Since monensin was dissolved in alcohol, an equal volume of alcohol was added to a separate set of slices to determine whether alcohol had any effect on their oxygen consumption. In this series of experiments the oxygen consumption of the slices under basal conditions was 29.2 ± 8.3 with an n=8.

Ouabain binding was measured using the technique previously described (Bull MDIBL 21:103, 1981). Monensin was added at concentration of .15, 1.5 and 15 x 10^{-5} M. The results are summarized in Table 3.

Table III

Effect of monensin .15, 1.5 and 15 x 10^{-5}M and stimulated with theophylline 10^{-3}M plus dibutyryl cyclic AMP 10^{-3}M on ouabain binding by shark rectal gland slices.

	Monensin x 10^{-5}		
	.15	1.5	15
Control	122.4±19.8	127.2±20.3	156.0±20.6
Stimulated	170.1±70.6[a]	189.4±41.9[b]	208.8±15.0[a]
Monensin	137.7±31.4[d]	148.8±29.5[a,d]	100.6± 9.1[a,c]
Alcohol	133.0±27.0	142.9± 5.0[a]	153.2±12.5

Values are mean ± SD. p values were calculated by "paired t" test.
a $p < 0.05$ compared to control
b $p < 0.005$ compared to control
c $p < 0.05$ compared to alcohol alone
d not significantly different from alcohol alone

Monensin, an ionophore that increases the entry of sodium into the cell, does not increase ouabain binding to slices of shark rectal gland. This result contrasts with the effect of theophylline and cyclic AMP which regularly stimulate ouabain binding. The mechanism of the stimulation of ouabain binding by theophylline and cyclic AMP is therefore unlikely to be an increase in activity of the sodium pump, since monensin, which causes a 30% increase in the intracellular sodium concentration and a 66% increase in the rate of oxygen consumption, does not evoke an increase in the binding of ouabain to slices of rectal gland. These results are consistent with our previous findings that cyclic AMP and theophylline stimulated ouabain binding in the absence of sodium and chloride as well as in the presence of bumetanide or furosemide. Thus, an increased transcellular movement of electrolytes is not necessary for the induction of increased ouabain binding by cAMP, and an increase in the sodium pump activity produced by monensin, rather than by cyclic AMP, does not enhance ouabain binding.

EVIDENCE THAT SODIUM IS SECRETED VIA THE PARACELLULAR PATHWAY IN THE SHARK RECTAL GLAND

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The current model for chloride secretion by the rectal gland of the shark can be described as follows. Two chlorides enter the cell across the basolateral cell membrane together with a sodium and a potassium through a neutral chloride sodium, and potassium cotransport system. The energy for this entry step is provided by the downhill gradient for sodium directed into the cell. The gradient for sodium is maintained by Na-K-ATPase. Chloride leaves the cell via a conductive pathway in the luminal membrane. Potassium recycles across the basolateral membrane through a potassium channel that can be blocked by barium. Sodium cannot cross the luminal

cell membrane because of a steep electrochemical gradient opposing it, but must enter the lumen through the paracellular space. The present experiments were designed to test the accuracy of the last statement. 2.4.6-triaminopyrimidine (TAP) was used as a probe of the paracellular pathway (Moreno, Nature. 251:150-151, 1974) in isolated perfused rectal glands.

Rectal glands were perfused as previously described (Silva et al. Am. J. Physiol. 233:F298-F306, 1977) with shark Ringers containing glucose 5 mM, 2.5×10^{-4} M theophylline and 5×10^{-5} M dibutyryl cyclic AMP at a pH of 6.7. This pH was chosen because TAP inhibits sodium flux via the paracellular pathway only in its protonated form and its pK is 6.72. At a pH of 6.7, 50% of the TAP in solution is protonated. The concentrations of TAP given in this report are those of the protonated form. At a pH of 6.7, the secretion of chloride by the stimulated isolated perfused rectal gland is 50 to 60% that during perfusion at a pH of 7.6, the normal pH of shark blood. Glandular secretion of chloride rapidly decreases as pH is lowered, until at a pH of 6.0, where 85% of any TAP present would be in the protonated form, it is less than 5% of normal.

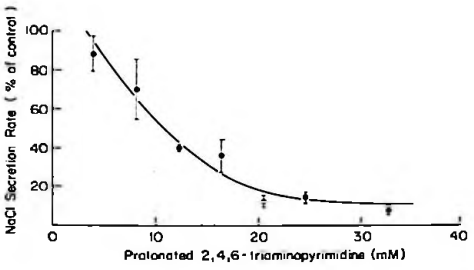

Figure 1.--Effect of 2.4.6-triaminopyrimidine on sodium secretion by the perfused rectal gland. The percent inhibition of sodium chloride secretion is plotted against the concentration of protonated TAP. Half maximal inhibition is found at about 11 mM. Inhibition is maximal at concentrations greater than 20 mM.

Figure 1 shows the effect of increasing concentrations of TAP on sodium chloride secretion by the stimulated shark rectal gland. The results are expressed as a percent of the rate of secretion in the absence of TAP. The half maximum inhibition was found at a TAP concentration of 12 mM. The effect was partially reversible. In glands perfused at concentrations of TAP of less than 16 mM it was always reversible. At 32 mM it was reversible only in 3 out of 9 glands.

The effect of TAP on rectal gland secretion is consistent with the view that sodium enters the lumen via the paracellular pathway. The inhibitory effect of TAP on rectal gland secretion was seen at concentrations 2 to 3 times higher than those at which it caused inhibition in the gallbladder or frogskin due perhaps to the fact that the sodium concentration in the perfusate of the rectal gland was two and a half times greater than that used in those experiments.

STOICHIOMETRY OF SODIUM CHLORIDE TRANSPORT BY THE SHARK RECTAL GLAND

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Previous work from our laboratory has shown that the ratio between chloride secreted and oxygen consumed by the rectal gland is approximately 25 to 30:1, greatly in excess of the 18:1 that can be calculated if 3 moles of sodium are transported per mole of ATP hydrolyzed by Na-K-ATPase in the basolateral membrane of the cells and the P:O ratio of the rectal gland mitochondria is 3:1 (Silva et al., J. Membrane Biol. 1980; 53:215-221). This has suggested, either that more than one chloride accompanies each sodium into the cell or that there is additional energy available from anaerobic metabolism. The latter explanation seems unlikely because lactic acid production by the gland is minimal.