CELL BIOLOGY

Chairman: Paul Mahlberg, Department of Plant Sciences, Indiana University, Bloomington, Indiana 47401

Lee F. Ellis, Lilly Research Labs., 346 East McCarty, Indianapolis, Indiana 46206 was elected Chairman for 1976

ABSTRACTS

Agglutination of Protoplasts from Crown Gall Tumors of Bean Leaves. Georgeanne Harris, C. Mayes, Carol L. Richardson, and D. James Morré, Purdue University, West Lafayette, Indiana 47907.——Protoplasts from normal bean leaf tissue and crown gall tumor tissue induced by *Agrobacterium tumefaciens* were compared. The tumor tissue shows increased tendency toward agglutination both with and without addition of concanavalin A. The results suggest an alteration in the properties of surface membranes in a plant tumor system.

Glycolipids of Plant Tumors. John F. Quinn, William J. Hurkman, Carol L. Richardson, and D. James Morré, Purdue University, West Lafayette, Indiana 47907.——Total glycolipids and glycolipids from membrane fractions of kohlrabi were analyzed. The total pattern of normal tissue was compared with that of tumor tissue induced by inoculation with *Agrobacterium tumefaciens*. Results indicate that plasma membranes of kohlrabi have a unique pattern of glycolipids and that membrane glycolipids of control and tumor tissues are similar.

Ultrastructural Characterization of Membrane Fractions from the Fungus *Gilbertella persicaria*. Martha J. Powell, Charles E. Bracker, and D. James Morré, Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907.——Mitochondria, vacuoles, plasma membranes, and vesicles with attached ribosomes from homogenized germlings of *Gilbertella persicaria* equilibrate at the 1.3/1.4 M interface in sucrose gradients. Differential centrifugation in sucrose and Ficol gradients results in the additional separation of the mixed membranes into three fractions: one containing predominately intact mitochondria, another composed of vacuoles and ribosomes-coated vesicles, and a third enriched in plasma membranes. Differences in thickness and symmetry of membranes and in contents of vesicles are used to distinguish these different classes of membranes. The abundance of ribosome-coated vesicles in the vacuole enriched fraction is surprising because rapidly growing hyphae of *G. persicaria* are practically devoid or rough endoplasmic reticulum. Reconstruction from micrographs of mitochondrial fragmentation and vesiculation show that most of the ribosome-coated vesicles originate from disrupted mitochondria. Superficial resemblance of mitochondrial vesicles to rough endoplasmic reticulum could lead to the fallacious attribution of mitochondrial properties to endoplasmic reticulum. So far, binding of

ribosomes to isolated mitochondrial membranes has been observed only in fungi.

Pectinase in the Serum Fraction of *Asclepias syriaca L. Latex.* KATHRYN WILSON, CRAIG NESSLER, and PAUL MAHLBERG, Indiana University.—— A pectinase with a pH optimum of 5.2 is present in the serum fraction of the latex of the common milkweed, *Asclepias syriaca L.* The intrusive growth and extensive elongation of the non-articulated branched laticifers of this plant suggest the necessity for such an enzyme system that can dissolve the middle lamellae of cells adjacent to the laticifer and that can loosen the cell wall material of the laticifer itself. Pectinase activity, which has been shown by others to be involved in cell wall softening in fruit ripening as well as in middle lamella dissolution during leaf abscission and during pollen tube growth, was postulated to have similar critical functions in the elongation and intrusive growth of all non-articulated branching laticifers. The enzyme was partially purified from the serum fraction of fresh latex by dialysis and ammonium sulphate fractionation. Enzyme activity was detected by a viscometry assay using either pectin or polygalacturonic acid as a substrate. Activity was confined mainly to the 40-50% ammonium sulphate fraction.

Laticifers in the Stamens of the Opium Poppy, *Papaver somniferum L.* CRAIG L. NESSLER and PAUL G. MAHLBERG, Indiana University.—— Articulated anastomosing laticifers were identified at both the light and electron microscopic levels in the stamens of the opium poppy, *Papaver somniferum* L. In thick sections prepared for light microscopy the walls of stamen laticifers stain darker than those of adjacent cells and their cytoplasm has a granular appearance. The ultrastructure of these cells is comparable to laticifers located elsewhere in the plant. They possess numerous vesicles of different sizes in their darkly stained cytoplasm. Cell wall perforation in stamen laticifers results from the gradual thinning of adjacent walls as it does in the laticifers found in other tissues of the plant. Laticifers in the stamen are located in the vascular bundle associated with the phloem where they form a continuous system from the filament into the anther.

