283. GILL Na-K-ATPase ACTIVITY AND CHLORIDE CELL FINE STRUCTURE IN A TELEOST ADAPTED TO VARIOUS HYPEROSMOTIC ENVIRONMENTS Karl J. Karnaky, Jr.*, Stephen A. Ernst, and Charles W. Philpott. Department of Biology, Rice University, Houston, Texas.

It is well known that the gills are the major site of sodium chloride excretion in teleosts adapted to hyperosmotic environments. The cellular site of this electrolyte excretion is the "chloride cell" and although the transport mechanisms are still incompletely understood, the sodium extrusion is thought to involve the enzyme, Na-K-ATPase (Epstein et al, 1967, Science 156, 1245). Fortunately, since the rate of sodium net outflux in double-strength seawater is several times the rate in seawater (Maetz, J., 1970, Mem. Soc. Endocr. 18, 3), it is possible, as in the present study, to examine ultrastructural and enzymatic features of the branchial epithelium under conditions of vastly different transport rates. Accordingly, specimens of the euryhaline teleost, Cyprinodon variegatus, were adapted for 5½ or 10½ days to half-strength seawater (½SW), seawater (SW), or double-strength seawater (2X SW) and the gills excised for enzyme assay or fixation. The specific activity of branchial Na-K-ATPase was 1.6 times greater in SW than in ½SW ($P<0.02$) and 3.9 times greater in 2X SW than in SW ($P<0.01$). The number of chloride cells appeared to remain relatively stable in the three environments and there were no apparent differences in the size and fine structure of chloride cells from ½SW- and SW- adapted fish. In contrast, chloride cells from 2X SW-adapted fish displayed a marked hypertrophy and a marked increase in cell surface area. These results suggest that there is an association between the four-fold increase in the specific activity of Na-K-ATPase in 2X SW and the proliferation of tubular extensions of the chloride cell plasmalemma. (Supported by NIH Predoctoral Fellowship (5-F01-GM-32,197-04) (to K.K., Jr.) and grants from the USPHS (AM-13705-06) and the Moody Foundation, Galveston, Texas).

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284. USE OF FERROCYANIDE-REDUCED OSMIUM TETROXIDE IN ELECTRON MICROSCOPY Morris J. Karnovsky. Department of Pathology, Harvard Medical School, Boston, Massachusetts.

An aqueous solution of osmium tetroxide may be treated with potassium ferrocyanide to yield a stable, clear, yellowish-brown solution. Presumably the osmium is partially reduced and forms a complex with the ferrocyanide. Thin sections from tissues fixed in aldehyde fixatives, then treated with the osmium-ferrocyanide solution, and processed thereafter routinely for electron microscopy, show interesting features. With or without light alkaline lead staining, contrast is greatly increased. With light alkaline lead staining, the trilaminar structure of unit membranes is readily appreciated, as the outer leaflets become intensely electron opaque. Glycocalyx is especially prominent. Glycogen particles are intensely opaque. Nucleoproteins do not stain and the nuclei have an empty, clear appearance, and ribosomes can only be faintly discerned. Counterstaining with uranyl acetate brings out these entities however. The reaction product of certain ultrastructural cytochemical methods e.g. peroxidase and oxidase methods utilizing diaminobenzidine, is much more electron opaque than with conventional osmium treatment, and thus the sensitivity of the methods is increased. For routine purposes, solid potassium ferrocyanide is slowly added to, and dissolved in, 1% osmium tetroxide in water to achieve a final concentration of potassium ferrocyanide of 1.5%. This solution is used at room temperature or at 4°C for 1-2 hours. The pH of this solution is about 10.5. Buffering to lower pH seems to have little effect on the staining. Decreasing the concentration of ferrocyanide decreases the intensity of staining. The chemical nature and mode of staining of the complex formed by treating osmium tetroxide with ferrocyanide is not yet established, but its use has proved advantageous in ultrastructural and cytochemical studies. (Supported by grant HE-09125 from the NIH, USPHS)