Cell Biology

Chairman: RALPH JERSILD, Indiana University Medical Center

DR. JERSILD was reelected chairman for 1968

ABSTRACTS

A Collodian-methacrylate Supporting Film. FRANK PADGETT and ALVIN S. LEVINE, Indiana University School of Medicine, Indianapolis.—A new supporting film for specimens has been developed for use in the electron microscopy of biological materials. The ingredients of collodian and butyl methacrylate impart properties to this film which are lacking in collodian or formvar substrates alone. The resistance of this film to the effects of air currents and moisture during its preparation and its resistance to the damaging effects of the electron beam are of special interest. The preparation and properties of this supporting film will be discussed.

Use of Plasma Fractions as Aids to Golgi Apparatus Isolation. D. JAMES MORRÉ, JOHN HORST, SALLY NYQUIST and WAYNE YUNGHANS, Department of Botany and Plant Pathology, Purdue University.—Golgi apparatus of plant and animal cells consist of dictyosome subunits made up of plate-like lamellae and a peripheral system of tubules. During isolation from cells, the tubular connections between adjacent dictyosomes are broken, followed by further decomposition of the tubules and loss of bonding substances between adjacent lamellae. Separated lamellae swell and often appear in section as large vesicles. Golgi apparatus breakdown is retarded by conditions of low shear homogenization, isotonic sucrose buffered at a pH between 6.0 and 6.5 and various additives including dextran and divalent ions. Even under these conditions, breakdown is rapid. Loss of structure is prevented by addition of 0.1 to 2.5% gluteraldehyde to the homogenization medium (Morre' et al., J. Exptl. Res. 38:672) but such chemically stabilized fractions show either greatly reduced (phosphoryl-choline-cytidyl transferase) or occasionally increased (acid phosphatase; cytidine triphosphatase) enzymatic activities.

With liver, plasma fractions added to the homogenization medium have made possible isolation of intact Golgi apparatus in a biochemically active state. With plant tissues, isolation media containing centrifuged coconut liquid endosperm (coconut milk) have yielded similar results. Dictyosomes isolated from onion stem or rat liver using plasma fractions appear suitable for detailed enzymatic studies.[1]

Nonspecific Neutral Esterase and Agranular Endoplasmic Reticulum. JOHN F. SCHMEDTJE, Department of Anatomy, Indiana University School of Medicine, Indianapolis.—Nonspecific neutral esterases are widely distributed intracellular enzymes that hydrolyze simple esters of alcohols, phenols, and naphthols, and that are most active at a pH approaching neutrality. Because they do not readily hydrolyze fats they

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are distinguished from lipases. The exact intracellular site and the physiological significance of this group of enzymes is unknown. Histochemical techniques for light microscopy indicate they are in the cytoplasm, but histochemical techniques aimed at electron microscopy have yielded contradictory evidence on their exact intracytoplasmic location.

In the present investigation esterase activity was visualized with the light microscope in the supranodular epithelial cells of the rabbit appendix. The fine structure of similar cells from adjacent and comparable sections was observed with the electron microscope. There was an association between the amount of neutral esterase visualized with light microscopy, and the amount of agranular endoplasmic reticulum visualized with electron microscopy.

These observations are interpreted as presumptive evidence that nonspecific neutral esterase is located in the agranular endoplasmic reticulum, either inside the membrane bound tubules, or in the membrane wall itself. This is in accord with other evidence on the organelle location of chemically related enzymes.

The Fine Structure of Human Leukocytes from Peripheral Blood. ITARU WATANABE, SHEILA DONAHUE, and NORMA HOGGATT, Department of Pathology, Indiana University Medical Center.—A procedure for the preparation of human blood for electron microscopic studies is described. Venous blood is taken into a test tube, mixed thoroughly with EDTA, and centrifuged at 1,000 r.p.m. for 9 minutes to obtain a buffy layer. After removing as much supernatant plasma as possible, chilled 6.25% glutaraldehyde solution in 0.1 M phosphate buffer is layered over the buffy coat. This fixative transforms the remaining plasma into a gel which is rubbery. The buffy layer in the solidified blood plasma is diced, osmicated, dehydrated in an ethanol series, rinsed briefly in propylene oxide and embedded in 27 parts of Araldite 502, 23 parts of DDSA and 1 part of DMP-30. Our sections are stained with uranyl acetate and lead citrate.

