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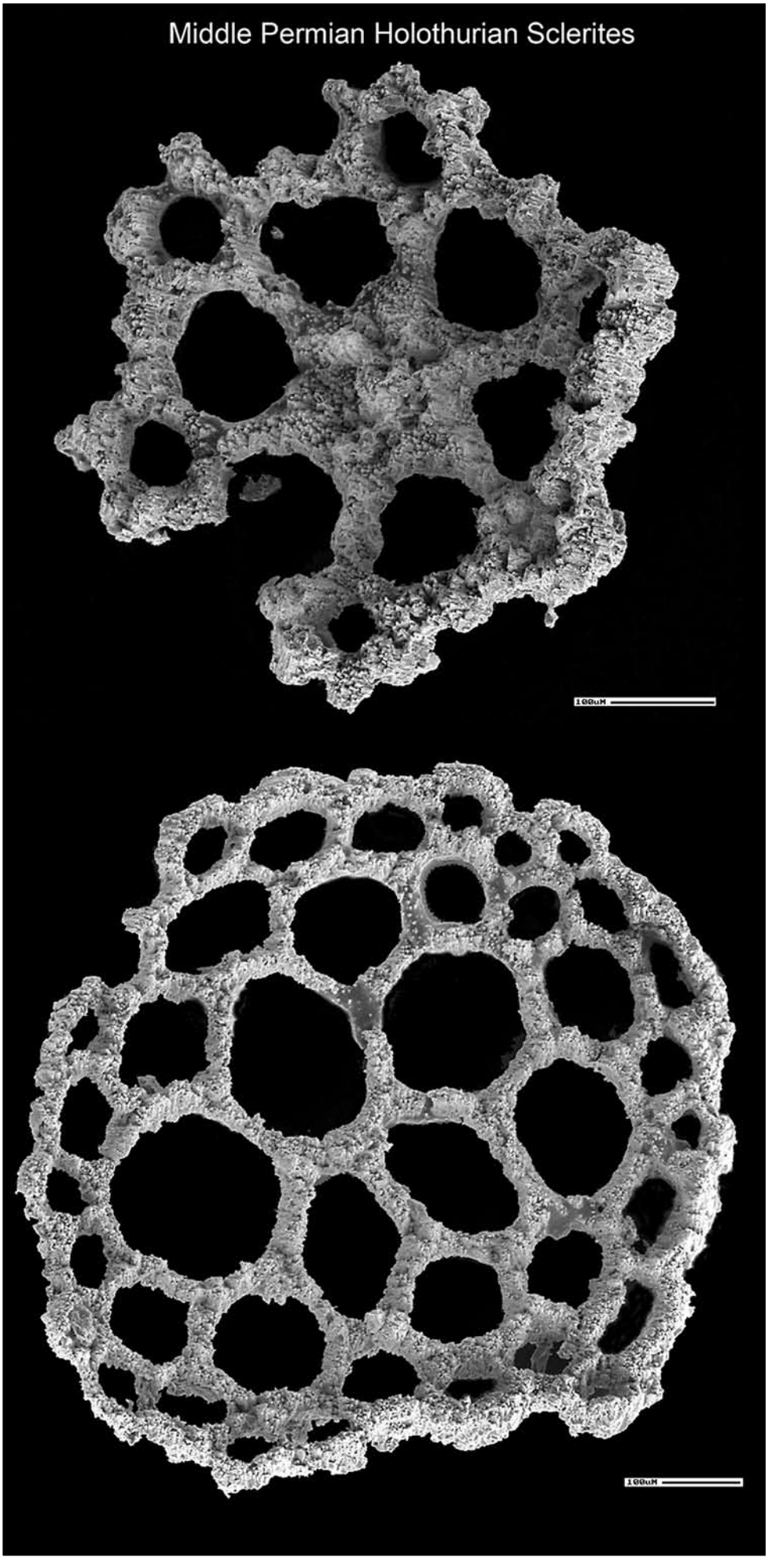

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Camelia G.-A. Maier, Editor
Department of Biology, Texas Woman's University, Denton, TX 76204

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ON THE COVER

Holothurian sclerites are small bone ossicles located in the skin of sea cucumbers. The ones on the cover are fossilized of approximately 300,000,000 years. The morphology of sclerites is used by taxonomists to identify various species of sea cucumbers. See also current abstract entitled 'Middle Permian Holothurian Sclerites from The Guadalupe Mountains, West Texas' by G. P. NESTELL[1], M. K. NESTELL[1], and M. GRACEY[2], [1]Department of Earth and Environmental Science and [2]Department of Biology, University of Texas at Arlington, Arlington, TX 76019. Plate prepared by Martha Gracey.

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President's Message

Thank you for electing me as your new President for the Texas Society for Microscopy. My association with TSM began in the late 1960's when I heard George Palade, Nobel Laureate, give a talk on his research on the transmission electron microscopy of the Golgi apparatus. I was a graduate student of Dr. Constantine Alexopoulos, in the Botany Department at the University of Texas at Austin. I arrived on campus to begin my doctoral work the same day that Howard Arnott began his postdoctoral work. Since then, my students and I have been warmly received over the years, traveling from El Paso to participate in the TSM meetings. Alice Stacey and I were planning a TSM meeting for October of 2001 when September 11th intervened.

Our society has had excellent leadership with very dedicated officers including Sandra Westmoreland, Past President; Robert Champaign, Past Secretary; Robert Droleskey, Treasurer; Jodi Roepsch and Pam Neill as Program Chairs; German Neal, Corporate Member Representative; and Camelia Maier, TSM Journal Editor. We are indebted to Becky Holdford, the TSM Webmaster.

Our enthusiastic new officers include Ernest F. Couch, President-Elect; Tina Halupnik, Secretary; E. Ann Ellis, Treasurer-Elect; and Phoebe J. Doss, Program Chairman-elect. Our membership is 240 including students and corporate members and at this point in time the Society is financially stable. Accomplishments of the society for this year have included a healthy increase in our corporate membership, online access for the *Texas Journal of Microscopy*, revisions of our by-laws and the expansion of the Arnott Award for best student presentation at TSM meetings to three awards, with a 1st place award of $100.00, a $75 2nd place award and a $50 3rd place award.

Sandra Westmoreland chaired our successful Spring 2006 meeting at Alcon Industries, Fort Worth, in April 2006. We are anticipating an excellent meeting at the Hilton Garden Inn in Allen for the October 19-21, 2006 Fall Meeting. Jodi Roepsch has planned this meeting to encourage a diversity of participants from both academia and industry. For next year, Ernest Couch, Pam Neill and Phoebe Doss are preparing our spring meeting on the campus of Texas Christian University, in Fort Worth for the weekend of April 13-15, 2007. Please plan to attend and bring colleagues and students!

The Society has lost a valuable member with the death of Richard Rebert, representative of Electron Microscopy Sciences, Hatfield, PA. We will greatly miss his cheerful presence at our meetings and his technical expertise.

My goals for this year include increasing membership, participation in meetings, participation in our journal advertising and publications, personal donations and support for students. To achieve these goals I will need the support of each member of our society.

Joanne (Judi) Tontz Ellzey
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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:

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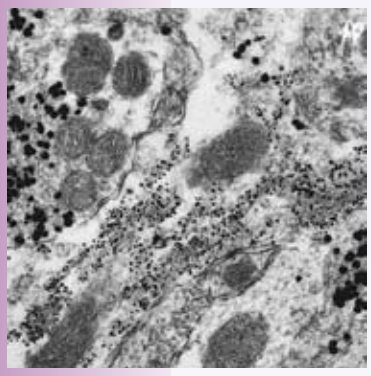

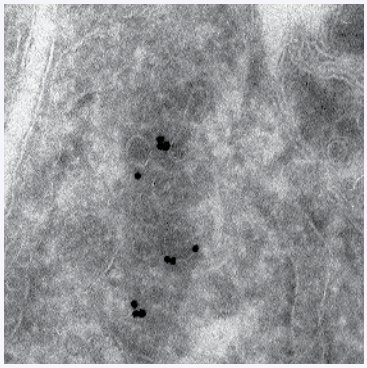

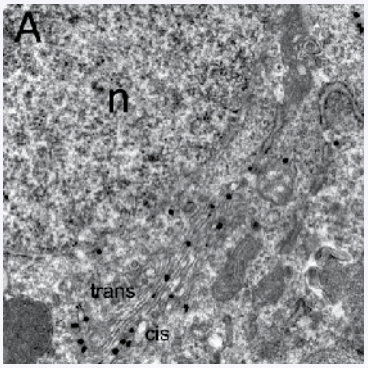

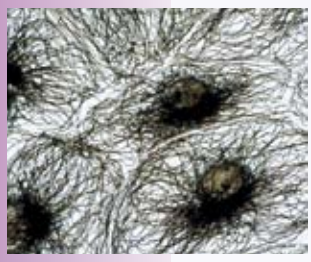

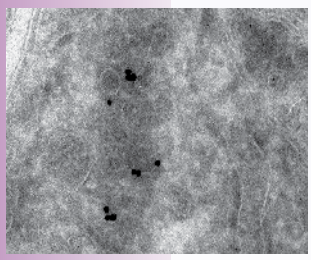

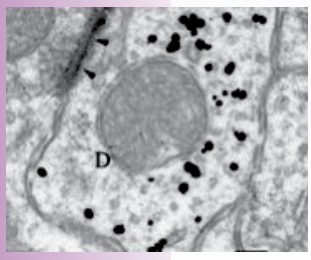

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IN MEMORIAM

RICHARD ROSS REBERT, 1920–2006

Rick Rebert passed away peacefully on July 27th, 2006 at 86 years of age. He was still actively working for Electron Microscopy Sciences with his usual energy and enthusiasm, love for life and compassion for all creatures, great and small. Friend to all, he loved his work and was especially fond of Texas, the state where he raised his family in the early '60's while working as a pharmaceutical research representative for Merck and Co.

To combine his love for travel and his desire to remain active both physical and mentally, upon retiring from Merck, he chose to stay involved with the scientific community through his travels for EMS & Diatome. For over 25 years, he traveled the U.S. extensively, visiting customers and supporting the microscopy societies. He enjoyed this work immensely, and found it enriching and rewarding because of the many wonderful people he was fortunate to meet.

As a young boy, his natural curiosity and intelligence brought him to the world of science, receiving his Ph.D. in Anatomy from Vanderbilt University, with a detour on the way to serve the country he so loved in WWII with the US Army (165th Combat Battalion Engineers).

Through several charitable and community organizations, he quietly worked on behalf of those who cannot take care of themselves – the handicapped, the elderly, those in the animal kingdom. He imparted love and caring to his family, friends and the many lives he touched until the day he left us. In giving of himself, he hoped to, and did indeed, leave us with a kindler, gentler world.

Prudence Rebert Irwin
Dr. Rebert's daughter

Rick was the longest continuous corporate representative of our Society. He hardly ever missed a meeting and he almost always saw to it that EMS gave some kind of support to our meetings.

Ann Rushing, Past President, honored him at the 40th anniversary meeting as being the longest serving corporate representative. He was always a true gentleman.

Robert Droleskey
TSM Treasurer

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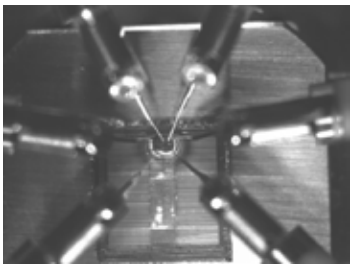

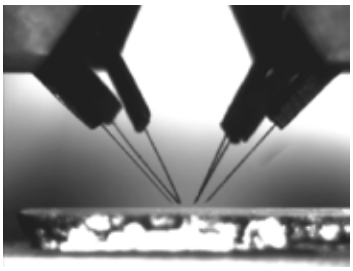

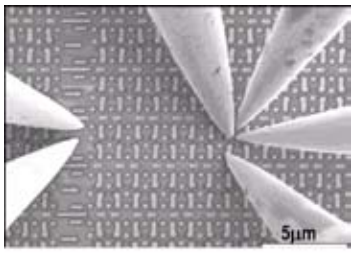

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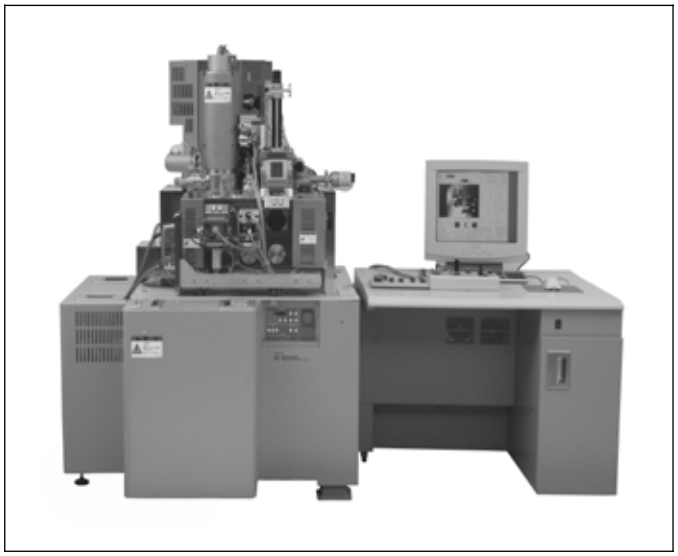

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EFFECTS OF ARSENIC TOXICITY AT THE CELLULAR LEVEL: A REVIEW

JAIME B. VIGO AND JOANNE T. ELLZEY

Analytical Cytology Core Facility, Biological Sciences, the University of Texas at El Paso, El Paso, Texas 79968-0519

ABSTRACT

Arsenic (As) environmental contamination has become a worldwide health concern. Exposure to As may occur through ingestion, inhalation, or skin contact from several natural and anthropogenic sources such as chemotherapy, homeotherapy and industrial applications. The toxicity of As can directly or indirectly affect the synthesis of carbohydrates, proteins, lipids, and DNA, cell morphology and division, detoxification mechanisms, the structure and functions of the skin, body fluids, immune, nervous and digestive systems. Exposure to As may lead to cancer and possibly diabetes mellitus. This review presents the major histological and ultrastructural changes associated with exposure to As in the liver, kidney, pancreas and red blood cells of humans and laboratory animals. Pathological changes include steatosis, inflammation, fibrosis and cirrhosis of the liver, glomerular and proximal convoluted tubular changes in the kidney, changes in insulin and glucagon production in the pancreas and hemolysis of red blood cells.

Keywords: arsenic, cirrhosis, fibrosis, kidney, liver, ultrastructure.

1. Sources of Arsenic Toxicity

Threats to human health from As environmental contamination have reached worldwide attention in the first decade of the 21st century. The United Nations World Health Organization (UN-WHO), acting as a coordinating authority on international public health, and the United States-Environmental Protection Agency (US-EPA), have established a maximum contaminant level (MCL) of As in the drinking water of 10 μg/L (10 ppb). Part of the justification for this decision was based on human epidemiological data that links illnesses with the effects of chronic occupational exposure or consumption of drinking water containing no less than 50 ppb of As[4,25,48,51,52,53,63].

Arsenic is a semi-metallic element that exists as arsenite [As(III)] and arsenate [As(V)] capable of forming inorganic and organic salts[50]. In reducing conditions, As(III) is the dominant form, whereas As(V) is generally the stable form under oxygenated conditions. Arsenic is found in igneous rocks at an average concentration of 2 ppm, forming inorganic salts in combination with other elements such as oxygen, chlorine, and sulfur[1,2,10].

The daily intake of total As from food and beverages is generally between 20 and 300 ppb. Any chronic daily dose equal or higher than 0.6 mg/kg/day may become lethal to humans, and an acute dose equal or higher than 100 ppm through food or water may be fatal. Seafood consumption may represent a significant exposure to As. Arsenic may be introduced into water systems through erosion, industrial effluents, and combustion of fossil fuels[1,50,71]. Arsenic exposure through drinking water has become a threat to public health[14]. The inhalation of 3-10 ppm of arsine gas causes toxicity within hours. Effects may include hemolysis, hypoxia, breathlessness, and tachycardia[10].

Corresponding author: Joanne T. Ellzey, Analytical Cytology Core Facility, Biological Sciences, the University of Texas at El Paso, 500 West University Avenue, El Paso, TX 79968-0519. E-mail: jellzey@utep.edu, Phone: (915)-747-6880. FAX: (9150-747-5808.

2. Overview of Arsenic Toxicity

Arsenic toxicity due to the trivalent state is greater than from the pentavalent state[20]. It seems that exposure to As(III) results in a better absorption and therefore higher concentrations in tissues than the exposure to As(V)[10]. Within mammals, As may accumulate in the brain, bladder[21], heart[25,34,37,63], lung[21], liver[73], kidney, pancreas, spleen, muscles, skin[21], hair, as well as in fluids such as bile, blood[28], and stomach juices[6,21]. Some of the toxic effects include disruption of the homeostasis mechanisms of blood, spleen and liver by interfering with the uptake of essential chemicals[62]. Mammalian chronic exposure to As has been associated with cardiotoxicity[32,34,37,60], peripheral vascular disease (PVD)[63], increased systolic blood pressure[25], skin lesions, and elevated urine As and glucose levels[19,49].

Arsenic is classified as a carcinogen, although a better classification is as a co-carcinogen or co-promoter as demonstrated in studies of mice exposed to inorganic As during gestation[66]. The carcinogenic effects of As have been shown by *in vivo* and *in vitro* studies of skin, bladder, kidney, liver, lung, and prostate cancers[1,2,8,11,35,36,38,61].

Numerous studies have been conducted on animal models to elucidate the biochemical and cellular effects of As toxicity. Arsenic can induce the biosynthesis of radical species, heat-shock proteins [7], nitric oxide as well as lipid peroxidation[12,25,40,72]. The toxicity of As is modified by metallothionein (MT) proteins[3,24,30,74] due to the ability of As to react with the sulfhydryl (SH) groups of the cysteine residues present in metallothioneins[20,26].

The oxidative stress generated during the metabolism of As produces hydrogen peroxide, dimethylarsenic peroxyl, hydroxyl radical, nitric oxide radical and dimethylarsenic radical[40,62]. These species may affect the signal transduction pathways of the nuclear transcriptional factors PPARs, AP-1, NFκB[27,62] as well as, the pro-inflammatory cytokines interleukin 8 (IL-8) and tumor necrosis factor alpha (TNF-α)[55]. Recent studies have found that the reactive oxygen species produced by As may induce hyperglycemia, hyperinsulinemia, and low insulin sensitivity in Wistar rats exposed to As (III) orally (12 hours) for a period of three

months[23]. The presence of As can disrupt the hepatic and renal enzymatic activity of catalase[44], copper/zinc superoxide dismutase (Cu/Zn SOD)[72], glutathione (GSH)[44], glutathione reductase (GR)[44], glucose-6-phosphate (G6P)[44], glutathione transferase (GST)[44,57], glucose-6-phosphate dehydrogenase (G6Pdh), Na+/K+ ATPase[40,56], nicotinamide adenine dinucleotide (NAD+)[6,16], and pyruvate dehydrogenase (Pyr-dh)[54]. Other cellular effects include apoptosis, cell proliferation, cellular growth, chromosomal aberrations[7,39,43], DNA demethylation[73], DNA replication and DNA repair[7,43], DNA strand breaks[7,13], genotoxicity, mutagenesis, and phosphate substitution[2,7,20,21,24,26,33].

3. Pathology of Arsenic Toxicity in Humans

Morphological and immunohistochemical effects of As have been documented in tissues at both the histological and ultrastructural levels. In general, As causes widespread endothelial cellular toxicity, resulting in capillary damage and tissue hypoxia[10]. Effects of arsenic damage to skin, gastrointestinal, cardiac, bone marrow, central nervous system, liver, pancreatic, gonadal, and renal tissues may be noticed at different stages of poisoning, particularly during chronic exposure[10].

3.1 Liver Pathology

In 1786, the tonic Fowler's solution (KH_2AsO_4) was developed to treat psoriasis, an immune-mediated disease affecting the skin. The disease manifests as red scaly patches called plaques that are produced by excessive skin cell production and inflammation. It has been documented that in humans, prolonged use of Fowler's solution in therapeutic doses caused hepatic ascites, non-cirrhotic portal hypertension, fibrosis, and cirrhosis[17]. Human liver fibrosis, cirrhosis and hepatoportal sclerosis due to As toxicity have been demonstrated to be linked to the disruption of the homeostasis of collagen, 4-hydroxyproline, phospholipids, cholesterol, and fatty acids[12]. Hepatic fibrosis is believed to develop from the oxidative stress induced by As[38].

Arsenical chemotherapeutic agents are used for the treatment of cancers such as chronic myeloid leukemia and Hodgkin's disease[58,59]. Both therapies have been documented to produce mild hepatic sclerosis, perisinusoidal fibrosis, portal triad fibrosis and narrowing of portal venules[59].

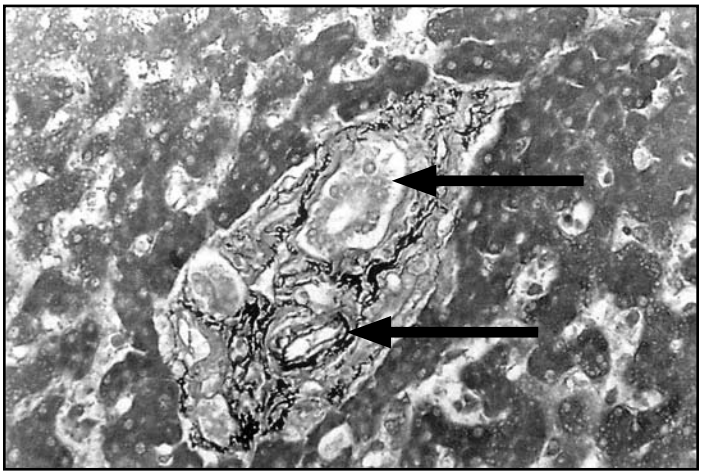

Figure 1. Light micrograph of liver portal triad containing a bile duct (top arrow) and the hepatic artery (bottom arrow) but no distinguishable portal vein due to increased fibrosis. (With permission from Journal of Clinical Pathology[59]).

