required when ouabain is applied to the exterior of the gill and inhibition by external ouabain occurs only when the drug gains access to the interior of the fish.

The functional effect of complete inhibition of gill Na-K-ATPase was tested by measuring the efflux of Na^{22}, Cl^{36} and tritiated water in seawater eels after intraperitoneal injection of 0.25 mg of ouabain per 100g body weight. After ouabain the efflux of Na^{22} was reduced to nine percent. The efflux of Cl^{36} was similarly inhibited to 11 percent of normal. These changes could not be attributed to the general circulatory effects of ouabain since the efflux of THO was much less reduced, to 60 percent of normal.

Inhibition of gill Na-K-ATPase by ouabain in intact salt water eels thus results in almost complete inhibition of Na efflux. Surprisingly Cl efflux is markedly inhibited as well though in other systems Na-K-ATPase is not thought to participate in the active transport of Cl or its carrier-mediated diffusion. The results are consistent with the theory that the Na-K-ATPase of chloride cells faces inward, lining intracytoplasmic tubular channels, and contributing to a high concentration of NaCl within these channels that in turn enhances the outward transport of NaCl across the gill.

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STUDIES OF RECTAL GLAND FUNCTION IN *Squalus acanthias* IN VIVO AND IN VITRO

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The role of the rectal gland in maintaining the chemical composition of the plasma of elasmobranchs is unclear. Following extirpation of the rectal

gland, plasma sodium and chloride have been found to rise significantly (Forrest et al. Bull, MDIBL 13:17, 1973) suggesting that the gland has a homeostatic function. In addition the fact that rectal gland fluid contains sodium and chloride at concentrations above both plasma and seawater would point to active secretion of these ions as the basis of this homeostatic role. The cellular mechanisms underlying these observations are undefined.

In an effort to delineate those cellular mechanisms, the response of the gland to pharmacologic agents known to be effective in modifying ion transport was tested *in vivo*. Previous attempts to study *in vivo* function of the rectal gland involved catheterization of its duct via transabdominal surgical intervention and confinement of the animal during the course of the experiment. We have been able to catheterize the duct without surgical intervention and to collect rectal gland fluid in the free swimming state.

Unanesthetized dogfish were placed on a shark board with gills immersed in running seawater. The rectum was carefully prolapsed through the anal opening using a curved clamp inserted into the rectum about three inches. Following prolapse, the opening of the duct could be identified and catheterized with P_{60} tubing. Sutures were carefully placed in the mucosa of the intestine to fix the catheter in place and the free end of the catheter, protruding from the anal opening, was attached to a collection balloon. Using this technique all animals lived until sacrifice.

Seventy-three collection periods in 17 dogfish under control conditions were made. The duration of individual collection periods ranged from one to 12 hours. Fourteen animals had three or more consecutive collections and some animals were followed for up to 12 consecutive days. Mean flow rate of rectal gland secretion was 0.40±.10 ml/Kg x hr., GF_{Na} was 469±15.0 mEq/L, and GF_K was 8.13±.3 mEq/L. Simultaneous plasma electrolyte measurements gave a

TABLE I

IN VIVO EFFECTS OF OUABAIN AND FUROSEMIDE

ON RECTAL GLAND FUNCTION

GROUP (N)	Rectal gland flow rate (ml/K/h)	GF Na (mEq/L)	GF K (mEq/L)	Na secretory rate (µEq/K/h)	K secretory rate (µEq/K/h)
CONTROL (6)					
pre	0.59±.2	469±24	8.75±1	280±111	4.97±2.2
post	0.57±.2	483±29	8.42±.32	289±94	4.45±1.2
OUABAIN (3)					
pre	0.47±.1	433±33	9.49±.9	213±74	3.98±1.6
post	0.38±.1 NS	438±28 NS	7.55±.8 NS	169±42 NS	2.86±.6 NS
FUROSEMIDE (4)					
pre	0.34±.1	508±6	7.94±1	171±46	2.52±.51
post	0.29±.1 NS	467±12*	7.42±2 NS	135±53 NS	1.92±.83 NS

NS not significant by paired "t" test

*p value less than .05 by paired "t" test

fluid/plasma ratio for Na of 1.74±.1 and for K of 2.07±.1. These data are similar to those found by Burger (Physiol. Zool. 25:205, 1962). Also as reported by him considerable variation in consecutive flow rates was noted in some but not all animals.

