of these compounds on Na/K ATPase activity at concentrations which inhibited Cl secretion. It seems reasonable to propose, therefore, that the action of diuretics of this chemical series on rectal gland secretion may be via blockade of the putative NaCl contransport system localized at the serosal surface of the tissue (see Silva et al., Am. J. Physiol. 233:F298, 1977).

As the rank order of potency of these chemically related derivatives is strikingly similar in the inhibition of chloride transport by the rectal gland, cation cotransport by the avian erythrocyte, and salt transport by the dog kidney it suggests these differing tissues may have pharmacologically similar diuretic-binding sites and possibly physiologically similar transport mechanisms. This analogy can be extended to other active Cl transporting tissues where it has already been shown that one of the most effective compounds of the series (bumetanide; Compound IV) potently inhibits a similar process in the amphibian cornea (McGahan et al., J. Pharm. Exp. Ther., 203:97, 1977) and in the squid giant axon (Russell, Ann. N.Y. Acad. Sci., in press, 1979). This agent was also effective in reducing Cl transport in isolated kidney tubules of rat and rabbit (Imai, Eur. J. Pharm., 41:409, 1977) where the site of action was shown to be the thick ascending limb of Henle's loop. In agreement with the present observations this compound was effective at much lower levels than furosemide in these tissues. If these transport systems do involve a common element, the new agents described here may be useful tools to selectively inhibit this component in physiological studies. Aided by NIH grant AM18078 and NSF grant PCM-77-01146.

CELL VOLUME REGULATION IN THE RECTAL GLAND OF SQUALUS ACANTHIAS: EFFECTS OF SODIUM AND CHLORIDE FREE MEDIA AND UREA FREE HYPOTONIC MEDIA

J. Goldstein, J.N. Forrest, Jr., A. Murdaugh and A. Kleinzeller, Department of Medicine, Yale University, New Haven, Connecticut and Department of Physiology, University of Pennsylvania, Philadelphia, Pennsylvania

We have extended our previous studies (Booz et al. Bull. MDIBL 18:23, 1978) of cell volume regulation in slices of the rectal gland of the dogfish shark (Squalus acanthias). The objective of the present work was to determine changes in cell volume and tissue electrolyte content in the rectal gland following aerobic incubation in media rendered free of either sodium or chloride or made hypotonic by the absence of urea.

Slices cut perpendicular to the long axis of the gland (mean thickness 0.3-0.4 mm) were employed. The slices were prepared free-hand by the method of Deutsch (J. Physiol. 87:568, 1936) or by a mechanical slicer, and the fibrous capsule was trimmed off. Slices were incubated in either: (1) standard dogfish Ringers (Forrest et al, Bull. MDIBL 18:10, 1978); (2) sodium free media containing choline; (3) chloride free media prepared by replacement of all chloride with nitrate salts ; (4) urea free hypotonic media. All Ringer's solutions contained ^{3}H-polyethylene glycol (PEG) (1 mg/ml; 1μci/ml) as a marker of the extracellular space. Usually 10-12 slices, each weighing approximately 10 mg. wet wt., were incubated in 2.5 ml saline for 60-120 minutes following gassing with 99% O_2, 1% CO_2. Tissue cations (Na^+ and K^+) were determined by flame photometry, Cl^- by potentiometric titration, and the activity corresponding to tissue PEG was assayed by scintillation spectrometry. Tissue concentration of urea was determined by the method of Fawcett et al. (J. Clin. Path. 13:156, 1960). The means and S.E. for each experimental group were determined and data are expressed in kg H_2O, meq. electrolytes per kg tissue D.W. and μ moles urea per g wet wt. The extracellular space E is given in kg/kg tissue W.W.

The effects of omitting all sodium (choline Ringers) an actual shrinkage (13%) of the tissue was observed compared to normal Ringers with a loss of 0.45 kg H_2O/kg D.W. at 60 minutes and 0.48 kg H_2O/kg D.W. at 120 minutes (both $p < 0.001$). A marked decline in the tissue content of sodium and potassium and a modest decline in tissue chloride occurred. Whereas the calculated intracellular sodium concentration $[Na^+]_i$ decreased markedly (58.2 ± 6.2 to 21.9 ± 4.2, $p<0.01$), $[Cl^-]_i$ declined only slightly (mean 91.3 ± 2.8 vs 84.2 ± 3.6, $p<0.05$) suggesting replacement of intracellular sodium by choline. In contrast to the omission of sodium, the absence of chloride from the media was

