This Week's Citation Classic®

Gonatas N K, Harper C, Mizutani T & Gonatas J O. Superior sensitivity of conjugates of horseradish peroxidase with wheat germ agglutinin for studies of retrograde axonal transport. *J. Histochem. Cytochem.* 27:728-34, 1979.
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In this study, we found that conjugates of wheat germ agglutinin (WGA) with horseradish peroxidase (HRP) are 40 times more sensitive than native HRP as in vivo markers of retrograde axonal transport. We proposed that WGA-HRP is an alternative and probably a better marker than native HRP in studies of neuronal connections. [The *SCI*® indicates that this paper has been cited in more than 260 publications.]

Tracing Axonal Transport

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In the late 1960s, Stratis Avrameas and his colleagues at the Institute for Cancer Research in Villejuif, France, were developing the immuno- and cytochemical methods that a few years later became standard techniques in laboratories throughout the world. Supported by a Guggenheim fellowship, I spent a year in the Avrameas laboratory in the early 1970s. At about the same time, R.B. Taylor and his associates wrote the seminal paper on the effects of anti-immunoglobulin antibody on plasma membrane immunoglobulins of lymphocytes.[1] Stratis, Jean Claude Antoine, and I decided to reexplore the effect of anti-immunoglobulin antibodies on lymph node cells with the immunoperoxidase method, which allowed morphologic studies in both light and electron microscopes. The results of this study were indeed astonishing because, for the first time, it was demonstrated that the Golgi apparatus of certain cells may be involved in receptor mediated endocytosis.[2]

We returned to Philadelphia, and in collaboration with Stratis, we showed that HRP covalently linked with lectins or toxins, which bind to numerous cell surface receptors. The HRP underwent endocytosis in lysosomes as well as in a compartment at the trans aspect of the Golgi apparatus of neurons in culture. In contrast, native HRP alone was internalized only in lysosomes.[3,4] Furthermore, in subsequent quantitative studies, we showed that the uptake of ricin conjugated with HRP by cultured neuroblastoma was 100-200 times greater than native HRP.[5]

To test whether the observations made in cultured cell systems were relevant to in vivo conditions, we exploited several in vivo systems as well as the classical experiment of the retrograde transport of native HRP in neurons.[6] The results of the in vivo studies were in agreement with the in vitro results; HRP covalently linked with WGA (WGA-HRP) was internalized in the Golgi apparatus of neurons, and it was much more sensitive than HRP in tracing the origin of axons with synaptic terminals in the rat submandibular gland. We thought that neuroanatomists might be interested in WGA-HRP and wrote the *Citation Classic* paper in which we described, in detail, a simple and inexpensive method for the conjugation of WGA with HRP.

In brief, this is the history of the development of WGA-HRP as a tool for neuroanatomists.

The significance of the uptake of ligands in a compartment at the trans aspect of the Golgi apparatus is not yet fully understood; but that is another story.

Since the publication of this paper in 1979, the use of WGA-HRP in more than 600 neuroanatomic studies involving the retrograde, orthograde, or transsynaptic transports of the conjugate has contributed to the elucidation of several interconnected neuronal systems. The original paper describing the properties of WGA-HRP has been cited in about half of these papers.[7] The partial obliteration of the citation of this method paper may be attributed to the commercial availability of the conjugate. Vendors and certain users of WGA-HRP may not be aware or appreciative of the studies that preceded its development as a tracer of neuronal connections.

1. **Taylor R B, Duffus W P H, Raff M C & De Petris S.** Redistribution and pinocytosis of lymphocyte surface immunoglobulin molecules induced by anti-immunoglobulin antibody. *Nature New Biol.* 233:225-9, 1971. (Cited 1,400 times.)
2. **Antoine J C, Avrameas S, Gonatas N K, Stieber A & Gonatas J O.** Plasma membrane and internalized immunoglobulins of lymph node cells studied with conjugates of antibody or its Fab fragments with horseradish peroxidase. *J. Cell Biol.* 63:12-23, 1974. (Cited 90 times.)
3. **Gonatas N K & Avrameas S.** Detection of plasma membrane carbohydrates with lectin peroxidase conjugates. *J. Cell Biol.* 59:436-43, 1973. (Cited 95 times.)
4. **Gonatas N K, Kim S U, Stieber A & Avrameas S.** Internalization of lectins in neuronal GERL. *J. Cell Biol.* 73:1-13, 1977. (Cited 160 times.)
5. **Gonatas J, Stieber A, Olsnes S & Gonatas N K.** Pathways involved in fluid phase and adsorptive endocytosis in neuroblastoma. *J. Cell Biol.* 87:579-88, 1980. (Cited 60 times.)
6. **Kristensson K & Olsson Y.** Retrograde axonal transport of protein. *Brain Res.* 29:363-5, 1971. (Cited 335 times.)
7. **Itaya S K.** Trans-neuronal transport of WGA-HRP in immature rat visual system. *Develop. Brain Res.* 38:83-8, 1988.

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