

and thus a constant cellular electrical potential gradient in the absence of urea. Since the section of the rectal gland contains only 12-30 mM urea, the data indicate that the secretory or apical membrane of the rectal gland tubule is impermeable to urea. On the other hand, the basal-lateral membrane appears to be freely permeable to urea as indicated by the diffusion equilibrium between plasma urea and the cytosol content of urea. This investigation was supported in part by USPHS Grant AM 17433 and the Whitehall Foundation.

EFFECT OF CHANGES IN SALINITY ON SURFACE ULTRASTRUCTURE OF GILL FILAMENTS OF *FUNDULUS HETEROCLITUS*

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Transfer of freshwater adapted euryhaline teleosts to seawater results in a number of structural changes in chloride cells of gill filament epithelia. These include an increase in the number of mitochondria and in the volume of the membranous tubule system, cell enlargement, a deepening of the apical pit and a decrease in apical membrane folds, and an increase in low-resistance chloride cell-chloride cell-junctions. Scanning electron microscopic observations of the gill filaments of mullet revealed that pores which likely represent the openings of chloride cell apical pits on the epithelial surface, exhibited obvious ultrastructural modifications which paralleled changes in ouabain binding sites resulting from changes in salinity (F.E. Hossler et al., *J. Exp. Zool.*, **208**:399-406, 1979). In freshwater the pores were broad (3-6 μm) and exhibited numerous cellular extensions in their interiors; and in seawater the pores were narrower (1-3 μm) and deeper, and lacked the cellular extensions. In the present study we report similar surface ultrastructural changes in *Fundulus heteroclitus* following seawater adaptation.

Gill arches from seawater killifish (*Fundulus heteroclitus*), or from seawater killifish transferred to freshwater 7 days prior to examination, were used. Killifish were killed by pithing and gill arches were removed and fixed at 5°C by immersion in 0.1M cacodylate buffer (pH 7.2) containing 2.5% glutaraldehyde and 1.8% p-formaldehyde.

After 3 days in fixative, the gill arches were dehydrated through a graded ethanol series, critical point dried in liquid CO_2 in a Samdri PVT-3 (Tousimis Res. Corp.) drying apparatus, fixed to a specimen stub with double stick tape, coated with a thin layer of gold-palladium in a Hummer II (Technics Inc.) sputter coater, and observed in an AMR 1000 scanning electron microscope.

Although the filament surfaces were essentially the same ultrastructurally in all four gill arches, for the observations reported here, filaments from only the first or second gill arch were used (Figs. 1-4). As with most other teleosts examined (*J. Exp. Zool.*, **208**:379-398, 1979) the filament surfaces were covered with ridged-epithelial cells (pavement cells) which measured about $4 \times 8 \mu\text{m}$. The ridges measured about 0.1-0.2 μm in width. On the opposing surfaces of the two rows of gill filaments (adjacent to the afferent arterioles) and on the filament surfaces between the respiratory lamellae, but very rarely on other filament surfaces, numerous pores opened along the borders of adjacent ridged-epithelial cells. The location of these pores was identical with that previously observed in mullet, and was in good agreement with the reported location of chloride cells. The possibility, however, that some of the pores represent the sites of mucous cells cannot be eliminated without additional transmission electron microscopy or light microscopy. A comparison between epithelial pores in fish adapted to seawater (Figs. 1 and 2) and freshwater (Figs. 3 and 4) revealed obvious differences which closely mimicked those seen in mullet. In seawater the pores were rounded pits, measured 1-4 μm in diameter, and contained no obvious internal structures. In freshwater the pores measured 3-6 μm in diameter, appeared shallower than in seawater, and contained numerous cellular extensions in their interior. Although the pores have not been quantitated, they appeared to be reduced considerably in number in freshwater fish. While it appears likely that these observations represent structural alterations in chloride cell apical pits in response



Figure 1. Micrograph of gill filament surface from seawater killifish. L, respiratory lamellae. Arrows, epithelial pores. x1040

Figure 2. High magnification micrograph of gill filament surface from seawater killifish. R, ridged epithelial cells. Arrows, epithelial pores. x5050

Figure 3. Micrograph of gill filament surface from freshwater killifish. L, respiratory lamellae. Arrows, epithelial pores. x2100

Figure 4. High magnification micrograph of gill filament surface from freshwater killifish. R, ridged epithelial cells. Arrows, epithelial pores. x5150

No salinity change, their functional significance is not as yet clear. However, deepening of the apical pits and increasing their number could allow an increased number of chloride cells access to the epithelial surface and thus enhance NaCl extrusion. This work was supported in part by NIH grants #5S07 RRO5764 and #5RO1 GM24953, by NSF grant #PCM77-01146, and by Alumni funds from Louisiana State University Medical Center, New Orleans, La.