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A Protocol for Silver Staining Ion Transport Epithelia of Whole Animals and Excised Organs

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Those of us who study the structure and physiological function of ion transport cells in animals or their excised organs (gills, for example) are always interested in "quick and dirty" screening methods to identify ion transport epithelia. Having identified epithelial areas rich in putative "ionocytes", we are also interested in looking at the mix of non-transport cells and "ionocytes" in the tissue. The so-called "silver staining" method can satisfy both of these desiderata quickly and easily.

Silver staining selectively blackens areas of the organism's body surface which are very permeable to chloride and/or silver ions; such areas are often the sites of ion transport epithelia (Croghan, 1958; Holliday, *et al.*, 1990; Kikuchi & Shirashi, 1997). The method is based on the precipitation of silver chloride in the integument: chloride ions diffusing out of the animal's integument combine with silver ions diffusing into it from a dilute solution of silver nitrate. Microscopic grains of elemental silver are then precipitated *in situ* from the silver chloride by immersion of the animal or excised organ in photographic developer. The method may be used on intact small animals such as brine shrimp or on excised gills or other discreet organs covered by an integument. The method presented here is slightly modified after a technique used by W.T.W. Potts, Department of Life Sciences, University of Lancaster.

Protocol

Rinse the animal or excised organ to be stained in at least three changes of deionized water; this is best accomplished by transferring it through successive culture dishes of water. The goal here is to remove all adherent chloride and other anions from the animal's surface so as to avoid "false positives" and general staining of the integument.

After the last rinse, transfer the organism or organ to 0.5% (w/v) silver nitrate for a brief time (start with 30 seconds) and then rinse well in deionized water as before. The time of exposure to the silver nitrate must be determined by trial and error. The stain will be too weak if the exposure time is too brief and there will be general staining of the entire integument if the exposure time is too long. Thus, when silver nitrate exposure time is properly adjusted, the method stains only the areas of the integument which are very permeable to chloride and/or silver ions. The silver nitrate treatment does not usually kill the organism. If larger animals with gills housed in a gill chamber are to be used, it must be recognized that the animal may be irritated by the silver nitrate solution and may not ventilate its gills properly. This will cause uneven staining and give "false negatives." In such cases it is better to kill the animal, excise the gills and handle them with forceps or hemostats so that the gill cut "stump" does not bleed into the silver nitrate solution (Holliday, 1988).

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To develop the stain, the organisms or organs are transferred to Kodak D-19 developer made up for normal use in developing film; other developers will also work. Again, proper development time is determined by trial and error (start with 30 seconds). Underdevelopment results in a weak stain, while overdevelopment results in a general brownish staining of the whole animal or organ superimposed on the black stain from silver development. This treatment kills the organisms.

An alternative developing process for delicate organisms or to eliminate the slight brownish cast that the developer produces in the integument is to simply leave the organism on a window sill in full sunshine or under an incandescent lamp in a dish of water for several hours until the silver is deposited.

Those using this method should note that some animals stain much more intensely at a constant time of exposure to silver nitrate when they are acclimated to media in which they increase ion transport. Thus, brine shrimp acclimated in 400% sea water (4000 mOsm or about 150=94) show very intense staining of their gill ion transport tissue, while those acclimated in *50%* sea water (500 mOsm or about 17.2=94) show very faint staining when both are exposed to silver nitrate for 30 seconds. I interpret this to be the result of increased ion transport in the 400% sea water animals and, therefore, more intense deposition of silver chloride in the transport tissue. See Holliday, *et al.*, 1990, for a picture and discussion of this phenomenon.

Illustrated below are the results of this technique as used with the brine shrimp, *Artemia salina;* other pictures are available on the author's WWW page at: http://www.lafayette.edu/~hollidac/ silverstain.html.

Figure 1: Silver stained metepipodites (approximately 400 µm long) of brine shrimp phyllopodia. The putative "ionocytes" stain on all but the 11th pair of metepipodites. A portion of this picture has been published previously (Holliday, *et al.*, 1990).

Barra *et al.* (1983) and Kikuchi and Shiraishi (1997) have published excellent micrographs of the "ionocytes" of the gills of fresh water crustaceans using modifications of the silver staining technique.

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