ENZYME CYTOCHEMICAL OBSERVATION OF EXPERIMENTAL DIABETIC RETINOPATHY

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To elucidate the early pathological changes of the diabetic retinopathy, retina of streptozotocin (STZ) induced diabetic rats were observed with electron microscope following enzyme cytochemistry of glucose-6-phosphatase (G6Pase).

MATERIALS & METHODS: Wistar inbred rats were made diabetic by intravenous administration of 50mg/Kg STZ. Eyes were fixed in cacodylate buffered glutaraldehyde and the enzyme activity was detected in the incubation medium of Wachstein and Meisel.

RESULTS & CONCLUSION: Higher enzyme activity was detected in the retina of diabetic rats. Electron microscopically, G6Pase was localized in the ER of all the retinal constituent cells. As the ER of the Müller cells were characteristically small and dense, this enzyme proved to be a good marker of Müller cells. Three months after STZ injection, Müller cells underwent lamellar transformation and at six months later, formed onion skin lesions. The center of the lesions seemed to degenerate during prolonged hyperglycemic states.

Changes not only of the retinal vascular structure but of the Müller cells are suggested to be important in the development of diabetic retinopathy.

Ferric colloid-labeled IgG, its preparation and application in histo- and cytochemistry for light and electron microscopy.

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Antibodies labeled with specific ligands such as fluorescein isothiocyanate and peroxidase have been developed for light microscopy by Coons, Nakane, Pierce et al., and those labeled with ferritin and colloidal gold for electron microscopy by Singer, Faulk, Roth, Hainfeld et al. The methods proved of great value in the study of biological and medical problems. However, the labeled-antibody which gives distinct picture like peroxidase conjugate under light microscope and also the fine picture as clear as ferritin or colloidal gold under electron microscope has been longed to appear. This time we have succeeded in preparing such an ideal labeled-antibody conjugating IgG with cationic cacodylate ferric colloid which has well retained antibody specificity and gives a distinct Prussian blue reaction for light microscopy and electron dense grains for electron microscopy. The Fe-Cac-labeled IgG were easily formed by mixing with excess of Fe-Cac at pH 7.4, and can be separated from free Fe-Cac by using column chromatography on Amberlite ion exchange resin.

Ischemic changes of cytoskeletons in the gerbil brain, especially actin.

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The object of this study was to investigate the changes of cytoskeletons, especially of actin, after brief ischemic insult. Adult Mongolian gerbils were subjected to bilateral common carotid occlusion for 7 min. From 6 h to 7 days following ischemia, the gerbils were sacrificed and then removed their brains. Ischemic damage and cell proliferation were determined by immunocytochemistry, using antisera against tubulin, grial fibrillary acidic protein(GFAP) and actin of rat brain.

Though nonfrozen floating method could demonstrate reaction products in the cytoplasm of astrocytes in anywhere of the gerbil brain, a reproducible reaction took place in the astrocytes of white matter in the case of paraffin section. Immediately after ischemia, the antigenicity of tubulin decreased or disappeared in the vulnerable portion of the hippocampus, e.g. CA2 and those immunohistochemically demonstrated ischemic lesions expanded rapidly after reestablishment of circulation. The antigenicity of actin was preserved until 12 h after ischemia. 2 to 3 days after ischemia reactive astrocytes were clearly identified by their intensive expression of GFAP, which were recognized as early as 24 h after ischemia by actin staining.

Lectin-binding Patterns of the Normal and Malignant Endometrium.

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Binding patterns of seven different kinds of lectins in the normal proliferative and secretory, and the malignant endometrium were examined by light and electron microscopy using HRP-labeling methods. ConA, WGA and RCA stained almost all glandular cells in the normal and malignant endometrium. The positive sites of PNA were observed only at the apical surface of normal endometrial glands. On the other hand, the cytoplasm of endometrial adenocarcinoma cells was often positive for PNA. UEA-1 strongly stained cancer cells, especially well-differentiated adenocarcinoma, but scarcely normal endometrium. DBA and SBA strongly stained secretory endometrium, but only slightly proliferative endometrium as well as adenocarcinoma. These light microscopic observations indicate that sugar residues recognized by PNA and UEA-1, such as D-galactose and L-fucose, changed in the course of malignant transformation and N-acetyl-D-galactosamine, reactive to DBA and SBA, changed due to the cyclic change of the sexual hormones. By electron microscopy, reaction product of these four lectins were observed in the Golgi and plasma membrane of normal and malignant cells.