27). It was also clear that the raphides were square or rectangular in cross section. This could be seen with optical microscopy but of course was made extremely clear in TEM views of thin sectioned raphide crystals. However, there was a question about how the twin plane related to the square or rectangular profile. Horner and Cody (1983) and again Cody (1987) put forth the view that the twin plane extended from one vertex of the cross section to the opposite vertex. If this were true, then each twin would have a triangular cross section while the two would fit together to make a rectangle.

Over the next few years we were troubled by their model of the twin structure. Our study of raphide crystals of several species of *Vitis* seemed to differ from their view. It appeared to me that the twin plane ran from one flat surface to the opposite flat surface rather than from vertex to vertex as they believed. With TEM we were able to show a variety of views in which it was clear that the twin plane ran from one surface to the opposite side (Plate 24:5; Arnott and Webb, 2000). It may seem trivial to be concerned with which direction the twin plane runs but it was extremely important in understanding twinning in the raphide crystals. In this case, we showed that "twinning is rotational about the twin plane along the length of the raphide." In other words, raphide crystals of *Vitis* are rotational twins.

Figure 29. Wood models of *Vitis* raphides made by HJA in 1999.

On several occasions, Mary Alice and I made models to demonstrate the nature of the two ends of the *Vitis* raphides. The first model was carved out of chop sticks saved from a Chinese restaurant. We also made models out of clay (Mary Alice was doing ceramics at the time), paper and finally I used my wood working skills to produce scale models of the raphides in which one twin was made of pine and the other twin of mahogany; thus we had one dark and one light twin. In particular these models showed the interesting nature of the "forked" end of the raphide twin. The arrow head seen in profile views was the result of attenuation of each twin in the opposite direction at the forked end. In the final analysis, model construction was the only effective way in which I could understand the structure (Figs. 28, 29).

When we began isolating the raphides from *Vitis* using absolute ethanol, we were surprised to find that the raphide crystals came out of the leaf in packets (Plate 24). Examination of the leaves in the living state showed a dense matrix surrounding each group of raphide crystals within the grape idioblasts (Plate 2:6). It was this vacuolar matrix surrounding the crystals that formed the isolated packets. Isolation of packets only happens when absolute ethyl alcohol is used; water or even 70% ETOH will dissolve the packet matrix and thus individual crystals will be isolated. If you place the isolated packets on a microscope slide and add a cover slip using 100% ethanol as the mounting medium you can easily observe the characteristics of the packets. However, if a small drop of water is added at the edge of the cover slip the matrix will dissolve and the crystals will separate into oriented group (Plate 25) of individual raphides. This process of matrix dissolution can be observed and followed across the slide as the water reaches the isolated packets. Obviously the matrix is soluble in water. With this understanding it becomes easy to separate the packet matrix from the crystals by using centrifugation.

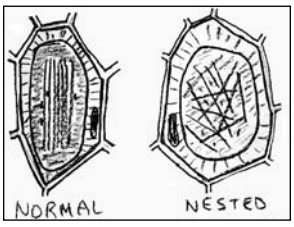


Figure 30. Illustration of the difference in crystal orientation between normal and nested crystal idioblasts. Drawing by HJA.

Packets isolated from grape leaves have the shape of an elongated cylinder. Individual packets may have long pointed, rounded or blunt ends. Each mature packet is usually longer than the crystal bundle contained inside; sometimes mature packets were almost twice the length of the crystal bundle. It seems likely that this variation is caused by exact shape of the idioblast from which packets arose. The exact shape of an idioblast may be determined by the cells surrounding it; pointed idioblasts produce pointed packets. The packets are usually thicker in the middle and the crystals bundle is almost always located in the middle of the packet. When the isolated packet surface

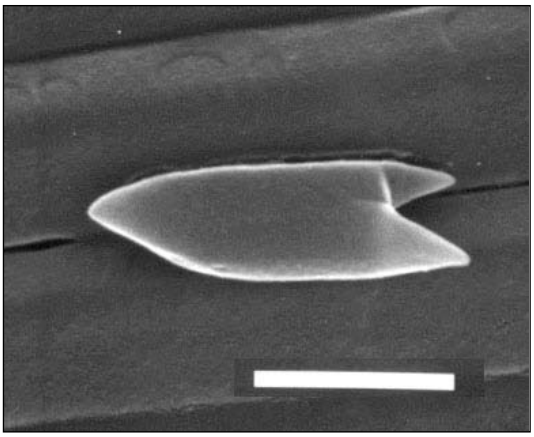


Figure 31. It's a space ship! No, it's a bullet! No, it's not either one, it is just a very young calcium oxalate twin crystal from *Vitis vulpina*. Notice that, even at this early stage, the twin has a pointed end and arrowhead end showing an excellent reentrant angle. Adapted from Arnott, Lopez and Webb, 1994.

is seen in SEM, its surface is relatively smooth with occasional small indentations (Plate 24). Occasional tears in the surface of the packet reveal the crystals inside and indicate that the packet matrix has considerable thickness and integrity. In many packets a large round hole penetrates through the packet "wall" down to the level of the raphide crystals (Plate 24). These holes can be seen with both scanning and light microscopy. As yet, a definitive relationship between these holes and the process of packet or crystal formation has not been established. The presence of these holes logically leads one to speculate that they are part of the mechanism of crystal formation; perhaps they are caused by a placenta-like connection between the crystal chambers and the cytoplasm. Isolation of packets from immature leaves often shows crystal bundles that are quite small, in fact maybe only one sixth of the packet length.

Since establishing her lab at Purdue, Mary Alice Webb has taken the lead in attempting to determine the nature of the packets and the crystal chambers in *Vitis* using molecular biology techniques (Webb *et al*., 1995). Using SDS-PAGE analysis, she and her colleagues established that the water soluble matrix contained proteins and carbohydrates. The structure of the carbohydrates was determined using GC-MS; a novel polymer with glucuronic acid linkages was found in the carbohydrate fraction. Demineralization/demineralization studies showed that the crystal chambers were distinct from the surrounding matrix and provided direct evidence that the chambers can promote crystal nucleation. Their investigation showed that the proteins of the packet matrix and those of the crystal chamber are different. I spent 8 months in 1990-91 at Purdue "helping" with these studies. As stated earlier (Part II), the molecular biology techniques are not my forte. So it seems, just like Adriance Foster, I hesitated to take a step forward, preferring to work with more familiar techniques. Mary Alice's work on calcium oxalate continues to the present; she is exploring the new techniques of proteomics and genomics in studying calcium oxalate in plants.

When we examined isolated groups of *Vitis* raphides (Plate 25) we found that most twin crystals were approximately the same length. Each crystal showed a forked (arrow tail or fishtail-like) and a pointed end. This means that the crystals are "polarized" in that each has a plus and minus end. This is interesting in light of the purported rotational twinning mechanism in which each end should be equal. The individual crystals inside the packets are oriented in either direction, but always parallel to the axis of the crystal bundle. This elicits the question of how the orientation in the bundle and then in the packet is accomplished. Since parallel orientation is normal and occurs in the vast number of cases, it seems probable that this arrangement is under genetic control, as most things in a cell. We have seen a few cases of "nested crystals", so called because they are arranged "higgley-piggley" like the array of twigs and branches in a bird nest but still within the vacuole (Fig. 30). The nested crystal arrays may be explained by the malfunction of the orientation mechanism; perhaps the orientation control mechanism in these cells has gone *kaput*. Whatever, it seems clear that there is a mechanism responsible for the orientation of crystals within the bundle and the bundle within the packet. Perhaps, the orientation mechanism relates back to the long axis of the crystal idioblast. Actin, as filaments, or tubulin, as microtubules, or some other protein, may be responsible for the crystal orientation.

While viewing crystals separated from their packet matrix, it is possible to see some very short crystals. They can be seen with a light microscope, especially under polarized light, and very well by SEM. We termed these short crystals **initials**; we called them "initials" because we assumed that these were raphide crystals that had not completed their growth cycle at the time of harvest. We saw initials that were less than one micron in length. However, even very short raphide initials attain the average diameter of mature crystals and demonstrate forked and pointed ends (Fig. 31, Plates 25-27); initials of intermediate length are also seen in these preparations. From these observations, it appears that when the initials have reached their appropriate diameter, growth only occurs at their ends. We do not know whether the growth occurs at the pointed or the fish tail end; however, it may be that the reentrant angle end represents a discontinuity toward growth and the pointed end undergoes growth. This is consistent with the variation in pointed ends that is seen, whereas the fish-tail end seems not to vary. Early stages in the formation of raphides (raphide initials) have been observed (Plate 27). In these cases, it appears that each initial is contained in a sac which connects through a narrow "thread" to a large "membrane ball." In one case, we could see several initials attached to the "membrane ball" (Plate 27:1, 2). The "membrane balls" are also seen with TEM and show membrane association with developing crystal initials (Plate 27: 3-6).

Figure 32. Specimen box from Susan Pratt. Box is 1.75 by 1.25 inches; it contained the forest litter that I used in for my paper (Arnott, 1981).

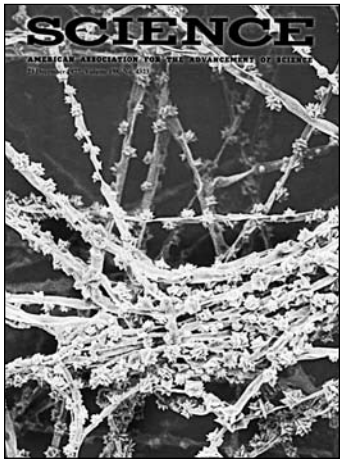


Figure 33. *Science* Cover. Original printed with a green tone, *Science* letters in red. Christmas edition. Adapted from Graustein *et al.*, 1977.

Some initials have defects which look like elongated holes in their sides (Plates 25:6, 26:2-6). These may be stages in the early growth of the twins or they may be the result of aberrant crystal growth. Demineralization of the crystals with EDTA often reveals holes in what previously appeared to be normal crystals. It may be that the nucleation of these twins involves development about a hole which is filled in during crystal growth.

Cindi Schwartz worked for several years as coordinator in our E.M. Lab and was a long time member of TSM (TSEM). During that time, she also worked on an MS in biology with me. She wrote an excellent thesis, in my opinion, which was equivalent or better than many of our Ph.D. dissertations. Her thesis concerned thermogenesis in the American lotus. She studied the cycle of heat production in the flowers of *Lotus* during the time of pollination. She found that despite ambient lower temperatures, the temperature of the lotus flower is maintained between 30-36°C during the time the stigma is receptive. Her "*in situ*" work required standing in three foot water for long periods of time at the Ft. Worth Nature Station. The lotus "swamp" has several well known animals including the Cottonmouth Water Moccasin, *Agkistrodon piscivorus*, and, ac-

Figure 34. Terminal rosettes of calcium oxalate. From a beetle gallery in *Pinus ponderosa* bark. Adapted from Arnott and Webb, 1983.

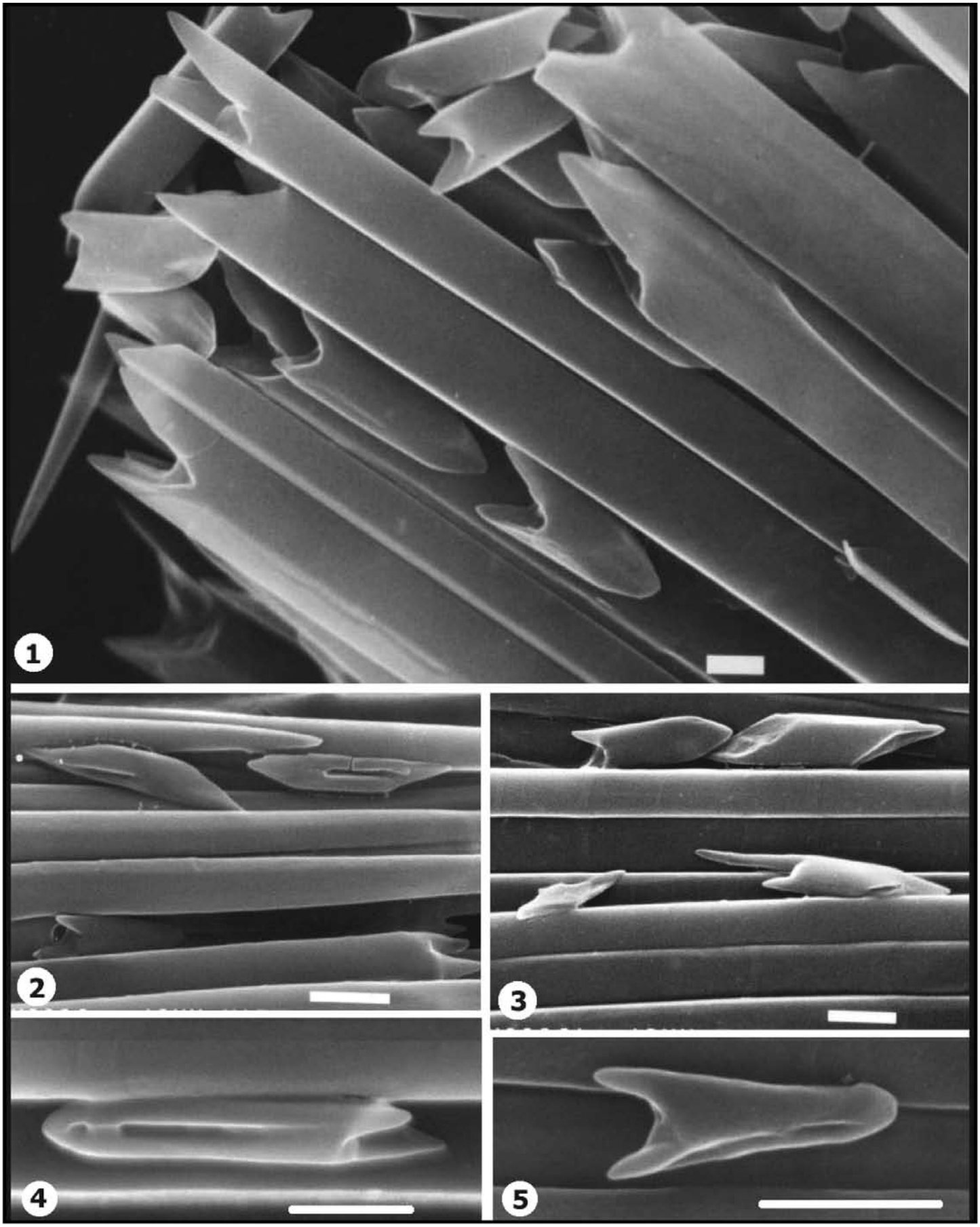


Plate 26. Isolated raphides from *Vitis vulpina* showing details of twin nature. Bars = 1 micron. 1. Surface of raphide twins. 2-4. Examples of raphide initials. 5. Very small raphide initial. Note hole in several initials. Adapted from Arnott, Lopez, and Webb, 1994.

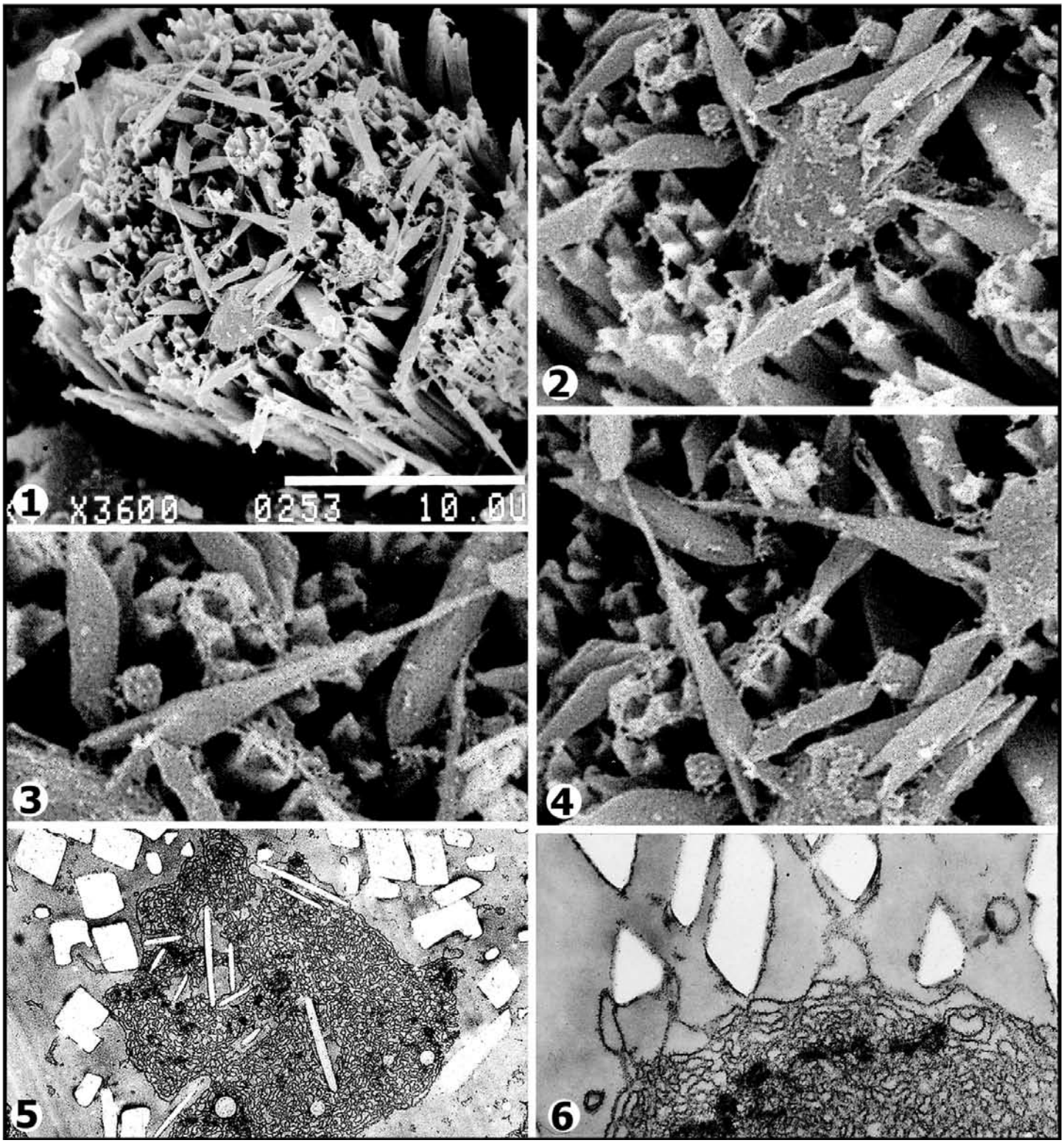


Plate 27. Early development in the raphide idioblasts of *Vitis vulpina*. 1. SEM of mature raphides and raphide initials *in situ*. At this low magnification, all the details within the developing raphide system could be seen. Note prominent membrane bundle in center of cell. 2. Enlargement of the cell center showing the membrane bundle with attached initial sacks. 3. Very young raphide initial contained within initial sac; note the elongate attachment fibril. 4. Several membrane-shrouded initials with attachment fibrils. 5. TEM of the membrane bundle with associated raphides in various stages of development. 6. Edge of membrane bundle showing membranes and attachment of membranes to the membrane-bound raphide initials. 1-4. Previously unpublished. 5-6. Adapted from Webb and Arnott (posters).

