CELL BIOLOGY

Chairman: Edward Hinsman, Purdue University

Edward Hinsman, Purdue University, was re-elected Chairman for 1970

ABSTRACTS

Response of the Duck Thyroid to the Administration of Thiouracil. J. R. Welser and W. W. Carlton, Purdue University.—The response of the thyroid gland to thiouracil was studied in day-old Pekin ducks fed a diet of duck mash supplemented with 0.2% thiouracil for 6 weeks. Thyroid glands were fixed with gluteraldehyde and formalin at days 1, 7, 15, 22, 28, 31, 38, 42 of the experimental period for observation with the light and electron microscopes. The initial response was a marked hypertrophy of thyroid epithelium with the normally squamous to cuboidal thyroid cells becoming tall columnar. Ultrastructural features of the response to thiouracil included a marked distention of the endoplasmic reticulum, proliferation of Golgi apparatus, increase in the number of mitochondria and microvilli and the formation of numerous large colloid containing vacuoles in thyroid cells. Hypertrophy was followed by a marked hyperplasia of the thyroid epithelium. The ultrastructural morphology of the hyperplastic cells was similar to the hypertrophic cells. In thyroids from ducks fed longer than 21 days, distention of the cisternae of the endoplasmic reticulum was greater and was accompanied by the formation of more numerous and larger colloid containing vacuoles.

Regeneration of Skeletal Muscle in Vitamin E-Deficient Rabbits. J. F. Van Vleet, B. V. Hall and J. Simon, Purdue University.—Weanling rabbits fed a semi-synthetic vitamin E-deficient diet developed hyaline degeneration of skeletal muscle in 20-30 days. Light and electron microscopic study of the subsequent events of regeneration of damaged muscle fibers in the diaphragm of affected rabbits showed the discontinuous type of regeneration to predominate. Myoblasts developed from surviving sarcolemmal nuclei and adjacent sarcoplasm. Myoblastic proliferation produced multi-nucleated giant cells that fused to form a syncytium or cords of cells that lay within the sarcolemmal tube of the regenerating fiber. Short fragments of thin (50 Å) myofilaments were observed adjacent to polysomes in proliferating myoblasts. Thick (100 Å) myofilaments were subsequently found in small bundles 2-3μ long in the sarcoplasm between a central row of myoblast nuclei and the sarcolemma lining the sarcolemmal tube. Sarcomere formation followed as thick and thin filaments become interdigitated and Z-band material formed discs at the ends of the sarcomeres. Numerous myofibrils were soon recognizable and sarcomere bands became aligned transversely to restore longitudinal and transverse striation to the regenerated fiber. Rows of nuclei surrounded by numerous mitochondria remained in the centers of regenerating fibers. Later, these nuclei were found in their normal position against the sarcolemma.

Deficient Myelination in the Brain of "Quaking" Mouse: an Electron Microscopic Study. ITARU WATANABE and GLENN BINGLE, Indiana University Medical Center.—The mutant gene "quaking" is inherited as an autosomal recessive trait. The homozygotes exhibit a life-long neurologic disorder manifested by tremulousness and frequent tonic seizures. The brain is small in size due to lack of myelin sheaths.

To obtain further information of the mechanism of the deficient myelin formation, electron microscopic studies were performed on the corpus callosum of homozygotes aged 10, 17, 19, 21, 35, and 40 days, namely at the period of physiological myelination in the normal mice.

The major alterations were restricted in the myelin-forming cells or oligodendrocytes. A number of unique lipid bodies were already present in the perinuclear cytoplasm as early as 10 days of age when myelination was not evident in the adjacent tissue. Furthermore, the process of myelination was markedly disturbed. Redundant oligodendroglial processes surrounded a single axon in an irregular fashion and eventually compacted to form a structure reminiscent of a myelin sheath. This resulted in a sheath of variable thickness and often a segment of the axon was not at all covered by these abnormal sheaths. The cytoplasm of the oligodendroglial processes harbored spherical vacuoles, which increased in size by liquefaction of oligodendrocytic cytoplasm and, further, disintegrated the abnormal myelin-like membranes.

These morphological changes suggest an inborn-error of metabolism in the oligodendrocyte.

The Identification of Gamma-A Globulin in Human Enteric Epithelial Cells. JOHN F. SCHMEDTJE, Indiana University Medical Center.—There have been discordant reports on the presence of gamma-A globulin in enteric epithelial cells—particularly in mucous type cells. In the present investigation, gamma-A globulin was identified in the mucous type epithelial cells of the human appendix.

Tissue blocks from normal human appendixes were quick frozen immediately after surgical removal. Frozen sections were cut on a cryostat. Some sections were set aside for H & E staining. Goat anti-human gamma-A globulin, conjugated with fluorescein isothiocyanate was used according to Coon's direct method. Non-specific reactivity of the conjugated antiserum was removed by adsorption with mouse liver and ox marrow powders. Unconjugated antiserum was used for control reactions.

Positive fluorescence occurred in numerous plasma cells located beneath the basement membranes of luminal epithelial cells and cells that lined the crypts of Lieberkuhn. Positive fluorescence also occurred in all epithelial cells, except those at the bottom of the crypts of Lieberkuhn. In mucous type epithelial cells, the mucinogen areas and nuclei were negative. However, positive fluorescence occurred in the cytoplasmic areas around the mucinogen. These results are interpreted as supportive evidence that gamma-A globulin is secreted into the appendiceal lumen.

