effect of rectal gland extirpation on blood Cl concentrations. That extra rectal-gland mechanisms may play a role in Cl balance in S. acanthias "pups" is supported by our earlier finding that rectal gland Cl secretion can account for only 8-21% of the measured Cl efflux (see above). These findings indicate that Na and Cl may be regulated quite separately by elasmobranchs--a proposition which should obviously be pursued. This research was supported by NSF PCM 77-03914 to DHE.

THE UPTAKE OF FUROSEMIDE AND BUMETANIDE IN DOGFISH RECTAL GLAND SLICES

Jill Eveloff, Eva Kinne-Saffran, Jussi Melartin, David Miller and Rolf Kinne, Max-Planck Institut für Biophysik, Frankfurt, Germany and Mt. Desert Island Biological Laboratory, Salsbury Cove, Maine

The rectal gland of the dogfish is an osmoregulatory organ which secretes a fluid with a high concentration of sodium chloride. The transport of chloride is active, sodium dependent and sensitive to the action of furosemide and bumetanide, diuretics which inhibit sodium chloride transport in the thick ascending limb of Henle's loop in the mammalian kidney. The model of sodium-chloride coupled transport in the rectal gland hypothesizes that a sodium-chloride cotransport system, sensitive to the diuretics, is localized only in the basal-lateral membranes of the cell but its exact localization is unknown. Thus, the binding of furosemide and bumetanide in rectal gland slices was investigated in an effort to optimize the conditions for future furosemide and bumetanide autoradiography and thus to determine the location of the sodium-chloride cotransport system within the rectal gland cells.

Dogfish rectal gland slices, 100 μm thickness, were incubated in 2 ml dogfish Ringer's (in mM): NaCl 280, KCl 6, $MgCl_2$ 3, $CaCl_2$ 2.5, NaH_2PO_4 2, Na_2SO_4 0.5, $NaHCO_3$ 8, urea 350, glucose 5, dibutyryl cyclic AMP 0.05, theophylline 0.25, Tris-OH 20 (pH 7.6), at 15°C with [^{35}S] furosemide, 145 μM (1 μC_i/ml) or [^{14}C] bumetanide, 27 μM (1 μC_i/ml) for varying time periods, up to 1 h. Furosemide and bumetanide uptake was also investigated at various diuretic concentrations and in the presence of 10^{-3}M ouabain or diamox in order to investigate a possible relation of the sodium-chloride cotransport system to the enzymes Na,K-ATPase or carbonic anhydrase. [^{3}H] inulin was added to the incubation medium (2 μC_i/ml) to determine the efficacy of the washout period and the size of the extracellular space. At the end of the incubation period, the slices were washed for a time period, from 5 to 60 min, in an incubation medium of the same composition without the isotopes or were placed in an incubation medium without the isotopes plus containing 10^{-4}M unlabelled diuretic. The slices were then weighed, solubilized in 0.5 ml NCS tissue solubilizer (Amersham/Searle) and the radioactive content of the slices was determined by standard liquid scintillation techniques. Furosemide and bumetanide content in the slices was expressed as pmoles/mg tissue and corrected for the amount of diuretic in the extracellular space, assuming identical distribution of the diuretic and inulin within this extracellular space.

The time dependency of furosemide and bumetanide uptake in the rectal gland slices is shown in Figs. 1 and 2. The uptake approaches steady state after 30 min of incubation at 15°C, no further significant increases in the diuretic tissue content were seen after 1 h of incubation. If the slices were then placed into a diuretic-free incubation medium, approximately 50 per cent of the furosemide or bumetanide was washed out within 1 h, whereas 87 per cent of the inulin, the extracellular space marker, was washed out of the slices, suggesting that part of the furosemide and bumetanide taken up by the slice was bound to the rectal gland tissue. If 10^{-4}M diuretic was added to the washing medium, the labeled diuretic was washed out faster from the slice. This phenomenon of tracer replacement by the unlabelled diuretic, suggests that at least a portion of the diuretic is bound to specific binding sites within the rectal gland tissue.

Increasing concentrations of bumetanide or furosemide, to 1000 μM, increased the uptake of the diuretic in the slice in a saturable manner, as shown in Figure 3. Additionally, the enzyme inhibitors, diamox and ouabain, at 10^{-3}M, had no effect on furosemide or bumetanide accumulation in the rectal gland slices.