The findings in human white blood cells are of interest. Neutrophil leukocytes have three kinds of granules: large dense spherical or ellipsoid, large pale cylindrical granules in which there is a crystal and small granules of intermediate density. Eosinophils and basophils both have crystalline structures in the cores of the granules with a repeat period of about 40 Å.

Fine Structure of the Canine Pinealocyte. J. R. WELSER, Department of Veterinary Anatomy, School of Veterinary Science and Medicine, Purdue University.—The pinealocyte, principal cell of the pineal gland of the dog, was studied with the electron microscope. The typical pinealocyte was round to oval with few cytoplasmic processes. The cytoplasm surrounded the large, round-to-oval nucleus in a narrow fringe.

Dark pinealocytes with smaller, darker nuclei, as well as an increase in ribosomes and cell organelles in the cytoplasm, were found.

Orangelles indicative of an active cell were in the cytoplasm of the pinealocyte. Individual ribosomes and polysomes were scattered through

the cytoplasm. Rough and smooth endoplasmic reticula were seen in short, isolated segments, as well as long, undulating layers forming networks in the cytoplasm. A Golgi complex was present in most pinealocytes, and many vesicles of several sizes and electron densities were in close association with it.

Mitochondria of many shapes and sizes, as well as giant mitochondria (4 to 5 μ in diameter), were present in the cytoplasm. Microtubules (with a banded appearance), lysomes, and groups of large lipid droplets were also seen. Cilia were observed in many electron micrographs, indicating that single cilia probably existed on most pinealocytes. Subsurface cisternae were observed underlying a considerable portion of the cytoplasmic membranes.

The endothelial cells had pinocytotic vesicles.

Studies on the Hyphal Wall of the Fungus *Pythium ultimum*.[1] MICHAEL MCCONNELL, STANLEY N. GROVE, CHARLES E. BRACKER, Department of Botany and Plant Pathology, Purdue University.—In accordance with the general concept of cell walls, the hyphal wall of the plant pathogenic Oömycete *Pythium ultimum* Trow is a two-phase system in which a network of fibrils is embedded in an amorphous matrix. This information has been gained from electron microscopic analyses of mechanically isolated, heavy metal shadowed wall fragments and from ultra-thin sections of chemically fixed cells. The wall varies in thickness from less than 0.1 μ to over 0.4 μ depending on age. In primary walls (i.e. those laid down in the hyphal tip region) the outer surface of the wall is largely amorphous with little evidence of fibrils, whereas the inner surface is highly fibrillar with minimal amounts of matrix material. The fibrils are oriented in a random manner with respect to each other and do not assume a preferential orientation with respect to the hyphal axis. The outer surface of frozen-etched cells is coated by a layer of blunt spines. This wall component is not seen using other preparative conditions. In aged hyphae, secondary wall formation is evident. The centripetal portion of the secondary wall is pitted by a series of small radial channels (less than 0.03 μ diam.), some of which are filled with densely staining material. The fibrils in these walls are oriented in such a way that they avoid certain regions of the wall, thus forming the channels. The channels may be segmented by bands of fibrils which serve as septations. The channels do not extend to the outer wall surface. Walls formed centripetally during cell regeneration in response to injury are intermediate in some respects between the primary and secondary walls. We find no consistent evidence to indicate that lomasomes function in the formation of fungal walls. Instead, vesicles, of the type derived from the Golgi apparatus, are suggested as carriers of wall material in the hyphal tip region.

Studies on the Effects of Ethionine on Intestinal Fat Transport. RALPH A. JERSILD and PHILIP S. GIBBS, Department of Anatomy, Indiana University Medical Center, Indianapolis.—Ethionine treatment has been shown by others to inhibit protein synthesis and to interfere with in-

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testinal fat transport, which is thought to result from an impairment in chylomicron formation. Fat accumulation in the absorptive cells results. A study of these effects by electron microscopy forms the basis of this report.

Fatty chyme, obtained from fat-fed donor animals, was injected into ligated intestinal segments of recipient rats for 30 minute absorption periods. Both normal and ethionine-treated rats were used. Morphological aspects of fat absorption were distinctly altered from normal in animals with reduced protein synthesis, as determined by leucine-H^3 incorporation. Fat droplets, which typically form in the ER lumen, were fewer in number. Accumulation of droplets in the Golgi apparatus varied from negligable to moderate amounts. Extracellular droplets, or chylomicrons, were considerably reduced in number. Most striking was the abundance of large fat globules in the cell matrix in proportion to ER-enclosed droplets. The results support the concept that ethionine interferes with chylomicron formation and therefore with the transport of fats from the absorptive cells. The evidence suggests that the ER may be the site of this interference, and that the chylomicron's protein component is normally supplied here.