Effects of Adenosine Diphosphate on the Morphology of Heart Mitochondria. N. E. WEBER and P. V. BLAIR, Indiana University Medical Center.—Ultrastructural studies have been made of beef heart mitochondria in various metabolic steady states. Adenosine diphosphate promotes a highly condensed morphological arrangement of the inner mitochondrial membrane. Although oxidation rates are altered (and adenosine triphosphate is produced) upon the addition of oxidizable substrate and inorganic phosphate, the appearance of the inner mitochondrial membrane is essentially unchanged. The effect of adenosine diphosphate was observed not only at 30°C but also at 0°C when mitochondria were exposed to rotenone, potassium cyanide and anaerobic conditions. The adenosine diphosphate promoted the condensed morphology in all cases with the possible exception of mitochondria exposed to rotenone. These observations suggest that large ultrastructural transformations are not required for energy transduction and that the morphology of the inner mitochondrial membrane may depend primarily on osmotic changes created by experimental conditions and nucleotide binding during steady state metabolism. The width of the most condensed double-layered cristae is approximately 110 Å. Thus a reinterpretation of the negative staining of mitochondrial membranes consisting of 'tripartite elementary particles' must be considered with these measurements in mind. (Supported by USPHS Grant HE060308 and the Indiana Heart Association.)

The Isolation of Nuclei and Endothelial Cells from Brain. A. N. SIAKOTOS, Indiana University Medical Center.—Pure preparations of organelles and individual cell types are required for determination of their chemical composition and metabolic characteristics. A procedure is offered for preparing highly purified endothelial cells (capillaries) and nuclei from human and bovine brains. Phospholipid compositions of typical preparations demonstrate, in particular, a lack of species variability for capillary structures.

The isolation procedure employs modifications of differential and density gradient centrifugation commonly used for subcellular separations. Special features of the procedure are: large-scale preparation; Sephadex G-25 used for the separation of nuclei and endothelial cells; and foam concentration used for further purification of endothelial cells.

Ultrastructural and Enzymological Observations of Isolated Kidney Microvilli. SAKAE YUMOTO and SHINJI ISHIKAWA, University of Tokyo.—Essentially pure microvilli of rat kidney cortex were isolated by isopicnic centrifugation on a discontinuous sucrose density (0.72-0.92 M). Electron microscopic examination of the obtained fraction showed the presence of cylindrical structures of 1.3 to 2.0μ in length and 0.1μ in width. When negatively stained with phosphotungstic acid, globular particles of 40 to 60Å in diameter were seen on the margin of microvillar membrane. These globular subunits were also observed *en face* on the surface of the membrane. This fraction showed phosphatase activity against ATP,

AMP and p-nitrophenylphosphate as a substrate. Presence of Mg ion is required for ATP hydrolysis but Ca can replace Mg. This adenosine triphosphatase activity is not stimulated with the addition of Na and K ions and not inhibited by ouabain. The microvilli fraction also hydrolyzes other nucleotide triphosphates such as GTP, CTP, UTP and ITP. The activation energy of adenosine triphosphatase is 9.200 cal/mole. It is concluded that kidney microvilli do not have ($Na^+ + K^+$)—stimulated adenosine triphosphatase but possess very high specific activity of Mg^{++} adenosine triphosphatase. This Mg^{++} adenosine triphosphatase might play an important role on the active transport mechanism of kidney microvilli.

NOTE

Ultrastructural Features of the Calcifying Epithelial Odontogenic Tumor. J. B. WHITTEN, JR., Indiana University Medical Center.—The calcifying epithelial odotogenic tumor (CEOT) was described in 1955 by Pindborg. This is a benign, locally aggressive, epithelial tumor which probably arises from the odontogenic apparatus. The lesion most often occurs in the mandible usually in the posterior aspect, does not exhibit a sex predilection, and involves the same age group as the ameloblastoma. Radiographically, this disease presents as an ill-defined radiolucency usually with multiple radiodensities. Histologically, the tumor is composed of sheets or cords of polyhedral cells which have dense eosinophilic cytoplasm and large single or multiple nuclei often with multiple nucleoli. Focal droplet calcification resembling Liesegang's rings is often found interspersed within the epithelial cells as well as from calcification of the surrounding connective tissue.

The tissue for this project was secured from a 68-year-old Caucasian male with a large 3.5 x 4 cm. expanding lesion of the anterior mandible. The tissue was hemisected, a portion processed for light microscopy and the remaining tissue prepared for electron microscopy. The tissue for electron microscopy was fixed in phosphate buffered 4% glutaraldehyde, post-fixed in phosphate buffered 1% osmic tetraoxide, dehydrated in ascending concentrations of ethyl alcohol and propylene oxide, embedded in epoxy resin, sectioned at about 600 Å, stained with lead citrate and uranyl acetate, and examined with a RCA EMU 3H electron microscope.

The fine structure of the CEOT showed the lesion to be composed of two cell types. The outer (Type A cell) was papillary in outline demonstrating many microvilli and closely resembled the papillary layer in developing teeth. The Type B cells which resembled the stratum intermedium, were more regular in outline and were "packed" with mitochondria. These Type B cells were remarkably similar to the previously reported structure in oncocytes. The Type B cells were responsible for the bulk of the neoplastic process. In association with both the Type A and Type B cells were large quantities of fibrous protein with the period and width of amyloid. This amyloid appeared to be produced by the Type B cells. In many odontogenic tumors there has been reported an inductive effect. In this example of CEOT this inductive effect is found to be composed of elaborate arrays of basal lamina.

OTHER PAPER READ

Methacrylate Embedding: With Good Results. FRANK PADGETT, Indiana University Medical Center, Indianapolis, Indiana.