Figure 1 shows a light micrograph of As induced portal fibrosis in a human patient who was receiving chemotherapy. In Figure 2, collagen inclusions between the endothelium and a hepatocyte are shown in an electron micrograph from a human patient[59]. Generated reactive oxygen species from oxidation reactions react with cellular lipids to produce a number of reactive aldehydes, which stimulate the synthesis of collagen mRNA. This toxic effect has been documented

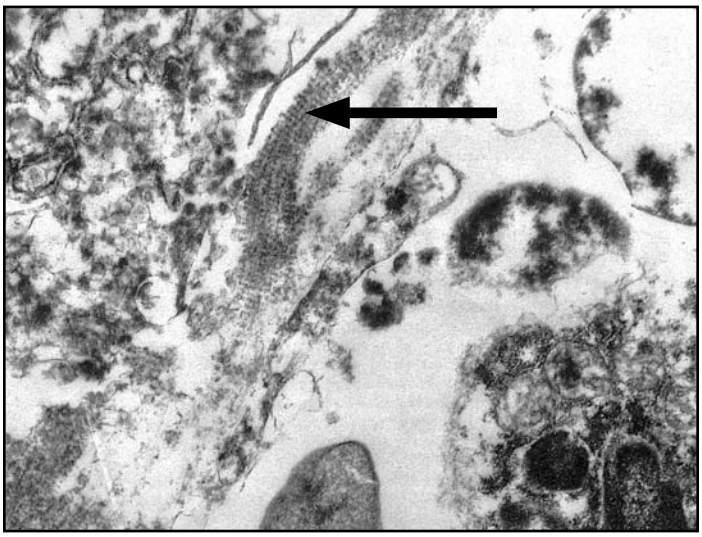

Figure 2. TEM of liver showing collagen deposits (arrow) between the endothelium and a hepatocyte in the space of Disse. (With permission from Journal of Clinical Pathology[59]).

for arsenic, ethanol, copper, and iron, in which the lipid peroxidation produces free radicals that induce an excess of collagen deposition[12].

Prolonged human consumption of drinking water contaminated with 0.05 mg/L of arsenic in West Bengal has demonstrated hepatic pathology including hepatomegaly, non-cirrhotic portal fibrosis, and a small number of cases with portal hypertension[17]. It appears that in humans the most compromising liver damage from arsenic toxicity involves hepatomegaly, non-cirrhotic portal hypertension, and jaundice[18,22,47]. Jaundice, a yellowing of the skin, sclera and mucous membranes caused by increased levels of bilirubin, may evolve into cirrhosis, which may be accompanied by ascites, especially after prolonged arsenical medication[18,22].

3.2 Arsenic and Diabetes

Epidemiological reports have suggested correlations between the chronic consumption of water contaminated with As and the incidence of Type 2-diabetes, primarily in Bangladesh, Sweden, and Taiwan[31,41,51,52,53,67,68]. However, Navas-Acien *et al.* (2005) concluded that there is inadequate experimental and epidemiological evidence to demonstrate causality between arsenic consumption and the onset of Type 2 diabetes mellitus[46]. Further research is needed.

3.3 Other Pathological Characteristics

Blood samples from arsine-exposed individuals showed increased hematocrit, a decreased hemoglobin concentration to <10-g/100 ml, erythrocyte leakage of sodium and potassium, and disruption of the cell membrane ultrastructure. Scanning electron micrographs also revealed intravascular erythrocyte fragments, ghost cells, anisocytosis, poikilocytosis, and reticulocytosis[70]. Arsenic toxicity has also

been documented in the disruption of the mitotic spindle in mitosis of human diploid fibroblasts. Fibroblasts observed under light microscopy showed an accumulation of membrane blebs and/or cytoplasmic vacuoles[65].

Chakraborti *et al.* (2003) published case studies of patients who had taken homeopathic remedies containing arsenic that resulted in pathological side effects including melanosis, keratosis, gastrointestinal illness followed by leukopenia, thrombocytopenia, diffuse dermal melanosis, and polyneuropathy[9].

4. Pathology of Arsenic Toxicity in Animal Models

The toxic effects of arsenic have been documented in animal models. Fowler *et al*., (1979) utilized morphometric and biochemical techniques to establish correlations between the ultrastructure of mitochondria from male Charles River rats and the specific enzymes that were affected by 40 ppm sodium arsenate provided in drinking water for six weeks. They found a 1.2-fold increase in the relative mitochondrial volume density and a 1.4-fold increase in the relative mitochondrial surface density of the inner mitochondrial membrane with a concomitant increase in enzymes associated with this membrane[16].

4.1 Liver Pathology

In rats exposed orally to 20–60 ppm of arsenate for up to 6 weeks, the hepatic pathology included swollen mitochondria with altered functioning, fatty infiltration and degenerative lesions (such as vacuolation), liver parenchymal cell degeneration, inflammation, focal necrosis, and proliferative lesions (adenoma and foci of cellular alteration)[16]. In male rats exposed to 0, 20, 40, or 85 ppm of arsenate in the drinking water for 6 weeks, the hepatic pathology included swelling of the liver mitochondria as the most prominent pathology[16].

BALB/C mice exposed for 15 months to 3.2 mg/L of arsenic showed hepatic pathology such as fatty infiltration at 12 months and hepatic fibrosis at 15 months. It was concluded that the predominant pathology was hepatic fibrosis caused by arsenic induced oxystress[17]. In male mice exposed to 0 or 45 ppm of arsenite in the drinking water for 48 weeks, the liver pathology included hepatocyte steatosis and overt hypertrophy[11].

Multidrug resistance gene knockout [mdr1a/1b(–/–)] mice, which were deficient in P-glycoproteins, (a family of proteins playing a role in the extrusion of intracellular As), were exposed to 0–80 ppm of arsenite in the drinking water for 10 weeks. The liver pathology for both the wild-type control and knockout groups included hepatocellular degeneration, focal congestion, foci of necrosis, apoptosis and cell proliferation, observed in both strains exposed to 40 or 80 ppm of arsenic. The effects were more severe in the knockout mice[72].

In MT-I/II knockouts and sv129 wild-type controls exposed to either arsenite or arsenate through subcutaneous injections for 15 weeks or drinking water for 48 weeks, the hepatic pathology included fatty infiltration and liver degeneration after chronic oral exposures, and inflammatory cell infiltration with a few areas of focal necrosis in the MT-knockouts[38].

When arsenic was supplied intraperitoneally (ip) to rats having free access to drinking water containing 10% ethanol, the group arsenic plus ethanol produced more pronounced liver pathology. The arsenic-exposed rats showed decreased hepatic eosinophilea and degeneration of the renal parenchyma. The renal pathology appeared to be caused by arsenic and not by ethanol. The results suggested that rats exposed simultaneously to arsenic and ethanol become more vulnerable to As toxicity[15].

4.2 Renal Pathology

Although the renal toxicity of arsenic appears less frequently documented than hepatic toxicity, chronic exposures produced significant renal pathology including tubular cell vacuolation, inflammatory cell infiltration, glomerular swelling, interstitial nephritis, and tubular atrophy in both MT-I/II knockouts and sv129 wild-type mouse controls exposed to either arsenite or arsenate through subcutaneous injections for 15 weeks or drinking water for 48 weeks. Interstitial inflammation appeared more severe in MT-knockouts[38].

4.3 Pancreas Pathology

In male Wistar rats exposed to 1.7 ppm of arsenite orally for 90 days, the pancreas pathology included the absence of insulin immunolabel in the Beta cells, and a discontinuous peripheral pattern of glucagon[23].

4.4 Cardiovascular Pathology

Sprague-Dawley rats exposed to arsenite in drinking water at 0, 2, 5, 10 and 25 ppm for 4 weeks had a dose-dependent increase of platelet aggregation and thrombus formation. These are contributing factors to cardiovascular disease[32].

4.5 Other Pathological Characteristics

Some of the pathological effects associated with As exposure are demonstrated in diabetes[29,62,69]. Mechanisms responsible for the toxic effects of As including oxidative stress[62], demethylation of DNA[68] and inhibition of the glucocorticoid receptors[5,46,62] are also associated with diabetes and other chronic diseases.

In rabbits exposed orally to 1.5 ppm/body weight/day of arsenite for 30 consecutive days, the pathology included diabetic levels of blood glucose, glycosylated hemoglobin, and islet cell damage due to oxidative stress[45]. In white rabbits (sixteen males and sixteen females) exposed intraperitoneally to 2.5 mg/Kg/day of arsenite for 2 or 4 weeks, the pathology included apoptosis of vascular smooth muscle cells with decreased endoplasmic reticulum, undetectable nucleoli, and condensed chromatin near the nuclear envelope producing a large apoptotic body[75].

Acknowledgments

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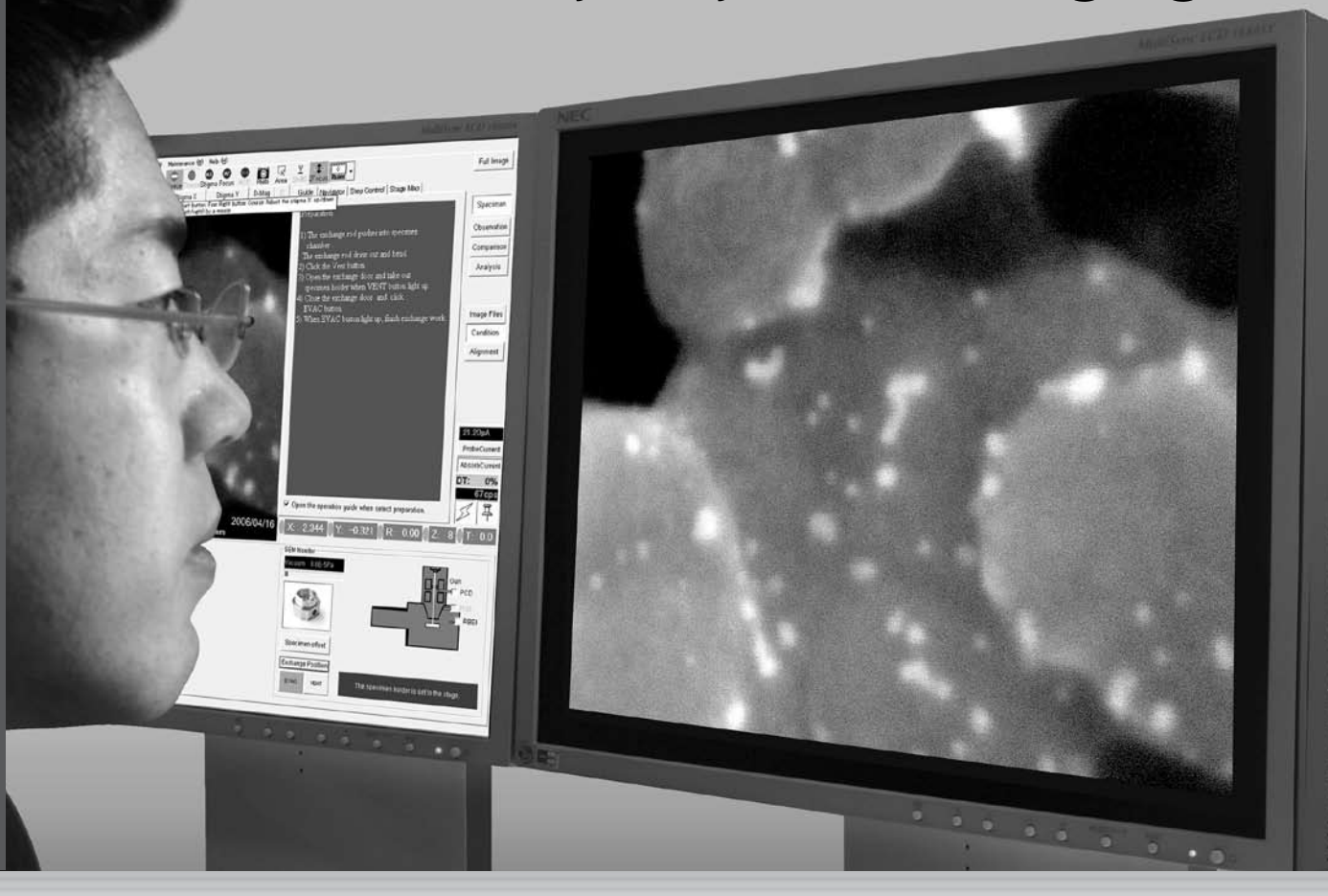

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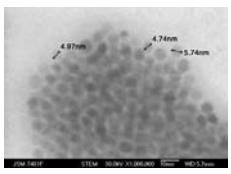

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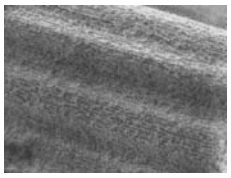

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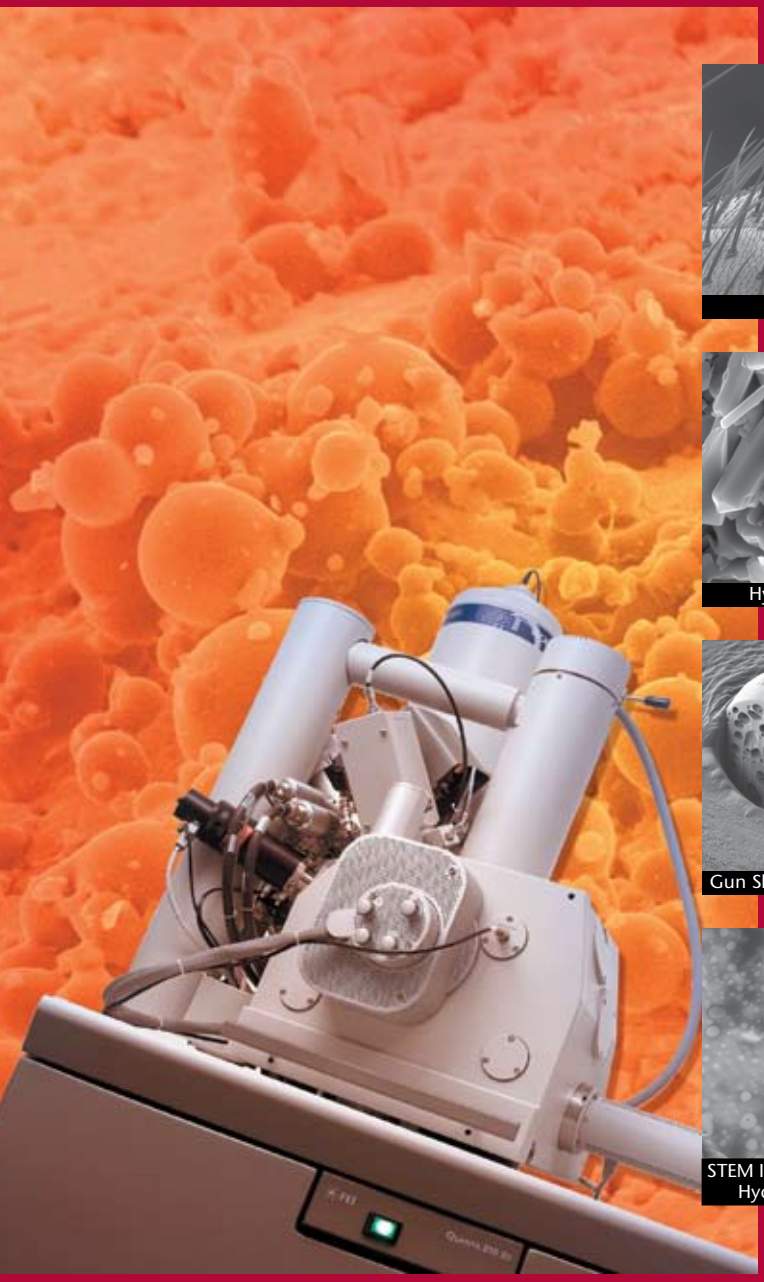

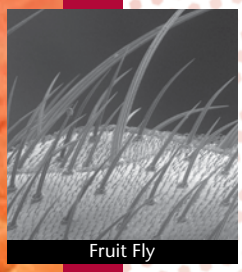
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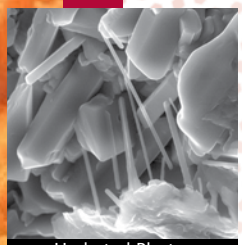
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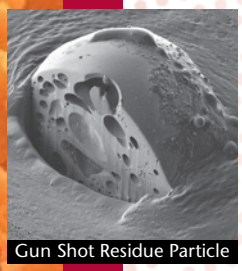
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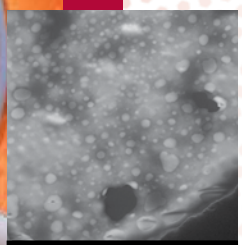
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Abstracts

BIOLOGICAL SCIENCES
FALL 2006

ULTRASTRUCTURE AND PATHOLOGY OF TWO DIFFERENT BACTERIA IN PIERCE'S DISEASE IN GRAPE PETIOLES. E. ANN ELLIS[1], B. GREG COBB[2] and GEORGE R. MCEACHERN[2], [1]Microscopy and Imaging Center and [2]Dept. of Horticultural Sciences, Faculty of Molecular and Environmental Plant Sciences, Texas A&M University, College Station, TX 77843.

Pierce's disease, an economically important plant pathogen, infects the xylem of wine producing grapes in the southeastern states, Texas and California. A transmission electron microscopy study of Pierce's disease in the xylem of grape (*Vitis vinifera*) petioles was performed on specimens fixed in the field in glutaraldehyde-paraformaldehyde followed by post fixation in osmium tetroxide and embedding in epoxy resin in the laboratory. Gross pathology showed partially yellowed leaves and the abscission zones formed between the base of the leaf and the petiole rather than at the base of the petiole adjacent to the stem.

Thick (1 micrometer) sections stained with toluidine blue were used to screen longitudinal and cross sections of petioles prior to cutting ultra thin sections. Thin sections demonstrated two different bacteria correlating with different xylem pathologies. The first rod-shaped bacterium, probably *Xylella fastidiosa*, was found in some xylem vessels and a few vessels appeared to be occluded by dense masses of the bacteria. A second rod-shaped organism had an electron dense glycocalyx with spikey projections. This organism was associated with tyloses and gum deposits, which appeared to occlude some xylem elements. There were only a small number of infected vessels in individual vascular bundles and longitudinal sections provided a better understanding of the pathology.

There are reports of two or possibly three bacterial species associated with Pierce's disease. We have demonstrated two organisms in close proximity in the xylem with different pathologies; however, it has not been possible to determine which pathology is more important. It is probable that the vascular occlusion with the tyloses and gum production associated with the second organism contributes to a slow, but significant water stress on the plant. The first organism may contribute to water stress in a less significant manner.

FLUORESCENT MICROSCOPY ON TEN SPECIES OF PHYTOPLANKTON FROM THE PALO DURO CANYON STATE PARK. MANDY WHITESIDE, LISA DYER and NABARUN GHOSH, Department of Life, Earth and Environmental Sciences, West Texas A&M University, Canyon, TX 79016.

Ten species of phytoplankton were identified in the samples collected from the water crossings of Palo Duro Canyon State Park. Fluorescent microscopy was used to characterize phytoplankton's autofluorescence. Very small samples of phytoplankton were obtained from each collection vial using forceps or droppers, and were mounted on microscopic slides. In some cases staining with Fluorol Yellow 88 (Sigma Cat. # F5520-25G) revealed better morphology of the samples by enhancing the contrast. The slides were observed under an Olympus BX40 microscope equipped with FITC and TRITC fluorescent filters and a mercury lamp source, and pictures were taken with an Olympus DP-70 digital camera with Image Pro 6.0 software. Fluorescence microscopy proved to be a rapid and reliable technique for detection of nitrogen fixing structures such as heterocysts and akinetes or asexual spores in *Anabaena*. Other organisms like *Euglena acus,* mixed Diatoms*, Volvox, Scenedesmus, Micrococcus, Gleocapsa,* other *Euglena* specie*s, Cladophora and Spirogyra* showed interesting characteristics under the fluorescent microscope. The entire cell of *Euglena* exhibited autofluorescence excepting the eyespot and the nucleus. The eyespot and the nucleus appeared as the dark spots with FITC and TRITC filters. The phytoplankton concentrations from the water bodies of the six water-crossings in the Palo Duro Canyon State Park were determined bimonthly. Using sterile droppers a few drops of water samples were transferred into a cell counting device (SEDGEWICK-RAFTER CELL S50, WARDS Cat# 21-0225) holding 100 cubic millimeters of liquid over an area of 50x20 millimeters. Concentrations were reported as number of phytoplankton organisms per cubic centimeter. Phytoplankton concentrations are indicative of water quality. Excessive accumulation of phytoplankton can cause death of aquatic animals, and therefore ecosystem degradation. One of the objectives of this study was to determine the health of the water body supporting the wildlife in the Palo Duro Canyon State Park. This study shows that FM, by enhancing the view of certain phytoplankton structures such as heterocysts, can be used to help characterize the phytoplankton that determines the water quality and sustainability of the wildlife.

FLUORESCENT MICROSCOPY STUDIES OF AEROALLERGENS CAUSING ALLERGIES AND RHINOSINUSITIS IN THE TEXAS PANHANDLE. MANDY WHITESIDE[1], LISA DYER[1], C. SAADEH[2], MICHAEL GAYLOR[2] and NABARUN GHOSH[1], [1]Department of Life, Earth and Environmental Sciences, West Texas A&M University, Canyon, TX 79016. [2] Amarillo Center for Clinical Research/ Allergy A.R.T.S. 6842 Plum Creek Drive, Amarillo, TX 79124.