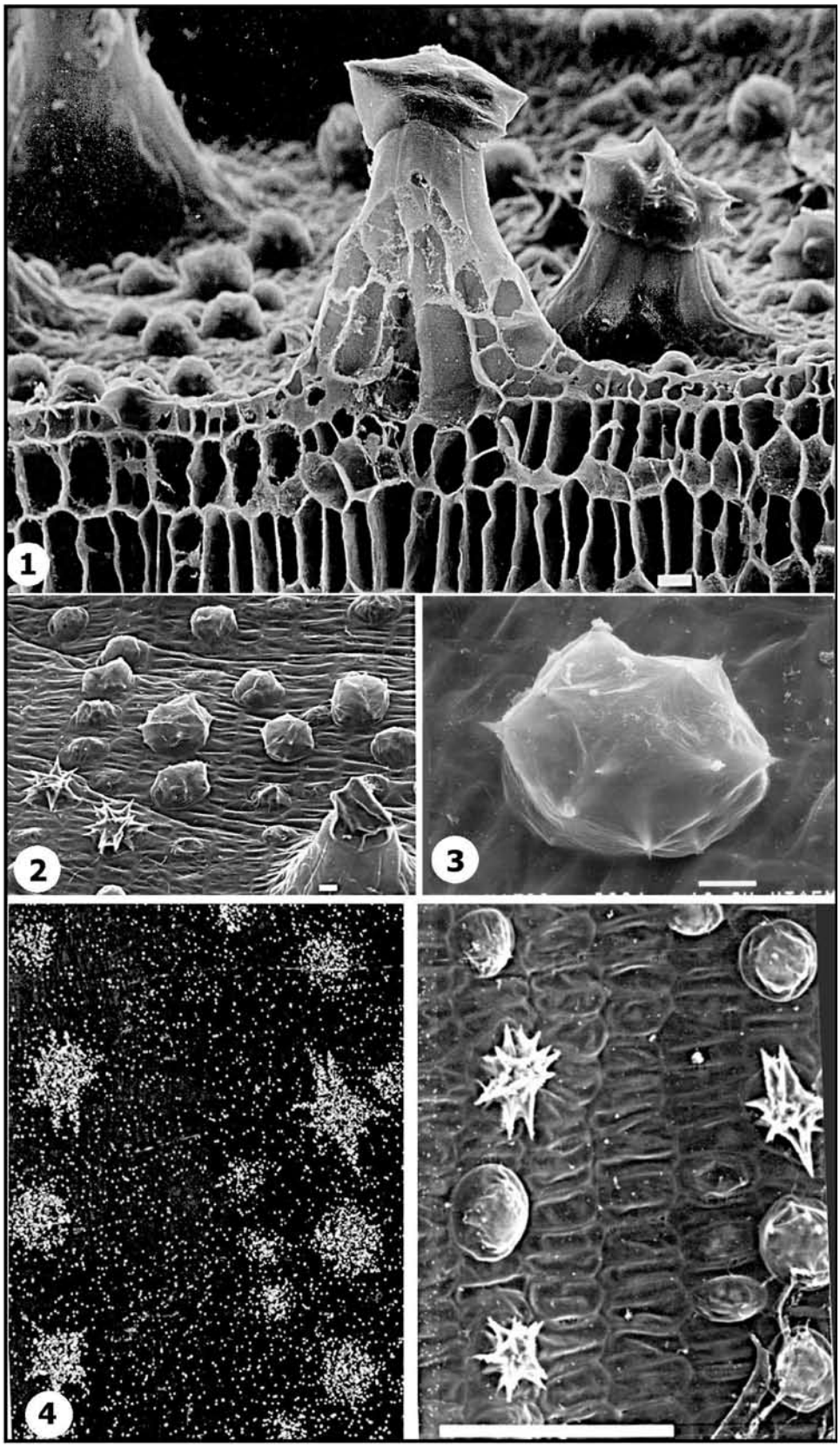


Plate 28. Epidermal trichomes with calcium oxalate crystals in the leaf of Lotus. 1. Two multicellular epidermal trichomes with terminal crystal cells, each showing a druse crystal; bar = 10µ. 2. Surface view of many single-cell epidermal trichomes, each containing a druse crystal; the cell walls surrounding two of these crystals are absent; bar = 10µ. 3. Single epidermal trichomes showing how the internal druse shapes the cell wall; bar = 10µ. 4. X-ray dot map showing the signal for Ca (left panel). Right panel, SEM of the same area, showing cells with both complete and absent cell walls; bar = 100 µ. Adapted from Arnott and Schwartz.

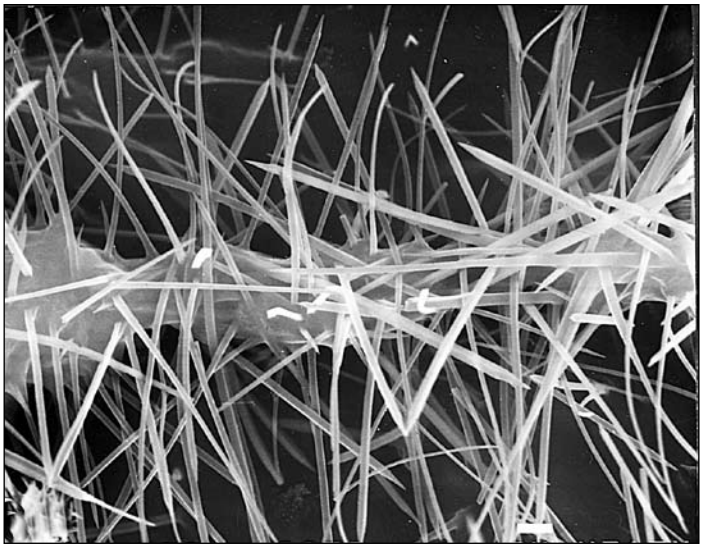


Figure 35. Narrow crystals of calcium oxalate attached to a central hyphal strand. The individual crystals have extremely sharp pointed ends. Adapted from Arnott and Fryar, 1984 .

cording to some reports, even alligators. Luckily, Cindi's father participated as a guard during her swamp sojourns.

Anyone who has looked at virtually any part of *Lotus* work knows that crystals are big time abundant throughout the plant tissues. Crystals of CaOx were not the focus of Cindi's research, nevertheless she saw many crystals in different parts of the plant. Examples of crystals on various parts of the leaf are shown in Plate 28. Perhaps, the most interesting thing about this system is that many CaOx crystals are produced in epidermal cells and hence readily seen with the SEM.

THE CRYSTAL TRAIL MEANDERS UNDERGROUND

Like Quatermain and his companions we now face the test of looking underground, luckily for us there is no witch like Gagool to deal with. Enormous amounts of calcium oxalate are found in, on or associated with the fungi on (leaf) litter. Unfortunately, litter is a term that is not understood by everybody. This was forcefully brought home one day early in 2000. After obtaining a collection permit from the Ranger Station in Bishop, Jean and I were heading up to the Patriarch Grove in the White Mountains of California to collect some litter samples. This was to be a part of a larger collection of litter samples I was collecting from areas in several western states. On the way up to the collection site, we stopped in at the Schulman Grove Ranger Station. From there it is 12 hard miles on a rough dirt road to the Patriarch Grove and the station is the last place to get water. While at the station, I struck up a conversation with one of the rangers and we talked about several things while looking at the familiar bristlecone demonstrations. Leaving, I casually mentioned to her that Jean and I were going to collect some litter up near the Patriarch Grove. The Ranger replied, "It's so good of you to clean up after people who litter our mountains." Even professionals confuse "people litter" with "natural litter!"

My first experience with calcium oxalate on leaf litter came from a small specimen box! Dr. Susan Pratt heard my talk about CaOx crystals and spoke to me afterward about some similar things she had seen in forest litter. I, of course, replied that, "it would be great to look at some of your material." A few days after arriving back in Arlington, a small package arrived from Dr. Pratt. In that package I found a specimen box which contained a mixture of aspen, spruce and fir litter from the Wasatch Mountains east of Salt Lake City (Fig. 32). The material was a treasure trove of fungi that produced needle like crystals of calcium oxalate. Soon I published "Calcium oxalate (weddellite) crystals in forest litter" (Arnott 1982) based on the SEM investigation of the material that came in that box. This **is** the box I mentioned earlier in the text. The fungi from the small white box captured my interest, and over the last twenty five years, I examined many other litter fungi which produce calcium oxalate. From that time on, I collected natural litter samples wherever I traveled. Sometimes I used litter collection as an excuse to visit important places such as the "Prometheus Site" in the Great Basin National Park (later).

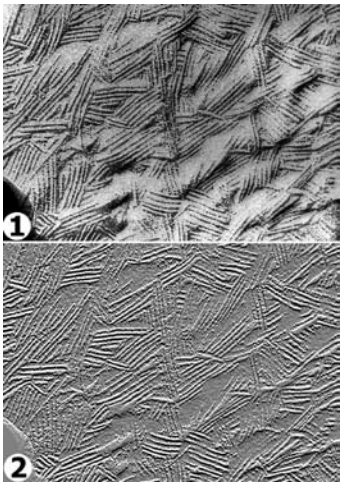


Figure 35A. 1. Freeze etch preparation of rodlets from Utah fungus. 2. Same image treated with a embossed filter to emphasize the rodlet pattern. Original by HJA.

However, let's continue the story about litter. An interesting paper on forest litter was published in 1977 (Graustein *et al*., 1977) (Fig. 33). This article showed SEM pictures of fungi growing in forest litter. It was published in the Christmas edition of *Science* and had a magnificent cover; forest litter printed in light green with 'Science' printed in red. It seems that even the Editors of *Science* were keen on Christmas spirit in 1977. The article dealt with calcium oxalate on fungal hyphae; the authors believed that the calcium oxalate precipitated on the surface of the hyphae. My material (from the box) showed that the CaOx crystals, identified as weddellite by x-ray diffraction, although attached to the surface were produced by the fungi, not precipitated on their surface. In fact, when I studied the *Science* cover, it seemed to me the CaOx crystals were dispersed on the hyphae in an orderly manner, a manner inconsistent with mere precipitation. At that time and now, I believe that the CaOx crystals in the SEM published on the cover of *Science* were produced by the fungi, and not a mere precipitate from the environment.

On a collecting trip, Mary Alice and I casually examined a pile of ponderosa pine stems cut for firewood. We noticed that under the bark there were many beetle galleries (tracks); I returned with some of this material to study it. As it turned out, the beetle galleries were filled with fungi, and you guessed it, every hypha was very well decorated with CaOx crystals. That fungus produced druse-like crystal of CaOx and we were able to observe the earliest stages in crystal production (Fig. 34)(Arnott and Webb, 1983). This was another example of the SEM power. Without processing, other than sputter coating, as the fungi were already desiccated, we could examine the details of crystal development. This is another case, in which I was not troubled by looking at an issue that most people in all probability considered outside my jurisdiction (Plate 29).

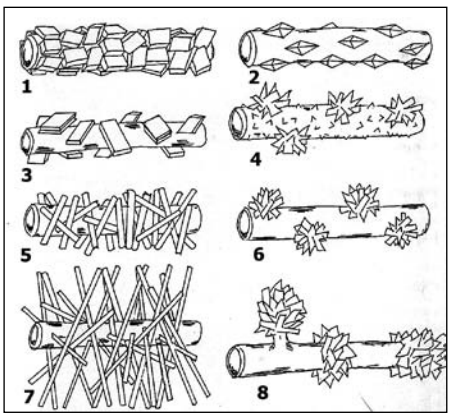


Figure 36. Variation in types of calcium oxalate crystals found in different fungal hyphae. Adapted from Arnott, 1995.

Anne E. Fryar was a student in the 1982 SEM class. When she found out about my in-

Figure 37. Prometheus, (*Pinus longaeva*) the 4900 year old living tree in Great Basin National Park. Donald Currey is standing in the tree. Photo courtesy of The National Park Service.

terest in litter, she brought me some from her own compost pile in Arlington, Texas. The material in that compost consisted of cultivar fragments (pecan, peach, cottonwood, mimosa and St. Augustine grass) common in Arlington. The compost pile was about 10 years old and some parts formed a dark, almost black, "mat." The mat's surface was covered by an ash-white layer of fungal hyphae. I told Anne that I thought this material would make a good paper and that we should cooperate on it. The mat had integrity and stability, apparently because the various component parts were "stuck" together by the myriad of fungal hyphae running through the mat. In this litter, many of the fungi had very elongate narrow crystals of CaOx extending in every direction from the hyphae, thereby mimicking the structure of a bottle brush (Fig. 35; Plate 30). Using x-ray diffraction and crystal solubility we determined that the crystals were calcium oxalate monohydrate (Arnott and Fryar, 1984). These crystals were very elongated and in that sense were similar to the raphides found in higher plants. Both fungi and higher plants are able to make elongated narrow crystals of CaOx, but chemists have not yet been able to duplicate this structure. Later, in the litter mat, we found shorter, more robust crystals which were clearly twins as they showed fish tails characteristic of reentrant angles found in twin CaOx crystals in higher plants (Fig. 36; Plate 30: 5).

In a laboratory culture of *Armillariella tabescens*, we saw the very early development of raphide-like crystals on hyphae. These crystals began to form only on an area located at a short distance from the hyphal tip. The micrographs in Plate 31 show that the crystals arise from inside the hyphae, which therefore implies that the fungus must control the formation of these CaOx raphide-like crystals.

In 1983 I went through a "training program" in the use of the Million Volt Microscope located in a special building on the University of Colorado campus, in Boulder. The laboratory was supervised by Dr. Keith Porter. (Incidentally Cindi Schwartz (see above), is currently employed in that laboratory). Visiting that lab was an interesting experience in itself, especially for being able to look at yucca crystals in very thick sections. I was able to have a brief discussion with Dr. Porter regarding CaOx crystals in plants, a subject he and Ledbetter had investigated as well (Ledbeater and Porter, 1970).

Following that training session, I traveled on to Provo, Utah to visit the laboratory of Dr. Bill Hess, who was experienced with freeze-etch studies of fungi. I first met Bill in the microscopy training program at UT Austin and we became friends. On the way between Boulder and Provo I stopped several times and collected litter samples. I also collected litter samples in and around Provo, Utah. I came to Hess' lab to use his freeze-etch machine with the hope to gather relevant data for the purpose of establishing the relationship between CaOx crystals and the fungal hyphae that produced them. It was my hope that freeze etch would clearly show that the crystals were formed within the fungal cell wall. As it turned out, while there I learned a lot more than expected. I found that both the hyphal cell walls and crystals are covered by a layer of **rodlets**. Rodlets had been noted in other fungi as the outer layer of the cell wall and were believed to be made up of proteins; however, they had never been associated with CaOx crystals. This was really quite astonishing, and it completely cinched the idea that crystals are formed by the fungi inside their cell walls (Plates 32, 33).

The litter fungi that I studied at Provo had both the druse-like and the raphide-like crystals. Both types of crystals had rodlets on their surface. As it turned out, there in Provo, I was lucky enough to settle the question of calcium oxalate's origin in those fungi: the crystals developed within the hyphal cell wall. The rodlets on the elongate raphide-like crystals tended to be oriented with their long axis aligned with the long axis of the crystals. Druses, on the other hand, usually had a "herringbone" pattern of rodlets distributed over their external facets. In some lucky cases, you could see that the rodlets on the surface of the crystals were continuous with those on the hyphal cell walls. I always thought that Bill Hess was very surprised with these findings. I am sure some people thought I had more good fortune that one person should expected – if that is true, all I can say is "three cheers for lady luck." I made a series of measurements on the diameter of the rodlets. Ninety measurements from 9 micrographs provided data that indicate that the rodlets had a diameter of 5.4 ± 1.3 nanometers. In one preparation, I was able to see that the longitudinal structure of the rodlets consisted of strands wound together to make up a rodlet. The strands were approximately ½ of the 5.4 nanometer diameter rodlets. That is something near the dimensions of a double helix of DNA (Plate 33). All the micrographs used in these studies were taken with either a Phillips 400 or JEOL 1200 microscopes, therefore these dimensions are well within the resolution capacity of the microscopes.

Virginiae Blackmon, an engaging lady from Ft. Worth, Texas came to work in my lab during the 1990's. In addition to photography Virginiae (this is the correct spelling) had many interests, especially on the artistic side of life. As a part of her MS work, Virginiae examined many collections of litter. By that time, I had over a hundred collections of litter, mostly collected west of Arlington but some from north and east. Virginiae made several collections in South Texas that were used in her study (Blackmon, 1992). Blackmon's thesis research narrowed the study to the examination of 28 well documented collections from five states in the Southwestern U. S. Her investigation found two basic types of CaOx crystals associated with the fungi from 25 of the 28 sites. One group of fungi had elongated CaOx raphide-like crystals associated with their hyphae; the crystals were somewhat like those seen by Arnott and Fryar (1984). The second group had druse-like crystals of CaOx on the fungal hyphae, which were similar to those seen by Graustein *et al.* (1977) and Arnott and Webb (1983). Of the 28 samples she studied, elongated raphide-like crystals were found in fungi from 14 sites. Eleven sites had druse-like (twin) crystals on the hyphae while the remaining 3 sites had no clear cut crystals but rather only blister like areas on the hyphae. The nature of the last group is still undetermined, however, similar blisters have been found in other investigations of litter samples from areas where calcium oxalate was plentiful.

