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SYMPOSIUM ON THE ENZYME-LABELED ANTIBODY METHOD

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Paul K. Nakane, Department of Pathology, University of Colorado, U. S. A., and Laboratory of Cell Biology, Tokai University School of Medicine, Kanagawa. Recent progress with peroxidase-labeled antibody method.

Kei-Ichi Hirai, Department of Cytochemistry, Chest Disease Research Institute, Kyoto University, Kyoto. Diaminobenzidine in cytochemistry

Akira Kawaoi, Department of Pathology, Nihon University School of Medicine, Tokyo. New method for peroxidase-protein conjugation.

Noboru Yamamoto, Department of Anatomy, Kitasato University, School of Medicine, Sagamihara, Kanagawa. Fundamental study of immunohistochemistry.

Hiroshi Hirano, Tatsuro Irimura and Fumiaki Nishiyama, Department of Anatomy, Kyorin University School of Medicine, Mitaka, Tokyo, and Division of Chemical Toxicology and Immunochemistry, Faculty of Pharmaceutical Science, University of Tokyo, Tokyo. Comparison and joint use of the peroxidase- and ferritin-labeling methods on the cell surface membranes.

Ikuo Suzuki, Laboratory of Ultrastructure Research, Aichi Cancer Center, Research Institute, Nagoya. Complement enzyme antibody method and application of nucleic acid research by enzyme antibody method.

Hiroyuki Miyamoto, Department of Pathology, Research Institute for Microbial Diseases, Osaka University, Osaka. Vaccinia virus infection in vitro studied by peroxidase-labeled antibody method.

Recent Progress with Peroxidase-Labeled Antibody Method

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Almost a decade has passed since the initial application of enzymes as labels in immunohistocytochemistry. More recent improvements with the conjugation of peroxidase to immunoglbulin, development of a new fixative for immunohistochemistry and studies on the penetration of various size of immunoglobulin labeled with peroxidase into fixed tissues should facilitate application of the method.

Aspects on the newly developed method of conjugation will be introduced by Dr. Kawaoi and will not be discussed at this time.

The stabilization of antigens *in situ* without destroying their antigenicity while

retaining relatively good tissue and cellular morphology is a common problem in immunohistocytochemistry. The majority of investigators have adapted fixation methods used for conventional light or electron microscopies with varying degrees of success. The conventional fixatives interact strongly with proteins and thus denature protein antigens. In order to overcome this problem, a new fixative which stabilizes primarily carbohydrate moieties was developed for immunoelectron microscopy. This fixative contains periodate, lysine and paraformaldehyde. Theoretically, the carbohydrate are oxidized by periodate and cross-linked by lysine. Paraformaldehyde is added at relatively low concentrations to achieve some stabilization of proteins and lipids.

In this study, preservation of ultrastructure and retention of antigenicity in tissues fixed in periodate-lysine-paraform-aldehyde solutions are compared with those in tissues fixed in glutaraldehyde and paraformaldehyde solutions.

At the light microscopic level, intracellular penetration of antibodies has not been a significant problem since the need for antibody penetration through cell membranes is usually avoided by the use of tissue sections for the staining procedure. However, at the ultrastructural level, it is considered essential that the labeled antibodies gain access to the intracellular antigens by passing through cellular membraneous systems unless ultrathin sections are used. Using perietal yolk sac carcinoma, fixed for three hours in periodate-lysine-paraformaldehyde containing 2% paraformaldehyde, we have studied the penetration porperties of various unlabeled and peroxidase-labeled antibody fragments. It was found that quick freezing and thawing enchanced anti-B.M. penetration when Fab', 1/2IgG, IgG, and Fab'-peroxidase were used. However, the freezing and thawing did not consistently enhance the pentration of IgG-peroxidase. We have found that unlabeled and peroxidase-labeled Fab' fragments to be especially effective for immunocytochemical localizations carried out on frozen sections of fixed tissues.

We currently feel that the use of frozen sections of fixed tissues, the periodate-lysine-paraformaldehyde fixative for retention of antigenicity and ultrastructure, antibody fragments labeled with peroxidase for their rapid penetration properties, and the efficient periodate oxidation method for the conjugation of peroxidase to proteins will be a powerful tool in cell biology.

DIAMINOBENZIDINE IN CYTOCHEMISTRY

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In enzyme-labeled antibody techniques the most popular marker enzyme is horse-radish peroxidase (HRP) (Nakane and Pierce, 1967). Antibodies con-

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