Fluorescence Microscopy (FM) has become one of the most useful approaches to understanding the structure and function of the microscopic objects. We used FM as part of our continuous study on the characterization of aeroallergens in the Texas Panhandle (2000-2006). Collections of pollen and spores were obtained from air by employing wax-coated Melinex tape in a Burkard volumetric spore trap. The aeroallergens were studied with an Olympus BX40 microscope equipped with FITC and TRITC fluorescent filtersand a mercury lamp source, identified, counted, and photographed using an Olympus DP-70 12.5 mega pixels digital camera. The images captured under bright field, FITC, and TRITC were analyzed using the Image Pro 6.0 software. We identified and characterized different grass species pollen (*Poaceae*), Short Ragweed (*Ambrosia artemisiifolia*), Scotch Pine (*Pinus sylvestris*), Common Sunflower *(Helianthus annuus)*, Hairy Sunflower *(Helianthus hirsutus*), Buffalo Bur *(Solanum rostratum),* Purple Nightshade (*Solanum elaeagnifolium*) and Lamb's Quarters (*Chenopodium album*). Fungal spores were identified for *Alternaria*, *Dreschlera*, *Stachybotrys*, *Cladosporium*, *Curvularia, Torula* and ascospores from *Peziza*

les. The septa and storage granules of fungal spores exhibited autofluorescence under FITC filter. The colpi and pores were very conspicuous with TRITC that were not visible in bright field. The storage protein and oil granules inside the pollen, and the layer of sporopollenin in the pollen's exine exhibited autofluorescence. The specific morphological parameters revealed by employing FM could be used for characterizing and identifying the pollen from different plant species.

COTTON POLLEN TRANSITION THROUGH THE DIGESTIVE TRACT OF FLOWER BEETLE, *CARPOPHILUS* SP. (COLEOPTERA: NITIDULIDAE). [1]DAVID C. GARRETT and [2]CAMELIA G.-A. MAIER, [1]University of North Texas, Department of Materials Science and Engineering and [2]Texas Woman's University, Department of Biology, Denton, Texas 76201.

Cotton pollen is a source of nourishment for the flower beetle, *Carpophilus* sp. (Coleoptera; Nitidulidae), a small group of black beetles of approximately 3 mm in length. Although both adults and larvae feed on pollen, it seems that they do not cause a decrease in the fertility of cotton flowers. The pollen grain (plant male gametophyte) contains nutritive reserves as lipid and protein bodies. The elaborate extracellular pollen wall, mostly the exine, is not easy to break down since it contains sporopollenin, an exceptionally stable biopolymer made of acyl lipid and phenylpropanoid precursors. It seems a paradox that flower beetles feed on pollen when it is difficult to digest it. In order to visualize the transformations suffered by pollen grains in the digestive tract of the *Carpophilus* insects, frass particles were studied by LM and SEM. Most of the pollen in the frass particles was crushed, but retained intact walls. It seems that *Carpophilus* feeds on pollen grains by biting on them, which releases their nutritive content. Some pollen grains showed bubbly surfaces, most probably due to the action of the insect digestive enzymes. Surprisingly, when frass particles were fractured, intact globular pollen grains were found inside, which indicates that flower beetles could also swallow intact grains, which were not digested. It may be possible that flower beetles make use of nutritive substances in the pollen coat as well, not only of those inside the grain. Further biochemical and microscopy studies on pollen grains inside the digestive tract of *Carpophilus* will be employed to evaluate pollen changes while in transit.

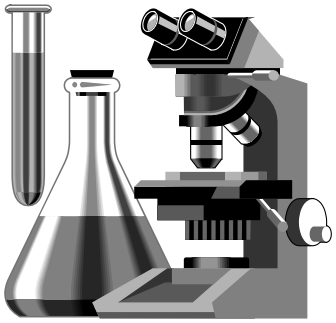
MINERAL DEPOSITS IN PLANTS OF THE MULBERRY (*MORACEAE*) FAMILY. [1]BRANDON ROBETRS, [2]DAVID C. GARRETT and [3]CAMELIA G.-A. MAIER, [1]University of North Texas, Texas Academy of Mathematics and Sciences, [2]University of North Texas, Department of Materials Science and Engineering, and [3]Texas Woman's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

We have previously reported on the mineral deposits in mulberry (*Morus alba*, *M. rubra*) and Osage-orange (*Maclura pomifera*) (*Moraceae*). Since mulberry contains a variety of mineral deposits from the point of view of morphology and chemical composition, we were interested in studying other plants from the *Moraceae* family for the purpose of getting a complete view of mineral depositions in this family. This study will help us better understand the genetics and environmental factors involved in mineral deposition in plants. The following species were studied: edible fig (*Ficus carica*), fiddle-leaf ficus (*Ficus lyrata*), paper mulberry (*Broussonetia papyrifera*), all perennial species, and mulberry weed (*Fatoua villosa*), 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 possess silicified trichomes, some of which have cystoliths inside their basal cells The trichomes are hollowed and some of them seem to deposit an opaque material. There are at least two types of hairs on both fig and paper mulberry leaves: short and sharp ones and very long, flexible, and entangled ones. The druses of mulberry weed were found inside cells on its flower organs, probably serving as protection against insect herbivores. The characteristics of the above mineral deposits can help in the identification of species in the mulberry family of plants.

MIDDLE PERMIAN HOLOTHURIAN SCLERITES FROM THE GUADALUPE MOUNTAINS, WEST TEXAS. G. P. NESTELL[1], M. K. NESTELL[1], and M. GRACEY[2], [1]Department of Earth and Environmental Science and [2]Department of Biology, University of Texas at Arlington, Arlington, TX 76019.

Holothurians or "sea cucumbers" are one of the classes of the phylum Echinodermata that has a relatively sparse representation in the fossil record. Complete impressions of fossil holothurians are known to occur in rock strata at some levels of the Phanerozoic. The fossil record of holothurian sclerites is from the Ordovician to Holocene, but perhaps they may be present even in the Cambrian. For example, impressions of specimens are found in ironstone concretions in clastic sediments such as the St. Francis Shale (Pennsylvanian) at a number of localities in the Mazon Creek area of the Illinois basin. More commonly, fossil holothurian remains are found as sclerites, microscopic calcareous spicules that are present in the body wall. These sclerites are so small that some living holothurians contain 10-20 million of them in one individual. They are very diverse in form and occur in the shape of hooks, tables, wheels, anchors, sieve plates, and anchor plates. The function of the sclerites is to strengthen the body wall.

Holothurian sclerites are known from Permian age strata in a number of places in the World, for example, from Kansas in the U.S.A., and from Italy, Germany, Iran and China. Abundant holothurian sclerites were recently discovered in some samples taken from Middle Permian strata of middle and late Guadalupian age (Hegler, Pinery, Rader and Lamar members of the Bell Canyon Formation) in the Guadalupe Mountains for the study of other microfossils such as conodonts, foraminifers, and radiolarians. The holothurian sclerite assemblage contains representatives of the genera described as *Eocaudina*, *Achistrum*, *Calclyra*, *Theelia* and *Microantyx*. Recently, this last form has also been suggested to belong to another group of the Echinodermata, the Class Ophiocistioidea. It is very important that these holothurian sclerites have been found in the same samples together with abundant conodonts that are the basis for precise dating of Middle Permian strata worldwide.



RELATIONSHIP BETWEEN PHYTOLITHS OF FORAGE SORGHUMS AND HERBIVORE PREFERENCE. [1]MICHAEL W. PENDLETON, [2]BONNIE B. PENDLETON, and [3]FREDERICK R. MILLER, [1]Microscopy & Imaging Center, Texas A&M University, College Station, TX 77843-2257 [2]Department of Agricultural Sciences, West Texas A&M University, Canyon, TX 79016-0001, and [3]MMR Genetics LTD., Box 60, Vega, TX 79092.

The presence of phytoliths (hydrated silica particles) in grass tissues has been postulated to deter vertebrate grazing because of abrasion of teeth by the silica (Baker, Jones and Wardrop, 1959). High concentrations of silica in forage could also deter grazing because silica may prevent access to carbohydrates and nitrogen during digestion by cattle (Van Soest and Jones, 1968). Gallego and Distel (2004) determined that the morphological differences in phytolith assemblages of several grasses native to Argentina differentiated between preferred (palatable, high forage value) and avoided (non-palatable, low forage value) grasses grazed by vertebrate herbivores. However, they cautioned that these results were preliminary and required study using many more species to determine if the relationship was true for most grasses. Sorghum is a grass used extensively for cattle feed and forage. Field evaluation determined that cattle in our study prefer certain sorghum genotypes that seem to have a greater concentration of phytoliths on the epidermal surfaces than do other sorghum genotypes, a preference behavior not predicted by the previous research hypotheses cited. Phytolith abundance were quantified and phytolith morphologies characterized for the sorghum genotypes studied to determine if these attributes are related to grazing preference behavior of cattle.

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MICROSCOPIC STUDIES OF HAWTHORN LEAVES AND STEMS HOSTING PARASITIC *GYMNOSPORANGIUM* FUNGI. [1]PATRICK PETERSON, [2]DAVID C. GARRETT, and [1]CAMELIA G.-A. MAIER, [1]Department of Biology, Texas Woman's University, Denton, Texas, 76201; [2]Department of Materials Science and Engineering, University of North Texas, Denton, Texas, 76201.

Species of the *Gymnosporangium* (Fungi: *Basidiomycetes*: *Uredinales*) are parasitic heteroecious fungi, needing two plant species hosts to complete their life cycle and inducing rust diseases on both host plants. The primary hosts for *G. clavipes* and *G. globosum* are species of *Juniperus*. The alternate hosts are mostly *Rosaceae* plant species. Infections are described as juniper-quince and juniper-hawthorn rust, respectively, and they are more harmful to the alternate host than to juniper. Because of their economic impact on timber, ornamental trees, and fruit crops, *Gymnosporangium* species has been studied since the beginning of the last century. Both *G. clavipes* and *G. globosum* undergo four different spore stages through their life cycles. Telia or spore horns are produced on the stems and leaves of *Juniperus*; teliospores will germinate to form basidia with basidiospores, which will infect nearby *Crataegus* trees. On the upper surface of the hawthorn leaf, basidiospores germinate and give rise to pycnidia or spermatia, which will grow through the leaf tissue. Later, aeciospores are produced and released from peridia on the underside of the hawthorn leaves and will infect *Juniperus*, thus completing the life cycle of the fungus. Peridia and aeciospores were collected from a hawthorn tree on the TWU, Denton campus and studied with LM and SEM. *G. clavipes and G. globosum* are very similar but differ mainly in having the aecia form on leaves (*G. globosum)* instead of stems and fruits (*G. clavipes)*. Other differences were observed in the shape and structure of the peridia (tubular protective sheaths). Peridia are composed of many colorless, rhomboid cells that are oriented vertically. The internal wall of the peridial cells is ornamented with short, coarse ridges (tuberculate type). The aeciospores were globose, with a thick, pale yellow wall with echinulate ornamentations. In mass, they appear bright orange. Based on the morphology of the aeciospores and peridial cells, we identified the hawthorn rust as being *G. clavipes*. Further studies of *Gymnosporangium* fungi will continue on samples of juniper.

ARSENIC TOLERANCE IN C57BL/6J MALE MICE. JAIME B. VIGO and JOANNE T. ELLZEY, Biological Sciences, The University of Texas at El Paso, 79968-0519.

Epidemiological and *in vitro* studies have suggested a link between ingestion of arsenic through drinking water and the onset of diabetes-Type 2. We designed two model experiments with C57BL/6J male mice to investigate these possible links. In the first study, 3 groups of mice were fed a diet with 6% fat content, and exposed to 0, 5, or 75 ppm of As(V) in the drinking water for 12 weeks. In the second study, 4 groups were fed with either a 4% or 11% fat diet, and exposed to 0 ppm or 22.5 ppm of As(III) for 40 weeks. During the last 10 weeks, the fat content of the 11% fat groups was increased to 14%. The group exposed to 75 ppm As(V) significantly consumed more food, but gained less weight than the group exposed to 5 ppm and controls ($p<0.05$). The blood urea nitrogen (BUN) levels increased over time for all 3 groups in the As(V) study. The aspartate aminotransferase (AST) was significantly higher in the 75 ppm *vs.* 5 ppm groups and controls. In the As(III) study, the average accumulated weights ($p<0.01$) of the high fat diet mice were significantly higher than the controls. In general, no significant differences due to arsenic were observed for water consumption, blood glucose, catalase, insulin, alanine aminotransferase (ALT), triglycerides, liver-to-body weight ratio, or kidney-to-body weight ratio. Hepatic, renal or pancreatic pathology was not observed. No arsenic-induced hyperglycemia, hyperinsulinemia, or diabetes was observed. In comparison to similar experiments with Metallothionein-I/II null mice and the corresponding wild-type 129 mice, which demonstrated liver and kidney pathology due to arsenic, our mice tolerated the same doses used by Liu *et al*., (2000) without pathological results.

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FROST RINGS IN TIMBER CORES FROM SPRING HOUSE, MESA VERDE, COLORADO

HOWARD J. ARNOTT[1] and REX ADAMS[2]

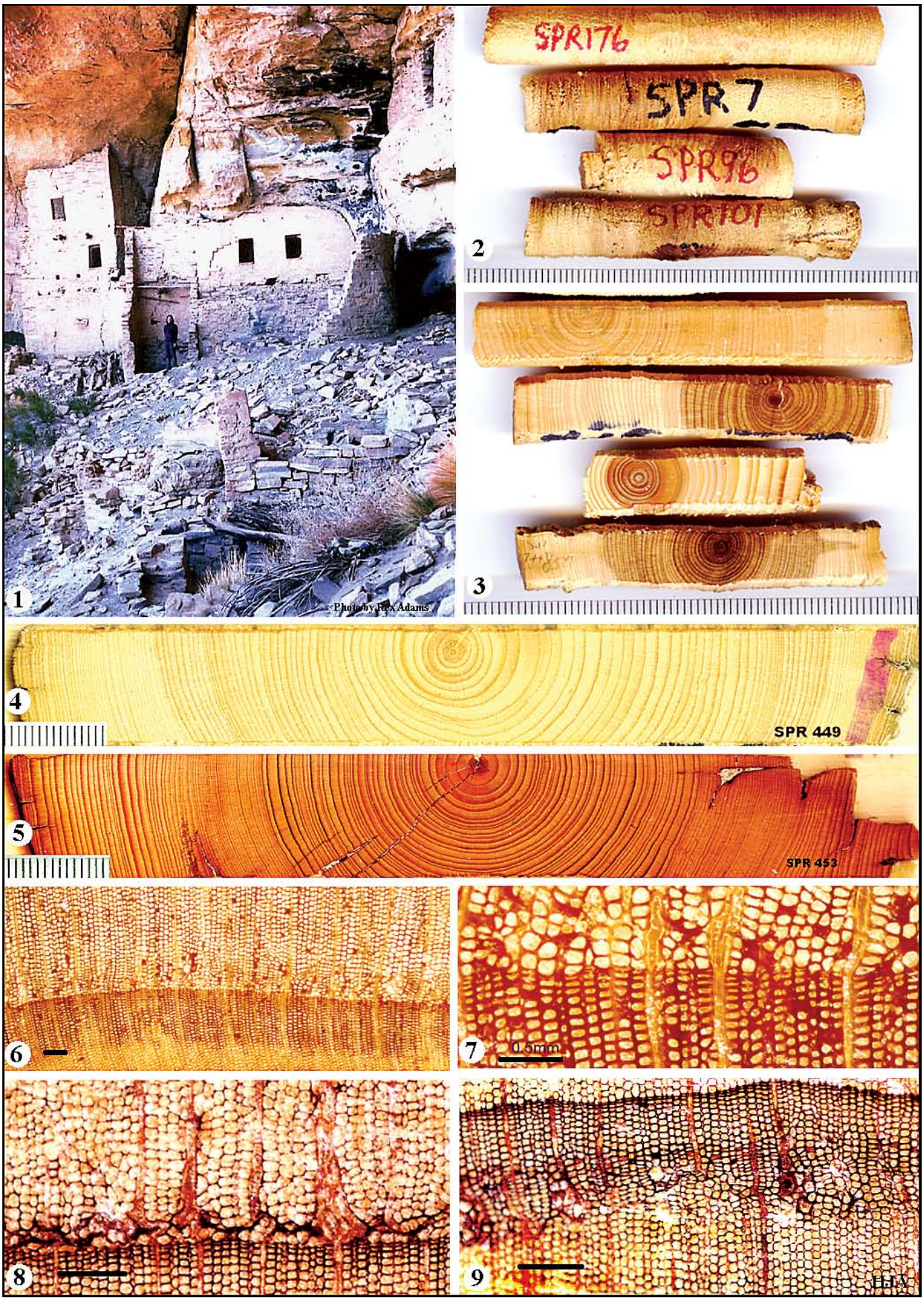
[1]Department of Biology and Center for Electron Microscopy, University of Texas at Arlington, Arlington, Texas, 76019 and [2]Laboratory for Tree-Ring Research, University of Arizona, Tucson, Arizona, 85721

Spring House (Fig. 1) is one of a many pre-historic cliff-dwellings found in the Mesa Verde region of Colorado. The cliff houses were the home of the Ancestral Puebloan people; the sites were abandoned in the 12-1300's. Most of the Mesa Verde cliff houses were built in the 1200's. The majority of cutting dates of timbers from Spring House are in the middle to late 1200's, however, a few earlier cutting dates are known, such as 1079 and 1087.

The presence of a frost ring in a Spring House timber core was first called to our attention by Dr. David J. Street. With the permission of Dr. Jeff Dean, Laboratory of Tree-Ring Research (LTRR), we have examined many other Spring House cores which are apart of the permanent collection of the LTRR. Figures 2 and 3 show examples that are characteristic of most of the cores in the collection. The accession data from LTRR gave the beginning date (often the pith date), the cutting date, location in Spring House, the species of wood, and the nature of each specimen in the collection. The three most numerous plant species from which the timbers in Spring House were made are pinyon pine (*Pinus edulis*), Douglas fir (*Pseudotsuga menziesii*) and juniper (*Juniperus osteosperma*). When received, each core had one well sanded surface, however, we routinely re-sanded them using 250, 400, 600, 1000 sandpaper and 2000 to 3600 Micro-Mesh cushioned abrasive pads; care was used to be sure that most surface imperfections were removed. Each core was scanned using an Epson 4900 photo scanner, and images of 300, 1200 and 2400 dpi were captured (Figs. 4, 5). The timber surface was best viewed using an 80i Nikon microscope with reflected light. Examination by 4X, 10X, 20X and 40X objectives was possible and frost rings and other details were easy to see. Images were captured using a Nikon DXM 1200F digital camera and Nikon ACT1 software. In some cases images were processed using the Extended Focus option of Image Pro 5.1. Images were sometimes sharpened using the Smart Sharpen filter of Photoshop CS2. Materials were sectioned using a sliding microtome, some sections were stained with saffarin and viewed in the optical microscope, and others were examined in the SEM. Cutting dates were determined by LTRR staff, frost rings dates were determined by HJA.

Out of 82 Spring House cores initially examined, 45 (55%) had frost rings. The 45 cores with frost rings are approximately equally representative of the three major timber species. In the 45 cores there were 69 frost rings, one core had 5 frost rings present; five other cores had 3 or more frost rings. In two Douglas fir cores, traumatic resin ducts were found in 6 and 8 annual rings, respectively. In this study, the dates of frost ring occurrence extended from the year 956 to 1267, however, the frost rings in the years 956 and 957 are extreme outliers. The years 1195, 1219 and 1256 were each represented in three cores. The collective data from 1100 to 1268 leads to the estimation that a frost ring would occur in one out of every 4 years. However, it is interesting to note, that the frost years are not equally spaced over the 17 decades, but rather they seem to be grouped in four or five major time periods. This study concludes that there is a wealth of frost ring data stored in archeological cores.

Figure 1. North end of Spring House, a portion of the Upper Alcove seen in the upper right; the "spring" for which Spring House is named is directly behind the wall where the woman is standing (Photo taken in 1996 by Rex Adams). ***Figures 2-5 have millimeter scales.*** **Figure 2.** Four selected 3/8 inch cores from the LTRR Spring House collection showing an external view with labels. **Figure 3.** The same cores in identical order as Fig. 2, showing the reverse sanded surfaces from which data is collected. SPR 176 is a pinyon pine core taken from a loose log in Room 41, it was cut in the year 1249 and has a 1205 frost ring; SPR 7 is a core of a juniper roof primary timber from room 6, it was cut in the year 1274 and has a 1189 frost ring; SPR 96 is a core of a Douglas fir roof secondary timber from room 49, it was cut in the year 1268 and has a 1255 frost ring; SPR 101 is a core of a juniper roof secondary timber from 51 West Pillar, it was cut in the year 1258 and has a 1167 frost ring. ***Figures 4 and 5 are specimens taken from 7/8 inch cores.*** **Figure 4.** SPR 449 is a core of a Douglas fir log found in the log pile of rooms 39/40, it was cut in the year 1087 and has a 956 frost ring. **Figure 5.** SPR 453 is a core of a juniper log found in the log pile of room the 39/40, it was cut in the year 1268 and a frost ring at 1123. ***Figures 6-9 are optical micrographs showing the cellular structure of frost rings, the scale line equals ½ millimeter.*** **Figures 6-7** show the 956early frost ring in SPR 449; note the irregularities in the ray cells in Fig. 7. **Figure 8.** The early frost of year 1123 ring in SPR 453, note the short period of growth before the formation of the frost ring. **Figure 9.** SPR 501 has a midyear frost ring in the year 1190, a pinyon pine, from the Red Horse Wood Pile.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CYSTOLITHS AND *NEBENCYSTOLITHEN (SECONDARY CYSTOLITHS)* IN WHITE MULBERRY (*MORUS ALBA* L., *MORACEAE)*

HOWARD J. ARNOTT[1] and CAMELIA G.-A. MAIER[2]

[1]The Department of Biology and the Center for Electron Microscopy, UT Arlington, Arlington TX 76019 and [2]Department of Biology, Texas Women's University, Denton, TX, 76204

Cystolith is the name given to stalked bodies containing amorphous calcium carbonate that occur in the leaves and stems of some flowering plants. Cystoliths result from a complicated series of cytoplasmic and cellular changes, which occur in specialized cells of the epidermis called lithocysts (F. M. Scott, 1946; Arnott, 1982). Lithocysts arise in the leaf epidermis but expand downward forcing their way in-between the sub-epidermal palisade parenchyma cells. In *Morus* leaves, lithocysts are cells elongated perpendicularly to the surface of the leaf, generally two to five times the height of the epidermal cells that surround them. The formation of cystoliths within lithocysts in *Morus alba* and *M. rubra* has been documented (Arnott, 1976, 1980, 1982); however, little attention has been given to the *nebencystolithen* or secondary cystoliths, which are commonly associated with the epidermal cells surrounding lithocysts. Both cystoliths and secondary cystoliths are common in mulberry and other members of the mulberry family (*Moraceae*). Here we report on the morphology and structure of isolated cystoliths and *nebencystolithen* from *M. alba* leaves viewed with LM and SEM. The cystoliths were isolated from "native" trees growing on the TWU campus in Denton, using the isolation technique previously reported (Maier and Arnott, 2002).