Virginiae Blackmon determined that the crystals on the fungi in her collections were CaOx crystals by comparing them with those in micrographs of known CaOx crystals presented in the lit-

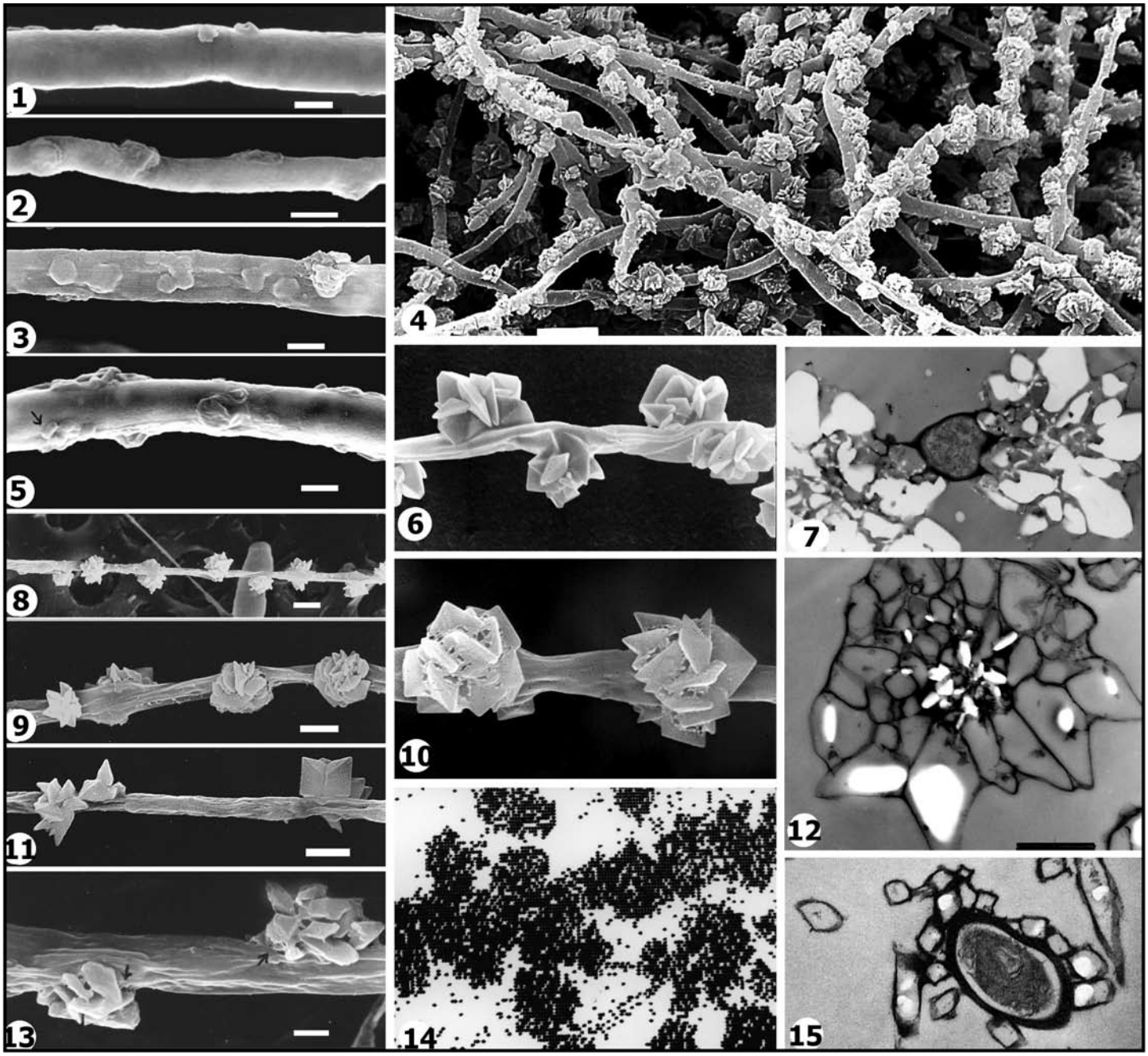


Plate 29. Fungal crystals. 1-14. Druse-like CaOx crystals on the fungal hyphae from beetle galleries in wood of *Pinus ponderosa*. 1-10. Stages in the development of the CaOx crystals on the hyphal surfaces. 1. Very early stage in crystal formation; note the smooth hyphal wall. 2-3. Small protrusions representing the early crystal growth stages. 4. Mass of druse-like crystals attached to numerous hyphae, which were associated with the surface of the wood lining a beetle gallery. 5, 6, 8-10. Additional stages in the development of the druse-like CaOx crystals on the hyphae. 11. Three developing druse-like crystals on a hypha; note the large prismatic twin at the right end. 7, 12. TEM showing the structure of druse-like CaOx crystal masses. 14. X-ray map dot for Ca of hyphae with druse-like crystals. 15. Cross section of hyphae showing the attached raphide-like crystals. Adapted from Arnott, 1992; Webb and Arnott, 1983.

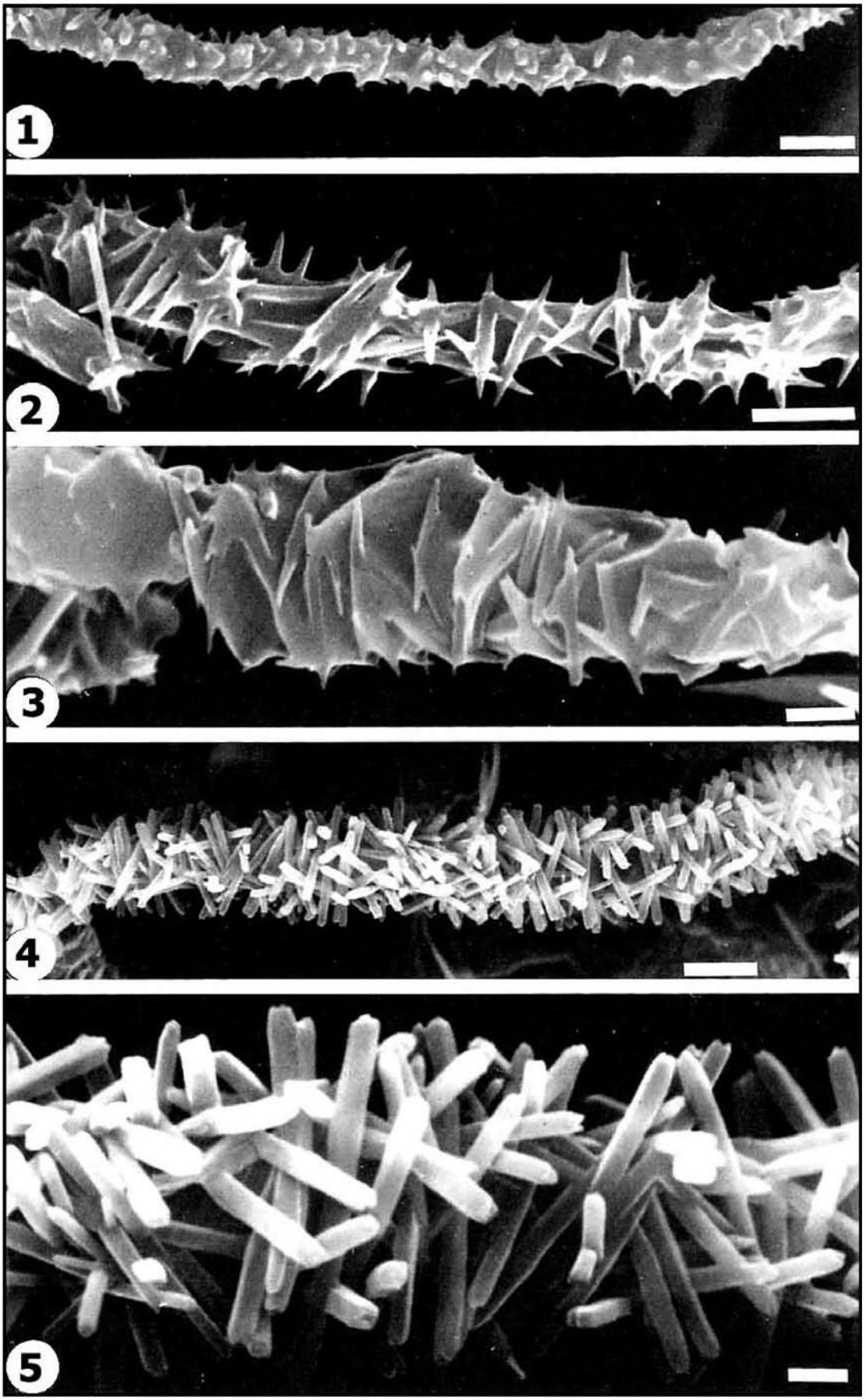


Plate 30. Calcium oxalate crystals on litter fungal hyphae. 1-3. Progressively older sharply pointed crystals associated with the cell wall of fungal hyphae. 4-5. Twin crystals with relatively blunt ends. Note the "fishtail-ends" in many of the crystals in these micrographs. "Fishtail-ends" are a characteristic indication that the crystals are twins. Adapted from Arnott, 1995.

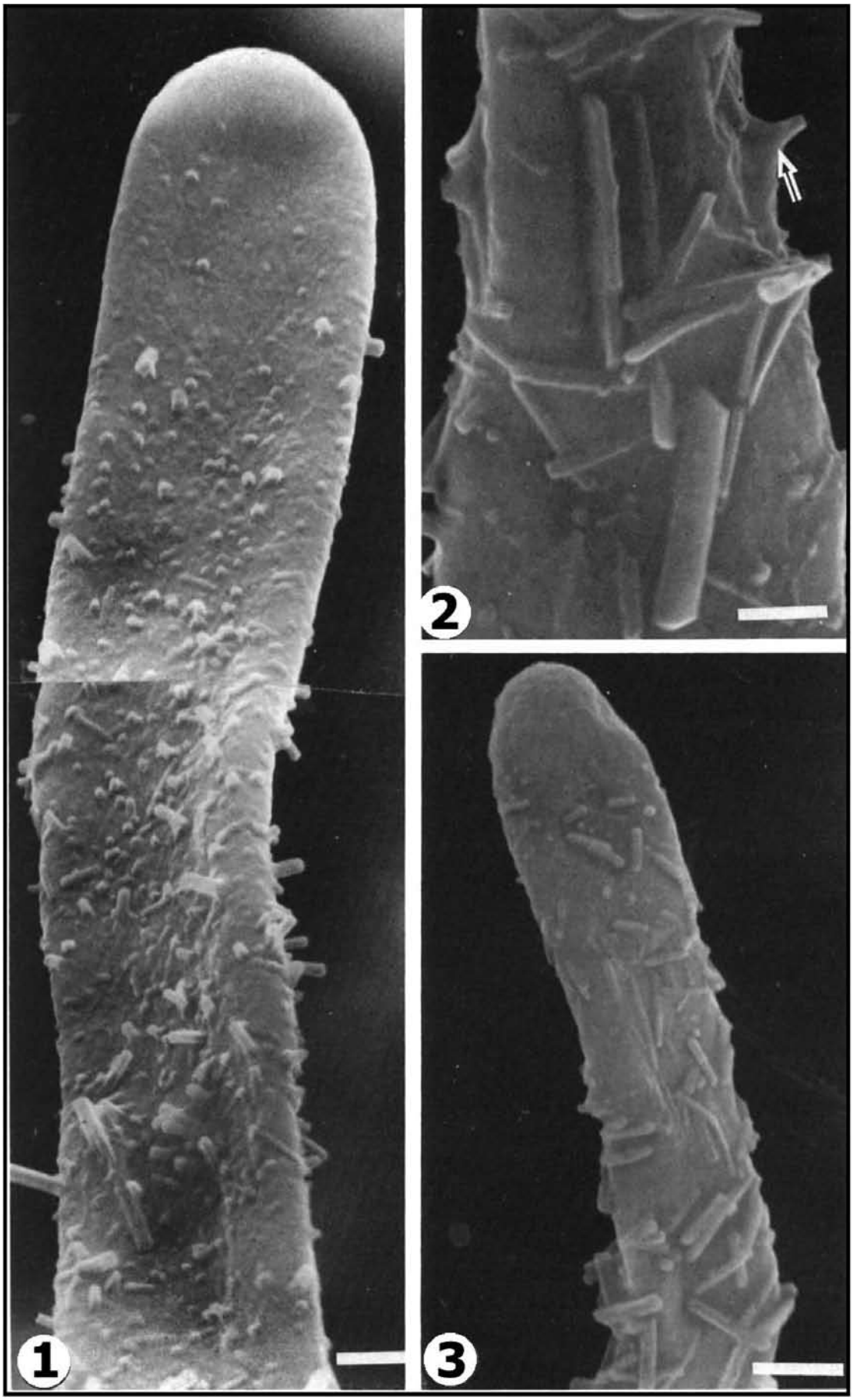
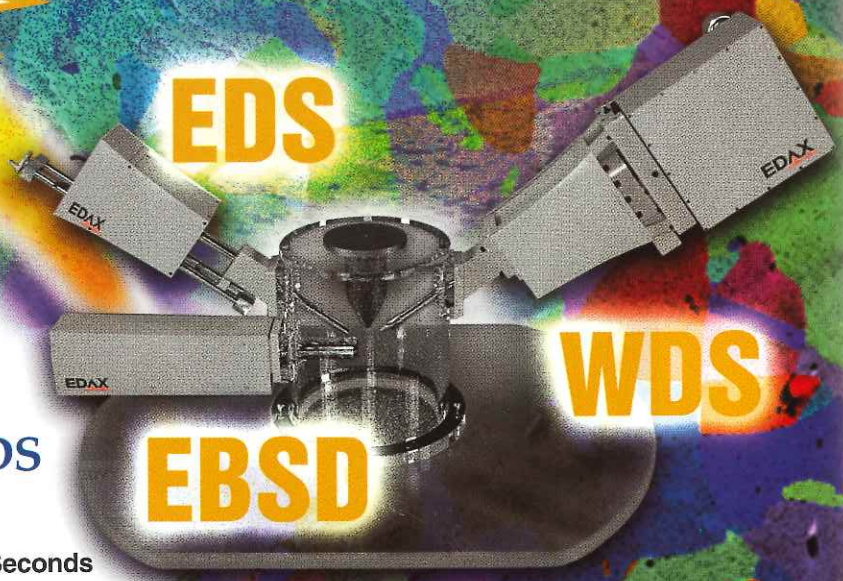


Plate 31. Fungal crystals. 1-3. Developing crystals of CaOx on the surface of leaf litter fungi. 1, 3. The apical dome is free of crystals, which arise in an acropetal manner. 2. CaOx crystals on the surface of hypha. 3. Developing raphide-like crystals on the basal part of this hypha. Adapted from Arnott, 1995.

Autobiography continued on page 73

Advancing Materials Characterization Once Again

New SDD



We've Expanded Our Product Range with the Most Powerful SDD for EDS

- ***New*** **Apollo Silicon Drift Detector Enables Complete Spectrum Maps to be Collected in Seconds**
- ***New*** **Hikari High Speed EBSD Detector**
- **Seamless Integration of EDS, EBSD, and WDS**

The original expectations of the development of the Silicon Drift Detector are now achievable with the EDAX Apollo SDD. Fulfilling the promise of the new technology, the Apollo SDD is capable of collecting high count rates with excellent resolution in an LN_2 free environment. The Apollo SDD, when seamlessly integrated with the LambdaSpec WDS and the Hikari EBSD Detector, provides the ultimate in high speed analysis.

Results with Confidence...Even Faster

The Apollo SDD is the latest innovation from EDAX. As the world's leader in Electron Beam Microanalysis, EDAX continues to raise the performance standard with features that provide faster results that you can trust to be complete and accurate.

For more information on EDAX's latest product developments visit our web site at ***www.EDAX.com/NEW*** *or call* ***1-201-529-4880****.*

EDAX TSL
advanced microanalysis solutions

AMETEK
MATERIALS ANALYSIS DIVISION

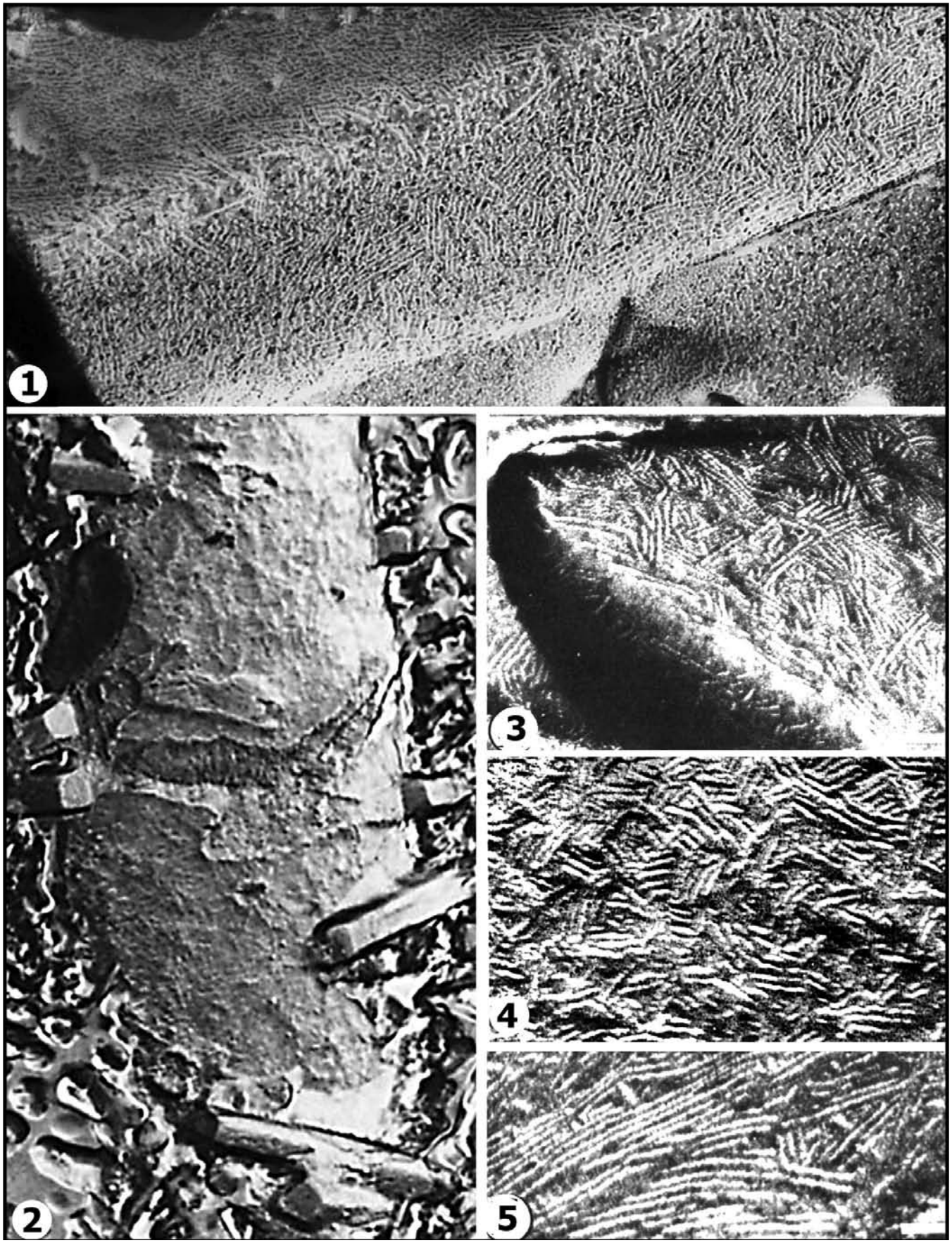


Plate 32. Fungal raphides. 1. Surface view of a freeze-etched fungal raphide. Note the large number of rodlets. 2. Freeze-etched fungal hypha with several attached CaOx raphides, many of which appear to be broken. 3. Freeze-etched druse crystal showing its rodlet-covered surface. 4. Rodlets on the surface of a druse crystal; note the herringbone pattern. 5. Rodlets of a fungal raphide; many are running in the same direction. Adapted from Arnott, 1995.