Furosemide Accumulation in Dogfish Rectal Gland Slices

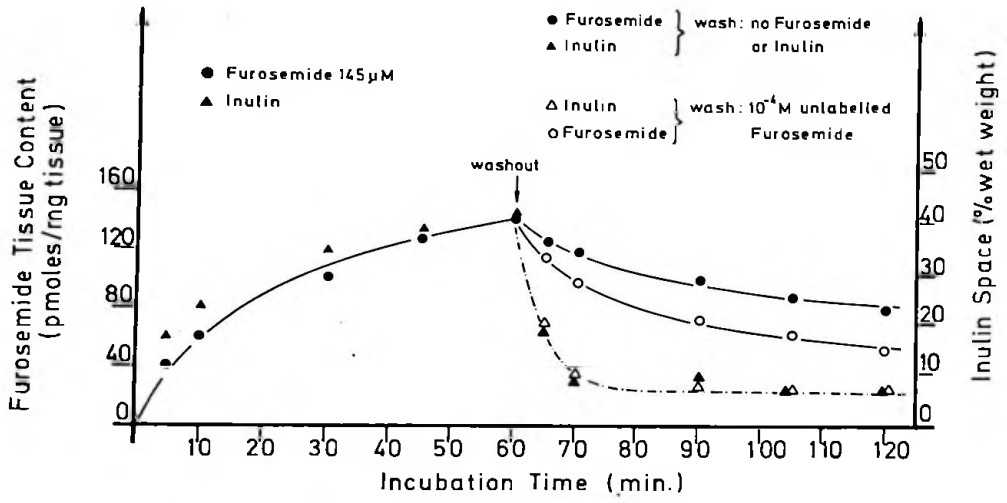

Figure 1. Furosemide uptake in dogfish rectal gland slices. Rectal gland slices were incubated at 15°C in dogfish Ringer's containing 145 μM [^{35}S] furosemide (O) and [^{3}H] inulin (Δ) for increasing time periods and then the wash out of the furosemide and inulin was followed for 1 h in an incubation medium containing no inulin or furosemide (furosemide tissue levels (O), inulin tissue levels (Δ)) or wash out was followed in an incubation medium containing 10^{-4} M unlabelled furosemide, furosemide tissue levels (O), inulin tissue levels (Δ). The values are the means of 4 experiments.

Bumetanide Accumulation in Dogfish Rectal Gland Slices

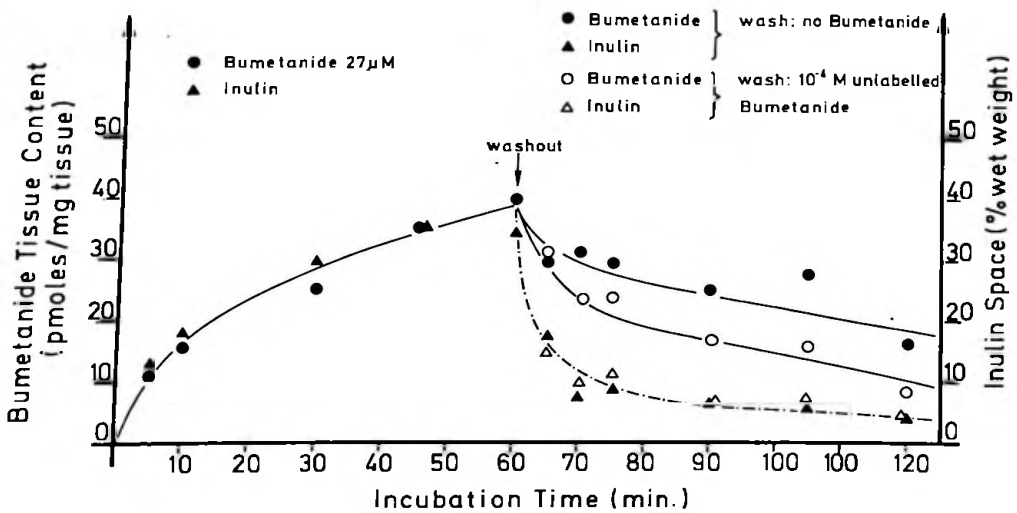

Figure 2. Bumetanide accumulation in dogfish rectal gland slices. Rectal gland slices were incubated at 15°C in dogfish Ringer's containing 27 μM [^{14}C] bumetanide (O) and [^{3}H] inulin (Δ) for increasing time periods and then wash out of the bumetanide and inulin was followed for 1 h in icubation media without radioactivity (bumetanide tissue content (O), inulin tissue content (Δ) or in an incubation medium plus 10^{-4}M unlabelled bumetanide (bumetanide tissue levels (O) inulin tissue levels (Δ)). The values are the means of 4 experiments.