The shape of *Morus* cystoliths varies from a prolate to oblate spheroid. *Morus* cystoliths average approximately 100 μ in length and 50 μ in diameter. Each cystolith is attached to the outer wall of the lithocyst by a short stalk that has been shown to be impregnated with silicon (Arnott, 1982). The main body of the cystolith may be relatively smooth with small "bumps" or "warts" on the surface or it may consist of a series of rounded protrusions which are also be covered with "bumps and "warts." Light microscopy reveals several organized regions within each cystolith; the stalk continues into the central portion of the cystolith and varies in shape sometimes having several branches (Fig. 1). X-ray analysis shows that the stalk region is high in silicon. Around the stalk, layers of organic matrix provide a scaffold for the deposition of calcium carbonate (Arnott, 1982). The concentration of cellulose, hemicelluloses, and pectins vary in different parts of the organic scaffold that supports the body of the cystoliths (Arnott and Webb, personal communication). In some cases, cystoliths have part or all of the amorphous calcium carbonate replaced by crystalline deposits (Arnott, 1982). Because they are highly birefringent, "crystalliferous cystoliths" can be differentiated easily from normal ones by using polarized light.

In mulberry, there may be several "rows" ("layers") of secondary cystoliths surrounding a single cystolith (Fig. 3). However, in some cases no secondary cystoliths were associated with an individual cystolith. Although the secondary cystoliths have many characteristics of the cystoliths, they have a much smaller volume (Figs. 4-7). Some secondary cystoliths have clear stalks while others seem to be attached by a broad base arising from the external cell wall of the epidermal cells hosting them. Secondary cystoliths vary in shape from thimble-shaped formations with relatively smooth sides and a rounded ends to almost cylindrical-shaped structures with rounded ends. The height of secondary cystoliths varies from 10 to 65 μ. Secondary cystoliths have a non-homogenous internal structure characterized by concentric rings (similar to those of cystoliths), dark granular areas, and areas with no clear pattern of internal structure. When placed in weak HCl, secondary cystoliths, as well as most cystoliths, give off bubbles, a clear indication that calcium carbonate is present. Preliminary X-ray analysis of secondary cystoliths indicates that they have a high concentration of silicon. The ontogeny, physiology, and molecular biology of the secondary cystoliths are fascinating topics, which await further research.

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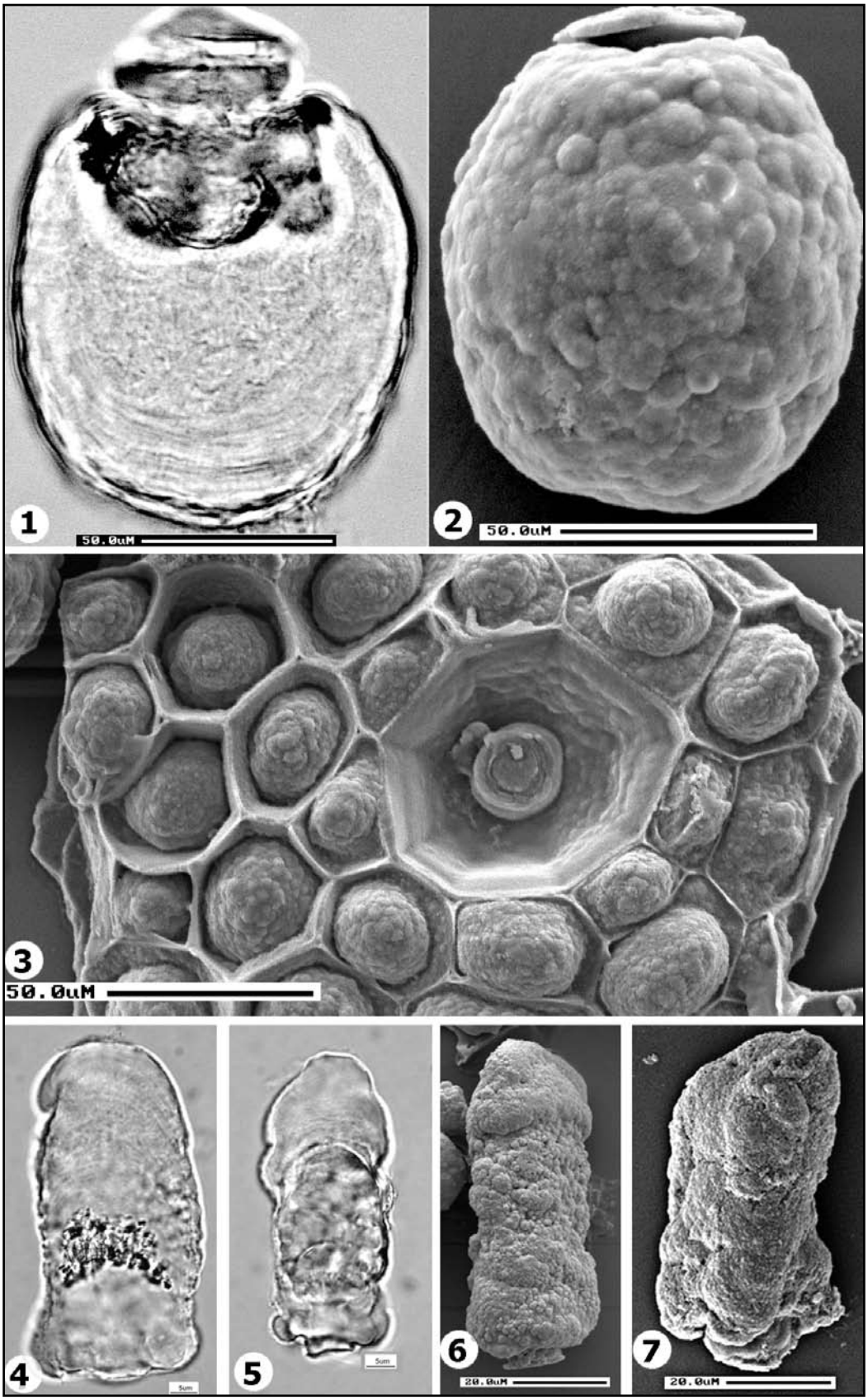


FIGURES 1-7. Isolated cystoliths from the leaves of white mulberry, *M. alba*, *t*rees growing on the TWU Denton campus. **Fig. 1.** Light micrograph of cystolith from a male tree. **Fig. 2.** SEM micrograph of cystolith from a female tree. **Fig. 3.** Fragment of the epidermis viewed from inside the leaf, showing (center) the upper part of a lithocyst with the remains of the cystolith stalk. Surrounding epidermal cells each contains a secondary cystolith or nebencystolithen. Note the thickened rugose (bumpy) cell walls of the secondary lithocysts. **Figs 4-7.** Isolated secondary cystoliths. ***Figures 4 and 5*** are light micrographs, and ***Figs. 6 and 7*** are SEM micrographs. Note the internal layered structure of the secondary cystoliths best seen in the light micrographs. Note also that each secondary cystolith has a short stalk by which it was attached to the cell wall of its secondary lithocyst (best seen in Fig. 6).

GaAs MMIC Resistor Deposition and Modification Via FIB

Milton Tam

Raytheon Company, 13510 North Central Expressway, MS 255, Dallas TX 75243

A precise high-resolution ion beam analysis technique, Focused Ion Beam (FIB) is described. Using high-energy Ga ions for materials ablation, in conjunction with low resistivity metal and insulator deposition has been shown to be a precise method to quickly modify GaAs. Monolithic Microwave Integrated Circuit (MMIC) resistors *in situ* on loose die, die assembled on test carriers or in packages. Prior to this technique, the design engineer would have to wait several months to get the new devices back from fab, then several more weeks to determine success after test carriers have been fully characterized. This process allows the MMIC design engineer to save on one mask iteration, in case the new design does not work, and 2 months of fabrication cycle time. Several Psuedomorphic High Electron Mobility Transistor (PHEMT) power and low noise devices have been modified and post electrical results have been in good agreement with simulation data.

The two premier US based GaAs foundries for X-, Ku- and mmW-Band products are Raytheon RF Components (RRFC) and Triquint Semiconductor (TQS). The MMIC fabrication cycle time varies between these 2 foundries. The typical RRFC cycle time varies from normal mode (3+ months) to for rocket lot (2 weeks). The typical Triquint cycle time varies from normal mode od less than 12 weeks to hot lot (4 weeks). If performance issues are found after design verification tests, the MMIC design engineer has few options but to submit another mask change and repeat the fab process to iterate his improved design.

A precise high-resolution ion beam analysis technique provides the ideal solution to quickly cut and reroute transmission lines but more importantly to change the value of existing resistors or to deposit new resistors within the MMIC layout (Figure 1). Field Effect Transistor (FET) gate widths, diodes and capacitors cannot be modified. Resistors, transmission lines and inductors can be easily modified. Resistors ranging from 5ĸ– 2.5Kĸcan be formed using low resistivity tungsten (5ĸSQ). Resistors ranging from 50ĸ- 10Kĸcan be formed using higher resistivity platinum (15-20 ĸSQ). These metals can be deposited on top of the existing SiNx passivation. Metal deposition in conjunction with insulator deposition and insulator etch enables dense circuitry to be accessed and modified. Resistances can be increased by trimming with the Ga ion beam depending on the available cross sectional area.

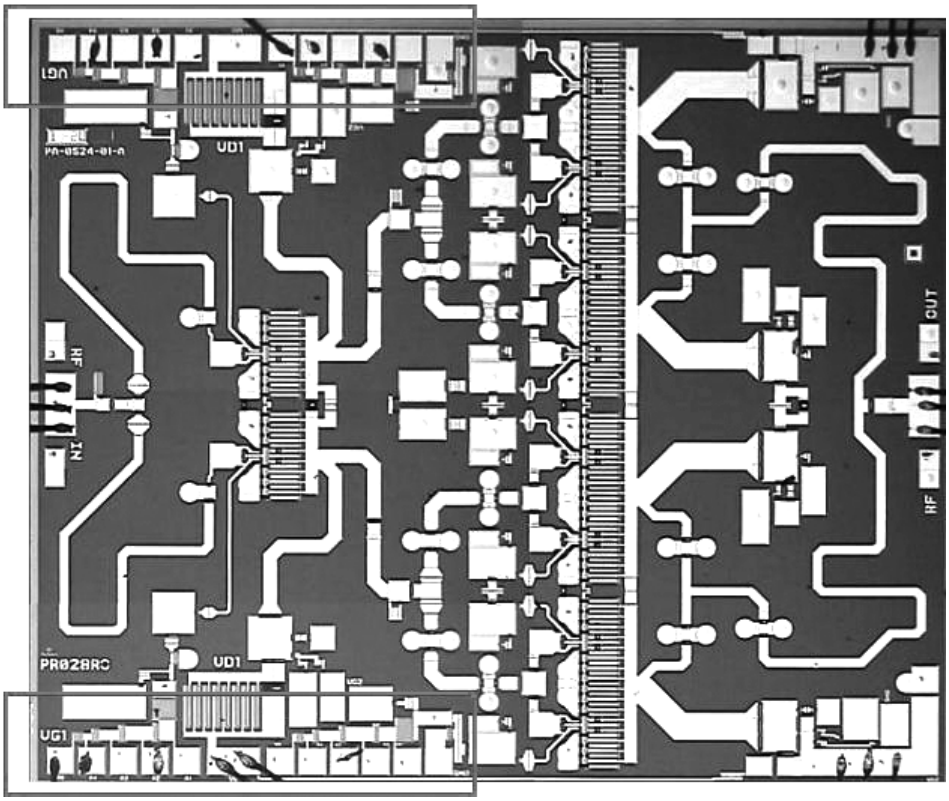


Figure 1. GaAs MMIC layout. Gate bias networks are highlighted.

FIB SYSTEMS: A CONTEMPORARY SAMPLE PREPARATION TOOL FOR SCANNING AND TRANSMISSION ELECTRON MICROSCOPY OF MATERIALS. [1]KULTARANSINGH N. HOOGHAN and [2]PAUL A. RODRIGUEZ, [1]Hooghan Consultancy and Services and [2]Reactive Beam Technology, Inc., 2051 Valley View Lane, Farmers Branch, TX 75234.

Semiconductor device geometries have continuously shrunk in the last decade, subsequently increasing their structural complexity many fold. Characterization and failure analyses of such devices have thus become more challenging than ever. Although the TEM is the ultimate characterization tool, it has only recently been introduced as a diagnostic tool of choice. A dramatic shift in sample preparation techniques has been a major challenge for TEM analysis for many years. Conventional methods work better with larger geometries, since one can see sample details, and know when to finalize its preparation. Currently, device geometries are beyond the optical resolution range, hence conventional methods are no longer feasible for today's site-specific sample preparation requirements. Nowadays, examining a prepared TEM sample is relatively easier and quicker.

The introduction of Focused Ion Beam (FIB) systems has been a major improvement for analytical characterizations due to the ability of FIB systems to repeatedly carry out site specific cross-sections, both for Scanning electron Microscopy (SEM) and TEM samples. Analytical capabilities on the TEMs have also advanced steadily to meet today's stringent demands primarily by the semiconductor industry. By using a FIB system and associated Lift Out (L/O) techniques, sample preparation time was reduced by half and throughput in the laboratory increased 350%. Success rate for TEM samples was more than 95% during the same time frame.

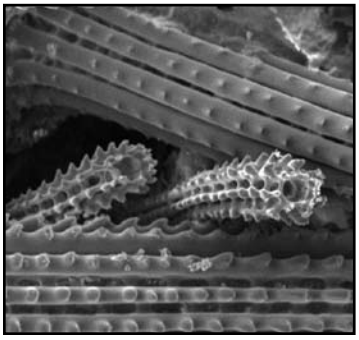
GEOLOGICAL SCIENCES
FALL 2006

SEM ANALYSIS OF GREAT SALT LAKE OOLITES. MANETTA DILLINGHAM, Department of Geology, Stephen F. Austin State University, Nacogdoches, TX, 75962.

Oolites from the Great Salt Lake, Utah, were viewed with a scanning electron microscope to determine their internal mineralogical structure. Oolites generally form in tropical to subtropical, subtidal or intertidal environments. Warm, shallow, supersaturated, marine or lacustrine water is agitated by tidal activity which causes sand grains or shell fragments to oscillate back and forth on the floor of the water body. Layers of calcite or other minerals grow in concentric rings around the oscillating particles. Modern examples are found in the Bahamas and the Arabian Gulf. Despite its inland setting and temperate climate, oolites are abundant on the shores of the Great Salt Lake. High concentrations of dissolved salts and minerals, as well as wave action, cause the development of oolites. Great Salt Lake oolites average 0.5 mm in size and form around either fecal pellets of brine shrimp or small sand grains. They grow larger by either the physical attachment of fine-grained material or by chemical precipitation of minerals to the nucleus. The result is a sequence of concentric lamellae that resemble tree rings. Great Salt Lake oolites are composed primarily of the mineral aragonite, distinguished by columnar crystals. Aragonite crystals are orthorhombic. When viewed in cross-section, they have a distinctive hexagonal shape. The crystals are oriented radially but may exhibit random orientations or even tangential orientations. These variations in orientation occur not only in separate ooids but also in discrete layers within the same ooid. Some ooids exhibit accretionary bands and growth interference. The rays may terminate distally at a major clay-rich discontinuity or at an area in which very fine-grained minerals have precipitated. Layers of randomly or tangentially arranged fine crystals are common immediately adjacent to the nucleus or just distal to major concentric discontinuities. Salinity levels in Great Salt Lake vary, sometimes significantly. Changes in salinity level and subsequence mineral saturation level may explain the presence of clay-rich bands found in many ooids. It is not uncommon for two or more oolites to become accreted to form a composite ooid through enveloping lamellae. The rolling motion that creates ooids, in conjunction with decreased mineral saturation levels, sometimes causes parts of the outer layer to be dissolved which can leave pits and scour marks on the surface of oolites.

Answer to "What Is It?"

from Texas Journal of Microscopy 37:1



The micrograph shows a detail of the external spines of sand dollars, Echinarachnius parma (Echinarachniidae) picked up on the Silver Strand State Beach in Coronado, California. Sand dollars are echinoderms related to sea stars and sea cucumbers. The term 'sand dollar' can also refer to the skeleton or test left when the animal dies. Air fractured samples of the sand dollars were mounted on aluminum stubs, coated with gold-palladium, and viewed on a JOEL 35C SEM.

Shawn Prapta, Sandra Westmoreland's student at The University of Texas at Arlington.

MY INTRODUCTION TO RESEARCH

Howard J. Arnott
Autobiography-Part Three

The concept of research was not part of my high school education. One might suppose that when the members of Dr. Zigenfoose's physics class measured our human horsepower that was research. Dr. Zigenfoose's measured our time (t) from the bottom to the top of bleachers (D) and we used our weight (W) to calculate our horsepower using the following equation:

$$HP = D\ W/t$$

A visit to the internet shows that schools still use this system to measure human horsepower. In my case, the study of our horsepower was never called research it was *just something we did*. Looking back, it is absolutely true that it was *research, simple research,* but nevertheless still *research*. If research was a "concept" to be taught in the 1940's Los Angeles High Schools curriculum, it completely "escaped" me, even though Dr. Zigenfoose was a fine teacher.

In actuality, it would be many years before research became a functioning concept for me; this confession is certainly distressing, but it is true. While I learned *many* things in the U.S. Navy, especially photography, the Navy did not supply me with an understanding of research. My science teachers in chemistry, geology and zoology at The University of Southern California, circa 1948-49, also did little to supply me and others with an understanding of what research was and its importance in science.

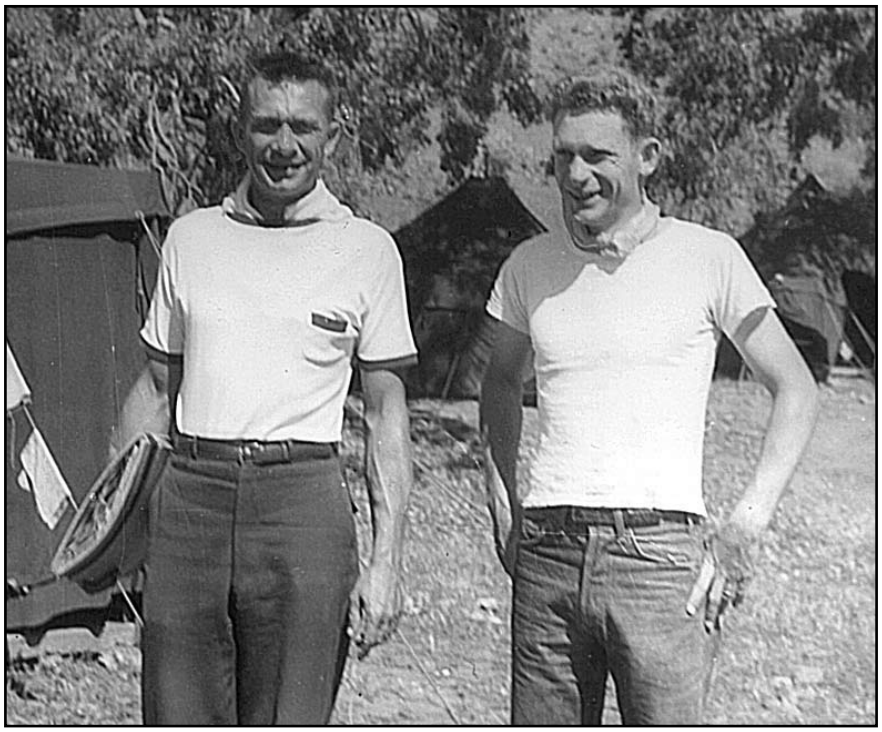


Figure 1. Thomas C. Fuller (left) and H.J. Arnott on Santa Catalina Island field trip in 1952. Note Fuller's home made plant press. Rattlesnakes were quite common on the island. Photo by Jean Arnott.

Oddly, the first time I came face-to-face with research was in a course in Music Appreciation. My instructor firmly insisted that we had to write a **research** paper, and he defined exactly what that meant. I chose to write about *Pipe Organs,* an off beat subject (pun intended), and soon found that there was a great deal of information in books and journals (primary sources) concerning almost every aspect of pipe organs. I was surprised at how easy it was to find information about them; in fact, how easy library research was (is).

The pipe organ exercise was the first time that I realized research could be fun (really cool). Thinking back I am sure that I wrote papers before, papers that might be classified as research papers (using the broadest definition), but what they were, was not clear, or really of any interest to me. Perhaps it was just lack of maturity; however, all the efforts of my former instructors had never defined research. Incidentally, I have no reason to think that things have changed today. The dictionary gives the following definition for research: from Old French: *re-* + cerchier, *to search*; studious inquiry or examination; *esp*: investigation or experimentation aimed at the discovery and interpretation of facts.

There is considerable difference between my pipe organ library research and "hands on research." In the latter there is a clear-cut involvement of individuals with the substance, concepts, mathematics, and/or instruments used to do (scientific) research. Of course "hands on research" also involves careful analysis of the literature in the area of interest.

This paper concerns my first years of experience in my world of "hands on research." It will cover from the beginning of my research career to the completion of my doctoral dissertation. I will make a few digressions to demonstrate the way research progresses.

