papilla which has the lower osmolality. Functionally this finding is difficult to explain since water presumably leaves the collecting duct by moving from a region with higher water activity to a region with lower water activity. Our current working hypothesis is that a hydrostatic pressure difference exists between collecting duct fluid and interstitial fluid causing the activity of the water in the compartments to be such that water can move down its gradient from collecting duct lumen to the interstitium. Experiments of the type reported in another paper (Schmidt-Nielsen, Patel and Patel, Bulletin, MDIBL, this issue) have shown that the papillary tissue as well as the salt gland tissue can withstand a hydrostatic pressure of 21 Atm without cell breakage.

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1973 #45

FUNCTIONAL CORRELATES OF THE DOGFISH RECTAL GLAND DURING *In vitro* PERFUSION

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In a previous study (Bull. MDIBL 12: 45 and 50, 1972) it was shown that during *in vitro* perfusion of the dogfish rectal gland with shark Ringer's solution the secretion of sodium remained relatively constant for at least two hours and was proportional to the perfusion flow rate and/or perfusion pressure. Sodium was secreted against an electrochemical gradient of 5.7 ± 1.4 mV (mean ± SE) and chloride against a gradient of 13.6 ± 1.2 mV. Using this model further studies were performed to analyze the composition of glandular fluid and to determine functional correlates of electrolyte secretion.

In these experiments rectal glands were perfused at a rate of approximately 4.0 ml per minute using gravity flow at a hydrostatic pressure of 30 mmHg. The perfusion solution was gently bubbled with 99 percent O_2 and one percent CO_2 in most experiments. In studies involving the influence of pH on sodium excretion either 100 percent O_2 or 95 percent O_2 and five percent CO_2 was used.

The composition of the perfusion solution (P) and glandular fluid (GF), under control conditions, is shown in Table 1. Sodium composition in GF was 475 ± 7 mEq/L and the concentration of chloride was 487 ± 9 mEq/L, indicating gradients (GF/P) across the glandular epithelium of 1.57 ± 0.05 and 1.53 ± 0.05, respectively. Associated with a high concentration gradient for sodium the level of urea in glandular fluid was approximately 14 percent of the perfusate concentration. There was no difference in total CO_2 content of glandular fluid compared to perfusate, and glandular fluid maintained a slightly but significantly lower pH.

It was of interest to compare the rate of secretion in these *in vitro* experiments with levels observed in collections from free swimming fish (Science 131: 670, 1960). In perfusion studies the flow rate of glandular fluid was 3.6 ± 0.5 µl/min/g of wet gland weight and sodium secretion averaged 1.73 ± 0.27 µEq/min/g. These levels compare reasonably well with rates observed *in vivo* as recalculated from Burger's data, 11 µl/min/g (glandular flow rate) and 6 µEq/min/g (sodium secretion rate.)

TABLE 1

COMPOSITION OF RECTAL GLAND FLUID DURING *In vitro* PERFUSION

	Perfusion Solution	Glandular Fluid	Glandular Fluid/Perfusate
Sodium mEq/L	300.3 ± 5.4 a = 7 n = 14	474.6 ± 27.7 * a = 7 n = 14	1.57 ± 0.05 a = 7 n = 14
Potassium mEq/L	6.6 ± 0.1 a = 7 n =14	10. 9 ± 0.2 a = 7 n = 14	1.64 ± 0.04 a = 7 n = 14
Chloride mEq/L	319 ± 9.5 a = 7 n = 14	486.5 ± 9.1* a = 7 n = 14	1.53 ± 0.05 a = 7 n = 14
Urea mg %	1806 ± 41 a = 7 n = 12	255 ± 33* a = 7 n = 12	0.14 a = 7 n = 12
Total CO_2 mM/L	6.8 ± 0.1 a = 9 n = 9	7.0 ± 0.7 a = 9 n = 9	
pH	7.79 ± 0.15 a = 5 n = 5	7.32 ± 0.05* a = 5 n = 5	

Values represent mean ± SE.
a indicates number of fish studied.
n indicates number of observations.
* $p < 0.001$ compared to value of perfusion solution.