Furosemide and Bumetanide Uptake in Dogfish Rectal Gland Slice: Concentration Dependency

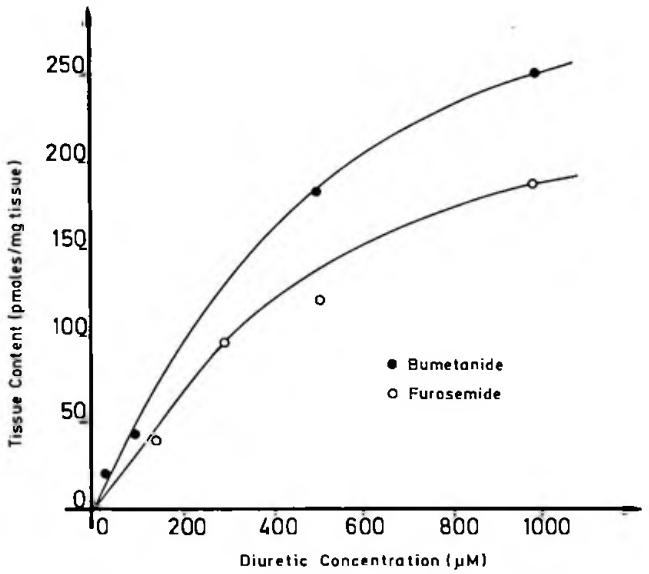

Figure 3. The concentration dependency of furosemide and bumetanide accumulation in dogfish rectal gland slices. Dogfish rectal gland slices were incubated at 15°C in dogfish Ringer's containing increasing concentrations of furosemide (O) or bumetanide (O) for 30 min followed by a 30 min wash period in dogfish Ringer's without the diuretic. Values are the means of 4 experiments and are corrected for the diuretic found in the extracellular fluid spaces as calculated using [^{3}H] inulin spaces.

Thus, it appears that bumetanide and furosemide are taken up into the rectal gland slices in a manner which suggests that some of the diuretic may be specifically bound to the tissue. Future autoradiographs will be attempted to localize the labelled diuretics. Supported in part by USPHS Grant AM 05841 and the Main Affiliate of the American Heart Association.

STRUCTURE OF TIGHT JUNCTIONS IN CHLORIDE-SECRETING EPITHELIA IN THE RECTAL GLAND OF SQUALUS ACANTHIAS

J. N. Forrest, Jr., J.L. Boyer, B. Ross, T.A. Ardito, D. Rieck, H.V. Murdaugh and J.B. Wade, Departments of Medicine and Physiology, Yale University School of Medicine, New Haven, Connecticut and University of South Carolina, Columbia, South Carolina

Tight junctions between epithelial cells play a critical role in recently proposed models of isotonic sodium chloride secretion in the rectal gland of the dogfish shark (Silva et al., Am.J. Physiol. 233:F298, 1977). In these models a neutral sodium chloride carrier in the basolateral membrane is responsible for active movement of chloride into the cell coupled to the inward movement of sodium. A favorable electrochemical gradient for sodium entry is maintained by the activity of basolateral membrane Na-K-ATPase. Whereas chloride then diffuses passively from the cell into the lumen down an electrical gradient, sodium is postulated to move from the extracellular fluid down its electrochemical gradient into the tubular lumen via the paracellular pathway. As part of the primary pathway for transepithelial sodium flux the tight junction must to some extent be permeable to sdoium, but must exclude urea since this solute is practically excluded from the secretion, even though urea is present in high concentration in the extracellular fluid. Although Bulger (Anat. Record 147:95, 1963) stated that tight junctions are present in the rectal gland, Van Lennup concluded that the rectal gland of the elasmobranch did not contain typical