The results of a single intra-arterial injection of ouabain (80 µg/kg) or furosemide (15-30 mg/kg) are shown in Table 1, along with data from a simultaneous control group which received an equal volume of shark's Ringers. Neither ouabain nor furosemide produced a significant change in flow rate or the rate of sodium or potassium excretion. While furosemide resulted in a decrease in GF_{Na} this may be artifactual, secondary to the high pretreatment levels recorded. Further observations on this point are necessary.

Finally, although the dose of ouabain did result in a significant rise in plasma potassium (mean rise of 2.03 mEq/L, N = 4 $p = < .05$), rectal gland Na-K-ATPase activity in the ouabain treated animals was not significantly different from control animals (47.54±1.5 μMP_i/mg/h compared to 50.68±2.0 μMP_i/mg/h) (micromoles P/mg protein/hr).

Similar pharmacologic studies were undertaken *in vitro* using an isolated rectal gland perfusion system. Rectal glands from 4-5 kg dogfish were catheterized (P_{60}) *in vivo* and then removed and perfused through its single arterial supply with oxygenated shark's Ringers with glucose (5mM) at 12-16°C and pH = 7.80. A constant volume (80ml) of shark's Ringers was recirculated using roller-type pumps which permitted perfusion fluid flow rates to the rectal gland of 3-6 ml/minute at perfusion pressures of 30-40 mmHg. Using this system, sodium concentration remained stable over 90 minutes of perfusion with gland fluid/plasma sodium ratios greater than 1.5 indicating active secretion (Siegel et al. Bull. MDIBL 13:45, 1973). Rectal gland fluid flow rate fell progressively during the course of perfusion.

TABLE 2

IN VITRO EFFECTS OF OUABAIN AND FUROSEMIDE ON RECTAL GLAND FUNCTION

GROUP	CONTROL PERIOD			EXPERIMENTAL PERIOD I			EXPERIMENTAL PERIOD II		
	V µL/m/g	GF Na mEq/L	GF Na V µEq/m/1	V µL/m/g	GF Na mEq/L	GF Na V µEq/m/g	V µL/m/g	GF Na mEq/L	GF Na V µL/m/g
CONTROL (5)	4.1±.1	491±6	2.0±.6	2.2±.5	487±5	1.1±.3	.8±.2	482±9	.4±.1
FUROSEMIDE (4)	8.6±3.1	412±9	3.5±1.2	3.9±1.2 NS	409±15 NS	1.6±.5 NS	2.2±.9 NS	415±16 NS	.9±.4 NS
OUABAIN (4)	5.5±1.5	502±9	2.8±.8	3.9±1.0 NS	494±1.5 NS	1.9±.5 NS	.7±.1 NS	491±2 NS	.3±.1 NS

NS not significant by paired "t" test of differences in treated groups in control.

Pharmacologic doses of ouabain (2.5×10^{-4} M) or furosemide (5×10^{-4} M) were added to the recirculating perfusion fluid after 30 minutes of control perfusion. Rectal gland fluid collections were made for 15 minutes prior to giving the drug (control period) and for two consecutive 15-minute periods thereafter (experimental period I and II). No significant effect on sodium or potassium concentration in gland fluid, gland fluid flow rate or rate of sodium excretion resulted from the addition of either ouabain or furosemide (Table 2).

The results of both the in vivo and in vitro studies indicate that the active secretion of sodium is not inhibited by ouabain in concentrations which are known to be inhibitory in other marine organs to active sodium transport. Inhibition of Na-K-ATPase activity with ouabain was not demonstrated in vivo. This may have resulted from insufficient amounts of ouabain reaching the rectal gland. Therefore a dose of ouabain sufficient to inhibit 100 percent of Na-K-ATPase activity in homogenized rectal gland was given to the perfused rectal gland. The failure of ouabain to result in inhibition of sodium transport in the rectal gland is interesting in view of the fact that ouabain has no effect on urinary excretion of sodium in this species. The rectal gland also appears to be insensitive to furosemide both in vivo and in vitro.

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ALTERATIONS IN RENAL CARBONIC ANHYDRASE IN RESPONSE TO SALINITY IN THE EURYHALINE EEL, *Anguilla rostrata*

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The absence of renal carbonic anhydrase activity appears to be a general property of marine fish, while its presence is characteristic of freshwater