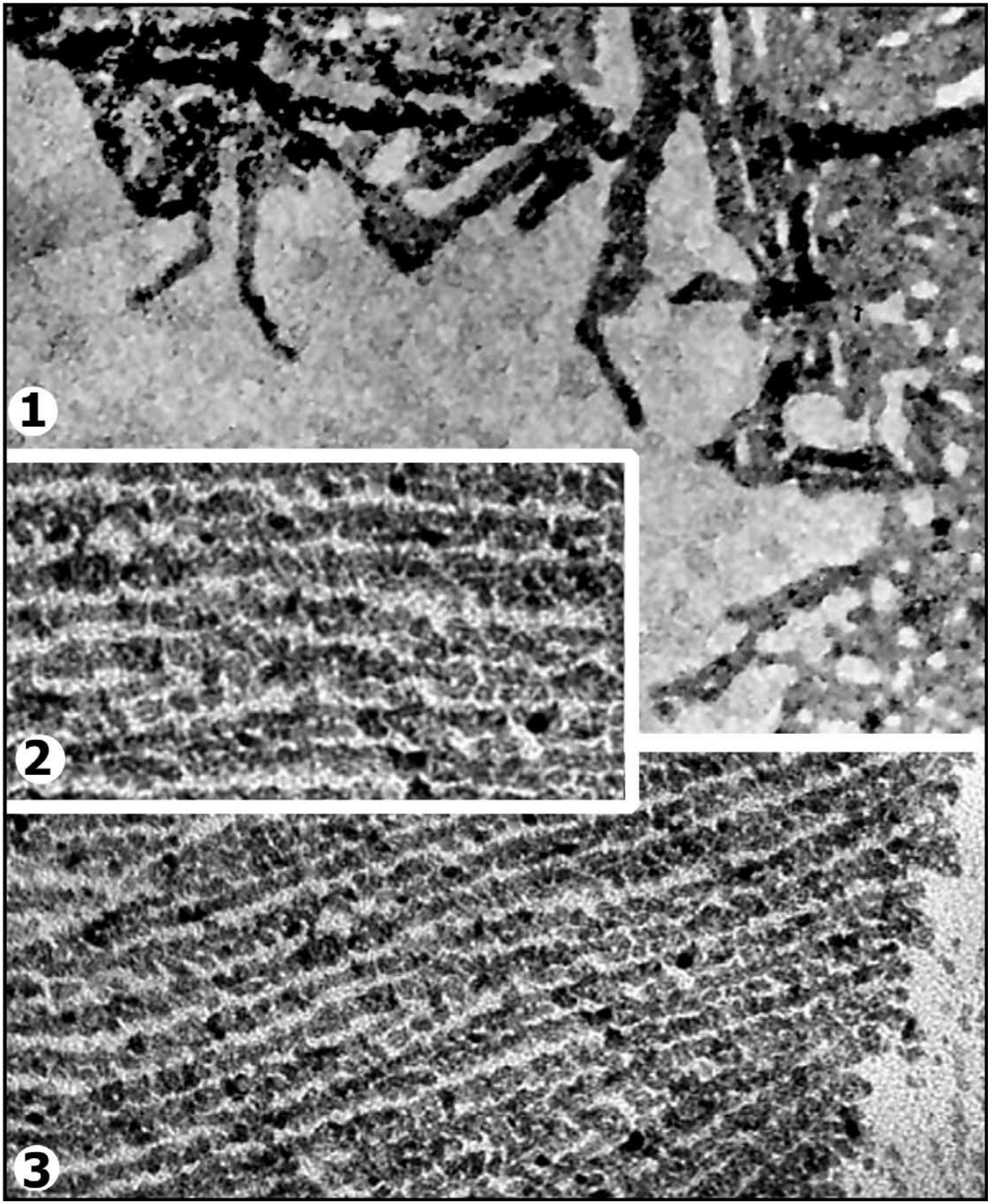


Plate 33. TEM of freeze-etched rodlets found on leaf litter fungal raphides. 1. Rodlets at the edge of a freeze-etch preparation showing the twisted nature of the two strands which make up a single rodlet. In some places the single strand (1/2 rodlet) can be seen clearly. 2, 3. Twisted proteins strands, which make up the rodlets. Strands are twisted in a double helix array. Rodlet diameter is 5.4 ± 0.27 nm. Previously unpublished micrographs by HJA.

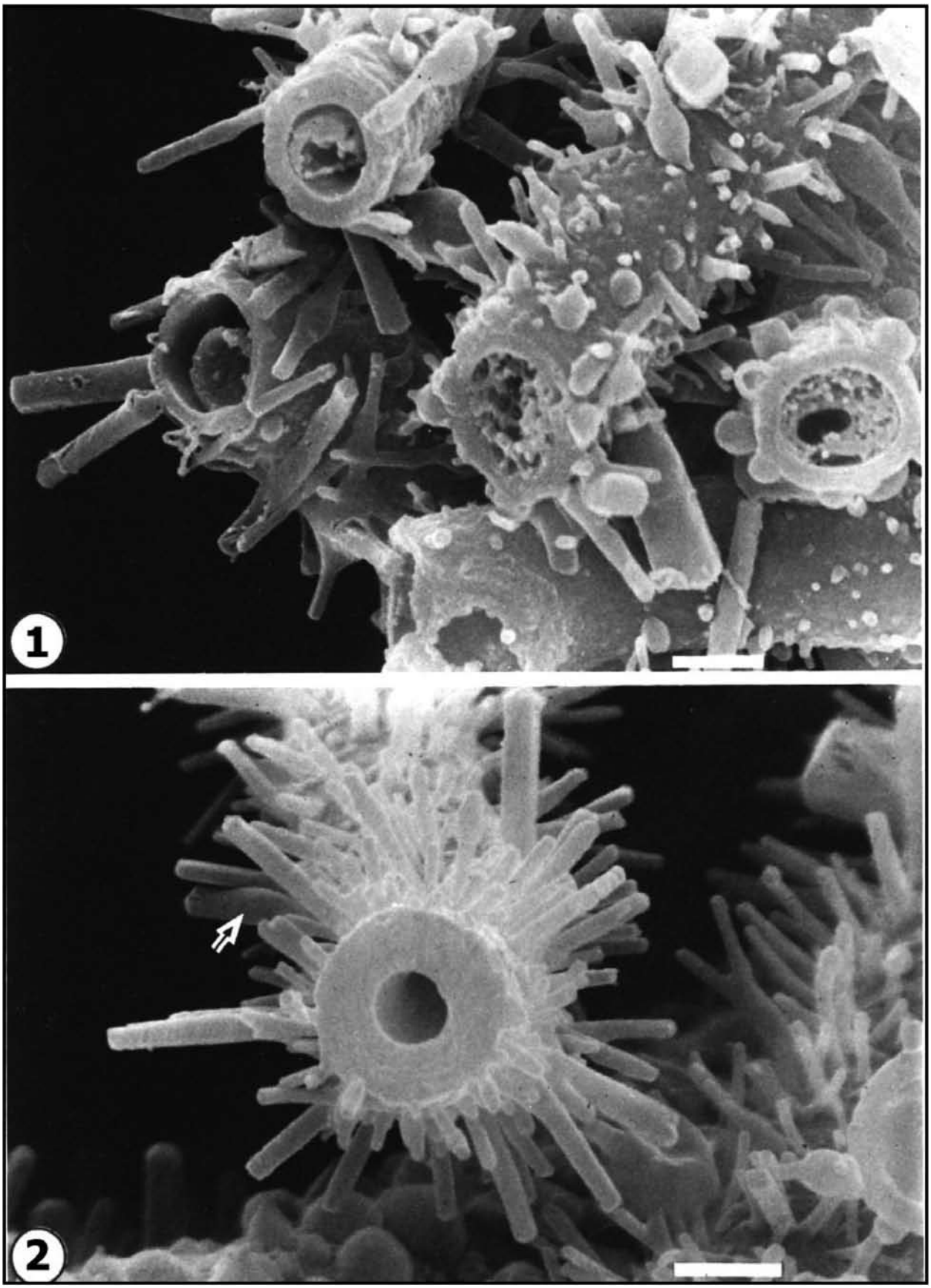


Plate 34. SEM of fungal hyphae collected near the Patriarch Tree in the White Mts. of California. 1. Several hyphae showing examples of "blister formation" of CaOx crystals on the hyphal surface. Note several examples of crystal formation on the central hypha. "Blisters" have crystals extending from their surfaces. Unlike other fungi, these show the crystals extending more or less perpendicularly on the hyphal surfaces. 2. Thick-walled hypha with numerous crystals extending from its surface. At the fractured surface you can see crystals extending directly from the cell wall. Adapted from Arnott, 1995.

erature, among those, several cases that I had identified using x-ray diffraction. Virginiae made countless SEM micrographs of the fungi in each site, over 80 being printed in her thesis. By careful examination of soil maps, she determined that about 2/3 of her collections came from calcareous (limestone) soils and the remaining from non calcareous (neutral) soil types. In her study she had 8 juniper litter samples, that is, samples collected from under juniper trees. Four of the juniper litter samples had fungi with raphide-like crystals and the other four had fungi with druse-like crystals. One sample from under a maple tree (her only broad leaf sample) had raphide-like crystals on the hyphae.

In the 1990's my daughter, Catherine Arnott-Thornton, and I found similar crystals on litter from the Prometheus (WPN-114) site in the Great Basin National Park of Nevada. Prometheus was the name a few Nevada ranchers used to call a bizarre living tree on the Wheeler Peak lateral moraine (Fig. 37). With the approval of the U.S. Forest Service, Prometheus was cut down by Donald Currey in 1964. He cut the tree in order to establish a chronology to help him understand the formation and evolution of the glacier at the foot of Wheeler Peak. When Prometheus was cut down in 1964, it was the oldest living tree ever found, of about 4900 years old. Around 2200 AD, Prometheus will loose its distinction at the oldest living tree ever found as there are some trees in California that are over 4700 years old and still growing. With the cooperation of the park officials, I was given permission to collect litter from the Great Basin National Park including the Prometheus site. On my first attempt to find the site of WPN-114, I fell, cut my leg, hit my head, and lost my fancy pair of sunglasses as I crossed the terminal moraine alone. On the second attempt, with better directions and accompanied by my daughter Catherine Arnott-Thornton, we were able to make the difficult climb to the upper lateral moraine; however, we did not find the Prometheus site. The next day, on our third attempt, we located the Prometheus' remains and stump. We collected litter from around and on the stump, as well as in other nearby areas. The site where Prometheus grew is at the foot of Wheeler Peak at 10,780 feet on the lateral moraine consisting mostly of medium to large boulders (38°59 93 N x 114°17 94 W). We published a short paper on this bristlecone pine litter (Arnott and Arnott-Thornton, 2000) (Plate 34).

Figure 38. Three sporocarps of *Geastrum*. They are generally known as earthstars and would be sitting on or in the litter underneath junipers. Photo by HJA.

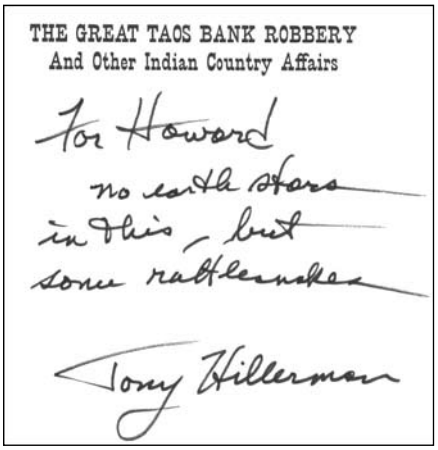


Figure 39. Hillerman dedication on the flyleaf of my copy of *The Great Taos Bank Robbery and other Indian Country Affairs*.

Figure 40. Part of my earthstar collection. Most specimens can be stored in small boxes together with some of the litter they grew on. Photo by HJA.

The problem with litter fungi is that their identification is difficult. Fungi are generally identified by the reproductive parts associated directly with the hyphae under study. Without a "fruiting body" any identification is problematic. However, litter samples do not often produce a clear cut association with reproductive structures. If the hyphae have clamp connections then it can be classified as *Basidiomycetes*, but that is a large and variable group. Litter is also difficult to study because the litter fungi cannot usually be cultured. Although they are very interesting, publication of litter research without specific names is very difficult. Perhaps, in a manner similar to 'naming' used by paleobotanists, litter fragments should be given "type" names such as: *Leafus literii, Composta arlingtonia* or *Juniper litterus utahensis*. A more likely scenario is that investigators will surmount the litter classification problem using genomics techniques; a little bit of DNA can tell a big story, and as far as litter fungi go, it would be almost like having bar codes on them!

Because of the classification problem, I began studying a group of litter decomposed by fungi called earthstars; they belong to the genus *Geastum* (*Basidiomycetes*) and experts can identify them with some certainty (Fig. 38). In the Southwest U.S.A., earthstars are frequently found associated with the litter of *Juniperus*. An earthstar anecdote came from an encounter with Tony Hillerman, the celebrated author of mystery novels set in the Indian Southwest, usually in the Navaho Nation. His books are "a great read" and he is the only author I am addicted to; I think I have read every book he has written! His autobiography relates his upbringing in the Southwest; however, his description of his activities in WWII relates war in a graphic manner few other authors have done! Back to the story, Jean and I were visiting Taos, New Mexico, and we met Hillerman at an evening event where he was talking. Afterward, around a "campfire," I was able to speak to him. I told him about earthstars and why we were in the Taos area collecting them. Later, I asked him to autograph one of his books and he wrote, "*For Howard, no earth stars in this, but some rattlesnakes. Tony Hillerman*." Signed on the flyleaf of my copy of "The Great Taos Bank Robbery and other Indian Country Affairs" (Fig. 39; Hillerman, 1973).

Collecting earthstars in some parts of the world is easy (Maier, 2006) but in the Southwest it is not easy or painless. In the first place, the earthstars are relatively small and often have colors that match the color of the litter they live on and therefore, are difficult to see. Secondly, especially when hunting earthstars associated with *Juniperus* (junipers or cedars), you have to crawl under the low hanging tree canopy in order to find them. In the last 20 years, I have looked under hundreds of junipers and pines, and maybe one out of 50 trees would have a crop of earthstars. In all my collecting sessions I have never seen a snake; but, occasionally, I have seen a lizard, rabbit or squirrel. It may be that I am "heavy-footed" and make a lot of noise as I walk, that is what my Chair, Jonathon Campbell, believes scared the snakes away. Clearly one does not want to meet a poisonous snake while crawling under a juniper or pine tree, whether searching for earthstars or not. Once earthstars are collected they can be easily dried and shelved (Fig. 40). I'm completely sure, that if my old friend Marion Cave were still alive and found some earthstars, she would exclaim, "Oh, aren't they cute."

Now let's have a snake anecdote. In 1990, TSEM met at the YO Ranch Resort in Kerrville, Texas. When the meeting was over,

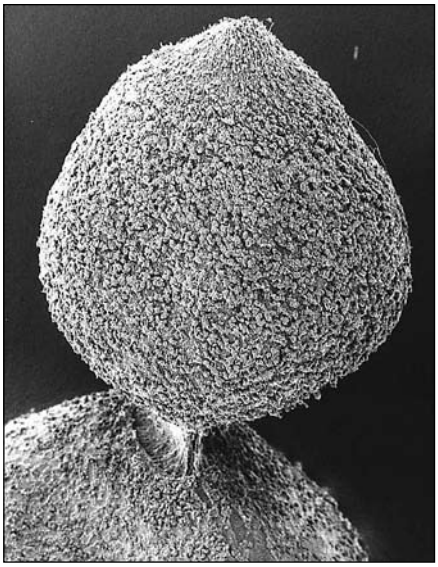


Figure 41. Spore sac of *Geastrum coronatum* covered with crystals of calcium oxalate. SEM by HJA.

I had time to look for earthstars. I walked a couple of blocks from the meeting site and spent about an hour on a grassy limestone hillside in the bright Texas sunshine. It was a fine day. On some of the rocky outcrops I found a few earthstars. After returning to the resort, I told Jean about some new houses at the top of the hill near where I was collecting. We drove up and looked at the houses but they were nothing special and we headed back down on a road that passed the spot where I was collecting. As we got to the collection area, a snake was crossing the road. **Safe** in the car, I drove up near it and we watched it for a few minutes before it headed back into the grass in the same area where I was collecting earlier. As Texas snakes go, it was a modest size western diamondback rattlesnake (*Crotalus atrox*) about 3.5 to 4 feet in length and 2.5 inches in diameter (I didn't really measure it). As I said above, "I never have seen a snake while collecting," but obviously, that snake lived in exactly the place from where I was collecting my earthstars. How many other snakes, poisonous or not, have I "missed?" Investigating "our" rattlesnake on the internet I was astonished to find out that a 92 inch (7.5 feet) western diamondback was captured near Cedar Hill, Texas (UTA Reptile website). I have spent many days looking for earthstars in and around Cedar Hill, in fact one of my prime collection areas was the Camp Wisdom Scout Camp in Duncanville which is only a few miles from Cedar Hill and unbeknownst to me, another prime location for western diamondback rattlesnakes! Hopefully, I will keep my record clean vis-à-vis snake sightings associated with collection (of anything).

Over the years I made a considerable collection of earthstars from Texas, New Mexico, Colorado and Arizona (Fig. 40). Visits to herbaria in the vicinity generally show few examples of these interesting fungi. The development of the fruiting bodies is interesting and can be followed in the laboratory with some species. Litter associated with mature sporocarps can be returned to the laboratory. If kept moist in glass dishes one often can be rewarded by the formation of sporocarps. Sometimes the juvenile sporocarps are already in the litter and the addition of water will hasten their development. The juveniles begin as small knots of hyphae which gradually form a ball sometimes only 1/4 inch in diameter.

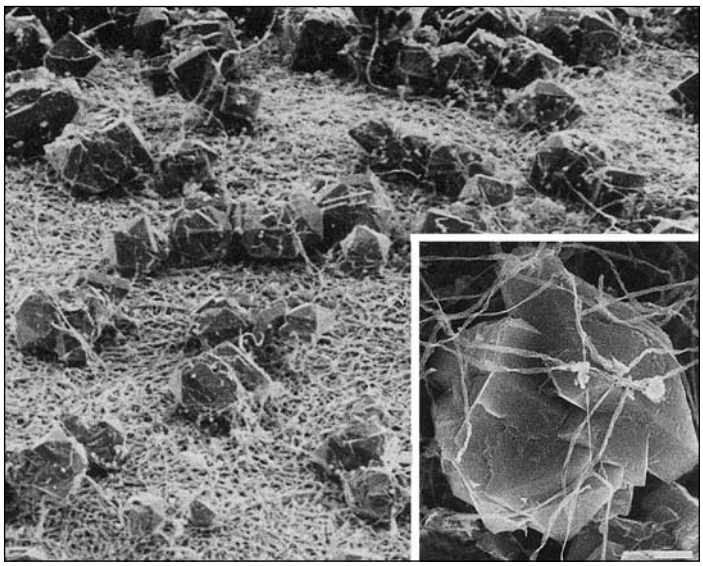


Figure 42. Calcium oxalate crystals on surface of *Geastrum coronatum*. Note, in inset, the hyphae which "tie down" the large crystals to the endoperidium surface. Adapted from Arnott, 1995.

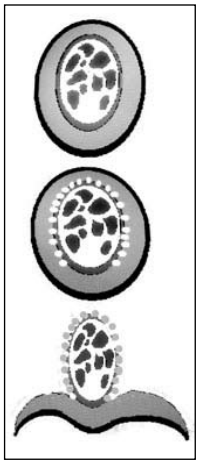


Figure 43. Sporocarp development of *Geastrum cornatum*. Computer drawing by James Arnott.

When the sporocarps of *Geastrum coronatum* are about 3/4 of an inch in diameter they will spontaneously open as mature sporocarps. In the process of opening they will be lifted above the litter in which they developed and their spore sac will be revealed (Fig. 41). *Geastrum coronatum* is the most common earthstar in the Dallas/Ft. Worth area according to my experience.