In the previous study (Bull. MDIBL 12: 45 and 50, 1972) the GF/P Na and GF/P Cl in glandular fluid fell to values near 1.0 in some experiments usually in association with a rise in flow rate. It was suggested that under these circumstances flow occurred through shunt pathways as a consequence of cell injury or death and represented passive diffusion driven by the hydrostatic perfusion pressure. To test this hypothesis the urea concentration gradient (GF/P urea) was measured as a marker of passive diffusion and compared to the GF/P Na. In 29 clearance periods from eight glands an inverse correlation was found (GF/P Na = [-1.38] [GF/P urea] + 2.4; $r = 0.93$, $P < 0.001$). At a GF/P Na of 1.5 or higher the GF/P urea was 0.16 ± 0.02. As GF/P Na fell progressively to lower values the GF/P urea was 0.86 ± 0.11 ($P < 0.001$). Since these data demonstrated that passive diffusion predominates when the sodium gradient is low a GF/P Na ≥ 1.5 was used as an index of active secretion in all subsequent experiments.

Since acidosis has been shown to reduce sodium chloride secretion by the avian salt gland (J. Phar. & Exper. Ther. 130: 401, 1960) the influence of hydrogen ion concentration on rectal gland function was examined. In six experiments pH was varied randomly between 6.6 and 8.0 by changing pCO_2 of the perfusate. The rate of sodium secretion was found to be directly proportional to pH of the perfusate solution (Na secretion [μEq/min/g] = 1.67 [pH] -9.89, $r = 0.67$, $P < 0.05$).

Further experiments were performed to characterize the mechanisms involved in active secretion of electrolytes. Since the rectal gland is rich in carbonic anhydrase (Physiol. Rev. 47: 595, 1967) the role of this enzyme in active NaCl transport was studied by comparing the gland flow rate and sodium excretion before and during perfusion of 14 glands with ethoxzolamide in a concentration of 0.1 mM. The total CO_2 content of the perfusate under control conditions was 6.6 ± 0.6 mM/L as compared to 6.8 ± 0.6 (P=NS) in the experimental period. Inhibition of carbonic anhydrase reduced neither flow rate nor the rate of sodium secretion. Glandular flow rate was 5.5 ± 0.9 µl/min/g control and 7.2 ± 1.1 experimental (P=NS) while the control level of sodium secretion was 2.7 ± 0.5 µEq/min/g compared to 3.3 ± 0.6 experimental (P=NS).

In an additional group of experiments the effect of thiocyanate, 10 mM on electrolyte secretion was examined since a recent report (Amer. J. Physiol. 224: 129, 1973) demonstrated that this compound inhibited active chloride transport in salt water teleost and that efflux of chloride and sodium were closely linked. Following the addition of NaSCN to the perfusate fluid in four glands, flow rate fell 36 percent from 3.9 ± 1.2 µl/min/g to 2.5 ± 0.7 ($P < 0.05$), and sodium secretion was reduced 64 percent from a control value of 1.9 ± 0.6 µEq/min/g to 0.7 ± 0.2 ($P < 0.05$).

These data indicate that the rate of formation and composition of rectal gland fluid during *in vitro* perfusion is comparable with values obtained *in vivo*. A sodium concentration gradient of 1.5 or greater serves as a useful index of active secretion since lower gradients are characterized by passive diffusion. Moreover while there was no evidence that carbonic anhydrase influenced the rate of electrolyte secretion, inhibition by thiocyanate supported the previous hypothesis that chloride is actively transported by the rectal gland.

1973 #46

THIOCYANATE INHIBITABLE ATPase IN THE GILL OF *Anguilla rostrata*

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The presence of a Mg-ATPase which is inhibited by thiocyanate (SCN) but not ouabain and requires neither Na or K has been described in a number of tissues in which active transport of anions may occur. (Kasbekar, D.K., and Durbin R.P. An adenosine triphosphatase from frog gastric mucosa. Biochim. Biophys. Acta 165:472, 1965.) The gills of teleosts are known to be a major regulator of ionic balance, particularly in sea water where the external environment contains four times the concentration of Na and Cl as the extracellular fluid. Chloride efflux is greatly increased in sea water and net transport of chloride occurs against both a concentration and an electrical gradient. Thiocyanate inhibits chloride efflux, suggesting that an active, carrier-mediated process is involved. It was therefore of interest to study the *in vitro* effects of SCN on ATPases.

Eels adapted to fresh water or sea water for at least three weeks were used. Gill filaments, removed immediately from eels were placed in an ice cold 20/1 solution (v/w) of 5mM EDTA, 0.25M sucrose, 10mM imidazole, 0.1 percent desoxycholate at pH 6.8. Homogenization was carried out in a glass homogenizer immersed in ice using a Teflon pestle at 1725 rpm. The homogenate, after