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The Mechanisms of Chloride Uptake by the Coniform Chloride Cells of Baetisca sp. (Ephemeroptera: Baetiscidae).

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Cytochemical evidence of the presence and position of chloride within the coniform chloride cells located on the gills of the mayfly genus <u>Baetisca</u> was obtained by tagging the chloride to be absorbed with silver ion.

Ultrastructure of the porous plate of the chloride cell has been described (Komnick and Stockem, 1973, J. Cell Sci. 12:665-681). The porous plate is composed of closely-packed, cross-linked cylindrical rods 20 nm in diameter, producing a grid of triangular holes. These holes allow only particles less than about 2.5 nm through, acting as a sieve selective for monatomic ions.

Fixation of mayfly gills was accomplished by perfusion with 1% glutaraldehyde in pH 7.4 phosphate buffer for 3 hours. Naiads were perfused by decapitation under fixative with subsequent removal of the gills themselves. Specimens were embedded in Spurr's resin and sectioned with a Sorvall MT-2B ultramicrotome and examined using a Philips 300 transmission electron microscope.

Figure 1 is a cross-section of a typical coniform cell. The mitochondria along the cell margins occupy vane-like projections, increasing the cell surface area and the number of active transport sites. When live-stained with silver ion (0.05 M AgNO3) for 15 minutes (Fig. 2), much of the AgCl precipitate is adsorbed onto the plate surface, and some diffuses into the cytoplasm. The active transport system cannot accomodate the addition of silver ion to the chloride, therefore the AgCl effectively blocks the active transport sites. This is shown by the line of silver ions present along the periphery of the cell. Cells fixed before treatment with silver ion (Fig. 3) show massive diffusion of AgCl into the cell itself, suggesting that the living cell admits only a fraction of the chloride available to it, while dead cells accumulate chloride, and act as a "sink".

It was found that uptake through the porous plate of the cell most likely occurs in two steps: (1) adsorption of chloride onto the surface of the plate, creating a high osmotic potential between the plate surface and the cytoplasm, and (2) diffusion of chloride ion into the cell, moderated by cell activity. Once within the cell, active transport pumps the chloride into the hemolymph, allowing the naiad to osmotically hyperregulate with respect to the surrounding water.

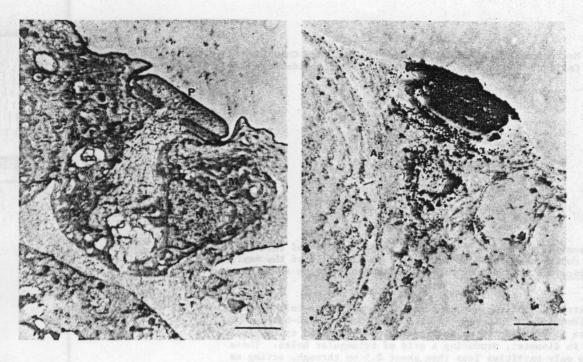


Figure 1.



Figure 3.

Figure 2.

calculated to plant the speciment buffer for I hours.

Fig. 1. Cross section of <u>Baetisca</u> sp. (mayfly) coniform chloride cell.

P - porous plate (note fine vertical striations on surface); M - mitochondria in vane-like cell projections;

N - nucleus.

Fig. 2. Chloride cell live-stained with AgNO₃ for 15 minutes. Note presence of silver ion within the cell. Cell boundary defined by line of silver ions (Ag).

Fig. 3. Pre-fixed chloride cell stained en bloc with $AgNO_3$. Cell is filled with silver ion. P - porous plate; N - nucleus.

Scale markers = 2 um.