

## India Ink as a Tracer for Circulatory System Study in a Ganglion, With a Note on Using Ferritin, Lanthanum, and Horseradish Peroxidase for the Blood-Brain Barrier

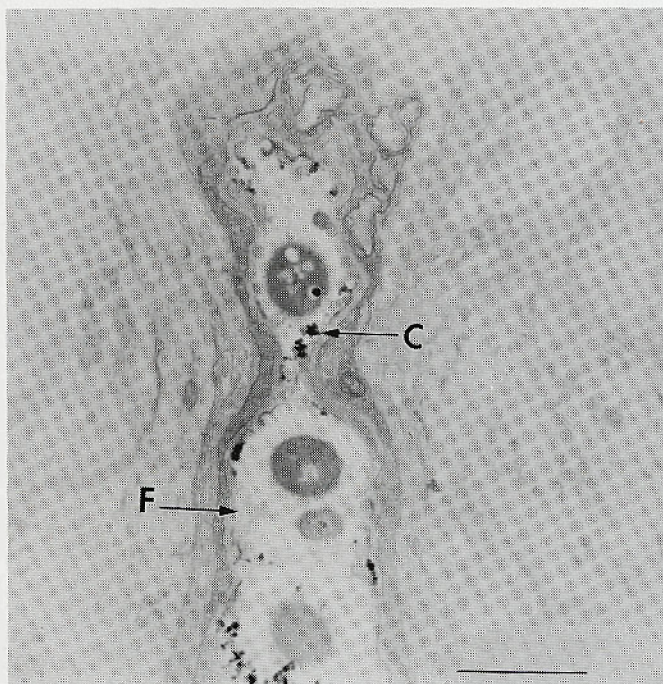
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Several summers ago, while working at the Marine Biological Laboratory in Woods Hole, Massachusetts, George Augustine (Duke University) and I wished to look at the circulation in the giant synapse of the squid, *Loligo pealei*. At that time, Dr. Eugene Copeland suggested the use of India ink in order to see the circulatory system. The ink contains carbon particles that are visible under the EM. In addition, it is visible to the naked eye.

The India ink was first sonicated for 15 minutes and then 0.1 mL of it was added to the fixative solution (2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 with 0.8 M sucrose). The squid was perfused through the abdominal aorta initially for 30 minutes at room temperature. The giant synapse was then removed and submerged into fixative overnight at 4°C.

In later experiments, we included ferritin (Calbiochem) to the perfusate to see if it crossed the blood-brain barrier. First, the ferritin was spun down for 30 minutes at 30 thousand rpm to concentrate the ferritin. Finally, 5 mL of ferritin and 2 drops of India ink were added to 5 mL of 2X artificial sea water (ASW). The final concentration of ferritin was 2.5% in ASW (low Ca<sup>++</sup>). After perfusing the squid for 30 minutes with this solution, the ganglia was removed and immersed into fixative overnight at 4°C.

Our main purpose was to be able to identify the blood vessels, and for this, the India ink worked very well. The carbon particles of the ink are larger and easier to see than ferritin. The ferritin did not cross the blood brain barrier, and demonstrated



Blood vessel of *Loligo pealei* filled with India ink carbon (C) and ferritin (F). Blood cells are visible within the vessel. Scale bar = 1  $\mu$ m.

the tight junctions of the pericytes which form the blood vessels. We also tried horseradish peroxidase (HRP) and lanthanum. The lanthanum did pass through the blood vessels, while the horseradish peroxidase (HRP) did not. ■

### Reference:

Sanchez, M. E., C. M. Nuno, J. Buchanan, and G. J. Augustine. 1990. Contractions of the squid stellate ganglion. *J. Exp. Biol.* 152, 369-387. (We were able to identify the blood vessels based on our previous work with the tracers.)



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