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This Week's Citation Classic

Sternberger L A, Hardy P H, Jr., Cuculis J J & Meyer H G. The unlabeled antibody enzyme method of immunohistochemistry: preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antihorseradish peroxidase) and its use in identification of spirochetes.
J. *Histochem. Cytochem.* 18:315-33, 1970. [Basic Sciences Dept.. Medical Res. Lab., Edgewood Arsenal, and Dept. Microbiol., Johns Hopkins Univ. Sch. Med., Baltimore, MD]

Antigen can be localized immunocytochemically without use of covalently labeled antibodies by the sequence of primary antibody from species A, antibody to the immunoglobulin of species A produced in species B and applied in such a way that one antibody combining site reacts with the primary antibody and the other site remains free, soluble peroxidase-antiperoxidase complex (PAP) affinity purified from antiseriim of species A, followed by histochemical reaction for peroxidase. The principle underlying this method yields high sensitivity because of inherently low background. [The *SCI*® indicates that this paper has been cited in over 1,280 publications since 1970.]

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"In the fall of 1968, I was participating in the teaching of microbiology to medical students at Johns Hopkins University. Part of the laboratory requirement was an independent research project to be carried out in six two-hour periods. I felt that an assignment suitable for so short a time could be an attempt at immunocytochemistry without use of labeled antibodies. Such a technique would be simpler than conventional methods that used covalently labeled antibodies. I assumed that the experiment probably would fail, for the approach was so obvious that if successful, it should have been adopted long before. I prepared the reagents for the students, including affinity-purified antiperoxidase to be followed by peroxidase in a four-layer staining reaction. To my surprise, the students were able to visualize erythrocytes and spirochetes on their first attempt. This work was published[1] but has been quoted infrequently, perhaps for good reasons. Despite its high sensitivity, the method had the drawback of partial loss of peroxidase from low affinity antiperoxidase.'

"We' spent the ensuing months in futile attempts to resolve the low affinity problem that resulted from difficulties in dissociation of antiperoxidase from peroxidase during its purification, until one day it dawned on us, in the middle of another attempt, to change the protocol and aim to obtain from the immune precipitate soluble peroxidase-antiperoxidase (PAP) complex instead of antiperoxidase. We found that the addition of a small excess of peroxidase during the otherwise ineffective acid dissociation of peroxidase from antiperoxidase led to immediate solubilization of the precipitate, and thus intrinsically stable PAP has become readily available. The immunocytochemical use of PAP proved sensitive, because staining background was negligible. Much of the characterization of PAP was carried out in collaboration with Howard Meyer, who more than anyone else mastered not only the knowledge of structure and composition of PAP, but also the art of preparing it consistently at high concentration and activity, whether from antisera or from monoclonal antibodies.

"We had some difficulty in. publishing these findings. One of the reviewers felt that the work was too insignificant to warrant a paper of its length. Influenced by such comments, I did not realize until 1974 that the method had the potential of wide applications (and frequent citations) because of its high sensitivity, low background, and consequent applicability to routinely fixed normal and pathologic tissue.

"Particularly interesting to me and my wife, Nancy, were the intriguing applications to neurobiology, so much so that we both decided to join the ranks of neuroscientists. Nancy soon discovered, during her work on myelin constituents, that with many (but not all) antigens, strong fixatives, such as osmium tetroxide, gave better structure and higher sensitivity, thus dispelling an old myth that in immunocytochemistry. fixation should be mild.

"My own interest in neurobiology was stimulated by the concept of the Scharrers[2,5] that peptidergic principles are fundamental to neuronal and vascular communication of cells. Our recent effort with monoclonal antibody immunocytochemistry confirms the universality of this concept and reveals a high diversity of such principles, sufficient to confer regional individuality to neurons. For a recent review, see reference 4."

1. **Sternberger L A.** Some new developments in immunocytochemistry. *Mikroskopie* **25**:346-61, 1969. [The *SCI* indicates that this paper has been cited in over 30 publications since 1969.]
2. **Scharrer E & Scharrer B.** Secretory cells within the hypothalamus. *Res. Publ. Assn. Nerv. Ment. Dis.* 20:179-97, 1939.
3. **Scharrer B.** Neuroendocrinology and histochemistry. (Stoward P J & Polak J M. eds.) *Hislochemislry: the widening horizons.* **New** York: Wiley. 1981. p. 11-20.
4. **Sternberger L A.** *Immunncytochemistry* second edition. New York: Wiley. 1979. 354 p.