The spore sac of *Geastrum coronatum* is covered with crystals of CaOx (Figs. 41, 42). Figure 43, drawn by my grandson, James Arnott, when he was 10 years old, accurately shows three developmental stages found in *Geastrum coronatum*. In the upper figure, a young sporocarp is shown in which the wall layers have differentiated. In the middle figure, the sporocarp has begun to differentiate a *crystal layer* between the inner and the outer wall layers. In the lower figure, a sporocarp has opened; opening comes about by the splitting of the wall into pie shaped segments which bend away from the spore sac. This splitting reveals the crystal layer which is then on the surface of the spore sac (Fig. 42). When the wall splits and bends away from the spore sac, the sporocarp becomes star shaped as viewed from above. In some earthstars, the "blades" may open and close depending on the humidity. Dr. Kenneth Whitney and I looked at the development of the sporocarp in *Geastrum saccatum* using the electron microscope (Whitney and Arnott, 1986). We were able to see the development of the sporocarp wall layers, called the exoperidium and the endoperidium which eventually turn into the spore sac containing spores. The CaOx crystals develop at the

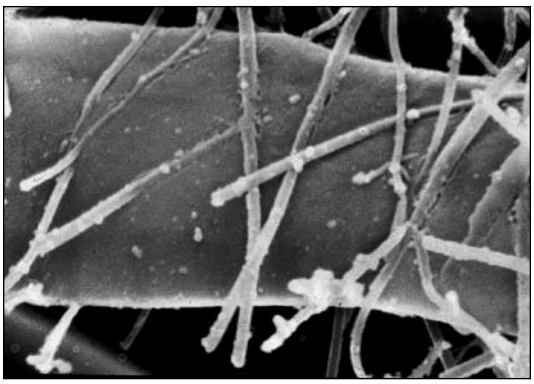


Figure 44. Young hyphae of *Agaricus bisporus* showing very young CaOx crystals, which are partly buried in the cell wall of the fungus. Adapted from Whitney and Arnott, 1987.

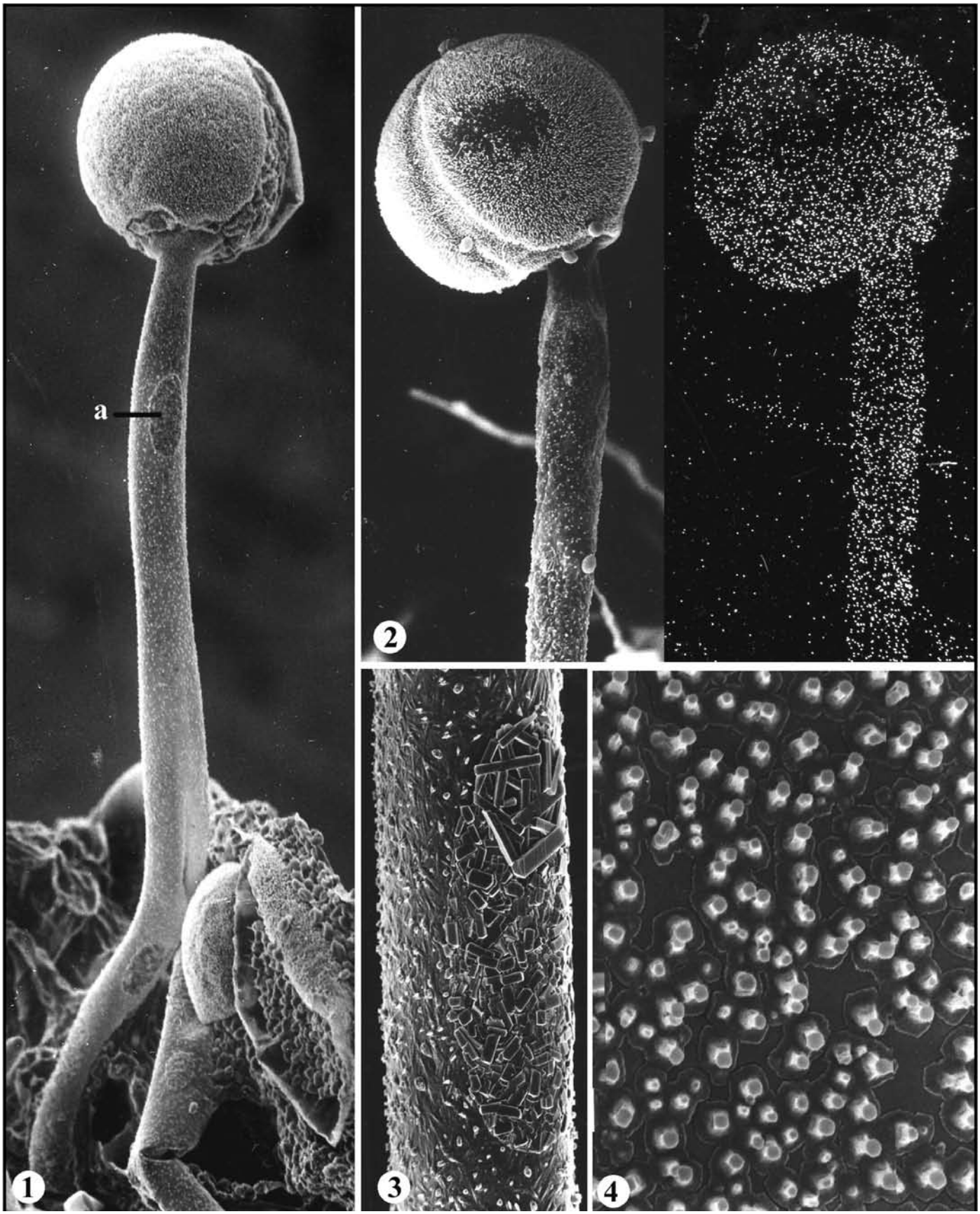


Plate 35. Calcium oxalate crystals on the surface of the sporangiophores of *Gilbertella persicaria*. 1. Sporangiophore extending from the substrate surface showing many CaOx dotting its surface; a = crystal plaques. 2. Sporangium with CaOx crystals covering its surface. Normal SEM on the left; X-ray dot map showing the position of calcium on the right. 3. Sporangiophore with a plaque of CaOx crystals (as in 1a). Note the individual crystal nature of the crystals in the plaque. 4. Surface of a sporangiophore showing the individual "spike-like" crystals attached to base plates . Adapted from Whitney and Arnott, 1986; 1988.

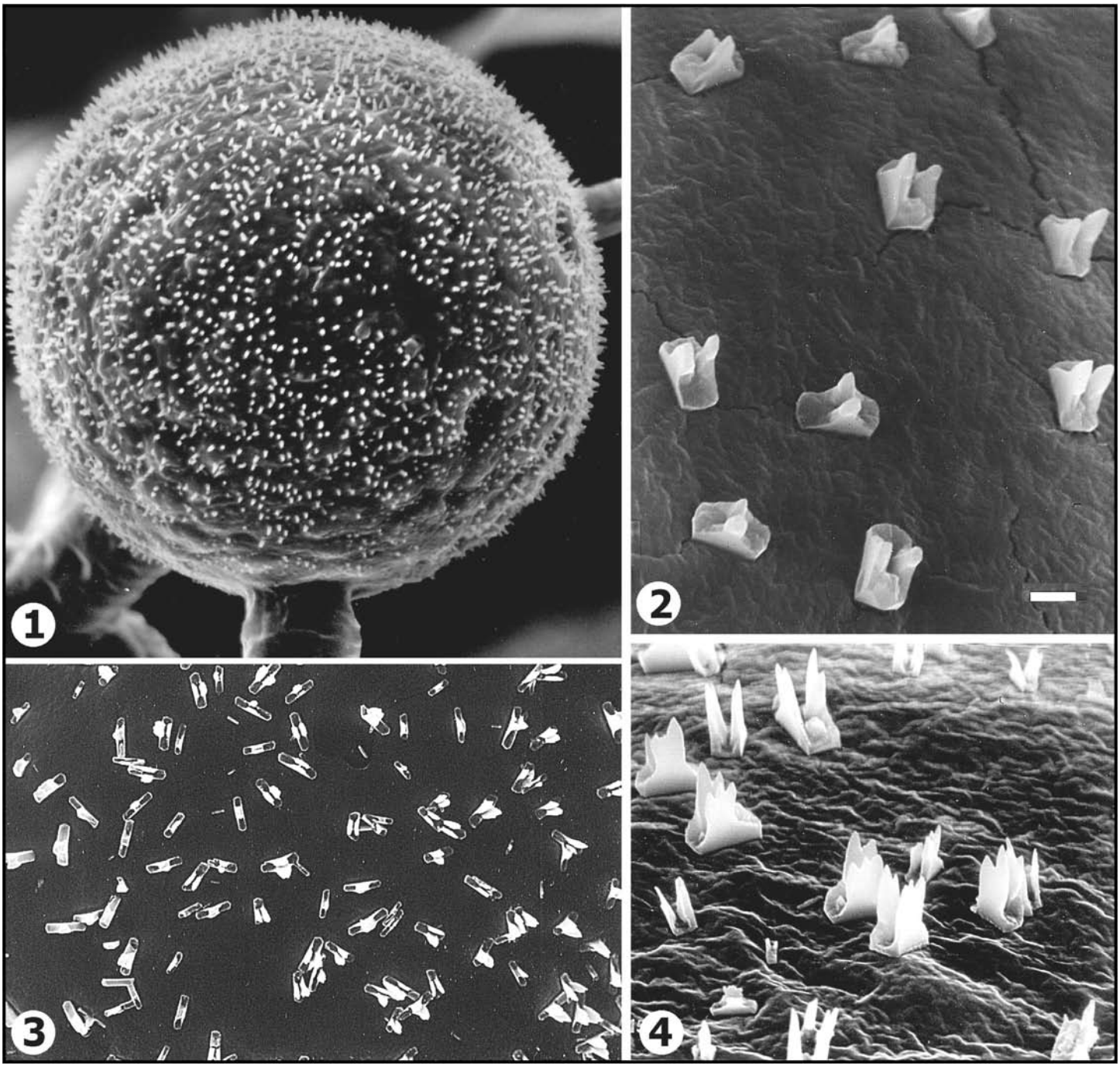


Plate 36. Calcium oxalate crystals on the surface of the sporangiophore of *Mucor hiemalis*, a member of the *Mucorales*. 1. Numerous CaOx crystals on the surface of a sporangium. 2. Bidentate teeth attached to a base plate. 3. Numerous bidentate CaOx crystals on the surface of the sporangium. 4. Bidentate crystal ornaments; each tooth is multi-spiked. Adapted from Powell and Arnott, 1985.

Autobiography continued on page 82

DiATOME
diamond knives

Development, Manufacturing, and Customer Service since 1970

What have we achieved in this period?

ultra 45° the first diamond knife with an absolutely score-free, hydrophilic cutting edge.

semi the first diamond knife for alternating sectioning ultrathin/semithin.

cryo the diamond knife for sectioning at low temperature.

histo the first diamond knife for semithin sections for light microscopy.

ultra 35° the diamond knife for optimized sectioning results in almost all applications.

STATIC LINE II the ionizer for eliminating electrostatic charging in ultramicrotomy.

cryo-P a cryo knife with a patented platform for section pick up.

cryo immuno the optimized cryo diamond knife for the Tokuyasu technique.

ultra sonic the oscillating diamond knife for room temperature sectioning.

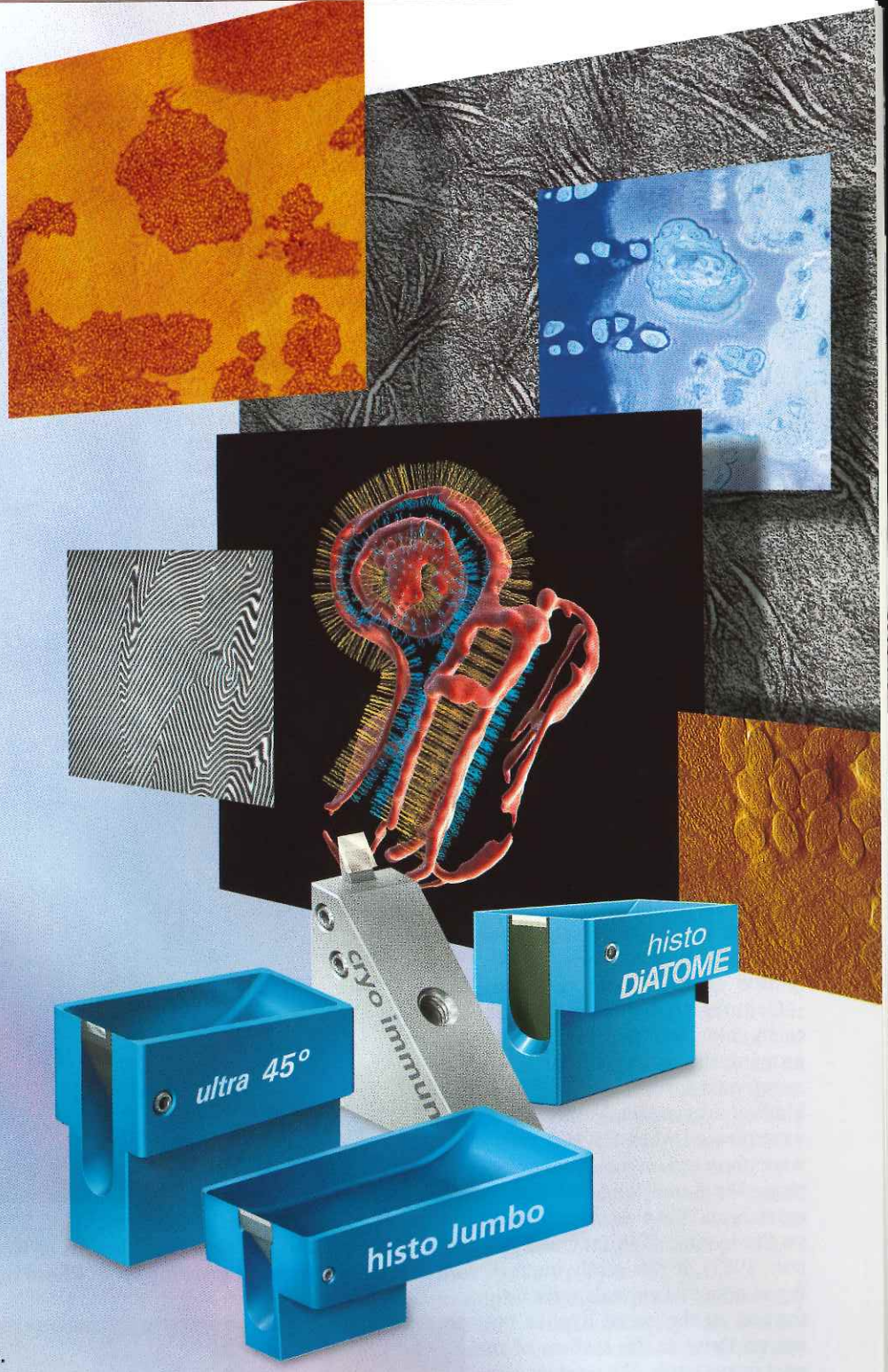
cryotrim 45 and 25 optimizing trimming with diamond blades.

ultra AFM & cryo AFM the first diamond knives for AFM at room and low temperatures.

cryo 25° for sectioning frozen hydrated specimens.

What services can we offer you?

- Technical assistance in all fields of ultramicrotomy.
- Free sectioning tests for all types of samples.
- Make use of our many years of experience in perfecting our knives.
- Custom knives, tools, and boats.
- Special purchase programs.
- Workshops and training.



For more information, please call or write us today, or visit us online at:

www.emsdiasum.com

DiATOME
for all your sectioning requirements

P.O. Box 410 • 1560 Industry Rd.
Hatfield, Pa 19440
(215) 412-8390 • Toll Free: 1-(800) 523-5874
Fax: (215) 412-8450 or 8452
email: sgkcck@aol.com • stacie@ems-secure.com
www.emsdiasum.com

exoperidium-endoperidium line and are formed on the surface of the hyphae. After sporocarp dehiscence, the crystals are exposed to the environment and are subject to erosion and recrystallization.

You may be still wondering, "Why collect earthstars?" The reason is not just that earthstars sometimes produce crystals of CaOx on their sporocarps (Figure 41, 42), but rather that they often produce CaOx crystals on their subterranean hyphae that are part of the litter layer. Since you can identify earthstar sporocarps you can then identify the litter hyphae that are associated with them.

KENNETH WHITNEY – EARTHSTARS AND OTHER FUNGI

As my interest in calcium oxalate associated with fungi increased, it was very reassuring to have Ken Whitney join my laboratory as a post doctorial associate. Dr. Kenneth Whitney received his doctorate from the Department of Botany at the University of North Carolina where he was trained in the traditions of modern mycology. Because of his knowledge and experience, it was my hope that we might be able to deal with the problem of litter identification. I have already mentioned the earthstar work but let's now examine some other fungi that produce CaOx crystals.

One of the first things Ken and I began to study was the CaOx crystals that occur on *Agaricus bisporus* (the common button mushroom commercially available). Calcium oxalate covering the hyphae of *Agaricus camprestris* was first described and illustrated by De Bary (1887); his drawings showed long crystals on the hyphae which, according to him, had "a chalky white appearance." You may remember that **Heinrich Anton de Bary of Strasbourg** is in my scientific linage (see Part III). We choose *A. bisporus* because it could be cultured on defined media in Petri dishes, completely free of any other organisms.

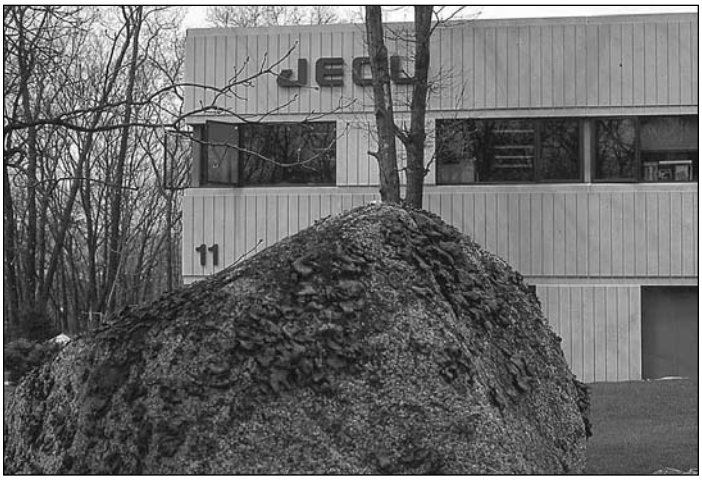


Figure 45. JEOL office in Peabody, MA. The large granit rock in foreground has many specimems of Rock Tripe, *Umbilicaria pennsylvanica*, growing on it. Photo by HJA.

Cultures of *A. bisporous* were easily obtained and Ken began growing the fungus in our laboratory on rye seeds and different kinds of agar media. Naturally, we were pleased when CaOx crystals were observed on the cultured hyphae. We found long rod-shaped crystals and flat plate-like crystals on the hyphae (Whitney and Arnott, 1987). It was really interesting to note that crystals were only formed on the aerial hyphae but not on those at the surface of the agar or rye seeds. We used both energy dispersive x-ray analysis and x-ray diffraction to show that the crystals had calcium, and secondly that the mineral was calcium oxalate dehydrate (weddellite). In this paper we were able to demonstrate by using both TEM and SEM that the CaOx crystals arise internally. Very young crystals were noted on the hyphae as they began their development within the wall (Fig. 44).

While Ken was at UTA, we investigated the development of CaOx crystals on the sporangiophore and sporangium of *Gilbertella persicaria* (*Mucorales*) (Whitney and Arnott, 1988). During the formation of sporangiophore and sporangium, a layer of CaOx crystals forms on their surfaces. This layer of crystals consists of a series of individual crystals attached to the sporangiophore and sporangium components of the fungus (Plate 35: 1, 2). There are four different classes of "crystals" found on *G. persicaria* surfaces: 1) simple, elongated, flat plates found throughout the sporangiophore, with or without an upright component; 2) simple spines, consisting of a flattened plate and a single upright portion; 3) complex spines, made up of three components: a flattened plate, an upright spine and a hexagonal cap; and 4) crystal plaques consisting of piles of elongate bipyramidal crystals with oval outline (Plates 35, 36). All of these crystals have their origin within the cell wall of these fungi. Some crystals appear to protrude through the thin wall component which overlies the crystal during its early development.

