PRINCIPLES OF IMMUNOCYTOCHEMISTRY*

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This outline will:
1. discuss the reasons for avoiding the covalent labeling of second antibodies in immunocytochemistry.

2. present a comparison between PAP and ABC methods.
3. discuss advantages and precautions in production and use of monoclonal antibodies for immunocytochemistry.
4. discuss advantages of using mouse or rat ClonoPAP made from monoclonal antiperoxidase.
5. show that immunocytochemistry with monoclonal antibodies permits dissection of biochemical processes in situ.
6. propose that the lesion in Alzheimer's disease and related disorders involves a disturbance in a specific neurofilament phosphorylation site.

All labels currently used in immunocytochemistry yield adequate staining intensity. The sensitivity of an immunocytochemical method does, therefore, not depend on the label itself, but rather on the manner in which a label is used (1). Second antibodies (for all indirect methods) have to be serum-derived, rather than monoclonal, in order to yield the broad applicability basic to the use of indirect methodology. Because of contaminants in

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immunoglobulin employed for production of second antibodies, the serum antibodies are likewise contaminated with nonspecific antibodies. These react directly with the tissue even in the absence of first antibody (2). The contaminating antibodies are present whether the second antibody is labeled, as in labeled antibody methods, or unlabeled, as in unlabeled antibody methods. The contaminants contribute to background if labeled. However the principle of the unlabeled antibody method (3) assures that only the specific anti-immunoglobulin is localized while nonspecifically reacting antibodies remain invisible. Basic to the unlabeled antibody method is the principle that, although second antibodies are heterogeneous, each individual antibody molecule possesses two identical idiotypic sites. Second antibodies in the unlabeled antibody method react twice, first with the first antibody (or with a tissue contaminant) and then again with the third layer reagent which is peroxidase-antiperoxidase (PAP) or another antigen-antibody complex (1). Since PAP is an affinity-purified reagent, it will react only with those components of second antibody which have reacted with immunoglobulin specifically and not with those components that have reacted with nonspecific tissue constituents. Furthermore, if it would have happened that PAP were contaminated with a constituent crossreactive with a tissue contaminant with which the nonspecific components of the second antibody would have reacted, again the reaction remains unvisualized. The reason is that any putative contaminant in PAP would, by definition, not be antiperoxidase and, therefore, even if reacting with a second antibody, it would not bind peroxidase and again not become visualized. Thus, the unlabeled antibody principle incorporates a dual specificity amplification.

Sensitivity of an immunocytochemical method is not evaluated by staining intensity. The true sensitivity is measured by the ratio of specific staining intensity to background staining intensity.