In 1951, my junior year at USC, I changed my major to Botany. Soon, I began to have interactions with my Botany Professors outside of the classroom; local field excursions, conversations or invitations to coffee were the first (Fig 1). Later Jean and I were invited to dinner or parties at faculty homes. I soon became aware of the advantages of being a student in a small department; the professors knew me by name and were actually interested in my future. On several occasions I went hiking with Dr. George R. Johnstone in the San Gabriel Mountains. The hiking involved many basic botanical lessons. For example, once he

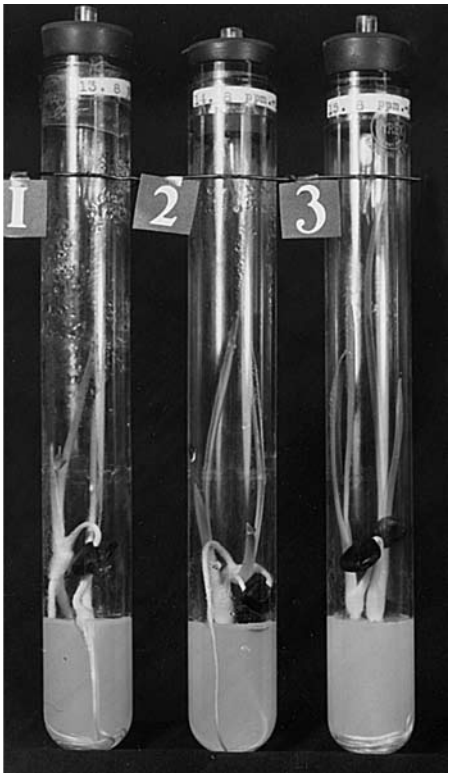


Figure 2. *Yucca brevifolia* seedlings growing in sterile culture. Note: dark black seeds and white roots circling around in the bottom of the tubes. Photo taken in 1952 by HJA.

sent me up a Coulter pine (*Pinus coulteri*) tree to get one of the very large cones characteristic of that species. I retrieved a cone but found when I got back to the ground that I was covered with small very sticky seeds. These, I soon found out were the seeds of the Dwarf Mistletoe (*Arceuthobium*); the seeds are forcefully ejected and can travel for many meters. By touching or shaking a branch a shower of seeds is discharged. The very sticky seeds stick to other trees, to animals, even humans, a unique distribution mechanism. That's one way to learn! Johnstone lived in a mansion on Mulholland Drive, the home of many 1930-60 era movie stars. Dr. Johnstone purchased his home during the depression when, as he told me, "If you had a job you could buy almost anything!"

Thomas C. Fuller (Fig. 1) and George R. Johnstone both became mentors and encouraged me to attempt research. My first "hands on" project involved culturing embryos of *Yucca*; the project was stimulated by research of Dr. Lewis Knudson on the culture of *Iris* embryos. The original publication was shown to me by Dr. Johnstone but Fuller pressed the issue. Dr. Knudson became famous for orchid culture; in part, his work created the orchid industry of today and stimulated plant tissue culture in many ways.

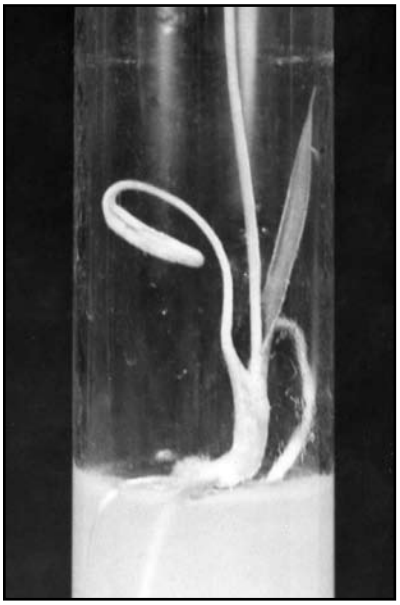


Figure 3. Cultured embryo of *Yucca brevifolia*. Note haustorial cotyledon in upright position. Photo by HJA. 1952.

The removal of embryos from the seeds of *Yucca* was not difficult but had to be done using sterile conditions, since the embryos were to be cultured *in vitro* on an agar/mineral substrate. I used the relatively large seeds of *Yucca brevifolia (Agaveaceae)*. The seeds were first surface sterilized using Clorox; then, inside a "clean box," using sterile forceps the embryos were removed and transferred to the culture tubes. I was able to grow both normal seeds and extracted embryos in the same manner; both under sterile conditions (Figs. 2, 3). I had a great success with both, but amazing success in raising embryos without being contaminated by molds or bacteria (Fig. 2, 3). The embryos, of course, were without the seed tissues which contain much of their food reserves.

Germination in *Yucca brevifolia* is epigenous, that is, the seed is lifted out of the soil during the germination process. It was interesting to note that in culture the seeds also lifted above the media (Fig. 2). However, it was even more interesting to note that the cotyledon of each plant (derived form the isolated embryos) also lifted above the culture media, as if still contained in a seed (Fig. 3). The culture tubes were supplied with light and both seeds and extracted embryo "seedlings" began to grow and produce green leaves. They continued to grow in the 1"x6" tubes for almost 6 months during which they almost filled the tubes (Fig. 2). The seedlings that developed from extracted embryos grew at about the same rate as those derived from sterile seeds.

As an additional experiment, Dr. Johnstone, a plant physiologist, suggested that I should add a low concentration of penicillin to half of a set of 20 tubes. This experiment would test whether penicillin was a plant growth promoter; a subject had not yet been investigated. At that time, around 1950, penicillin was still very scarce, but with his connections, Dr. Johnstone was able to get some from the campus infirmary. The results showed no difference in growth when the penicillin was added compared to the control without it.

In those days, students (and perhaps even professors) were not explicitly encouraged to publish, so the growth of *Yucca* embryos *in vitro* remained unpublished until my dissertation. It would have made a good paper and I think it would have been published if submitted. However, the climate was different in the late 1940's; the USC campus was not yet submerged in the "publish or perish" *modus operandi*. This "brainchild" would overwhelm USC and most other American universities in the next few decades. It would, in fact, change the very nature of American universities and eventually bring "Big Brother" into control of every aspect of university life.

Meanwhile I started another research project with *Yucca*. It was well known that some Joshua Trees (*Y. brevifolia*) were quite big; the one in the photograph (Fig 4) was over 80 feet (24m) in height and 9 feet (2.7m) in diameter according to McKelvey's "Yuccas of the Southwestern United States" and had an "estimated age of 1000 years." The size and possible understanding of its age was lost when it was deliberately burned down in 1938. The question of size and age, however, are still appropriate. Richard Doornbos of Hesperia, CA has devised a method of estimating the age of Joshua Trees (vvdailypress.com; 2004) as follows: "Measure the circumference of the trunk 3 inches above ground level and multiply that figure by 10." Using that method the tree in Fig. 4 would be in the neighbourhood of 4000 years of age. I think that may be a substantial overestimate.

How long does it take a Joshua Tree to attain that size? Is size directly related to age? Since, at that time, nothing was (perhaps is) known about the growth rates of the Joshua Tree, I decided to measure, photograph and mark a number of trees, so that I could return in 20 years and determine how much they had grown (Fig. 5, shows two of the marked plants).

Six locations (plots) were selected in the Mojave Desert; in each I measured and marked all the trees. A total of sixty-four trees in these locations were marked, measured, and photographed in the summer of 1952. The trees were marked using copper tags as follows: "These tags were made by inserting a 1/16 inch copper wire about three feet in length in a short (1 inch) piece of ¼ inch copper tubing. The tubing was pressed flat in a vice securing it to the copper wire. Numbers were etched into the flattened tubing; USC 1 to USC 64. The wire was placed around a tree and the ends of the wire were

Figure 4. Large tree of *Yucca brevifolia* photographed by Earnest Branton in 1925. In her *Yucca* monograph, Susan McKelvey says this tree was over 80 feet in height and over nine feet in diameter. The tree was maliciously burned in 1938. This is a copy from a print in the UC. Berkeley Herbarium.

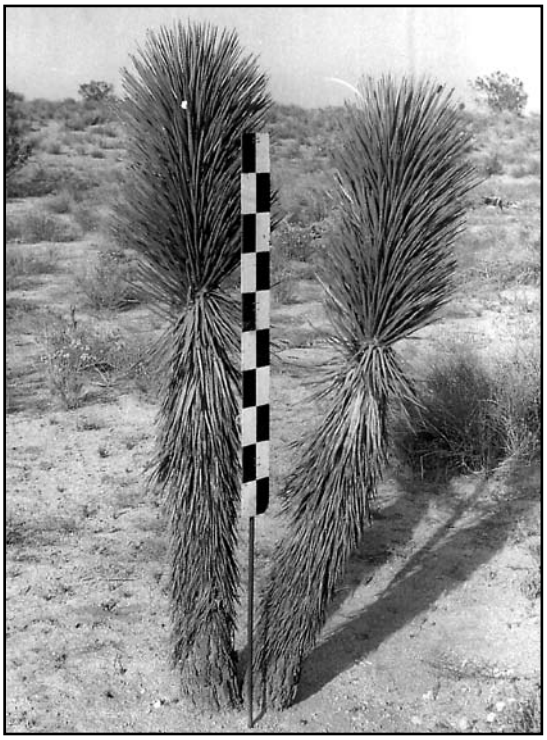


Figure 5. *Yucca brevifolia* trees 6 and 7, Plot 6, June 1956. These trees were measured both in 1952 and 1956 but showed little change in height. Meter scale, Photo by HJA.

secured together; since the length of the wire on each marker was much larger than the diameter of any of these trees, the tagging should not cause any stress. A standard decimeter scale was placed by each tree for photography. The diameter at three feet was measured in inches using home made callipers. The number of dichotomies and the number of offshoots was recorded for each plant (Table 1). The plot sites were marked on maps current in 1952. The entire project was written up, bound with my thesis and kept in my library. I did not tell any of my professors about this "experiment," and it was done with out supervision. In retrospect that was probably not wise; quite possibly they might have helped me better establish the location of my plot sites, and/or suggested other improvements.

In 1956 I revisited plot 6 and photographed the trees a second time; little difference could be seen. In, 1972, sixteen years later, I attempted to relocate the sites. Regrettably, I never have found any of the plots again; by 1972, everything had changed. Roads had been relocated and the landscape altered by extensive development. Wouldn't it have been fantastic to have had a GPS in 1952! I suppose this project is still ongoing, since someone, might find them even now. I still have hopes of finding some of them; even today I have been re-examining my maps in the light of modern aerial (space) photography.

		1952 Measurements of *Yucca brevifolia*				
Plot #	# of Plants	Height in Feet	Diam. in Inches	# Basal Offshoots	# Major Branches	Location
1	20	7.9	5.7	0.3	3	East of Neenach
2	4	21.5	21.5	2	3.2	SW Victorville
3	5	21.8	19	0.2	4.2	SW Victorville
4	15	7.1	5.3	0.5	1.46	Denis Siding
5	10	10.1	5.9	0.4	1.4	SW Hesperia
6	10	11.8	9.7	0.1	1.2	Near Hesperia

Table 1. Data from measurements of *Yucca brevifolia* made in 1952. 64 trees were measured and tagged by H. J. Arnott.

My next hands on research project became part of my Masters program at USC. The research involved a study of the embryology of several species of *Yucca*. The research was done under the direction of Dr. Thomas Fuller (Fig. 1). By tradition, plant embryology involves more than just the development of the embryo. It involves the growth of the ovule, the embryo sac (female gametophyte), pollen grains (male gametophytes) the embryo proper, and even the development of the seed. Such research requires careful preparation and examination of serial (LM) sections.

I had taken a course in microtechniques and knew how to fix, embed and section animal tissues. However, I had to learn how to fix and section plant tissues; because of the rigid structure of their cell walls the plant tissues are more difficult to section; they require heavy microtome knives rather than razor blades often used for animal tissues. Be that as it may be, I was able to cut excellent sections. I settled on FAA (Formal, Acetic Acid, and Alcohol) as a fixative and used paraffin from the grocery store mixed with 5 percent bee's wax for embedding; xylene was used as a transition fluid. With Dr. Fuller's help we collected yucca flowers from specimens growing in the very *ritzy* Huntington Botanical Gardens, San Marino, California. I remember hauling a very long "collecting pole," with which we were able to cut off the flowers from tall plants like *Yucca australis.* I also collected specimens from local species of *Yucca*.

My microscope research at USC was done before the broad use of photo-microscopy; authors had to record and illustrate their papers with drawings often made with a *camera lucida*. *I learned to* draw embryo sacs, embryos and pollen grains with pen and ink. By using stippling, I was able to shade the structure of the embryo

Autobiography continued on page 66

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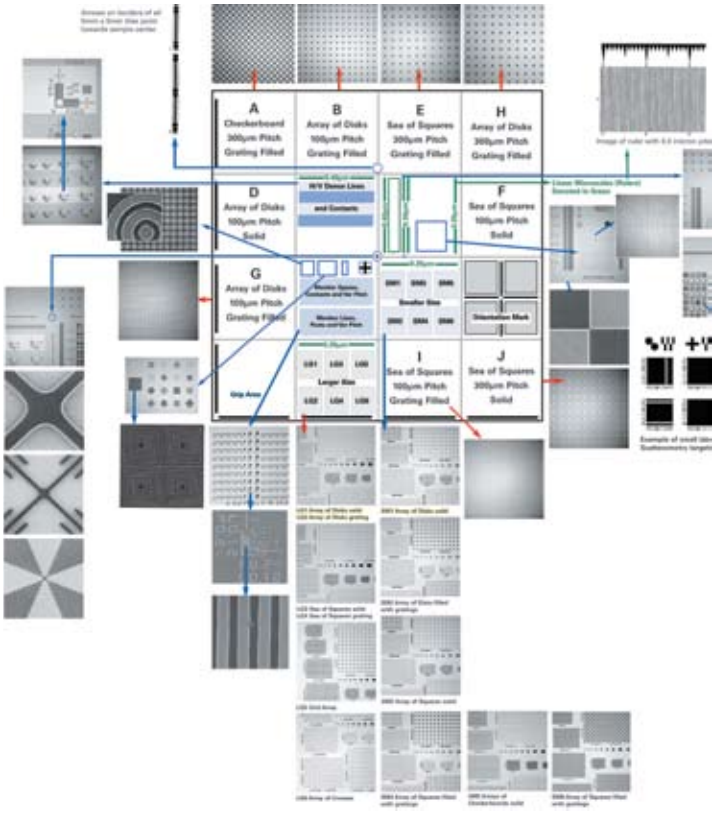

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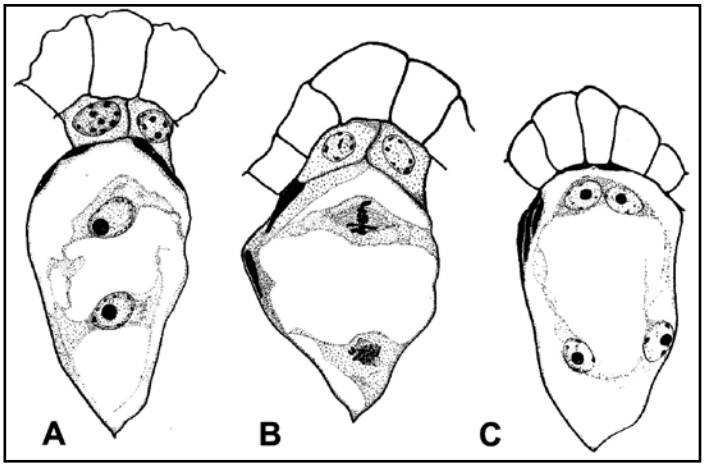

Figure 6. Drawings of the embryo sacs of Yucca *whipplei*. A. Two nucleate stage. B. Mitotic divisions forming the four nucleate stage. C. Four nucleate stage. Outer parietal cells visible in A and B. From H. J. Arnott, "Sporogenesis and Gametogensis in Some Spices of *Yucca*." Thesis (M.S.), University of Southern California, 33p. 1953.

sac (Fig 6). None of species used in my thesis research had been previously investigated; however, I noted that their development was similar to other species shown in the literature. One thing learned in that research was that specific megaspore which gives rise to the embryo sac varies in different species of yucca. My research was documented in my Master's Thesis submitted in June 1953 and signed by Thomas C. Fuller, George R. Johnstone, and Louis C. Wheeler, three fourths of the USC Botany Department. The Thesis represents my first "published" research. Some argue that a thesis or dissertation is not actually a publication. Even yesterday, my current (2006) Chair seemed to hold to that position. Many biologists agree that they are publications. I list the Master's Thesis as my first bibliographic citation.

Despite having some success in research, other than their general encouragement, the USC professors didn't coach me in the next step of a botanical career, namely, getting into a Ph.D. program. I got into U.C. Berkeley on what I have always thought was a totally "**unplanned stroke of luck**." I actually wanted to go to work with either Dr. Vernon Cheadle at the U.C. Davis or Dr. Lee Lenz at the Rancho San Ana Botanical Gardens, Claremont University. I missed getting into the Claremont Program on the basis of a poorly written application (entirely my fault). I missed getting into U.C. Davis for a reason unknown to me. Jean and I traveled to Davis and I met with Dr. Cheadle. We stayed with Bob Lambe, a USC ex, and his wife at Davis. I even got in some pheasant hunting while we were there. I imagine that incompatibility was the problem with Dr. Cheadle—it was too bad since we had a number of common botanical interests. Nevertheless, in some inexplicable "turn of the cards," a letter arrived at our apartment in West Los Angeles indicating that I had been admitted to the Botany Ph.D. program at U.C. Berkeley. It was pretty outrageous! Even today, I am not sure that I applied for entrance at U.C. Berkeley. However it was one of the **luckiest days** of my life.

Figure 7. Howard Arnott working in the dough room of Langendorf United Bakery, 1952. (unknown photographer).

As things worked out, in the fall of 1953 I went north to U.C. Berkeley. I went without any idea what graduate school would be like or who I might work with. I went without any support; my GI Bill funds had run out; I knew that I could support us working as a baker as I had at USC (Fig 7). Jean and I knew very little about U.C. Berkeley, just that the campus had a good reputation. Unbeknownst to me, at that time, Berkeley had one of the top botany departments in the U.S., or in the world for that matter. Only five students were admitted in the fall of 1953, four eventually finished and all of us subsequently established careers in Botany.

When I got to Berkeley in the fall of 1953 it was still a picturesque community rising from San Francisco Bay and extending up to the Berkeley Hills. The intersection of Telegraph Ave. and Bancroft Way at the south edge of campus was the center of student activities. The area was bursting with small college oriented businesses. There were used book shops, clothing shops, food shops of many types, Chinese and Italian restaurants, coffee houses and characters galore. The long axis of the University campus ran from west to east and it sloped gently upward to the Campanile (Sather Tower) and Memorial Stadium at its eastern end. Farther up into the hills was the Cyclotron and U.C. Botanical Garden. From the upper end of campus you could easily see Alcatraz and the Golden Gate Bridge across the bay. The mixture of town and gown *seemed* almost idyllic.

Jean arrived in a couple of weeks and we moved into the first floor of a duplex at 2438 Hilgard Ave, a few blocks up from the North Gate. The street was named after Dr. Eugene W. Hilgard, the founding Dean of Agriculture at U.C. Berkeley; Dr. Hillgard began teaching botany at a time when U.C. Berkeley had less than 300 students. The duplex was owned by an elderly widow, Mrs. Emma Hoffman; she occupied the upstairs and we lived on the first floor for the entire five years in Berkeley. Mrs. Hoffman, a widow, was a landscape painter and had done sketches of Albert Einstein when she lived in the east.

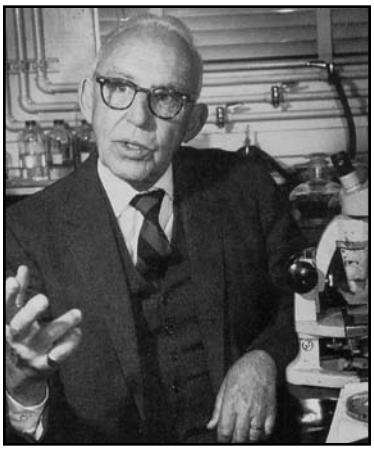

Figure 8. Dr. A.S. Foster in his office/lab. Ca. 1956. Photo by Marion S. Cave.

In the advance papers sent to me I was directed to meet the graduate advisor *as soon as possible.* So I lost no time in visiting Dr. Adriance S. Foster, who was the Graduate Advisor at that time (Fig 8). We met in a small office, used only for advising, and small talk aside, it was clear that there was only one item on the agenda, namely, was I interested in the s*tructure* or the *function* of plants*?* In those days there were just two paths, and almost literally those who followed one hardly ever interacted with those that choose the alternative. Our talk "confirmed" that my interests were in *structure;* thus in roughly five minutes the overall direction of my work at Berkeley was settled. Two botany courses were immediately put on my schedule, Plant Anatomy and Plant Morphology. My appeal that I had recently taken both courses at a creditable university received only raised eyebrows (Fig. 8). I learned later that those courses were Dr. Foster's major contribution to the botany program. Later I found from personal experience they were very well done and not easy, even though I had been over the material before. I also came to realize that those courses were Foster's "pride and joy!"

Next I want to give you an idea of how my doctoral program evolved. All Ph.D. programs require a demonstration of independent

research. True as that is, like most curricula, course work generally takes up a great share of the student's time. I found out, talking to Dr. Foster, that I was also lacking in physics and organic chemistry; and that I needed to take plant physiology over (again my appeal was ignored); these were courses that *must* be taken. I greatly enjoyed the physics labs and lectures. You may remember (Part II) I mentioned my high regard for the teaching of my physics professor, Dr. Harvey White. I found that the organic chemistry course was really bad, horribly bad, an abomination; my professor just didn't cut it. Plant physiology was pretty much a repetition of what happened at USC.

As my second semester started, I learned that there was a job open in the U.C. Berkeley Herbarium. I applied and was given the position. At that time it was not obvious what a great opportunity it was. The U.C. Herbarium is the largest herbarium west of the Mississippi; at that time it occupied 9 floors in the five story Life Science Building, the additional floors were designed to increase the space used for storing specimens. Over time, I met many people who worked in and/or made the U.C. Berkeley Herbarium their "home." The variety of people was large, interesting and included professors, curators, visiting professors, students, lay visitors, herbarium staff, graduate and undergraduate students, etc. I worked for Annetta Carter, who worked under the Herbarium Director, Dr. Herbert Mason.