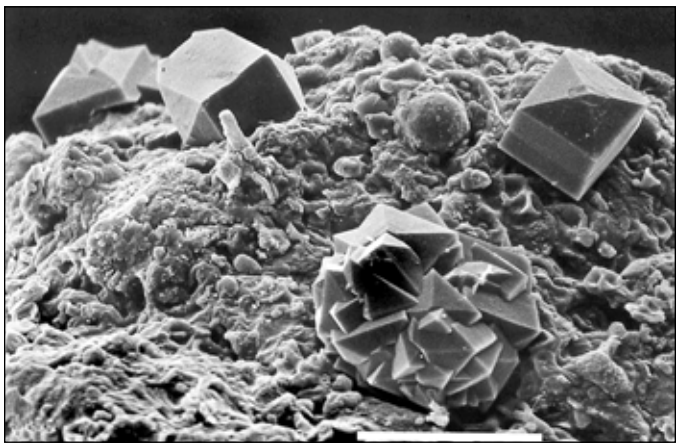


Figure 46. Large calcium oxalate dihydrate crystals on the surface of Washington's Rock Tripe, *Umbilicaria pennsylvancica*. Unpublished micrograph from collection of HJA.

Ken and I published a second paper on *G. persicaria* which dealt with the response of this fungus to calcium (Whitney and Arnott, 1988). Using liquid rather than agar cultures we found that the hyphae without added calcium oxalate soon began to form aberrant and fewer crystals on the sporangiophores. The same culture media with added calcium supported the normal production of CaOx crystals with normal morphology. In the liquid culture without added calcium the fungus grows very well but does not form the normal complement or shape of crystals. Presumably, the hyphae soon use all the calcium in the liquid medium and hence can no longer form a normal complement of crystals. The small amount of calcium in the normal medium is used in other "more important" cellular functions. One can also assume that, with low concentrations of calcium in the medium, there is no need to detoxify the surrounding environment through the formation of CaOx crystals. When calcium is added to the medium, crystals are formed in the normal manner. In our first paper on *G. persicaria*, we suggested that the calcium oxalate mineralization may play a role in sporangial support. That contention is supported by the fact that in liquid medium without added calcium the sporangiophores bend and do not seem to be as stable as those that are normally mineralized. Clearly, the amount of calcium in the medium is reflected in fewer crystals, aberrant crystals, and in "drooping" sporangiophores.

Mike Powell, a microbiology student in our department, and I became interested in the mineralization of the reproductive structures of certain members of the *Mucorales* (Powell and Arnott, 1985). We investigated the reproductive structures of *Mucor hiemalis* and *Rhizopus oryzae*, and found CaOx crystals on the sporangial walls, which were very different in the two species. We suggested that the CaOx crystal configuration might be useful in the *Mucorales* taxonomy.

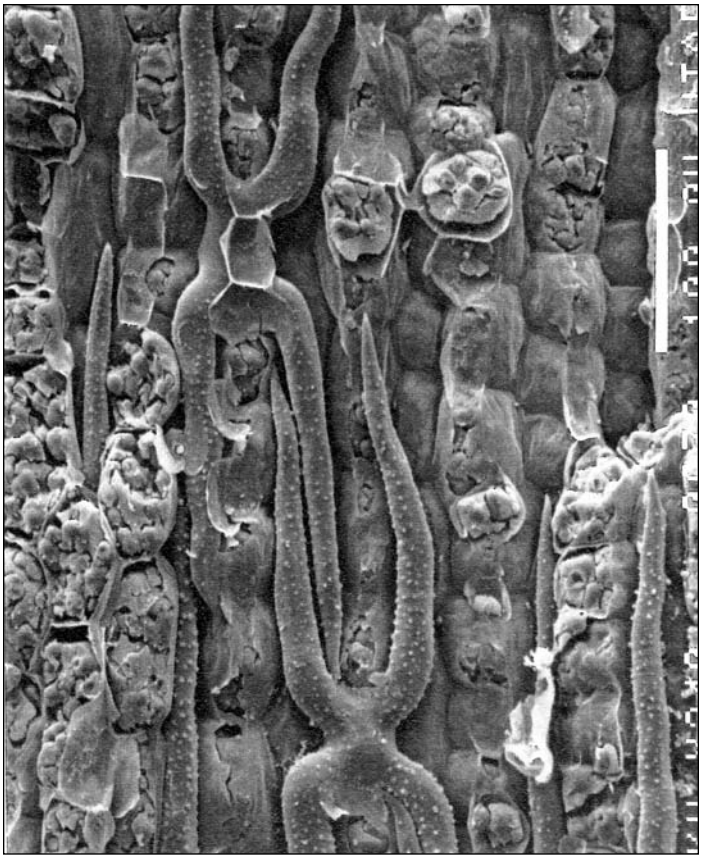


Figure 47. Crystal sclereids of *Nymphaea mexicana*. Note CaOx crystals imbedded in the cell wall of four different sclereids in the storage root. Adapted from Van De Veire, 1997.

On a visit to the JOEL office in Peabody, Massachusetts, I discovered some interesting leaf-like "plants" growing on the large granite boulders which were frequent in the area (Fig. 45). At the time, I was taking a course on the EMS 1200 TEM that we had just purchased. I took a few of these leaf-like structures back home and determined that they were *Umbilicaria pennsylvanica*, a lichen species. I also found that one of its common names was "Washington's Rock Tripe." This lichen is associated with George Washington because when the Revolutionary War soldiers he commanded at Valley Forge were hard pressed for food, they boiled and ate the rock tripe.

I was fascinated by rock tripe, not only because they had many crystals of CaOx, but because of their "reputation" as "emergency food." Large multi-interpenetrant twins and single crystals were found on the surface and in the interior of these interesting "plants." On a later trip, I collected a large number of the leaf-like thalli for SEM studies (Fig. 46). I studied both the dried thalli and hydrated fixed specimens. I also boiled them for some time and found that the plentiful crystals of CaOx did not dissolve. Calcium oxalate is insoluble in water (Merck Index), even in boiling water. Soldiers of the Revolution and/or others that have eaten *U. pennsylvanica* would have got a good dose of oxalate when the crystals dissolve in their stomach acids. On another occasion, while in Massachusetts, I had the opportunity to work with (then) the newly developed ESEM, and among other things, I collected *U. pennsylvanica* to look at the thalli in a hydrated state. The walls were much thicker and wrinkles in the cell contours were erased when hydrated. In some cases, it appears that the CaOx crystals form in the fungal component of the lichen since examples of crystals attached to the hyphae were found. The crystals appear to be embedded on the surface of the thallus and thereby partly surrounded by hyphae. However, some crystals seem to be free within the "tissues" of this "plant." Although twinning is common, many single large prismatic crystals of CaOx dihydrate were also present (Fig. 46).

One of the last students to work on plant crystals in my laboratory was Jackie Van De Veire. The subject of Jackie's thesis, completed in 1997, was on the large multi branched crystalliferous sclereids which occur in the storage roots of *Nymphaea mexicana* (Van De Veire, 1997). This water lily produces unique storage roots in which large masses of starch are stored in the cortical parenchyma. In addition, these roots produce very large sclereids which have crystals embedded in their cell walls (Fig. 47). The sclereids are unique as they may have five or more cellular branches, all of which run in a parallel direction and more or less in one plane. The arms of these sclereids extend by intrusive growth forcing their way between other cells in the cortex. The sclereids are also notable for the large CaOx dihydrate crystals that are embedded in the cell wall. Jackie worked out the morphology and development of these unique cells and she established the role these storage roots play in the life history of *N. mexicana*.

Figure 48. Sandra Westmoreland, "The egg shell lady", about 1998. Photo by HJA.

Crystals are not just found in association with specialized cell types. They are often found in the cytoplasm or in organelles such as microbodies, mitochondria and chloroplasts and even in the cytoplasm proper. I studied them in corn phloem (Plate 37) and in *Malpigia glabra* flowers, which have cells with crystals in their mitochondria. These crystals may occupy much of the volume of some mitochondria. The lattice planes of these crystals can be seen with the TEM (Plate 37: 1-6). The line to line measurements can be on the order of 50-100 Å. Proteins are probably the only molecules which are large enough to form lattice planes of these dimensions. I always have assumed that the above crystals were proteins which underwent crystallization being present in excess in the mitochondrion. These "protein" crystals often seem to be nucleated on mitochondrial membranes. *Malpigia* also produces large "protein crystals" in the cytoplasm (Plate 37: 4).

Crystals are also found in single cell organisms. The bacterial magnetosomes are partly crystalline iron sulfide crystals and are found in a few bacterial species. *Euglena granulata* is a eukaryotic single cell organism that produces crystals. The cells of *Euglena* are somewhat complex; they have a nucleus, mitochondria, chloroplasts, an intricate folded external structure called the pellicle which allows them to change shape, a very large Golgi apparatus with 30 to 50 or more cisternae, a contractile vacuole, and two flagella, one which is associated with the large eyespot. The cells have the ability to change their shape from spherical to elongated and back, and to swim backward or forward. These characteristics certainly establish *Euglena* as one of the "one-cell wonders".

E. granulata also makes crystals, hence the specific name *granulata*. The crystals can be seen as bright spots when viewing cells under polarized light (each spot representing a single crystal). The *E. granulata* crystals are believed to be calcium oxalate as they are extracted in the same manner as the CaOx crystals in higher plants; however, unambiguous identification of these crystals has not been achieved yet. The specimens I studied came

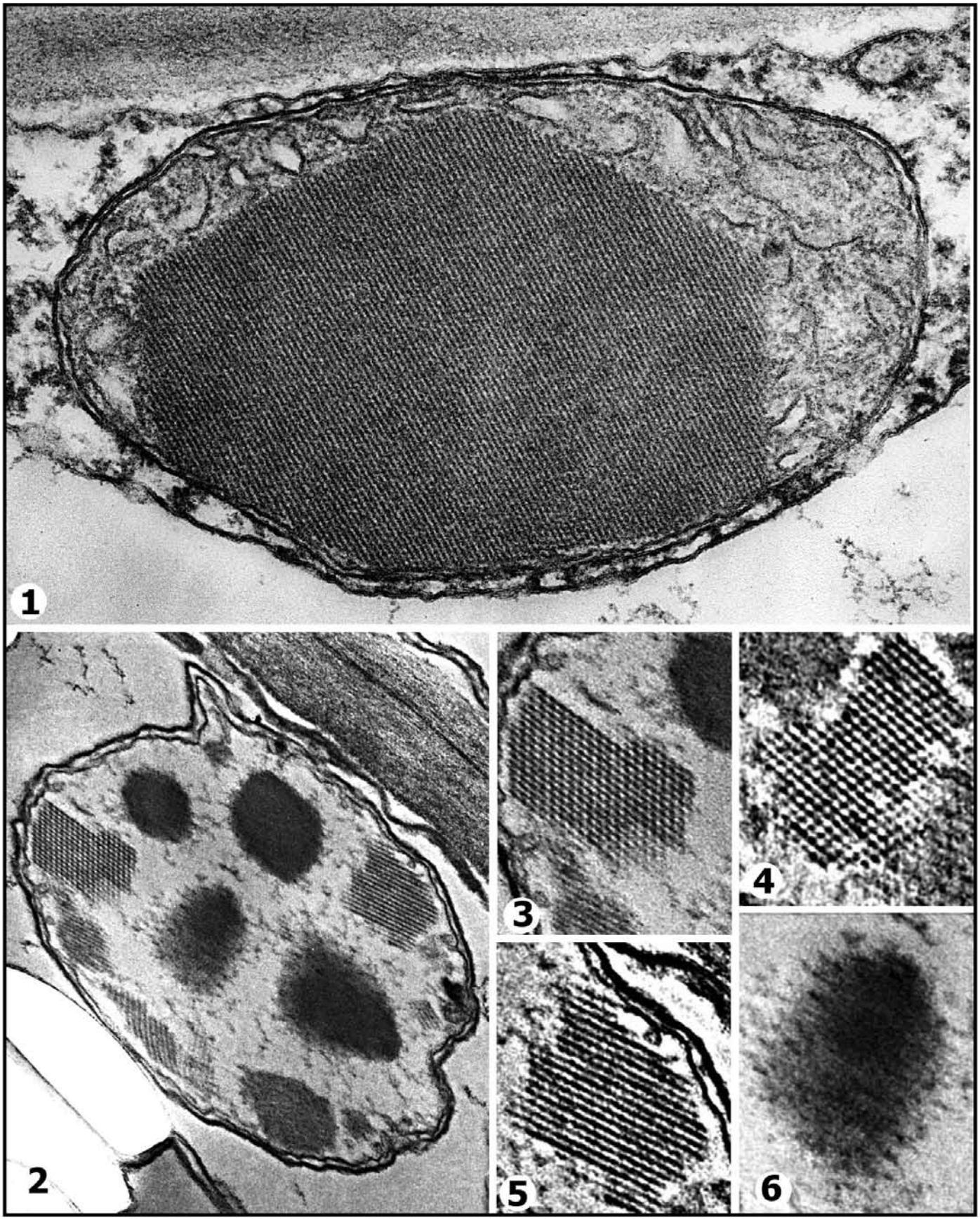


Plate 37. Protein crystals in cellular compartments. 1. Protein crystal in a mitochondrion of *Malpigia glabra*. Lattice lines are clearly seen and the overall shape of the crystal is clear with relatively sharp facets seen on four sides. Note also the close association with the inner membrane of the mitochondrion. 2. Plastid in the phloem of corn; the lattice lines are sometimes clear. 3, 5 and 6. Enlargements of phloem plastid crystals seen in 2. 4. Protein crystal in the cytoplasm of *M. glabra*. Unpublished micrographs by HJA.

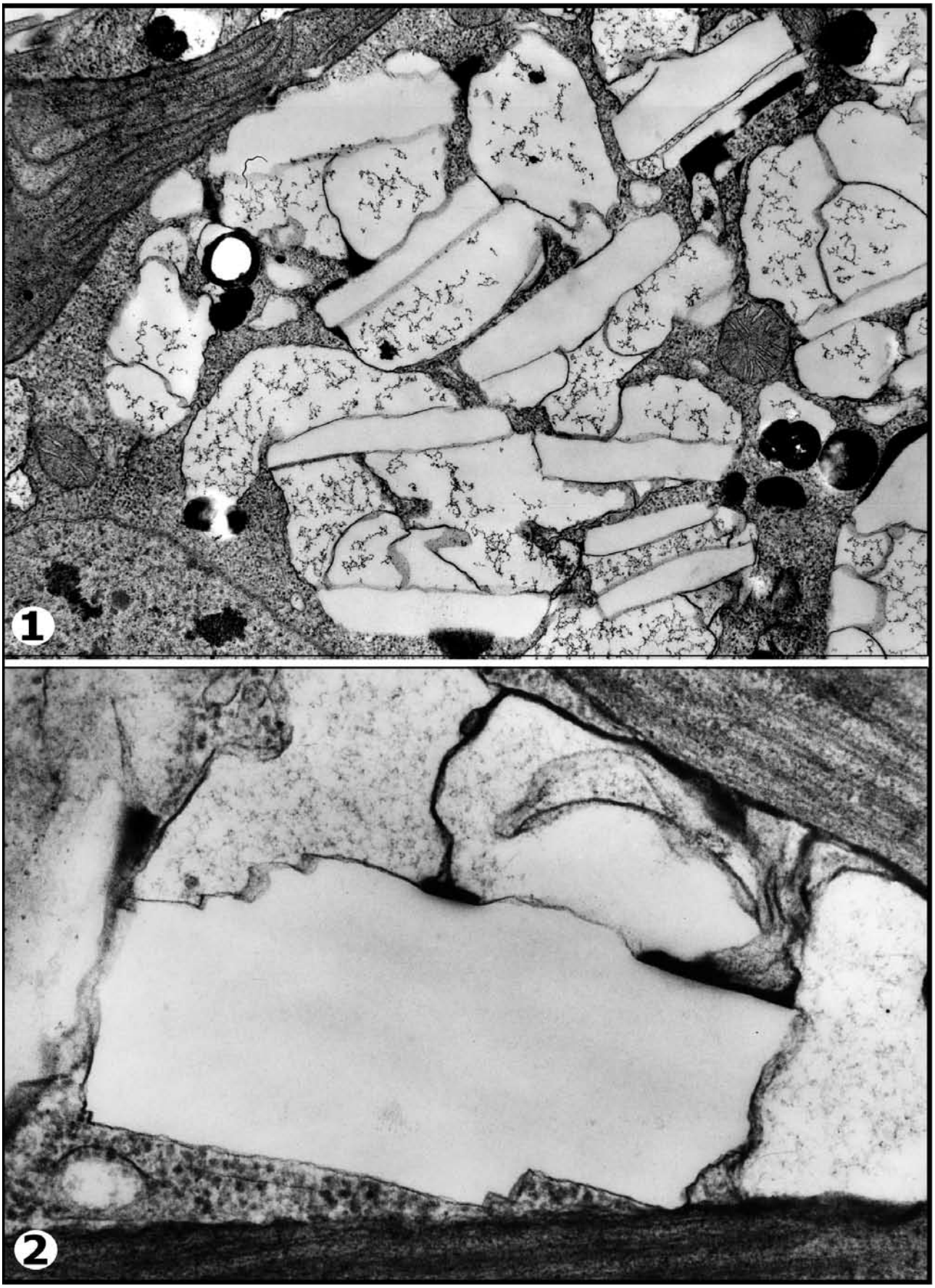


Plate 38. Crystals of *Euglena granulata*. 1. TEM of a *Euglena granulata* cell containing numerous crystals. A portion of the chloroplast, the nucleus and several mitochondria are visible. Note that the individual crystals are each associated with a vacuole and each possesses a crystal chamber of its own. 2. Enlarged view of a crystal chamber membrane, which follows the structure of many crystal facets; the crystal was extracted from the chamber during fixation of the specimen. Note the membrane connections that cross the vacuole from the cytoplast to the surface of the crystal chamber. Unpublished micrographs by HJA.