The Chromatographic Migration of Cannabinoids as Resolved by Thin-Layer and Gas Chromatography. PAUL G. MAHLBERG, JOHN K. HEMPHILL and JOCELYN C. TURNER, Indiana University.——Studies of the chromatographic migration of cannabinoids extracted from a Mexican strain of *Cannabis sativa* L (marijuana) are described. Thin-layer chromatograph was employed to characterize the extraction procedure for routine analysis of known geographical strains of *Cannabis.* At least three 1-hour extraction periods were required for complete extraction of total cannabinoids. Concentration of these aliquots was adequately performed by either a vacuum oven (60) or nitrogen evaporation (ice bath) and were compared with results derived from flash evaporation. Variation of cannabinoid migration and order of resolution on reversed phase plates was observed to be influenced by the duration of air drying and/or the impregnation of chromatographs with various ratios of N' N' methyl formamide: carbon tetrachloride.

Gas chromatographic analysis of eluted cannabinoids derived from regions of the thin-layer chromatographs will be discussed.

Chemically Induced Cytoplasmic Inclusions in Canine Hepatic Cells. STEPHEN D. BARNARD and STANLEY D. WARNER, The Dow Chemical Company, Indianapolis, Indiana 46268.——Routine toxicologic screening of an experimental imidazoline compound revealed hyaline cytoplasmic inclusions in canine hepatic cells when examined at the light microscopy level. Additionally, histochemistry indicated these chemically-induced inclusions to be lipoprotein. Treatment withdrawal appeared to result in reversal to normal morphology.

For subsequent ultrastructural investigations, four adult Beagles, two per sex, were given daily intravenous imidazoline doses of 10 mg per kg of body weight. After dosing for ten consecutive days, the animals were removed from drug treatment; this post-dosing period varied in duration per individual. In addition to hepatic tissues obtained at necropsy, liver biopsies were performed at prescribed time periods during the experiment. Ultrastructural examinations of these tissues disclosed large (6-12μ), predominantly finely-granular cytoplasmic inclusions in hepatic cells. These were found to be, at least in part, membrane limited. Explanation by sequestration appears to be contraindicated due to the absence of glycogen and paucity of organelles in these inclusions. Hepatic cell inclusions do, however, contain mitochondrial remnants, lysosomal elements and numerous myelin figures. Other hepatic cell response to imidazoline includes a proliferated smooth endoplasmic reticulum, dilated Golgi vesicles and apparent increases in numbers of microbodies.

Evidence for Fusion of Artificial and Natural Membranes. CHARLES W. GOFF and WAYNE E. MAGEE, Indiana State University and The University of Texas at San Antonio.——The fate of antigenic determinants incorporated into the lipid bilayer of liposomes was studied by the antibody-peroxidase conjugate immunocytochemical method after interaction of the liposomes with HeLa cells. The artificial membrane markers were transferred to the cell membrane even at 4-7°C (well below the phase transition of all membrane lipids). Initial results indicate that label incorporated into relatively rigid liposomes (*i.e.* those containing cholesterol, sphingomyelin, and stearylamine) does not occur over as great a proportion of the HeLa cell surface as does that incorporated into lysolecithin-containing liposomes. Whether this is due to greater rates of fusion of the latter liposomes with cells or to greater rate of lateral diffusion of the liposomal marker after fusion is not yet known.

Effect of Drugs on Mouse Embryo Hearts in Organ Culture Visualized by Acoustic Microscopy. R. C. EGGLETON*, L. W. KESSLER**, F. S. VINSON* and G. B. BODER†; *Fortune-Fry Research Labs of the Indianapolis Center for Advanced Research and Indiana University School of Medicine, Indianapolis; **Sonoscan, Inc., Bensenville, Ill., and †Lilly Research Laboratories, Indianapolis.——A method has been developed for sustaining mouse embryo hearts in organ culture for extended

periods of time on the stage of an acoustic microscope. A culture chamber was fabricated to supply the hearts with a continuous flow of oxygenated culture medium to maintain contractile function. Simultaneous acoustic and optical viewing of the hearts permits more complete observation of the contractile events than heretofore possible. Anatomical features of the heart such as atria, ventricles, coronaries and valves have been observed. A group of hearts has been maintained simultaneously in the chamber for several days. The hearts may be in various physiological conditions, e.g. synchronous, asynchronous, rhythmic or arrhythmic. Drugs are introduced into the chamber either by pulsing (as short as 5 sec. exposure) or using a continuous perfusing. The effectiveness of these agents in restoring normal behavior can be evaluated. A videotape will be presented to describe the method and results.