Table 1. Tissue water and electrolytes in slices of dog fish rectal gland*

Conditions: Ringers/time	H_2O kg/kg DW	E kg/kg WW	Na meq/kg DW	K meq/kg DW	Cl meq/kg DW
Regular/60 min	3.65 ± .04	.21 ± .004	445 ± 11	421 ± 4	537 ± 8
Regular/120 min.	3.61 ± .04	.20 ± .006	446 ± 19	403 ± 4	528 ± 13
Na^+ Free/60 min.	3.20 ± .07	.18 ± .003	64 ± 3	180 ± 3	426 ± 5
Na^+ Free/120 min.	3.13 ± .03	.18 ± .01	67 ± 9	162 ± 4	411 ± 11
Cl Free/60 min.	3.68 ± .04	.21 ± .01	520 ± 19	317 ± 7	3.4 ± 2
Cl Free/120 min.	3.65 ± .04	.19 ± .004	753 ± 28	287 ± 4	---

*See text for analytic conditions. All values are Mean ± S.E.; n = 12 for each group.

not associated with cell shrinkage as tissue water content was identical to controls at both 60 and 120 minutes (Table 1). we have previously reported that replacement of chloride with isethionate produced shrinkage comparable to a sodium Free media. In further experiments, a specific effect of furosemide (10^{-4}M) on tissue water, sodium and potassium content could not be demonstrated. The present experiments thus demonstrate that: (1) regulation of cell volume in the rectal gland is critically dependent on the presence of sdoium in the media and a passive gradient for sodium entry into the cell; (2) nitrate, but not isothionate, can substitute for chloride in the maintenance of cell volume in this tissue; (3) cell volume regulation is not impaired by furosemide, an agent which inhibits coupled sodium-chloride transport in plasma membrane vesicles prepared from this tissue (kinne et al. Bull. MDIBL, 17:98, 1977).

Figure 1 depicts tissue water and tissue urea in fresh slices of rectal gland and the time course of changes in these parameters in slices incubated in regular and urea free hypotonic media. Whereas swelling is observed at 30 minutes following incubation in regular media, greater swelling (16%) was observed in urea free hypotonic media at 15 minutes and was maintained throughout 60 minutes of incubation. That this swelling was not due to cellular impermeability to urea is demonstrated by the prompt decline in tissue urea 20 21% of the control value by 15 minutes of incubation and to 6% of control by 60 minutes. It was of interest that the persistence of cellular swelling in urea free media was associated with a maintenance of the Donnan ratio for potassium, $\frac{[K^+]i}{[K^+]}$ as shown in Figure 1. These findings suggest that the maintenance of cell swelling in the absence of urea is not due to cellular retention of this solute, but may be due to an osmotic compensation between urea efflux and net potassium influx maintaining a normal Donnan ratio for potassium,

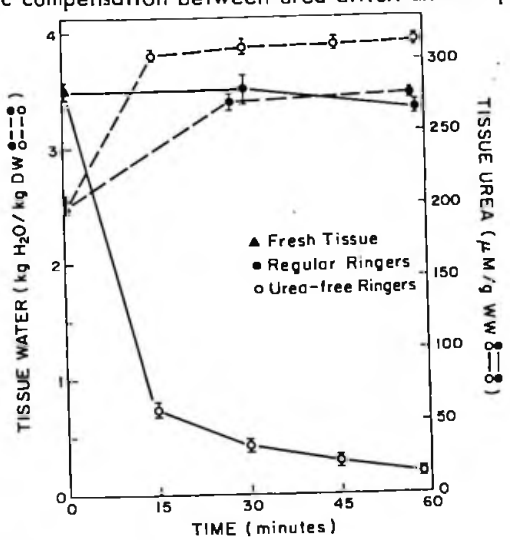

Figure 1. Time course of changes in tissue water and urea content in slices of dogfish rectal gland incubated in regular (reg) and urea-free hypotonic media.

$\frac{[K]_i}{[K]_o}$ Reg	30.2	27.0		26.0
$\frac{[K]_i}{[K]_o}$ Urea-free	25.8	28.6	29.0	29.0

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 upported 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

Fred E. Hossler, Franklin H. Epstein and Karl J. Karnaky, Jr., Department of Anatomy, Louisiana State University, New Orleans, La., Department of Medicine, Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts, Department of Anatomy, Temple University, Philadelphia, Pennsylvania

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 (Tousimus 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 x 8 μm. 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 3-6μm 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