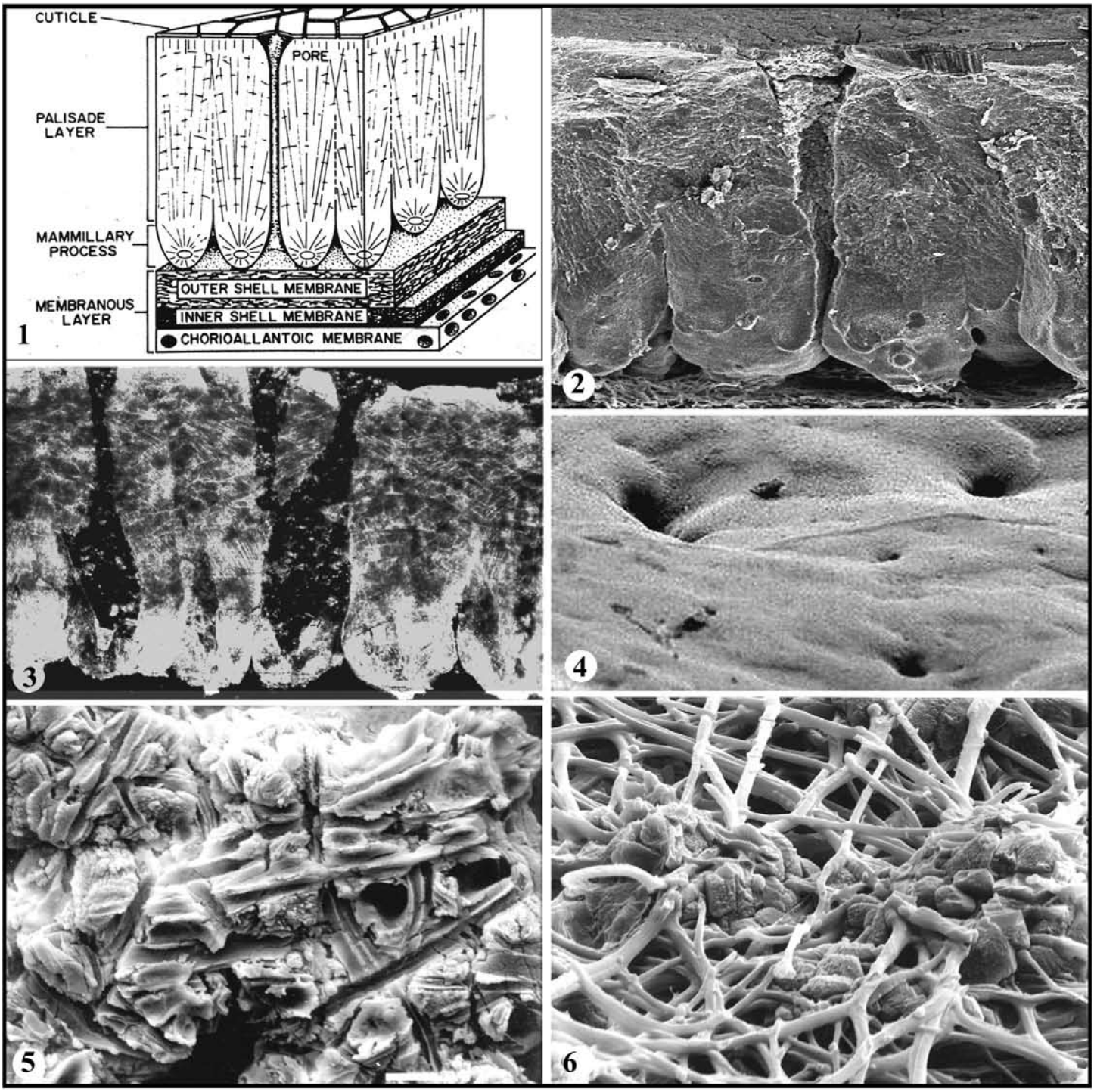


Plate 39. Crystal system of the chicken egg shell. 1. Diagram showing the various components of the egg shell. The palisade layer is made up of crystals of calcium carbonate. 2. Fractured egg shell showing an excellent example of a shell pore, which runs from the external surface into the internal surface of the egg shell. Shell thickness is approximately 500 microns. 3. Thin sections of egg shell showing seven large calcium carbonate crystals under polarized light. Two central crystals are at their extinction point. 4. Pores on the surface of the egg shell. 5. View of the mammillary processes with striations representing the position of the outer membrane fibrils, which have been extracted. 6. Two mammillary processes with the outer membrane fibrils still in place. Adapted from Westmoreland, 1998, 2001.

from the fish pond next to the Biological Sciences Building on the UT Austin campus and they were identified by Dr. Harold Bold. The same *E. granulata* cells were used for papers on the eyespot apparatus (Walne and Arnott, 1967), and another paper on the "apparatus of metaboly" (metaboly is the term used for rapid and reversible cell shape change exhibited by euglenoids) (Arnott and Walne, 1967). I investigated the "granules" (crystals) using TEM (Plate 38). As in higher plants, the crystals in *E. granulata* are produced in vacuoles. Within the vacuole, each crystal is formed within a crystal chamber defined by a membrane which shows the shape of the crystal. The crystal chamber may have connections with the cytoplasm. The micrographs of Plate 38 show that the crystalline material was extracted during processing specimens for TEM. If the crystals in *E. granulate* are CaOx crystals, then they would be classified as prismatic crystals. Obviously there is much more to learn about the crystals in this remarkable organism.

A recent group of my graduate students, Sandra Westmoreland (Recent Past President of TSM), Natalie Hubbard and Tina Halupnik (current Secretary of TSM) have been interested in the structure of avian egg shells. Egg shells have a crystalline component; hence, this makes their work a part of my crystal trail, albeit close to the end of the trail. Sandra Westmoreland (Fig. 48) investigated the nature of egg shells using light and electron microscopy for a good part of the last decade. Sandra began her avian work investigating the eggs of 19 species of birds as a project for my SEM class. This initial survey proved to be interesting and showed that there was still much to learn about avian egg shells (Westmoreland and Arnott, 1997). Sandra continued her studies of avian eggs both for her MS and Ph. D. research work, which produced the thesis entitled "Ultrastructural changes in the mammillary cones during development of fertilized eggs of *Gallus domesticus*" (Westmoreland, 1998) and the dissertation "Variation in the eggshell pore system of the white Leghorn chicken" (Westmoreland, 2001). With over 8500 species of birds (Ernst Mayr, 1946), why choose chicken eggs? There were several reasons for choosing the chicken egg but the following three are the most important: 1) chicken eggs are well studied, therefore representing the foremost model for the avian egg, resulting in a substantial scientific literature resource; 2) there were (still are) many things unknown about their structure; and 3) chicken eggs are a major part of a multibillion dollar poultry industry; hence the research has very good potential for obtaining financial support.

Sandra's work discovered many new features of the chicken egg shells, but here I can only review few of them (Plate 39: 1, 2). As pointed out above, there is a very strong crystalline component as calcium carbonate crystals in bird eggs. In fact, the main structural feature of shells is the columnar or palisade region which consists of large rod shaped calcite crystals (calcium carbonate) arranged tightly side by side. A cuticle layer covers the shell outer surface. The inner part of each calcite crystal forms a mammillary cone, which collectively forms the mammillary layer. Inside the mammillary layer the outer shell membrane is found and inside that, the inner shell membrane; both of the latter are complicated mesh-works of protein strands. The columnar region is made up of single crystals of calcite with their long axis orientated in a radial fashion. Using thin shell sections, the individual crystals making up columnar region are easily demonstrated using polarized light (Plate 39: 3). The inner end of these crystals is modified to form the mammillary cones which are partly imbedded in the mesh work of the outer shell membrane. The nature of this relationship was shown very clearly in Sandra's MS thesis (Westmoreland 1998).

The pores of the egg shell provide an avenue for gas transfer and are situated between the individual calcite crystals (Plate 39: 1, 2). One of Sandra's most important pieces of research was the design and development of an image analysis program to automatically count the pores per unit area. The number of pores per area is important because the health of the developing chick inside the shell depends on the gas exchanges through these pores. In checking the accuracy of her measurements, Sandra ran the traditional gas exchange experiments of similar eggs. The exact nature of the shell pores is difficult to determine by looking at either sections or fractured shells because it was only rarely that the pores followed the fractures. However, using an ingenious method of embedding the shell in plastic under high pressure, followed by the removal of the calcite by dissolving it in HCl solutions, it was possible for Sandra to demonstrate the exact shape, size, and orientation of the pores. Practically, the pores were studied as a plastic replicas (Westmoreland, 2001).

Using the SEM, Sandra found that the calcite crystals, after treatment with bleach, are permeated by small holes which form a complicated internal system of channels within the calcite crystals. Investigation of unbleached shells using histochemical stains for proteins showed that many protein strands extend through the inside of shell crystals. Likewise, dissolution of the shell crystals at pH 6.6 revealed a complicated set of fibers which appear to be a protein matrix embedded within the crystal structure. An obvious hypothesis as to their origin would state, "Shell proteins make up a matrix that provides a scaffold for the growth of the calcite crystals during egg shell formation." The holes, seen in SEM preparations, are merely remnants of the protein matrix involved in the control of crystal growth. Very recently, in her MS thesis, Natalie Hubbard examined dove eggs with the SEM and found similar channels within the calcite crystals making up the palisade layer of these egg shells (Hubbard, 2006). Tina Halupnik helped Sandra examine "eggs from space." These were eggs that were involved both literally and virtually with space travel via the U.S. Shuttle.

I am sure there were many times when Quatermain and his companions would have been glad to have any kind of eggs, most likely large Ostrich eggs, during the rigors of traveling on their trail to King Solomon's mines. At one point in their journey they were saved from starvation by finding a large patch of wild melons. I'm sure the melon leaves had CaOx crystals and maybe also cystoliths.

Before closing this expedition, perhaps we can spend a minute looking at our diets. Many edible plants contain CaOx crystals. One might wonder about other common diet items; for example spinach leaves, beet roots, grape leaves, grapes, pineapples, kiwis, etc. they all have CaOx crystals. Everyone knows that table salt is crystalline but few know that black pepper has a fairly large amount of CaOx crystals in each peppercorn (Fig. 49). As you travel your life's trail, crystals litter your environment and are even found in your food; it will be difficult for you to miss some of them. If you or your family have a disposition toward forming kidney stones you would be wise to examine what you eat.

Occasionally, a student gives you something and you just don't know what to do with it! For some inexplicable reason you can't throw them away. I'm not talking about class papers, exams, theses, dissertations, but rather something "out of the blue." I'm talking about something that has no category because it is so different. Circa 1968, when I was studying the development of CaOx crystals in different systems, I was given a short note attached to a mi-

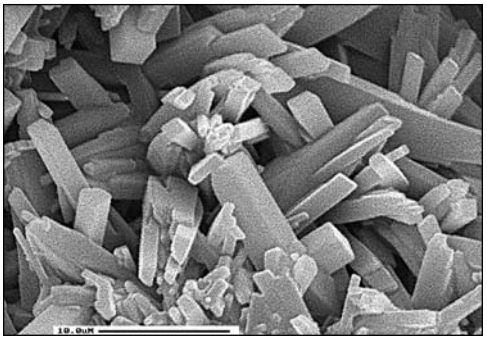


Figure 49. Crystals of calcium oxalate in the tissue of a black pepper (*Piper nigrum*) peppercorn. Unpublished micrograph from the collection of HJA.

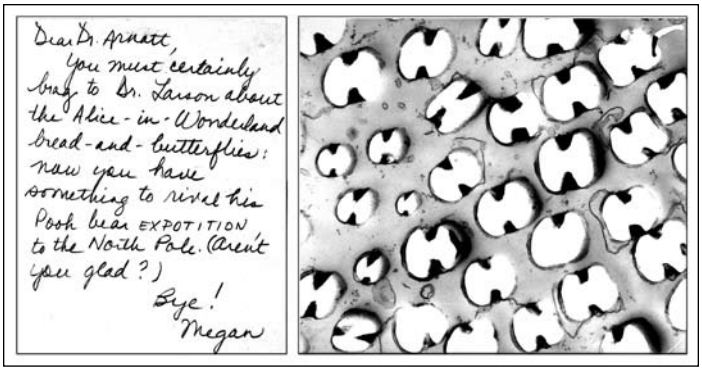


Figure 50. Mystery note and micrograph of crystal differentiation in the frond cells of *Lemna minor*. See text for more information.

crograph of developing *Lemna minor* crystals (Fig. 50). The note signed Megan and the micrograph kicked around in my office for close to 40 years. Many people have asked about it. Who was "Megan?" What does the note mean? Just the other day, I began to wonder! Megan is Dr. Megan Biesele, daughter of John Biesele, Emeritus Professor of Zoology at UT Austin. Dr. Megan Biesele is currently on the staff of the Department of Anthropology at UT Austin where she does research on "Medical Rhetoric" of different cultures. The "Alice in Wonderland bread-and-butterflies" allusion I think is self obvious; the "Dr. Larson" is Donald A. Larson, at that time, Professor of Botany at UT Austin. Why did I need something to "rival his Pooh Bear EXPOTITION to the North Pole?" That line was never clear to me, and still isn't. I do know that at that time, Larson and Mollenhauer were writing a paper about CaOx crystals in the aerial roots of orchids (Mollenhauer and Larson, 1966). Well, for all of you that have asked questions about the mystery note, I'll borrow a line from Looney Tunes Cartoons, "That's all folks!"

By now, you realize that this paper is as much a review of my studies of biological crystals as it is an autobiography. Regarding that conundrum, here are some bits and pieces for thought. First, I am not the only one who studied biological crystals. Second, there are hundreds of thousands of papers dealing with aspects of the subject. Think about the myriad of papers written about bones, teeth and kidney stones; all are important examples of biological crystals ***in action***. If you want to read a recent review of biomineralization I suggest you consult the following (Dove, De Yoreo and Weiner, 2003). When you judge Part IV, please differentiate between the general subject of biological crystals (or biomineralization) and the small number of systems I have studied. True, the latter are a part of the general literature, but they are also a part of my life; hence they belong in my autobiography. The papers and the crystal systems described in this "autobiography" are the gold and diamonds that I found on my crystal trail.

Quatermain and his companions were lucky to escape from King Solomon's Mines. They popped out of the volcano's surface like groundhogs out of their burrows. Unfortunately, they left behind "enormous wealth" in Solomon's storeroom. However, they arrived at the surface with their lives, and ***a pocket full of large diamonds***. In an analogous manner, my 50 years on the trail produced a pocket full of crystal papers (my diamonds). Left behind and undiscovered there is a wealth of crystal information still to be discovered. As others travel the crystal trail, I will be waiting to hear about their discoveries.

When trekking along your scientific trail, remember, you have turn over "stones" to find out what's under them! Success comes from turning, and turning, and turning some more. When turning over stones, please watch out for "rattlesnakes!" With the former thought in mind, I am reminded of a line from an old cowboy song which goes like this:

"A cowboy song is just like gold, it's anywhere it's found."

I want to thank all of those, living and dead, that helped guide me along the crystal trail. I have borrowed freely from the work of students in my laboratory; to them I'll always be indebted. I thank the following for their many suggestions and for their proof reading: Susan Garrett, Jean Arnott, Catherine Arnott-Thornton, Martha Gracey and Camelia G.-A Maier. Errors are of course my responsibility. One final comment. Writing in the first person seemed appropriate for an autobiography; however, it's hard to write a first person declarative or exclamatory sentence without "the pronoun **I**." Those of you with a quantitative bent can tally the number of "I's" as a measure of my self-image (ego). Is "My Crystal Trail" an ego trip? "**Whoa, big time!**" The latter is a "handy exclamation" often used by my friend and teacher, Jess Livesay.

The last part of my autobiography (Part V) is entitled, "**Wood – the Final Frontier**." With Part V completed in October 2007, it will bring the details of my fifty seven years in science to a close.

LITERATURE CITED

Arnott, H.J. 1962. The seed, germination, and seedling of *Yucca*. Univ. Calif. Publ. Bot. 35:1-164.

Arnott, H.J. 1964. Ultrastructure of the root of *Yucca*. Amer. J. Bot. 51:671. (Abstract).

Arnott, H. J. 1980. An SEM study of crystal idioblasts in pecan. Scanning Electron Microsc. III:563-569.

Arnott, H.J. 1982. Calcium oxalate crystals in forest litter. Texas Soc. Electron Microsc. J. 13:15. (Abstract).

Arnott, H.J. 1982. Three systems of biomineralization in plants with comments on the associated organic matrix: *In* Biological Mineralization and Demineralization, ed. by G.H. Nancollas. Dahlem Konferenzen. Springer-Verlag, New York, pp 199-218.

Arnott, H.J. 1995. Calcium oxalate in fungi. In Calcium oxalate in biological systems. Ed. Saeed R. Khan. CRC Press.

Arnott, H.J. and Catherine J. Arnott-Thornton. 2000. Crystalliferous fungi associated with bristlecone (*Pinus longaeva*) litter from the Great Basin National Park. Texas Journal of Microscopy 31: 41. (Abstract).

Arnott, H.J., A.C.G. Best, and J.A.C. Nicol. 1970. Occurrence of melanosomes and of crystal sacs within the same cell in the tapetum lucidum of the stingaree. J. Cell Biol. 46:426-427.

Arnott, H.J. and A. Fryar. 1984. Raphide-like fungal crystals from Arlington, Texas compost. Scanning Electron Microsc. 1984 (IV):1745-1750.

Arnott, H.J., M.L. Kelly, and M.A. Webb. 1982. Calcium oxalate crystals in the leaves of *Oxalis*. Texas Soc. Electron Microsc. J. 13:15-16. (Abstract).

Arnott, H. J., L. E. Lopez and M.A. Webb. 1994. The initial development of calcium oxalate crystals in the leaves of *Vitis mustangensis* and *V. labrusca*. Texas Soc. Electron Microscopy J. 25:63. (.)

Arnott, H. J. and C. G.-A. Maier. 2006. Cystoliths and nebencystolithen (secondary cystoliths) in White Mulberry (*Morus alba* L., *Moraceae*). Texas Journal of Microscopy 37:58-59.

Arnott, H.J. and J.A.C. Nicol. 1970. Reflection of ratfish skin (*Hydrolagus colliei*). Can. J. Zool. 48:137-151 + plates I-XI.

Arnott, H.J., J.A.C. Nicol, and C.W. Querfeld. 1971. Reflecting spheres in the eyes of weakfishes (Sciaenidae). Nature 233:130-133.

Arnott, H.J., J.A.C. Nicol, and C.W. Querfeld. 1972. Tapeta lucida in the eyes of seatrout (Sciaenidae). Proc. Royal Acad. Sci. (London) 180:247-271 + plates 12-20.

Arnott, H.J. and F.G.E. Pautard. 1965. Mineralization in plants. Amer. Jour. Bot 52:613. (Abstract).

Arnott, H.J. and F.G.E. Pautard. 1970. Calcification in plants. *In* Biological Calcification, ed. by H. Schraer. Appleton-Century-Crofts, New York.

Arnott, H.J., F.G.E. Pautard and H. Steinfink. 1965. Structure of calcium oxalate monohydrate. Nature 208:1197-1198.

Arnott, H.J. and K.M. Smith. 1967. Electron microscopy of virus-infected sunflower leaves. J. Ultrastruct. Res. 19:173-195.

Arnott, H.J. and K.M. Smith. 1968a. An ultrastructural study of the development of a granulosis virus in the cells of the moth *Plodia interpunctella* (Hbn.). J. Ultrastruct. Res. 21:251-268.

Arnott, H.J. and K.M. Smith 1968b. Ultrastructure and formation of abnormal capsules in a granulosis virus of the moth *Plodia interpunctella* (Hbn.). J. Ultrastruct. Res. 22:136-158.

Arnott, H.J., K.M. Smith, and S.L. Fullilove. 1968. The ultrastructure of a cytoplasmic polyhedrosis virus affecting the Monarch butterfly, *Danaus plexippus*. I. Development of the virus and normal polyhedra in the larva. J. Ultrastruct. Res. 24:479-507.

Arnott, H.J. and P.L. Walne. 1967. Observations on the fine structure of the pellicle pores of *Euglena granulata*. Protoplasma 64:330-344.

Arnott, H.J. and M.A. Webb. 1983a. Calcium oxalate crystal development in a fungus found in pine beetle mines in the cambial zone of *Pinus ponderosa* logs. Amer. Jour. Bot. 70:15. (Abstract).