Annetta Carter was the local administrator and she kept things organized and moving. Among many things, Annetta taught me to print herbarium sheet labels; it took some considerable effort on her part, as neither spelling nor printing were my *forte*. After Annetta developed some confidence in me she taught me to sort and file herbarium sheets. Filing is especially important since once a specimen is misfiled it can be missing for some time (it's not unlike the problem of misfiling library books). John Ingram, a friend from USC, who graduated a year ahead of me, was a Ph.D. student who also worked in the herbarium. John taught me the complicated protocol for wrapping packages of specimens for shipment; the wrapping paper, string and knots were all strictly defined.

Figure 9. Dr. Adriance S. Foster visits with John J. and Howard J. Arnott in our home at 2438 Hilgard, Berkeley in 1958. Photo by Jean Arnott.

John, in addition to sometimes supervising my filing, taught me a lot about plant taxonomy, his major interest. John and I went on a field trip to Baja California, California, Arizona and northern Mexico in 1955 to collect material for John's dissertation. The brand new U.C. Berkeley station wagon John checked out from the motor pool, had most of its paint removed or damaged during a sand storm in southern California. This situation was very difficult for John because he thought that the University would charge him for the damage. They didn't!

The following herbarium anecdote may be of interest: several times I watched the famous paleobotanist, Dr. Ralph Chaney, at work in the herbarium. Dr. Chaney had an illustrious career and was justifiably famous; he coined the popular name "Dawn Redwood" for *Metasequoia glyptostroboides.* He often worked in the herbarium during the time I was filing specimens and he paid absolutely no attention to my activities. He would come in with a box of fossils (rocks), and would go from case to case, and sheet to sheet, until he found some plant leaf or stem that resembled his current "rock". When he found something "pleasing," he wrote the name down on a small piece of paper and attached it to the rock (fossil). *Se la paleobotanic*!

The "herbarium people" always observed "tea time" in the afternoon. No matter who you were or where you worked everyone went to tea in the afternoon. At tea time you were able to meet people, including your colleagues, visitors and professors. I spent one and a half happy years in the herbarium. Retrospectively, it was time very well spent; it gave me a better understanding of plants and opened my eyes to the wide variation in plants and in people who study them. The herbarium experience has always been a useful resource in my teaching.

By my second year (1954), I had chosen Dr. Adriance S. Foster as major professor (Figs. 8, 9). Although I had made a good start on the embryology of *Yucca* while at USC, I spent a great deal of additional effort at Berkeley refining my understanding of embryo sac development in the four California species, *Y. whipplei, Y. brevifolia, Y. Schidigera,* and *Y. baccata*. These studies were included in my dissertation, however, they were never separately published (**A real mistake!**). Many years later I listened to a presentation on the embryology of *Y. whipplei* by Dr. Maynard F. Moseley. After the paper I told him about my dissertation and we agreed that we would jointly publish our story. I visited him at U.C. Santa Barbara and we went over each other's figures and he accompanied me on a *Y. whipplei* collecting trip. Somehow we never got the publication done.

Eventually, my dissertation involved many aspects of *Yucca* research. The most comprehensive part dealt with the structure and development of yucca seeds. Almost from the beginning of my time at Berkeley I was engaged in collecting, processing and viewing of yucca materials. The boundary that should have been drawn around my research on yucca seemed to have no restrictions. The research I did is documented in my dissertation and ranges from taxonomy, cytology, morphology to anatomy. Much of the breadth of my exploration arose from an unlikely source—*the language examinations*. We were required to translate passages in German and French. Foster gave the exam on French and I passed it without a problem.

The German exam, on the other hand, was given by Johannes Proskauer, a young professor (in his early 30's) with a German background. On my first two tries, I failed his German examination as given by Proskauer. When I found the third exam stuck in my mailbox and realized that I had failed it too, I was ready to fight. If Proskauer and I had met that afternoon, it would have unquestionably ended my college career. By the next day, I had cooled off, but I still believed he "had it in for me" (*how often have you heard that*?). The point of writing about this is that the German exam extended my stay in Berkeley by a couple of years. Between the exams, I continued to work on yucca research (that was fun) and on German (that was not fun).

Finally, to end this absurd situation, Foster decided to give me the German exam himself; I passed it on the first attempt. I believe that even then in the mid 50's these language requirements were an anachronism. Of course, now language requirements are not generally a part of Ph.D. programs. This is true because most of international scientific community began publishing in English in the 1950's. For better or not, I never used either German or French very much in my research. My first published paper came

Autobiography continued on page 69

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108 carbon/A	Carbon	A/M	120 x 120	No	Z axis	MTM-10
108C auto/SE	Carbon	A/M	150 x 165 (adjustable)	No	Optional RT or RPT	MTM-10
208HR	Cr, Pt:Pd, Ir, Au, Au:Pd, Pt, W, Ta, others	A/M	150 x 165 to 250	Yes	RPT standard	MTM-20 Controller
208C	Carbon	A/M	150 x 165 (adjustable)	No	Optional RT or RPT	MTM-10
308R-EM	Carbon or Metals Au, Au:Pd, Pt, Pt:Pd, Cr, Ta, W, and many more	A/M	305 x 305	Yes	Optional RT or RPT	MTM-10 or MTM-20

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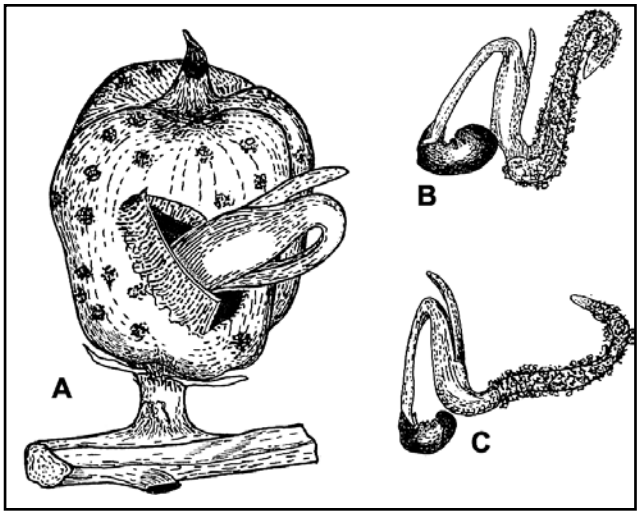

Figure 10. 1. A. fruit of *Cordyline australis* with viviparous seedling extending out of the fruit wall. B, C. Young seedlings with rough primary root; in the latter the root hairs had grown into the fruit wall and had to be broken out with force. Adapted from Arnott, H. J. Vivipary in *Cordyline australis* Hook. Madroño 15(3):71-73.

from some observations I made in the backyard of our Berkeley flat where specimens of *Cordyline australis* were growing. These palm-like plants have very large inflorescences with hundreds of white flowers. Later the flowers become hundreds of fruits. While studying them I noticed that some seeds had germinated, *in situ*, and were breaking out of the fruit coat (Fig 10). I told Annetta Carter about this and she suggested that I write a brief paper and submit it to Madroño, which I did. The phenomenon of seed germination while still in the ovary is called vivipary, thus the title of the paper was "Vivipary in *Cordyline australis*." It is a small paper but it was a start!

You have most likely noted that much of my early research involves the genus *Yucca*. Those interesting plants were obvious subjects for me. Some of my earliest memories involve viewing the extraordinary flowering stalks (inflorescences) of *Yucca whipplei* (sometimes called

Figure 11. Lena Alderman Donnelly, my maternal grandmother, with a flower stalk of *Yucca whipplei* on the porch of 2457 Pomeroy Ave., Los Angeles, Ca, 1909. The photo was taken and processed by Nellie Alderman, my maternal great grandmother.

"Our Lord's Candle") and the bizarre forms of *Yucca brevifolia* (The Joshua Tree). Pictures of my great grandparents, grandparents, mother, father, and aunts with these flowers are abundant in our photographic memoirs (Fig. 11). I continued to study *Yucca* in my dissertation work done under the direction of Dr. Adriance S. Foster at the University of California, Berkeley. My third Ph.D. student, Harry T. Horner, also studied *Yucca* seed ultrastructure as a part of his dissertation work. One of Horner's Ph.D. students, Albert Kausch, also worked on *Yucca*. Another of my Ph.D. students, Genie Brackenridge, studied the development of calcium oxalate crystals

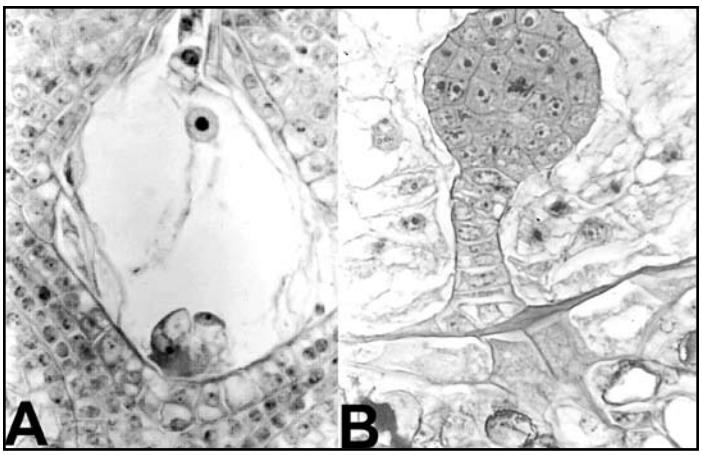

Figure 12. A. Embryo sac of *Y. whipplei* with egg apparatus, antipodals and fusion nucleus within the embryo sac. B. Young globular phase embryo with suspensor. Adapted from the dissertation of HJA.

in the roots of *Yucca torreyi* in her dissertation. Later, using crystals isolated from *Y. rupicola,* Fred Pautard, Hugo Stinfink and I worked out the crystal structure of calcium oxalate monohydrate. Even now I have two new yucca investigations which are on going.

Yucca embryology was the subject of my first professional paper given at a major scientific meeting. The paper was given at the 1957 American Botanical Society Meeting at Stanford University (Fig. 12). My paper (usually these presentations are called "papers") dealt with embryo sac development in several species of *Yucca* and used Kodachrome slides. Most botanists had never seen embryo sac development depicted in color. For its time it was unusual, it might even have been *high tech* for the time, and it got excellent reaction from the audience. I made the 35 mm slides using my own equipment: a Leica camera back attached to a photo tube with an in/out photo (light) detector. The detector, wired to a galvanometer, was used to determine the amount of light and hence calculate the exposure.

Naturally everyone's first paper is a landmark; however, for me the greatest moment of that meeting was hearing Dr. Katherine Esau present some of her research. We were in the same session and our papers were given in the same dreary room with several pillars making viewing of the screen difficult. Dr. Esau had exactly the same facilities for her talk as I did. It was bizarre, one of the foremost botanist in the world was doing just what I, a beginning graduate student, did; how very *cool.* Her book, "Plant Anatomy" had just come out in 1953 and it became an instant classic. I remember at the end of her presentation someone pointed out a "possible mistake." With complete composure and self confidence she simply said, "I stand corrected." End of controversy! Up to that time I had no idea that scientists could be that elegant.

The American Botanical Society's Meetings was where the "big boys" went to "pat" each other on the back. It was my first introduction to "*cigar smoking botanists with big names*." I got to meet many of them because my professor was one of them. At that time, most people still rode trains to meetings; three days and nights on a train cemented many a botanical friendship, even for teetotallers. The contacts made at meetings of this kind are (were) absolutely necessary; they are *indispensable* for anyone who wants to be an "upwardly mobile botanist." The internet and other means

Figure 13. HJA watering yucca seedlings in the Botany Greenhouse on the roof of the Life Science Building in 1957. Photo by Jean Arnott.

of instant communication have greatly diminished that aspect of botany. However, now as then, one's major professor can still make or break any student—the choice of a professor is not just important, it is **critical**!

Much of my work on yucca was self-guided. I knew the general direction I wanted to go and Foster was not one to look over your shoulder. From time to time I would show Foster what I had been doing, especially if it was exceptional. Mostly, I went on my own way pushing the yucca research boundaries in various ways. I raised seedlings in the Botany Greenhouse on the roof of Life Science Building (Fig. 13). During that time, however, I did some other research as the opportunity offered itself. I will describe two cases to illustrate the point. I have already mentioned *Cordyline* observations.

By my third year, I was working as Foster's teaching Assistant in both Plant Anatomy and Plant Morphology. On occasion, Foster and I would go together to lunch at The True Blue Cafeteria, which was in "downtown" Berkeley. The journey involved walking down from the Life Science Building, through the grove of gigantic *Eucalyptus* trees, along Strawberry Creek and then out into the city and to the cafeteria; a total distance of ca. 0.3 miles. In those days, there was no urgency and we would walk along admiring what ever we came upon. Near the lower edge of the *Eucalyptus* grove, there was a large *Ginkgo biloba* tree. On one of these "True Blue" occasions, I collected a few leaves from under this ginkgo. One of the leaves was especially interesting as it had two odd side lobes (Fig 14).

At that time, I was involved in a major "side project" of clearing plant material. Clearing is a process that uses chemicals (5% NaOH and saturated chloral hydrate) to remove pigments in plant parts so that their internal structure is exposed. Leaves, stems and flowers, often taken from herbarium sheets (with permission) and personal samples were cleared routinely; the many preparations were to be a central part of my projected teaching career. Routinely, I decided to clear that odd shaped ginkgo leaf.

In all the courses I had taken, including Foster's, and in my study of the literature, people stated categorically that *Ginkgo* had open dichotomous venation; ***the veins branched*** but ***they did not fuse***. With that background, I was shocked to find two vein anastomoses (fusions) in this odd leaf (Fig 14). I hastened to show it to Foster and he was, quite literally, astounded! No one had ever reported anastomoses in the veins of ginkgo. This simple discovery started me on a ten-year investigation of venation in *Ginkgo biloba* and other plants.

In the beginning, it was the venation of ginkgo, but soon branched out to *Ranunculus, Lemna* and other genera. While still in Berkeley I found out that *Ginkgo biloba* leaves have four

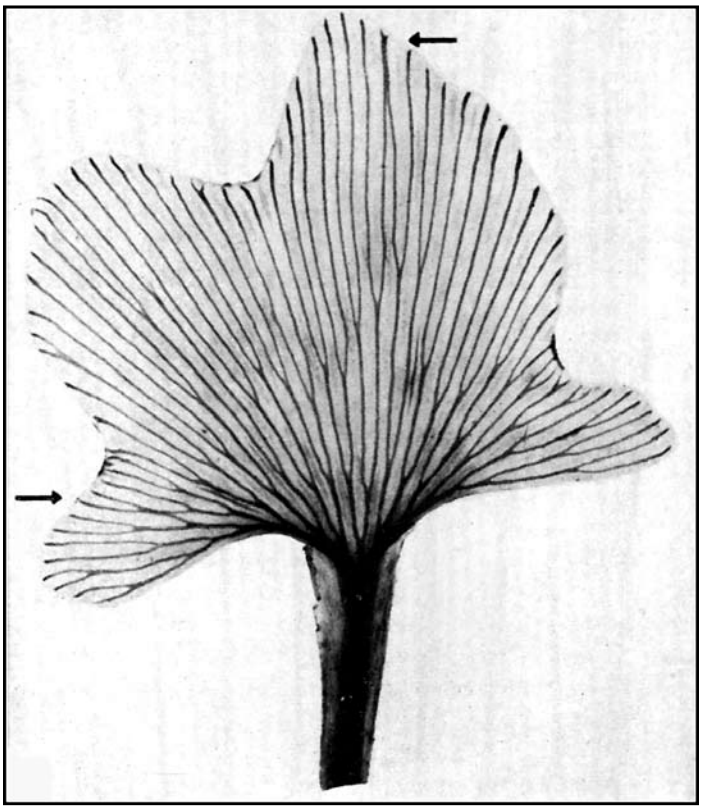

Figure 14. Cleared "True Blue" leaf of *Ginkgo biloba*. Note the small "ears" on each side of the lamina. Arrows point to anastomoses. With permission, from Arnott H, J. Anastomoses in the venation of *Ginkgo biloba*. Amer. J. Bot. 46:405-411.

different types of anastomoses; some involving one vein (types A and B) and others involving two veins (type C and D) (Fig. 15). Somewhat later, I found out that the four types of anastomoses are not distributed at random in the leaves of long shoots. That is, the type of anastomoses produced in ginkgo long shoot leaves is under physiological control. Since *Ginkgo* had been long considered a *prototype* for a primitive stage in leaf evolution these anastomoses were of importance when considering the potential evolutionary development of higher plant leaves.

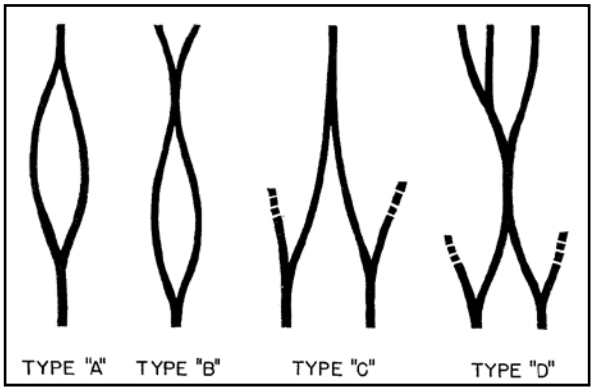

Figure 15. Four types of anastomoses found in the leaves of *Ginkgo biloba*. With permission from Arnott, H. J. Anastomoses in the venation of *Ginkgo biloba*. Amer. J. Bot. 46:405-411..

A second avenue of research also had its origin from clearing leaves. I cleared the leaves of several species of ferns, including *Botrychium*. Pieces of leaves were detached from herbarium sheets (with permission). In a single species of *Botrychium*, I found very unusual cells in the leaf mesophyll and along veins. Each cell looked like it had a series of washers distributed along the cell. The

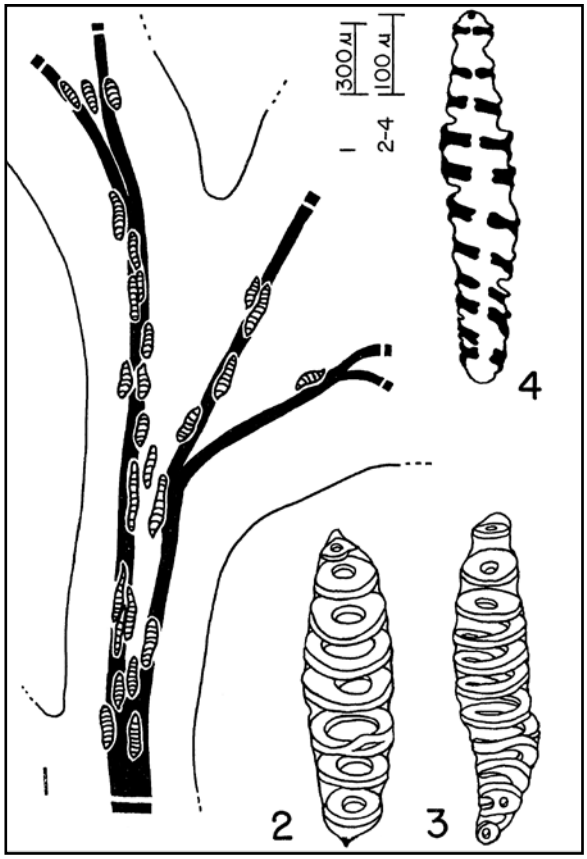

Figure 16. Tracheoidal idioblasts in *Botrychium*. With permission, from Arnott, H.J., 1960, Trans. Amer. Microscopic Soc. 79:97-103.

washer-like secondary wall thickenings supplied the support for a thin primary cell wall (Fig. 16). I named these cells tracheoidal idioblasts. They were another "fun discovery" made simply by clearing leaves. Dr. Herb Wagner, a U. C. Berkeley graduate and an expert on ferns, especially *Botrychium*, was always amazed that I found the tracheoidal idioblasts in what turned out to be an obscure species of a genus he had studied for some time.

I am including camera lucida drawings used in my illustration of the *Botrychium* work to show what line drawings look like (Fig 16). I have recently tried, using modern light microscopy with digital cameras, etc., to show the idoblasts more clearly. There is no question; my illustration shows the structure better than anything I can do with modern equipment.

As a student, I was assigned a cubical in room 2054 of the Life Science Building. The room accommodated two graduate students and Dr. Marion S. Cave (Fig. 17). We each had a cubical with a worktable, where microscopes and other equipment were set up, a stool, a chair and some bookshelves for storage (Fig. 18). Some common facilities were present such as sink (with water taps), a paraffin oven and Bunsen burner for making tea water. By the end of my occupancy, my shelves became filled with books and with many collection jars containing specimens of yucca in various solutions. I

Figure 17. Dr. Marion Stillwell Cave, Berkeley, 1954. From L. Constance, Botany at Berkeley, The First Hundred Years. Privately printed, 1978.

Figure 18. My cubical in 2054 Life Science Building about 1957. During earthquakes, I held my collection bottles on the shelf. Photo by HJA.

still remember frequent earthquakes and standing in front of my precious specimens hoping to keep them on the shelves (Fig. 18).

It was my good fortune to have Marion Cave, with 40 years of experience in plant cytology and embryology, in close proximity. We became good friends and shared many interests. She was an avid photographer and an excellent microtechnician. Every so often, in our quite office, she would sing out "Ooh"--meaning she had once again found another interesting object in her microscope—such an "Ooh" or "isn't it cute" always brought an obligatory visit to her end of the office to see her latest finding.

At that time Sherwin Carlquist occupied the middle cubical; he was also interested in cytology and micro techniques. I learned many things from him but the practical information about making line drawings was the most important. By observing him at work, I learned that his technique involved making very large drawings of plant components and then reducing them to page size, which caused many of the "flaws" disappear. In the 40 years since we were there, Sherwin has become a world expert in wood anatomy and on island biogeography. Like Marion, he was also interested in photography. When he left to do a postdoc at Harvard, Yos Shigemura took the middle cubicle. He was one of the "functional" students working tissue culture with John Torrey. He collected stamps, as Marion did, and followed the stock market with great enthusiasm.

