# CELL BIOLOGY

Chairman: Carl W. Godzeski, Eli Lilly Research Wing, Marion County General Hospital, Indianapolis, Indiana 46200

Carl W. Godzeski, Eli Lilly Research Wing, Marion County General Hospital, Indianapolis, Indiana 46200, was elected Chairman for 1974

## ABSTRACTS

**Preparation of Microbes for Scanning Electron Microscopy (SEM).** Lee F. Ellis and Linda K. Sampson, Eli Lilly and Company, Indianapolis, Indiana 46206.——Microbiologists grow bacteria and fungi in either broths or on agar surfaces, and each case presents problems for the electron microscopist. When broth cultures are used, components in the media form crystals or an amorphous coating on the cells. Generally cells were fixed and washed in buffer, but for higher resolution alcohol rinsing was tried. *Streptomyces cinnamonensis* fermentation cultures were used in this study.

Colonies of *Cladosporium* sp. and *Streptomyces cinnamonensis* were grown on Sabouraud's plates and were fixed with osmium vapor. Small pieces were removed and prepared for scanning electron microscopy with the following procedures:

1) Air dried
2) Graded alcohol dehydration and air dried
3) Graded alcohol dehydration and critical point dried
4) Graded alcohol dehydration, held overnight in 100 per cent alcohol and critical point dried

All the specimens were coated with gold and examined in the SEM. The best dehydration was attained when the agar pieces were held overnight in alcohol. Both the vegetative organisms and spores show wrinkles in their surfaces when dehydration was incomplete.

**Scanning Microscopic Comparison of Starch Grains from the Latex of *Euphorbia* Species.** Paul G. Mahlberg, Department of Plant Sciences, Indiana University, Bloomington, Indiana 47401.——A morphologic comparison of starch grains from latex of different species of *Euphorbia* shows that grain morphology differs between each species. In addition, the morphology of these grains in any one species differs from grains present in other cells within the plant body. Four classes of grains were identified in different latices: elongated, osteoid, osteoid-lobed, and discoid. The average length and maximal length of grains also can differ in species possessing a similar class of grain. The elongated grain was present in three non-succulent species examined, *E. heterophylla* L., *E. pulcherrima* Willd. and *E. terracina* L., where the maximal length of recorded grains was 158, 70 and 27 microns, respectively. Latex in succulent species most frequently contained grains with an

osteoid or variation of the osteoid morphology. In some species, the enlargement of the grain ends was minimal as in *E. valida* Br. (maximum length, 48$\mu$) and in *E. viguieri*, Denis (maximum length, 33$\mu$) while in *E. abyssinica* Gmel. (maximum length, 35$\mu$), *E. tirucalli* L. (maximum length, 49$\mu$) and *E. trigona* Haw. (maximum length, 62$\mu$) the grain ends are progressively more flared. The development of additional lobes along the mid-region of the grain characterized *E. inconstantia* Dyer (maximum length, 55$\mu$) and the osteoid ends were frequently lobed. The grains in *E. lactea* Haw. showed a complex development in that multiple lobes developed along the mid-region as well as the ends to form a discoid grain (maximum length, 36$\mu$). The variations in starch grain form suggests that their morphology is genetically controlled. This feature will make it possible to interpret the genetical basis for grain morphology and provide a criterion for interpreting the evolution of the laticifer within the genus *Euphorbia* and the family Euphorbiaceae.

**VLB Effects on CHO Cells As Seen By Scanning Electron and Phase Microscopy.** LINDA K. SAMPSON, GEORGE BORDER, and LEE F. ELLIS, Eli Lilly and Company, Indianapolis, Indiana 46206.——Control and VLB (vinblastine)-treated cell cultures of a Chinese hamster ovary line were examined by phase microscope time lapse photography and scanning beam electron microscopy for additional data on the mechanism by which VLB alters cellular division.

Many investigators document the growth inhibiting activity of vinca alkaloids to be due to metaphase arrest. We found correlation with that effect on the mitotic cycle of this particular cell line by use of phase time lapse photography and scanning electron microscopy.

For the scanning electron microscopy studies, non-synchronized control cells and cells subjected to $10^{-4}$ micrograms per milliliter of VLB for 5.0 hours were established as 24-hour cultures on glass coverslips. The cells were subsequently glutaraldehyde-fixed, ethanol dehydrated, critical point dried, and vacuum gold coated.

Time-potentiated metaphase accumulation is easily demonstrated during time lapse microscopy. This compares favorably with the surface morphology shown by scanning electron microscopy, which also demonstrates pronounced increase in metaphase-arrested cells of the VLB-treated cultures over the controls. Morphologically, the rounded-up, spherical cells in metaphase can be easily distinguished from the stretched or retracting surfaces containing numerous blebs and microvilli which are characteristic of $G_0$, $G_1$, S, and $G_2$ phases.

At present, this information suggests a possibly reliable system for use as a further aid in studying the *endogenous* mechanisms controlling the mitotic cycle.

**A Cytological Study of the Uptake of Liposomes by HeLa Cells.** J. D. SCHOKNECHT, W. E. MAGEE, and C. W. GOFF, Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809.——Phospholipid spherules, or liposomes, were prepared from mixtures of sphin-

gomyelin, cholesterol and stearylamine. The enzyme horseradish peroxidase was trapped inside the particles in the aqueous phase during their preparation, thus providing a cytochemical marker for the particles. The particles were allowed to adsorb to cells as described previously (Magee, W. E. and O. V. Miller, Nature 235:339 (1972)), and the fate of the particles was followed over the succeeding 24 hours. The enzyme was localized by the Karnovsky staining procedure following glutaraldehyde fixation. The cells were postfixed in $OsO_4$, embedded in an epon-araldite mixture, and examined by electron microscopy. Although no peroxidase staining could be detected in control cells, peroxidase positive material was observed in what appeared to be phagocytic vacuoles within 30 minutes after initial exposure to liposomes. Large aggregates of peroxidase-positive material apparently corresponding to liposomes were also present at the cell surface and in association with pseudopodia extending from the cell surface at 30 minutes and at all subsequent times. The cell membrane itself was stained over much of its free surface. The possible contribution of phagocytosis and of liposome fusion with the cell membrane in the acquisition of peroxidase staining at various loci will be considered.