An Electron Microscopic Study of Transmissible Gastroenteritis in Swine. M. PENSAERT, School of Veterinary Science and Medicine. Department of Veterinary Microbiology, Pathology and Public Health, Purdue University.—A sequential electron microscopic study was made on transmissible gastroenteritis (TGE) virus-infected small intestinal loops of pigs. A segment of the jejunum was removed from the small intestine of 7 day old pigs and, while still attached to its mesenteric blood supply, placed in a subdermal pocket. Continuity of the small intestine was assured by end to end anastomosis. The intestinal loop was infected with TGE virus and tissues were removed at different time intervals. Virus-like particles, 70-80 mμ in size, were detected in smooth walled cytoplasmic vesicles in the columnar epithelium cells on the villi. Early in the infection, the microvilli of the cells became short, uneven or were lost. The terminal web area decreased in size or disappeared and degeneration and desquamation of the cells then occurred. They were replaced by flat to cuboidal cells which showed short, uneven microvilli and contained numerous free ribosomes. Differentiation of these cells to normal mature columnar cells occurred between 2 and 3 days after infection.

Electron Microscopic Study of Human Islet B-Cell Adenomas.[1] CARL R. MORGAN and RALPH A. JERSILD, Department of Anatomy, Indiana University Medical Center, Indianapolis.—Three cases of insulinoma (excessive amounts of circulating insulin produced by a pancreatic islet B-cell tumor) were studied with the cooperation of the departments of Medicine and Surgery. Tissues for EM study were recovered at surgery and within minutes were fixed in glutaraldehyde and osmium tetroxide. Fixed tissues were embedded in epon. Tissue sections were double

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stained with uranylacetate and lead citrate. The typical granules of islet B-cells were sparse in the tumor tissues of these 3 patients. Numerous small vesicles of the size of B-granule enveloping membranes were observed. Of special interest were large amounts of amorphous and fibrillar material between and within some of the tumor cells, particularly in the area of basement membranes. This material has not been identified precisely, but it is probably amyloid. The sparsity of mature B-cells in the adenoma cells correlated well with the moderate insulin responsiveness of the patients to normal insulin releasing stimuli prior to surgery and the low insulin content of extracts of the adenomas. Portal and peripheral levels of insulin declined to normal levels following excising of the tumor. All of the evidence agreed with the concept that the adenomas were secreting insulin independently of normal physiological mechanisms.

Protochlorophyll Holochrome Participation in Photorearrangement of Tubular Membranes in Prolamellar Bodies of Etiolated Bean Leaf Proplastids.[1] ALBERT KAHN, Purdue University.—A close relationship between light absorption by protochlorophyllide and rearrangement of paracrystalline tubular membranes in etiolated bean leaf proplastids has been established previously. The photoreduction of protochlorophyllide to chlorophyllide a and membrane rearrangement in the prolamellar bodies have similar or identical light requirements and are closely sequenced or concomitant. Irradiation of etiolated leaves by a single, electronic flash elicits both phenomena.

Prolamellar bodies with tubular membranes were isolated from dark grown bean leaves (*Phaseolus vulgaris L.*). The prolamellar bodies were studied by phase contrast and fluorescence microscopy and by negative contrast electron microscopy. New evidence was obtained for solution-filled channels within the tubular membrane elements of paracrystalline prolamellar bodies. Protochlorophyll holochrome, a specific complex of photoreactive pigment and a protein macromolecule, was tentatively identified in the tube walls. Protochlorophyllide was localized in the membrane walls with certainty.

These results, considered together with older observations, led to the formulation of a speculative mechanism for light-elicited rearrangement of tubular membranes in paracrystalline prolamellar bodies: The photoreduction of protochlorophyllide to chlorophyllide a is coupled with the oxidation of a protein component (the protein portion of protochlorophyll holochrome) of the tubular membranes. Bonds of unspecified nature in the protein are made or broken as a result of the photo event, inducing the membranes to form a less-ordered configuration of higher free energy. Preliminary experiments on the photoreaction, using specific inhibitors that react with sulfhydryl groups, support this hypothesis.

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