Marion and I worked together on the embryology of *Paeonia*. A controversy had developed between a group of Russians and Professor P. Mashawari, a famous Indian botanist and author of the major text on plant embryology. The Russians, M. S. Yakovlev and M. D. Yoffe showed that in *Paeonia anomala* and *P. wittmanniana* the zygote develops into a massive coenocytic (multinucleate) embryo. That is to say, the embryo **is a large cell with many nuclei**. They showed this using a series of drawings, standard for the day. Dr. Prem Murgai, one of Mashawari's students, studied other species of *Paeonia* and maintained in her paper that the first division was separated by a cell wall and that the suspensor was coenocytic pushing the apical cell into the endosperm and producing normal embryo development; she showed this using drawings. The dispute is important because flowering plants, like peonies, are not supposed to have free nuclear (coenocytic) stages in their embryology. The presence or absence of free nuclear or coenocytic embryo is a major difference between angiosperms and gymnosperms.

Luckily, on a field trip in the Monterey region of California, I was able to collect many flowers and fruits of *Paeonia californica*. Marion and I prepared the material and were able to show that *P. californica* had a free nuclear stage in its embryo development

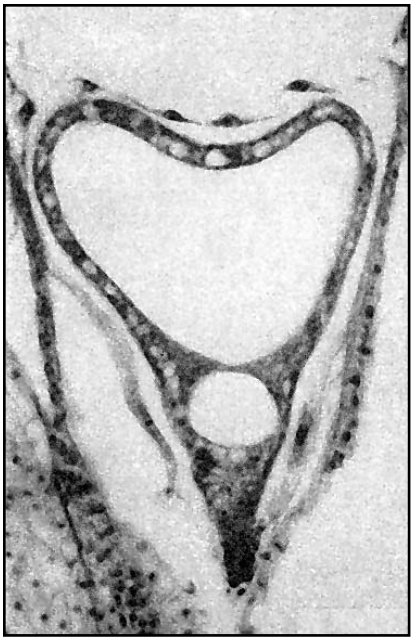

Figure 19. Free nuclear development of the embryo of *Paeonia californica*. With permission from Cave, Arnott and Cook, American J. Botany 48:397-404.

(Fig 19). We published our work using photomicrographs instead of drawings. Later, after I graduated, Professor Mashawari visited Berkeley, and even after viewing our preparations, he remained, "skeptical" and continued to support his student's view even though it was in error. At the 1958 International Botanical Meeting in Montreal, Marion and I met the Russian botanists and they were very "appreciative" of our support. I also met Dr. Poddubnaya-Arnoldi the Montreal meeting; later I received a major monograph (in Russian) on plant embryology written by her; I still have it. I also met Professor Mashawari at that meeting; he was the author of the major book on plant embryology in English. Drs. Mashawari and Poddubnaya-Arnoldi were two of the most important plant embryologists in the world. One needs say no more about how important international meetings can be to young investigators.

It may be of interest to note that during my tenure in Room 2054 I witnessed the founding of the "Index to Plant Chromosome Numbers. This was a scheme hatched by Marion Cave and Richie Bell, a botany professor at the University of North Carolina, on an afternoon in 1955 in the third cubicle. Marion edited the "Index to Plant Chromosome Numbers" for some years after its founding. The "Index" was a way of collecting chromosome numbers from the literature making it easier to gain chromosome information about specific plants. It was produced by offset press on an annual basis; the University of North Carolina underwrote the costs.

While at U.C. Berkeley I sometimes helped Foster with microtechnique. For example, I helped clear the leaves of Circaeaster *agrestis* (*Circaesteraceae; Ranunculales*) and worked with him when he mounted them. Circaeaster is one of a few flowering plants that have leaves with open dichotomous venation. As specimens of *Circaeaster*, were quite rare, they were handled with "great care." The leaves were not very large so they were easy to manipulate in the clearing process.

However, the level of technique difficulty was upgraded when it came to handling the leaves of *Kingdonia uniflora*. This pant, first collected in China by Kingdon Ward, was a second herbaceous flowering plant that had leaves with open dichotomous venation, *a la Ginkgo,* that Foster studied. The first specimen of *Kingdonia* that Foster got came from the **type** specimen contained in the Royal Botanical Garden in Edinburgh. Later Dr. Foster received additional material was received from China.

Clearing the entire leaf of *Kingdonia* was problematic because the leaves were much larger, almost 6 cm in width. *Kingdonia* is a relatively delicate herb with fragile leaves. The clearing process took place in Foster's lab/office and took several days. During the clearing process, especially when in chloral hydrate, the leaves were exceedingly fragile. Once the leaves were stained and dehydrated the leaves were less fragile. We devised a method of

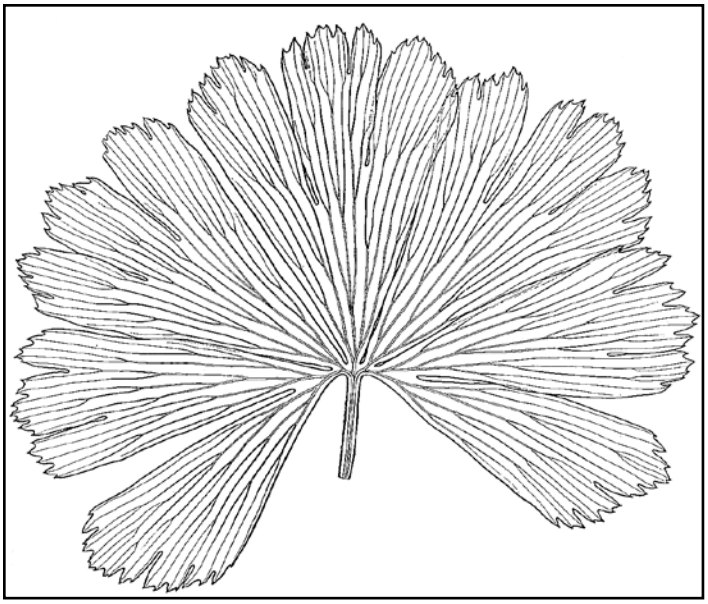

Figure 20. Drawing of a cleared leaf of *Kingdonia uniflora (Kingdoniaceae; Ranunculales)* with open dichotomous venation. With permission from Foster and Arnott, 1960, Amer. J. Bot. 47:684-698.

moving the leaves from one dish to another using a piece of glass. We also moved the leaves this was when they were so permanently mounted in piccolite (a resin). I had previously cleared large *Ginkgo* leaves and mounted them but they were not fragile. I used pieces of window glass for mounting ginkgo. We planned to mount *Kingdonia* the same way, but you could not lift the leaf with forceps. So we used a window glass and fine brushes to slide the leaf from the large glass Petri dish onto the mounting glass. A second piece of glass was used as a coverslip. With luck, a perfect specimen was made. Believe me, there was no one more particular than Foster, when it came to microtechnique! Later, I embedded and sectioned some of this precious material and we used my sections to work out the vasculature of the petiole and lamina-petiole junction. The cleared leaf was drawn by Emily Reid who often made drawings for Foster (Fig. 20). The photomicrographs for that paper, and many of those in my dissertation, were taken by Mr. Victor Duran, a master photographer working in U.C. Berkeley Library. We published the *Kingdonia* paper in American Journal of Botany in 1960. Of course it was fun working with Foster but he was so intense it was also sometimes disconcerting. In his middle life Foster took piano lessons; he played the piano in the evening to relax.

It is now almost 50 years since my dissertation was written. The original manuscript was written by hand and a rough copy typed by me. Jean typed the final copy; I remember how hard it was for her to type my dissertation since an original and three carbon copies were required for the library inspection. The library had to sign off on all degrees involving theses or dissertations. In 1958 it was before liquid paper and other correction methods had been invented; long before IBM correcting typewriters and current word processors were available. Correcting errors, when erasing didn't work, often required retyping the entire page and hoping that the changes would not disturb the overall page sequence. Today the word processing programs and digital images take all of that kind of drudgery out of writing.

Some of the remaining parts of this paper will survey the unpublished research completed as part of my dissertation. As stated earlier, there was a large percentage which was never published. A series of papers could have been written from the remaining materials. **Lost chances**! Chances misplaced by the pressures of moving, starting a new job and, most of all, the attraction of new research. I am sure some of you are thinking,

Autobiography continued on page 74

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"All this is water over the dam; why bring it up now?" My reason for including this is to set an example one should **not** follow. Research must have a clear publication plan. Research expends time and money, unpublished research wastes both.

The systematics of *Yucca* is taken up first in my dissertation. My thought was that taxonomy set the scene for the anatomical and histological studies that came later. The genus *Yucca* had been classified in the family *Lilliaceae* for over 50 years when in 1934, Hutchinson removed it and placed it in a different family, the *Agavaceae* (Hutch). Hutchinson's Agavaceae included *Agave, Yucca, Furcraea, Hesperaloe, Cordyline, Draceana, Sanseveria, Nolina, Dasylirion, Beschoneria, Polianthes, Doryanthes,* and *Phormium.* With the exception of *Phormium*, the group as a whole had some common characteristics; most important was the general habit. Hutchinson's taxonomic proposal was widely accepted and was *in vogue* as I worked on my dissertation.

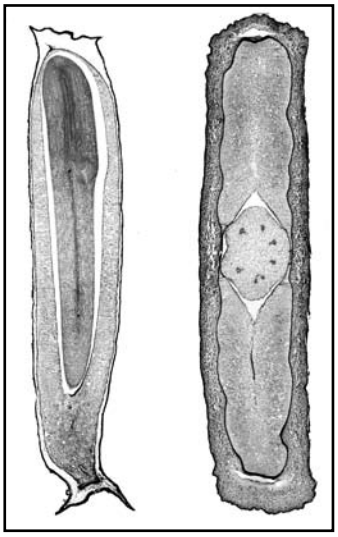

Figure 21. Longisection of a *Yucca whipplei* seed on left; a cross-section of the seed of *Y. brevifolia* on right. Note feather –like edges on *Y. whipplei* seed. Material surrounding the embryo in each is perisperm From Arnott, H.J. 1958. "The Morphology and Anatomy of Yucca L". Dissertation (Ph.D.), University of California, Berkeley, 600 p.

As I studied the seeds of these genera it soon became clear that Hutchinson's *Agavaceae* was not a unified group. *Yucca* is clearly related to *Agave, Furcraea, and Hesperaloe* with similar dark-black flat seeds (Fig. 21); they have a common chromosome karyotype (see later) and a similar habit. In fact, some species of *Yucca* and *Agave* are so similar it is difficult to tell them apart until they flower.

However, the seeds of *Draceana, Dasylirion Nolina* and a few other genera were round and light brown. They share a different karyotype than the *Yucca* and *Agave* group. *Phormium* (New Zealand Flax), because of its decussate phylotaxy is unique among the genera of the Huchinson's *Agavaceae;* also Phormium has karyotype different than the two previous groups.

I therefore proposed that Huchinson's Agavaceae should be divided into three families, namely: *Agavaceae. sensu lato*, *Dracenaceae*, and *Phormaceae*. As I worked up this proposal I was greatly helped through the literature and complications of nomenclature by none other than Johannes Proskauer. He and I traveled all over the campus libraries to find the correct documentation for such a scheme. Yes, by then I had served as his TA and I was no longer estranged.

I actually considered 30 sets of characteristics (see Dissertation p. 54) in developing this proposal. My taxonomic proposals were never published, apparently, dissertations do not count in taxonomy, and therefore my proposal was never given consideration by appropriate taxonomists. **Another lost opportunity!** However, the utility of such this classification scheme is still valuable.

A second general dissertation area that I investigated was the cytology of *Yucca* and other closely related plants. Marion Cave encouraged me to investigate yucca chromosomes as she was fascinated by the unique karyotype first published by McKelvey and Sax. The karyotype consists of five large and twenty-five small chromosomes in each haploid cell, that is N=30. I was able to compare the yucca-agave karyotype with that of some other members of the Agavaceae (*sensu* Hutchinson). The assemblage consisting of *Dracaena, Cordyline, Nolina, Beaucarnea* and *Dasylirion,* all have N=19; this distinction also supports their separation to the Dracaenaceae. The chromosome number in *Phormium* is N=16, different from both other groups and supports the separation of that genus into the Phormiaceae. One of Foster's passions was the shoot apex. His early research on the shoot apex in cycads is a landmark in plant anatomy. In *Cycas revoluta,* he found the largest shoot apices ever reported. Those apices were so unique he had to originate new terminology. I still have some of his original drawings that show some of his ideas on how to conceptualize cycad shoot apices. As you might imagine, his plant anatomy course spent considerable time on the shoot apex. I suppose then, that it was "natural" for me to look at the shoot apex in *Yucca*. Both yuccas and agaves have, in common with cycads, large, complicated perennial leaves supported by a central axis (stem). However, their apices are not large, at least in comparison to those of the cycads.

I investigated the shoot apex of yucca through stages of development, from the embryo to young seedlings and finally large mature plants. The organization of yucca apices was similar to those found in other monocots. Generally, they had a one or two-layered tunica covering a central corpus. However, unlike the apex in many angiosperms one could find zonation similar to that described by Foster for cycads. Thus in the corpus you could find a "central mother cell" zone with peripheral zones around it. *Yucca brevifolia* becomes a very large plant; however, the method of stem enlargement is unlike that of gymnosperms or dicotyledons. It is also different from palms since real lateral meristems (vascular cambium) occur in most species of yucca.

In the mature shoot of *Y. brevifolia,* the shoot apex is quite big while younger plants and seedlings have smaller shoot apices. The size of the shoot apex in the different species of yucca that I studied varied. *Y. brevifolia* the largest, *Y. whipplei* the smallest and *Y. schidigera* intermediate (Fig. 22). It was interesting to understand the growth of the apex in yucca seedlings. In young seedlings the production of a leaf uses up a big proportion (volume) of the shoot apex; progressively the relative volume of shoot apex given over to leaf production becomes less. The shoot apex in axillary buds is also interesting. These buds are dormant only have a few leaves present. A large prophyll (leaf) functions to protect the axillary shoot apex and other leaf primordia.

Branching originates with the growth of these buds. Branching is of course common in the Joshua tree. On the other hand, *Y. whipplei* varies at to whether it branches. For example, the subsp.

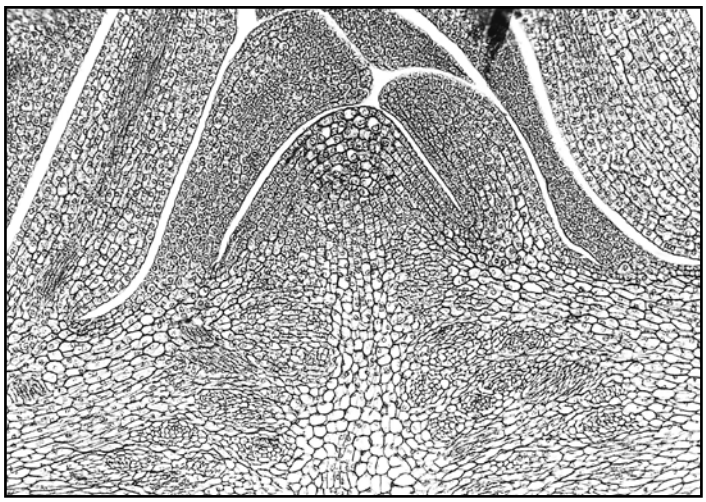

Figure 22. Mature shoot apex of *Yucca whipplei*. Note zonation in the center part of the apex. From: Arnott, H.J. 1958. The Morphology and Anatomy of Yucca L. Dissertation (Ph.D.), University of California, Berkeley, 600 p.

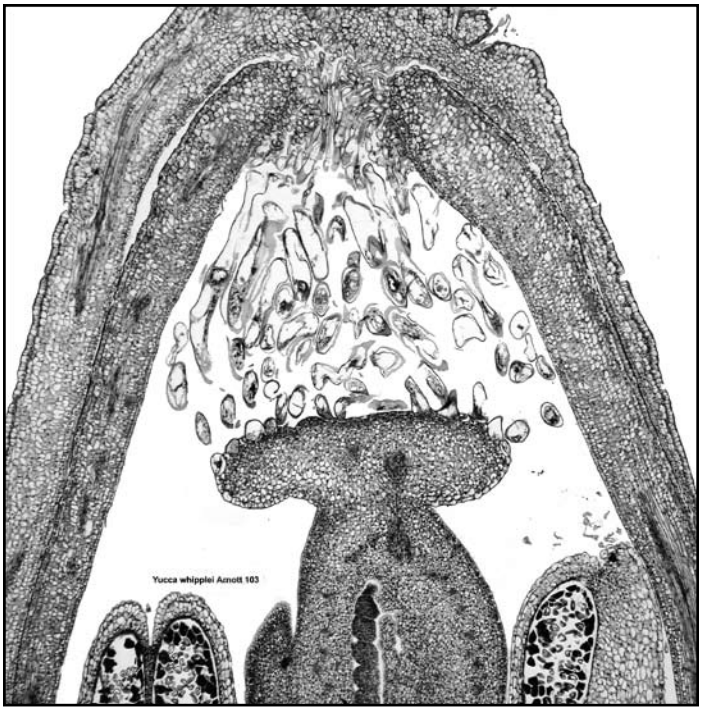

Figure 23. Capitate stigma of *Yucca whipplei* in longisection. Stigmatic hairs are still contained inside the tepels. Slide made in 1958, photographed by HJA in 2005.

caespitosa undergoes substantial branching above and below ground. Subsp. *Parishii* is monocarpic; each plant is unbranched during its lifetime and dies after flowering. This subspecies also produces the largest inflorescences, some measuring over 20 feet in height. Many agaves also have this habit.

One can not study embryology without concurrently investigating the structure and development of the flowers. The flowers of *Y. brevifolia* are much like those of the majority of species in the genus in that they have a long tapering style and stigma. The surface of the stigma is covered with bulging secretory cells. This is in strong contrast to the style and capitate stigma of *Y. whipplei* which has many upward directed stigmatic hairs (secretory cells). These cells are very large and have nuclei that are several times the size of **ordinary cells** in the stigma/style region (Fig. 23). These characteristics are best seen in SEM preparations, but of course they were not a part of my dissertation and it would be 35 years before I could see the flowers in SEM. I have included an SEM

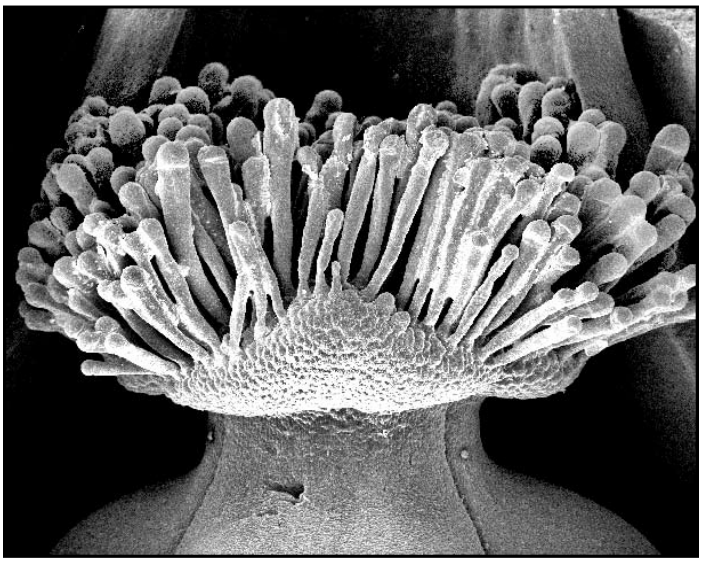

Figure 24. Capitate stigma of *Yucca whipplei* as viewed by Scanning Electron Microscopy. Micrograph by HJA.

view as it shows the structure of the capitate stigma very well. Each stigmatic hair bulges at its upper end and higher magnification shows how the stigmatic fluid is secreted (Fig. 24).

As I mentioned earlier the investigation of the development of the ovule, gametogensis, embryo sac development and finally the development of the embryo was studied in *Yucca. whipplei, Y. brevifolia* and in *Y. schidigera*. In each, as the flower develops, ovules are formed in six rows, two rows in each of the three carpels. When the fruit matures these ovules form the dark black seeds which remain stacked in rows. In each ovule a cell enlarges to form the megaspore mother cell. Meiosis in that cell produces the four megaspores, sometimes in a linear array and other times in a T-shaped arrangement. Generally the innermost of these cells will become the functional megaspore, and the others will be crushed. From the functional megaspore a mature embryo sac will develop. The development embryo of sac in yucca is "normal" or Plumbago type.

The mature embryo sac consists of an egg and two synergids at one end, three antipodals at the other end and a large cell initially containing two nuclei which later fuse to produce the "fusion nucleus" of the embryo sac (Fig. 12). After pollination the pollen tube reaches the embryo sac and one sperm nucleus enter the egg forming the zygote and the other sperm fuses with the fusion nucleus to form the triploid primary endosperm nucleus.

As yucca seeds germinate the embryo undergo changes that lead to the formation of the young seedling. In addition to becoming larger the embryo/seedling changes involve the formation of vascular tissue. While it is true that the development of vascular tissue can be followed by laboriously studying serial sections, the process of clearing can reveal these changes with some ease. I studied the process in *Y. brevifolia* and developed several plates showing stages in vascularisation for my dissertation publication, "The Seed, Germination, and Seedling of Yucca."

I was very pleased that Dr. Katherine Esau used illustrations from my work for the cover of her book entitled, "Vascular Differentiation in Plants," Holt, Rinehart and Winston, 1965. She also used eight of my illustrations in the body of her book. I also cleared whole seedlings of yucca with and without the seed coat attached. The complicated nature of the vascular system is difficult to picture, however, a large drawing helped me represent the nature of this system which shows both the cotyledonary node and the node of the first leaf (Fig 25); this is one of the illustrations Dr. Esau used in her book.

My fledgling time of research concludes with the writing and illustration of my paper on **"The Seed, Germination, and the Seedling of Yucca"** At that time it was my most important publication and gathering together the pieces was a major piece of work in itself. The paper consists of 163 pages of which 95 pages were text; it had 33 plates, 213 line drawings and 9 Tables. I was finished writing it in the spring and put the plates together in the summer of 1960 while at Berkeley. Mrs. Francia Chisaki-Hommersand, who then worked in the U.C. Berkeley Herbarium, was extremely helpful in the latter activity and I again, profoundly thank her.

