

Perfusion with 10^{-4} M amiloride, and incubation of excised pieces of mucosa with this drug causes derangement of the normal internal polarization of the cell. The granular endoplasmic reticulum hypertrophies and extends, atypically, into the apical cytoplasm as arrays of flattened cisternae. Transport is reduced but not totally blocked in one-half hour. Unlike the effects of ouabain, there was no evidence of cell swelling, the cytoplasmic matrix is normally dense and the interdigitations of lateral cell membranes are complex. Lateral intercellular spaces with cell projections occur, atypically, well into the apical regions but at the nuclear level are replaced by closely apposed membranes. In the perinuclear region there are multiple Golgi areas with abnormally increased numbers of flattened lamellae some of which are contorted to multilayered myelin whorls.

When the normal outflux mechanisms are deranged by treatment with ouabain or amiloride the endoplasmic reticulum gives rise to large intracellular vacuoles (or vacuoid spaces lacking membranes) which may fuse with lateral cell membranes resulting in bulk discharge of the fluid.

Little is known of the processes of transport between the cytoplasmic matrix and the interior of the Golgi-endoplasmic reticulum compartment. In normal preparations with active fluid transport our observations are consistent with an assumption of osmotic balance between these intracellular compartments. The observed morphological proportions and arrangements of these compartments are profoundly affected by both ouabain and amiloride but in distinctly different ways. The effects of amiloride warrant further investigation.

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CYTOLOGY OF CHLORIDE CELLS IN GILLS OF THE EEL, *Anguilla*; THE STICKLEBACK, *Pungitius*; AND THE ROCK-EEL, *Pholis*.

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The structure of the chloride cells in the gills of the American eel, *Anguilla rostrata*; the nine-spined stickleback, *Pungitius pungitius*; and the rock-eel, *Pholis gunnellus*, was examined in the electron microscope in relation to conditions of osmotic adaptation, cortisol treatment, and acute poisoning with thiocyanate.

In *Anguilla*, as described by Doyle and Epstein (Cytobiologie **6**, 58, 1972), the correlation shown between development of Na-K-ATPase and development of chloride cells left open the question of a possible effect of the position of the cells in the gill epithelium on the lag in ability to regulate serum electrolytes upon transfer to sea water. Subsequent experiments with freshwater adapted eels, treated for an additional ten days with cortisol and transferred to seawater for 24 and 72 hours respectively, showed that a sufficiently large number of mature chloride cells were exposed at the surface of the epithelium to rule out the time required for a shift in position of chloride cells to the surface as a cause of the delay in physiological regulation of serum electrolytes. There were however some fine structural differences between the 24 and 72 hour specimens. The 24-hour specimens showed a more highly reticulated tubular system than the 72-hour ones, suggesting that some intracellular reorganization process may be involved which requires two or three days to be accomplished.

The stickleback was examined in order to determine the characteristics of the apical tubular system in its chloride cells. This system, which we described for freshwater *Anguilla*, has been reported to be more prominent in three-spined stickleback *Gasterosteus* (Bierther, Ztschr. f. Zellforsch. **107**, 421, 1970) after gradual adaptation to freshwater. The nine-spined stickleback, *Pungitius*, was found in moderate abundance in Northeast Creek in association with *Fundulus*. In the laboratory it withstands abrupt changes in salinity which may make it a preferred species especially for study of specific ion effects on configurations of the intracellular tubular membrane systems. The morphology is well suited to electron microscopic study.

The rock-eel, *Pholis gunnellus*, was collected from beneath rocks exposed at minus low tides at the laboratory point. It lives well in laboratory aquaria. Its gills have abundant chloride cells of the usual seawater configuration as found in *Anguilla*, *Fundulus*, and *Pungitius*. This normal configuration can be summarized as consisting of an evenly spaced distribution of the abundant mitochondria in intimate association with the highly branched tubular reticulum which is characteristically present in the cytoplasm of chloride cells. In *Anguilla*, Epstein has reported that 7mM NaSCN in seawater adapted eels causes a 65 percent fall in chloride efflux within a few minutes and a similar but delayed response to 10mM SCN in the external medium. Rock-eels in 10mM SCN in seawater became sluggish in 20-24 hours and died in 30-36 hours. The fine structure of their chloride cells taken at 2.5 to 18 hours of exposure to thiocyanate was essentially normal including normal Golgi areas in the 2.5 hour specimen. Specimens taken at 23 hours showed marked alterations in all chloride cells. Some, located beneath the superficial epithelium in the distended intra-epithelial lymph space, were maximally rearranged and probably moribund. These cells were rounded, with the mitochondria and matrix cytoplasm clumped centrally and surrounded by a broad peripheral zone entirely composed of parallel arrays of *unbranched* tubules in dense packing. Cross sections of such arrays showed up to 800 densely-packed tubules and sections of longitudinal arrays showed 20-30 tubules of uniform diameter. These tubules are about twice the diameter of those in normal cells. Other cells of the thiocyanate specimens, which were still in the superficial epithelium, showed transition stages in dissociation of tubules from mitochondria, loss of branching, and association in small parallel arrays. This response is unlike other varieties of degenerative changes seen in chloride cells and probably represents a response to specific ions. Although this morphological response is delayed several hours beyond the onset of the physiological effect, it would seem to implicate the tubular reticulum of the chloride cell as a site of action of thiocyanate.

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THIOCYANATE INHIBITION OF ADENOSINE TRIPHOSPHATASE IN GILLS; POSSIBLE RELATION TO CHLORIDE TRANSPORT.

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Thiocyanate specifically inhibits the efflux of chloride across the gill of European eels (*Anguilla anguilla*) adapted to seawater, blocking the net extrusion of NaCl and eventually producing