Arnott, H.J. and M.A. Webb. 1983b. Twin raphide crystals in the mustang grape (*Vitis mustangensis*). Texas Soc. Electron Microsc. J. 14:20 (.)

Arnott, H.J. and M.A. Webb. 1986a. A histochemical and electron microscope investigation of *Morus* cystoliths." Bot. Soc. Amer. at AIBS Meetings, San Antonio, Texas. (Poster).

Arnott, H.J. and M.A. Webb. 1986b. "Isolation and characterization of the calcification matrix in *Vitis* raphide idioblasts." Bot. Soc. Amer., AIBS Meetings, San Antonio, Texas. (Poster).

Arnott, H.J, M.A. Webb. 2000. Twinned raphides of calcium oxalate in grape (*Vitis*): Implications for crystal stability and function. Int. J. Plant Sci, 1611:133-142.

Arnott, H.J. and C. Workman. 1981. An SEM and x-ray diffraction study of crystals in okra leaves. Scanning Electron Microsc. 1981(III):293-298; See also in the "discussion with reviewers" of Horner and Franceschi on page 250.

Brackenridge-Eilert, Genie. 1974. An Ultrastructural Study of the Development of Raphide Crystal Cells in the Roots of *Yucca torreyi*. Dissertation, University of Texas at Austin.

Cody, A.M. and H.T. Horner. 1983. Twin raphides in the Vitaceae and Araceae and a model for their growth. Bot. Gaz. 144:318-330.

Cody, A.M. and R.D. Cody. 1987. Contact and penetration twinning of calcium oxalate monohydrate. J. Crystal Growth 83:485-498.

Crick Rex, (ed.), 1985. Proceedings of the 5th International Symposium on Biomineralization.

De Bary, H. A. 1887. Comparative Morphology and Biology of the Fungi, Mycetozoa, and Bacteria.

Dove, P.M., J.J. De Yoreo, and S. Weiner, 2003. Reviews in Mineralogy and Geochemistry Vol. 54. Biomineralization. Mineralogical Society of America Geochemical Society

Foster, Adriance S. 1956. Plant idioblasts: remarkable examples of cell specialization. Plant Science J. 46:184-193.

Franceschi, V.R. and H.T. Horner 1979. Use of *Psychotria punctata* callus in study of calcium oxalate crystal idioblast formation. Z Pflanzenphysiol 92: 61–75

Frey-Wyssling, A. 1968. Crystallography of the two hydrates of crystalline calcium oxalate in plants. Amer. J. Bot, 68:130-141.

Gianni, Gary and Mark Schultz. 2006. Hal Foster's Prince Valiant. King Features Syndicate.

Gottsch, John. 1974. The tapetum lucidum of *Didelphus virginiana*. Master's Thesis. University of South Florida.

Graustein, W.C, K. Cromack, Jr., and P. Sollins. 1977. Calcium Oxalate: Occurrence in soils and effect on nutrient and geochemical cycles *Science*: Vol. 198. no. 4323, pp. 1252 – 1254.

Grimson, M.J. 1984. An electron and light microscopy study of development and ultrastructure of crystal sand idioblasts in the stem of *Solanum tuberosum*. Master's Thesis, the University of Texas at Arlington.

Grimson, M.J., H.J. Arnott and M.A. Webb. 1982. A scanning electron microscopic study of winged twin crystals in the bean legume. Scanning Electron Microsc. 1982(III):1133-1140.

Haggard, H. Rider. 1885. King Solomon's Mines. Puffin Books, Penguin Books, London.

Hillerman, Tony, 1973. The great Taos bank robbery. Univ. of New Mexico Press, Albuquerque.

Horner, H.T., A P. Kausch, and B. L. Wagner. 2000. Ascorbic acid: a precursor of oxalate in crystal idioblasts of *Yucca torreyi* in liquid root culture. Int. J. Plant Sci. 161:861–868.

Hubbard, N. 2005. The eggshell structures of ringnecked turtle dove (*Streptopelia risoria*). Master's Thesis, the University of Texas at Arlington.

Kassanis, B. 1982. Biographical Memoirs of the Royal Society, 28: 451-477.

Koch, H.M. and H.J. Arnott. 1979. Reflecting crystals and their development in the tapetum lucidum of *Dorosoma cepedianum*. Proc. 37th Annual EMSA Meeting, pp 288-289.

Koch, H.M. 1974. Reflecting Crystals and their development in the Tapetum Lucidum of *Dorosoma cepedianium*. Master's Thesis.

Ledbetter, M.C. and Keith R. Porter. 1970, Introduction to the fine structure of plant cells. Springer. 188pp.

Maier, C. G.-A. 2006. Personal communication.

Mayr, E. 1946. History of the North American Bird Fauna. http://www.wku.edu/~smithch/biogeog/MAYR946B.htm.

Merck Index. 1983. Merck and Co., Rahway, N.J.

Mollenhauer, Hilton. 1959. Permanganate Fixation of Plant Cells. The Journal of Cell Biology, 6:431-436.

Nicol, J.A.C. and H.J. Arnott. 1972. Riboflavin in the eyes of gars (Lepisosteidae). Can. J. Zool. 50:1211-1214

Nicol, J.A.C. and H.J. Arnott. 1973. Studies on the eyes of gar (Lepisosteidae) with special reference to the tapetum lucidum. Can. J. Zool. 51:501-508 + plates I-IX and a color plate.

Nicol, J.A.C. and H.J. Arnott. 1974. Tapeta lucida in the eyes of Goatsuckers (Caprimulgidae). Proc. Royal Acad. Sci. (London) 187:349-352 + plates 30-32.

Nicol, J.A.C., H.J. Arnott and A.C.G. Best. 1973. Tapeta lucida in bony fishes (Actinopterygil): a survey. Can. J. Zool. 51:69-81 + plates I-V.

Powell, M.D. and H.J. Arnott. 1985. Calcium oxalate crystal production in two members of the Mucorales. Scanning Electron Microsc. 383:183-189.

Rivera, Ezequiel. 1974. *Echinomastus intertextus* (Cactus). An ultrastructural physiological and biochemical study. Dissertation, University of Texas at Austin.

Scott, F.M., Karl C. Hamner, Elizabeth Baker, Edwin Bowler. 1956. Electron Microscope Studies of Cell Wall Growth in the Onion Root. Amer. Journal of Botany, Vol. 43:313-324.

Teigler, D.J. and H.J. Arnott. 1972. X-ray diffraction and fine structural studies of crystals in the Malpigian tubules of silkworms. Nature 235:166-167.

Van De Veire, J. 1997. The vegetative reproductive structures of *Nymphaea mexicana*. Master's Thesis, The University of Texas at Arlington.

Walne, P.L. and H.J. Arnott. 1967. The comparative ultrastructure and possible function of eyespots: *Euglena granulata* and *Chlamydomonas eugametos*. Planta 77:325-353.

Wattendorf, J., 1970. On the formation of the pattern of crystal idioblasts – in *Canavalia ensiformis*. DC Planta 95: 202-217.

Webb, M.A. and H.J. Arnott. 1981. An ultrastructural study of druse crystals in okra cotyledons. Scanning Electron Microsc. 1981(III):285-292.

Webb, M.A. and H.J. Arnott. 1982. Mineralized layers in seed coats. Texas Soc. Electron Microsc. J. 13:22. (Abstract).

Webb, M.A., J.M. Cavaletto, N.C. Carpita, L.E. Lopez, and H.J. Arnott. 1995. The intravascular organic matrix associated with calcium oxalate crystals in leaves of *Vitis*. Plant J. 7, 633–648.

Westmoreland, S.L. and H.J. Arnott. 1997. Changes in mammillary cones during incubation of fertilized eggs of *Gallus domesticus*. Texas Soc. Electron Microscopy J. 28:45 (Abstract).

Westmoreland, SL. 2001. Variation in the eggshell pore system of the White Leghorn chicken. Dissertation, The University of Texas at Arlington.

Westmoreland, SL. 1998. Ultrastructural changes in the mammilary cones during development of fertilized egg of *Gallus domesticus*. Master's Thesis, The University of Texas at Arlington.

Whaley W.G., H.H. Mollenhauer and J.H. Leech. 1960. The ultrastructure of the meristematic cell. Amer. Jour. Bot. 4: 401-449.

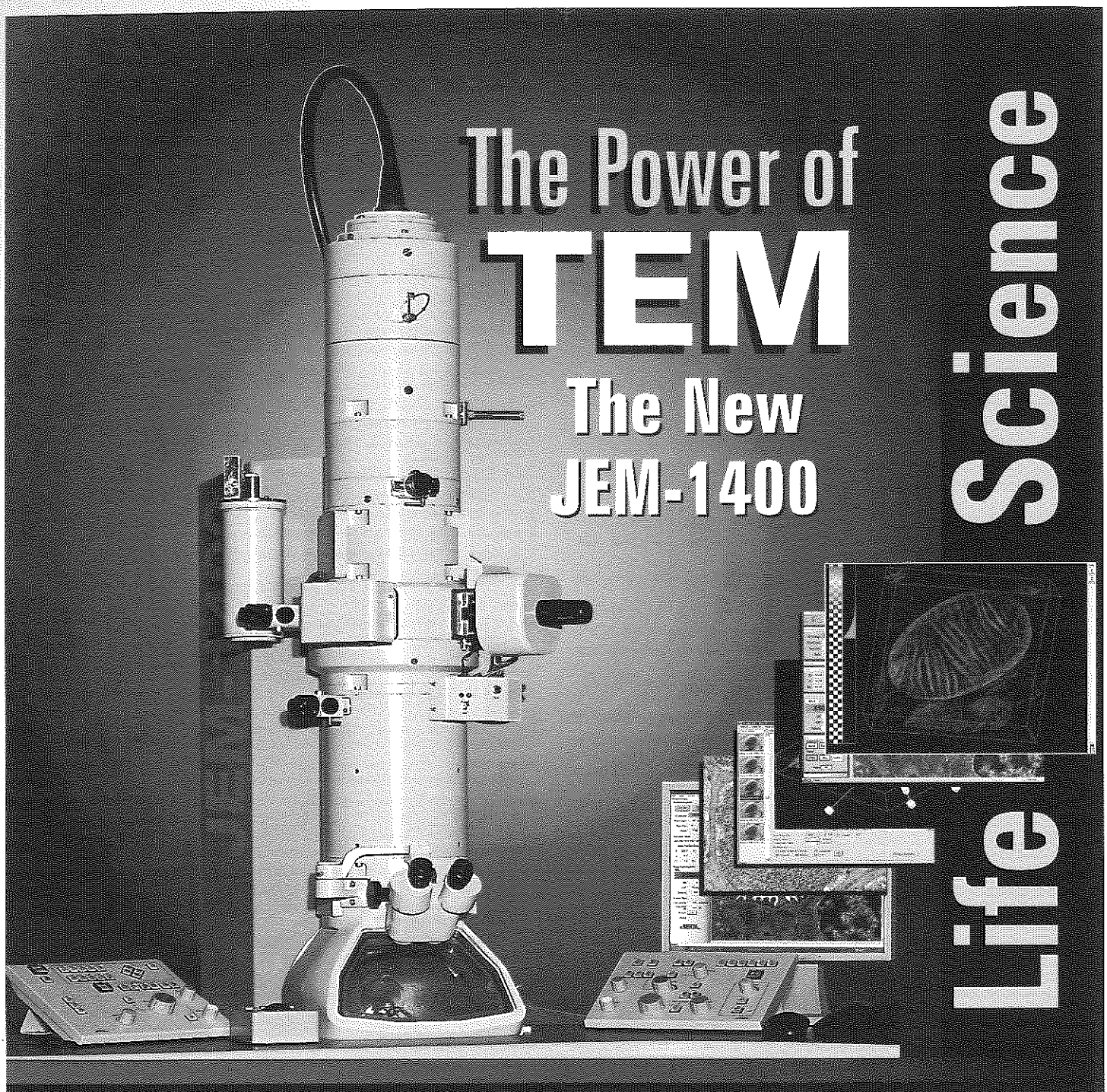
Whitney, K.D. and H.J. Arnott. 1986. Calcium oxalate crystals and basidiocarp dehiscence in *Geastrum saccatum* (Gasteromycetes). Mycologia. 78:655-662.

Whitney, K.D. and H.J. Arnott. 1986. Morphology and development of calcium oxalate deposits in *Gilbertella persicaria* (Mucorales). Mycologia 78:42-51.

Whitney, K.D. and H.J. Arnott. 1988. The effect of calcium on mycelial growth and calcium oxalate crystal formation in *Gilbertella persicaria* (Mucorales). Mycologia. 80:707-715.

Wilson, E.B. 1928. The Cell in Development and Heredity. 3rd ed. Macmillan Co. NY

Zyznar, E. S., F. B. Cross and J. A. C. Nicol. 1977. Uric Acid in the Tapetum Lucidum of Moon-eyes Hiodon (Hiodontidae Teleostei) Proceedings of the Royal Society of London. Series B, Biological Sciences, Vol. 201: 1-6.



Get ready for a whole new TEM experience — the new JEOL 120 kV TEM with advanced automation for multiple users and every level of expertise. The JEM-1400 meets all your TEM and STEM imaging needs including cryomicroscopy, elemental mapping and 3D tomography. Advanced electron optics, automation, application and training assistance, plus remote operation — all in a single compact TEM.

Learn what the experts are saying at www.jeolusa.com/JEM-1400Highlights.

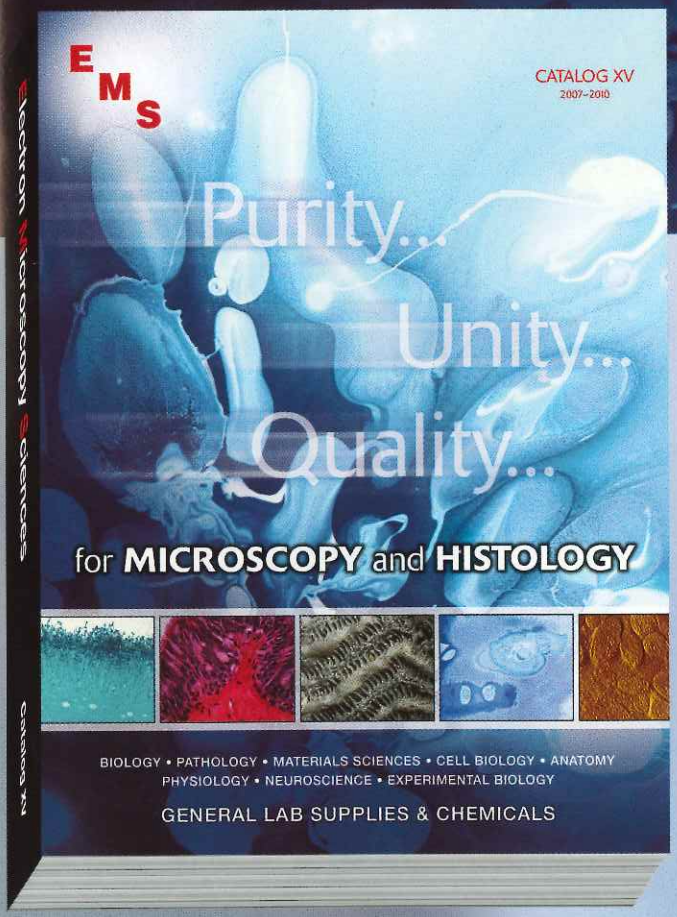
Another
Extreme
imaging
solution from

JEOL

Stability • Performance • Productivity

www.jeolusa.com salesinfo@jeol.com
978-535-5900

The new **2007-2010 EMS CATALOG** is now available!

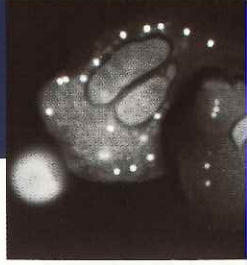
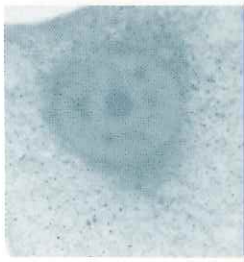
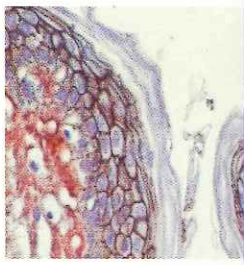
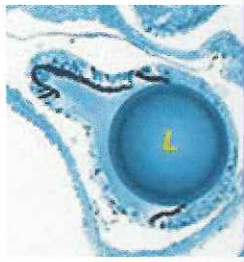


Exacting Research
demands only the
Highest Quality Products.

Introducing the 2007–2010 EMS Catalog, your comprehensive source for chemicals, supplies, accessories, and equipment for Microscopy, Histology and all fields of biological and materials research.

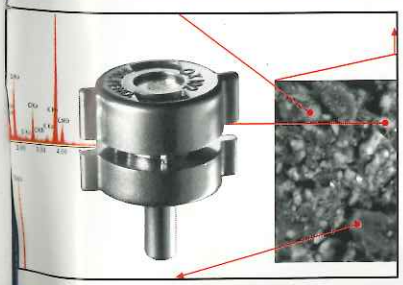
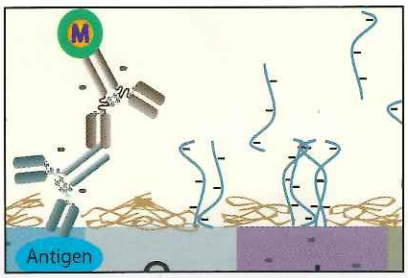
Featuring new and revolutionary products, including:

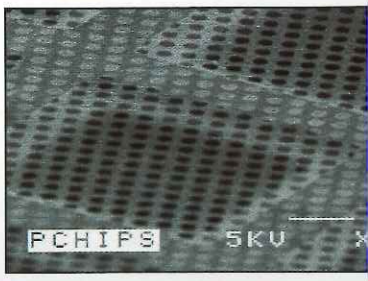
- C-flat™ Holey Carbon Grids for cryo-TEM
- Ilford Photography Papers and Photochemicals
- WETSEM™ Capsules for Hydrated SEM Samples
- DuraSIN™ Substrates for TEM & X-ray
- Diatome Oscillating Diamond Knife
- PathScan Enabler III
- Ultra-Thin Carbon Tabs
- Plunge Freezer
- EMS 9000 Precision Pulsed Laboratory Microwave Oven
- State-of-the-Art Oscillating Tissue Slicers
- NioProbe and TipCheck for AFM
- Aurion ImmunoGold Reagents and Accessories
- EMS LYNX Tissue Processor
- MAG*I*CAL®
- EMS Carbon Coaters and Sputter Coaters


Your one-stop shop for the latest products and solutions for Microscopy and Histology!

Application Notes • More Technical Support • Enhanced Product Lines • Revolutionary Products



QuantomiX WETSEM™ • AURION Newsletters • Diatome Diamond Knives • C-flat™ Holey Carbon Grids

To request our new catalog, please call or write us today,
or visit us online at **www.emsdiasum.com**

Electron Microscopy Sciences

P.O. Box 550 • 1560 Industry Rd. • Hatfield, Pa 19440
(215) 412-8400 • Toll Free: 1-(800) 523-5874
Fax: (215) 412-8450 or 8452
email: sgkcck@aol.com • stacie@ems-secure.com
www.emsdiasum.com