PASSING THE TORCH

I became an Assistant Professor of Biology at Northwestern University soon after graduation. Part I and Part II of this series touch on that time to some extent, see part II for my "Northwestern Interview." On entering this stage of university life you soon come face to face with *students*, first *undergraduate students,* then *graduate students.* These are not somebody else's students, they are yours—you give the grades, you can pass or fail them. You can teach them well or you can ignore teaching as an unimportant drudgery that keeps you from research, reading or golf. It is an awesome responsibility. I chose to take teaching seriously.

Autobiography continued on page 77

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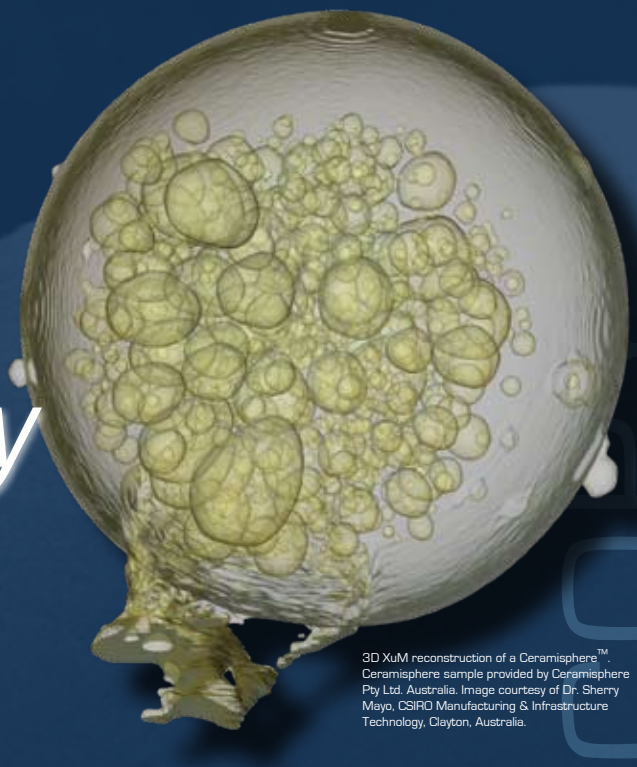

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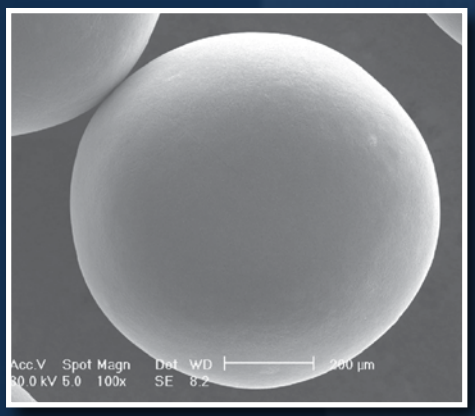

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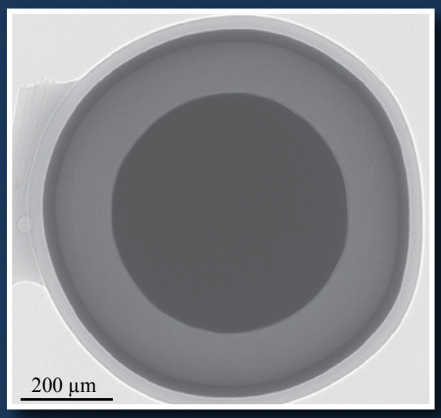

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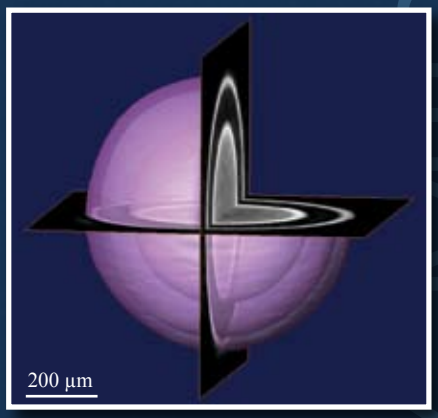

Images: SEM and XuM imaging of an intact multi-layered micro sphere. Left: Conventional secondary electron image. Middle: 2D XuM image showing composite internal structure. Right: 3D XuM tomographic reconstruction with cutaway section to expose internal core.

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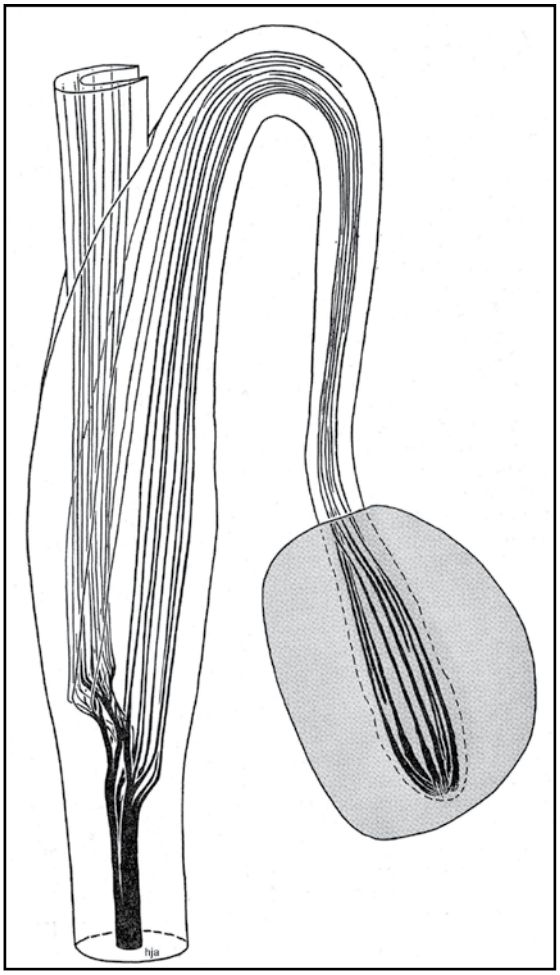

Figure 25. Diagram of a cleared seedling of *Yucca brevifolia* showing the vascular system as it forms the cotyledonary node and the node of the first leaf. The haustorial portion of the cotyledon is shown within the seed. From: Arnott, H.J. 1958. The Morphology and Anatomy of Yucca L. Dissertation (Ph.D.), University of California, Berkeley, 600 p.

Unfortunately the quality of your teaching has little to do with job security, promotion or financial rewards. With classes of over 800 undergraduate students it was indeed a serious business.

Graduate courses at Northwestern were generally relatively small. However, it is important to remember that the graduate students are professors real "bread and butter." They usually have the responsibility for the beginning laboratories, a major chore released from the faculties shoulders. When they choose to work with you, they can amplify your research efforts enormously. At this point they come seeking your guidance and may come to depend on your advice. This is the ultimate stage of research and it becomes your job to transfer your interests, enthusiasm and knowledge to students. It's a natural event in the progression of research. Unfortunately, there is no *bona fide* training for this component. With each student you pass through unknown territory; the journey is an exciting part of the learning-teaching-research process.

This part of my autobiography ends with a few comments and anecdotes about my first three Ph.D. students: Elizabeth Gantt, Frances N. Fletcher, and Harry T. Horner. Each started as a student in my Plant Anatomy class; a class that I really like to teach; perhaps my enthusiasm showed. Don Kaplan who later became Dr. Adriance Foster's replacement at Berkeley was also in that same class. At that time I was not much older than any of the three (Fig. 26).

Figure 26. Frances Fletcher, Jack Horner and Beth Gantt (L to R) on the roof of Swift Hall, Northwestern University, summer 1963. Photo by HJA.

Elizabeth Gantt finished her Ph.D. in 1963 working on the spore germination in *Matteuccia struthiopteris*, the ostrich fern. Beth was interested in cell development and the process of fern spore germination offered an excellent opportunity to study cellular differation. Using multiple staining techniques and thin paraffin sections she followed the controlled way in which the spores transform during germination. Beth learned TEM from Robert King and also applied the technique to her spore studies.

I remember several occasions which give some insight into our relationship. Soon after she decided to work with me, we visited a local Forest Preserve west of Evanston, IL in order to find the fertile fronds containing the precious spores of the ostrich fern. It was still winter and we were blundering through 6-12 inches of snow looking for a plant neither of us had ever seen, except in illustrations. At one time we became separated. My imagination quickly kicked in and I was sure that I had lost my first graduate student. I even imagined the headlines, *"Professor loses graduate student in forest preserve*." I still remember yelling over and over "Mrs. Gantt, Mrs. Gantt" louder and louder with each increase in my level of panic. Eventually, I found her, or she found me. We found the fertile fronds necessary for her research and there was no need for headlines. In the end she used spores which came from Minnesota. I soon began to call her Beth.

The second anecdote involved no panic attack but just a solution to a technical problem. Beth had embedded her fern spores in paraffin (normal for the day) and the microtome sections she was getting were not thin enough to see all the elements within the spores. I remembered using expanding liquid CO_2 for freezing material to use on the freezing microtome and a possible solution evolved. By directing the expanding liquid CO_2 at the paraffin block we could make the paraffin much harder, hard enough to cut one micron sections. With these sections she was able to resolve the internal structure more accurately.

An anecdote pertinent to transmission electron microscopy also comes to mind: One afternoon soon after Beth began electron microscopy, she came in with a handful of recent micrographs. I protested that they were all out of focus and proceeded to tell her that she should, "**focus** the microscope . . . it's just like a camera and you must adjust it for proper focus." Ha ha! At that time I had never even looked in an electron microscope, let alone tried to focus it. Big mistake! Some things you have to learn the hard way and that's what happened. She exclaimed, "It isn't that easy!" She then took me down to the Hitachi 1 TEM, and I looked at the image which seemed pretty well impossible to focus. I did finally find out that by moving the specimen stage up and down you could get

what looked like a better image (that is not an approved method of focusing). As I learned to operate a TEM, focus became the real bugaboo and a source of continual annoyance especially when trying to show someone what focus is. Eventually, in the fullness of time, I learned that there is **focus** and then there is **TEM focus.** But not before I apologized to Beth.

After Beth graduated, she did a postdoctoral at Dartmouth where she became interested in the photosynthetic apparatus of red algae. She followed that subject with tenacity down to the molecular level and beyond. Dr. Gantt developed a remarkable career; currently she is a Distinguished University Professor at the University of Maryland, College Park. In 1996 she was elected to the U.S. National Academy of Sciences. She is the 1994 recipient of National Academy of Sciences' Gilbert Morgan Smith Medal and the 1981 recipient of the Botanical Society of America's Darbaker Prize. She was the President of the American Society of Plant Physiologists in l988-1989

N. Frances Fletcher Ekern came to Northwestern University from Wellesley College where she had completed her undergraduate work. She earned a Master's degree and completed her dissertation, entitled, "An Experimental Study of the Venation of *Lemna minor* L.," in 1964. After establishing sterile cultures, Frances grew *Lemna* plants in flasks on liquid media and under controlled conditions of light and temperature. To various tissue culture media she added different growth substances. The most effective in modifying the venation was TIBA (2, 3, 5-triiodobenzoic acid). Mature plants were cleared and stained in order to determine the effect of growth promoters on venation. Using TIBA she was able to increase both the number of dichotomies and anastomoses in the fronds; thus she experimentally produced a more complicated venation. She was able to show statistically that the degree of vein complication was directly related to the concentration of growth substances present in the cultures. In her dissertation Frances also displayed electron micrographs showing the internal structure of *Lemna* fronds including the veins in various planes of section.

Fran Fletcher was extremely helpful in my study of the venation of *Ginkgo biloba* which over time evolved into a major study involving thousands of leaves and careful analysis of the venation in both long shoot and short shoot leaves. Using punch cards and tape technology, she helped put all our data into shape for a statistical assessment of the venation. Unfortunately, that happened just before I left for Texas and when I again looked at the tapes they were partly disintegrated and the data was gone. The leaves and the photographic renditions of the leaves are still in my closet awaiting a "new student of venation." **Another lost opportunity**? Perhaps not, I still have hope.

After graduation Frances joined the Plant Biology Department at Michigan State University. She specialized undergraduate teaching and in aquatic plants and retired as Emeritus Professor in 2002.

Dr. Harry T. (Jack) Horner received his Ph.D. at Northwestern in 1964. Previously he graduated with biology major from there. For his master's work he investigated the distribution of micro and mega sporangia in the cones of many species of *Selaginella*. His dissertation work was entitled, "An Ultrastructural Study of Germination in Yucca Seeds." He carefully detailed the changes that occur during germination in the complicated protein/oil rich yucca seed cells. In the spring of 1964 Jack gave a presentation on his dissertation work at the American Botanical Society meetings in Amherst, MA. On this unique occasion four members of the National Academy were in attendance, Jack remained calm, and as usual, made an excellent presentation

After graduation, Dr. Horner won (earned) a post doctoral position in electron microscopy at Iowa State University. Soon there after he was appointed to a faculty position. Currently he is a University Professor and the Director of the Bessey Microscopy Facility, a component of Center for Life Sciences & Biotechnology Research and Teaching. Jack served the Botanical Society of America as President, 1994-1995; Treasurer, 1986-1992; he was Chair of the 1993 Gordon Research Conference on Calcium Oxalate; President, Iowa Academy of Science, 1982-1983. Jack teaches a unique yearlong class in all phases of microscopy for selected graduate students.

Jack has contributed in many areas involving both light and electron microscopy. He completed a variety of important studies on plant cells which produce calcium oxalate crystals. He has also made important contributions to the understanding of male-sterility in corn and soybeans. The latter has extreme economic consequences. Jack has published over 125 peer-reviewed papers.

I remember Jack as a student who was hard working and eager to please. His father, Dr. Harry T. Horner, Sr., a physician, took a serious interest in his son's education: They traveled together on collecting trips in the southwest looking for *Selaginella* specimens to be used in his Master's work. Jack is an admired scientist, a skilled technician and excellent teacher. His motto is, "If it is worth doing, then do it right." When Horner was engaged in clearing massive numbers of *Selaginella* cones we developed the "**multiple-compartment tray for processing many specimens at one time."** This is a plastic ice cube tray with small holes drilled in the bottom so that fluids could drain out without loosing the specimens. The idea may sound simple or even pedestrian but it was a great time saver. We published this technique in "Stain Technology."

WHERE DID THE TORCH COME FROM?

You already know that I was a student of Adriance S. Foster; he was a student of Irving W. Bailey at Harvard, who in tern was a student with Edward C. Jeffery at Harvard, who was a student of William G. Farlow at Harvard who was a student of Heinrich Anton de Bary in Strassburg, and so it goes back through time. I met Irving W. Bailey, my academic grand father, when he visited U.C. Berkeley to give a seminar. I remember two interesting things about his visit. First, he walked up from the Berkeley railroad station to the Life Science Building caring his suitcase (leather bound, 20 x 24 x 6 inches in size). That was no mean feat for a man of 72 years since, the distance from the railroad station to the University is 2.5 miles and it is uphill. Although taxi were readily available, the "Independent Yankee Spirit" was still a part of his everyday life (Fig. 27).

Figure 27. Dr Irving W. Bailey in the Botany Seminar Room at U C Berkeley circa 1955. Photo by HJA.

That night after his seminar Dr. Bailey, Member of the National Academy of Science and an internationally distinguished plant anatomist, was hosted at a reception given by Helen and Adriance Foster at their home in the Berkeley Hills. Many faculty, graduate students and wives attended. Of course this was my opportunity to speak to Dr. Bailey, a real hero of mine. I got to talk to him for about three minutes, however, my wife, Jean, talked to him (one on one) for about 45

minutes. From afar I heard Bailey discussing the "Centrosperme*" and other plant groups with Jean, I am pretty sure she had never heard of those groups. However, she is a **master of listening**, it only requires a yes here and there, or a head shake at the right time and people, *even great botanists*, will just talk on and on!

***Centrospermae* is a botanical name, meaning "with the seed in the center" referring to free-central placentation.**

Recently, I have been rethinking what it means to say "the torch is passed." In our minds we have the symbolism of the Olympic Torch being pasted from one runner to another. Is there anything like that in science? Here are my observations on the relationship between Foster and myself regarding the "torch." Foster was interested in the shoot apex, thus I became interested in the shoot apex. Foster was interested in venation and thus I became interested in venation. Foster was interested in idioblasts and their development and I became interested in idioblasts. Those things followed like night follows day.

My venation studies were different than Foster's in that they involved statistical analysis. Even though I worked at venation for sometime it did not become a life time activity; in part this was because very few other people were interested in this area of study. My shoot apex work involved *Yucca* and didn't extend beyond graduate school. My interest in idioblasts however, survived most of my career changes and is still of significant interest, especially the development of crystal idioblasts. Although Foster was interested in crystal idioblasts but he never focused on them; being completely dedicated to venation studies during the time I knew him.

So what did Foster pass on to me? Or what does any professor pass on to their students. Instinctively, I believe it is not the subject matter but the *modus operandi* of Foster's research and life which is the real nirvana. My emulation of Foster's attention to detail, his intensity in research and finally the excitement of his research is "what was passed on." To some extent in this instalment you found out what I passed on to the next generation of my students. In the next instalment you will find out quite a bit more.

A NOTE ABOUT THE LOST JOSHUA TREE PLOTS OF 1952.

On July 13 and 14, 2006, my daughter, Catherine and I searched for the Joshua Trees plots that were marked in 1952. Although the temperature was running about 105° F we spent some time looking for Plots 2, 3, and 4 without real success. Plots 2 and 3 were not reached because of the Los Angeles Aqueduct which stopped our progress about 0.8 miles short of the purported sites. The Aqueduct was not there in 1952. We were perhaps more successful in regard to Plot 4. Plot 4 was located about 1/3 of a mile east of the Denis Siding of the Union Pacific railroad line that parallels the Sierra Highway in North East Palmdale, Los Angeles County, California. Unfortunately, we were not able to see the actual trees marked in 1952; however, I could see that the trees in the appropriate area appeared to be in good shape. Fortunately for those trees, they are on the property of U.S. Air Force's Plant 42 on the east side of Palmdale, CA. They are about ½ mile from the west end of the east/west runway on that facility. Being so located, the Joshua Trees may be saved from malicious damage by the public, and are potentially protected from new urban developments (Fig. 28). Given more time perhaps we will find the plots in the future.

Figure 28. Purported site of Plot 4 (Arnott, 1952) on the U.S. Air Force's Area 42, northeast of Palmdale, CA. Photo taken July, 2006 by HJA.

A SCREEN SAVER!

You will remember Dr. Marion Cave (Fig. 17) from my previous comments. In closing Part Three, I want to introduce you to Marion's husband, Dr. Roy C. Cave. When I knew him Roy was a Professor of Economics at San Francisco State University. Roy loved life but seemed to love limericks still more, much to Marion's distaste and consternation. Every afternoon after work Roy would arrive to take Marion home. He generally swept into our office with a flourish and always made some kind of small talk. Often he would relate a "new" limerick or joke. He was a "perpetual character" and loved to "embarrass" Marion with off color remarks or terrible puns, or both. Incidentally, Adriance Foster and he got on extremely well and the limerick rubric was very much part of their mutual admiration. Foster always cherished the way words sound, and he would often "converse" with colleagues or strangers in Spanish, French or German just for the fun of it! This activity is completely foreign to my ideas of "fun." Even today, fifty years after my difficulties with German, I find foreign languages a difficult pill to swallow.

It was sometime before Roy Cave learned that I was working on yucca. I have always thought it was in 1957, but it may have been earlier. When he learned about the subject of my research he quickly originated his "*magnum opus catch phrase*:" **"Yucca Yucca Winnemucca,"** I heard it over and over whenever he came or when he was leaving our office. Sometimes he said it seven or eight times in a row; you try it! However, it obviously caught my attention.

I added quotation marks to "Yucca Yucca Winneamucca" and Roy Cave, 1957 to make the closing line of my dissertation. Since the time I became "computer literate," I have used Roy's catch phrase as a Screen Saver on all my home and lab computers. I have to admit, it does have a certain "flavour."

I present the "Roy Cave Screen Saver" to you! If you care to use it in Windows you simply go to Start, Control Panel, Display, Screen Saver, choose Marquee, in the Text Box type "Yucca Yucca Winnemucca" Roy Cave, 1957; choose blue background, random position, medium slow speed and format text: Arial, bold, 72 color: yellow. This gives you the "Roy Cave Screen Saver" in U.C. Berkeley colors.

I express thanks to the following for their help in preparing this manuscript: Catherine Arnott-Thornton, Susan Garrett, Jean Arnott, Martha Gracey and Camelia G.-A. Maier.

"On the crystal trail" is the subject for Part Four, the penultimate segment of My Autobiography.

CORPORATE MEMBERS

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(520) 745-0001 FAX (520) 745-0004
dave@boeckeler.com

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Madison, WI 53711-5373
(805) 523-1882 FAX (805) 523-1896
Alan_Hollaar@bruker-axs.com

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313 S Jupiter Road, Suite 105
Allen, TX 75002
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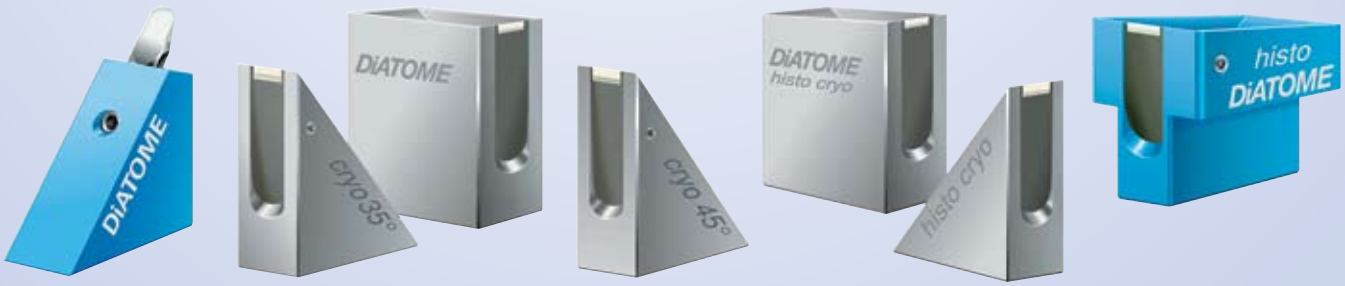

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