At 10^{-3}M TFP, however, neither a concentration of 10 μg of VIP nor the higher concentration of cAMP-theophylline stimulated rectal gland secretion (n = 2 for each).

Because calcium ionophores did not stimulate, and verapamil did not inhibit rectal gland secretion, these experiments suggest that an influx of external calcium is not required for cAMP-induced secretion by the dogfish rectal gland. The results with trifluoperazine, on the other hand, raise the possibility of a role for calcium, since this compound binds to and inhibits the calcium-calmodulin complex that is thought to mediate the intracellular regulatory actions of calcium in almost all animal cells (C.B. Klee, T.H. Crouch and P.G. Richman, Ann. Rev. Biochem., 49:489-575, 1980). Calmodulin has been identified in rectal gland cells (H.C. Palfrey et al., this issue) and it is possible that the release of calcium from intracellular stores modulates at least a portion of rectal gland secretion. The results may be compared to those obtained by P.C. Smith and M. Field (Gastroenterology, 78:1545-1553, 1980) who found that calcium ionophores served as a submaximal stimulus to chloride secretion by rabbit small intestine, while trifluoperazine inhibited, though incompletely. The effect of trifluoperazine in the rectal gland may indicate a role for calmodulin in mediating secretion in this organ, but does not prove the case since it may also reflect other cellular actions of this drug.

OXYGEN CONSUMPTION OF CELLS ISOLATED FROM THE RECTAL GLAND OF THE SPINY DOGFISH SQUALUS ACANTHIAS

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The use of tissue slices for studies of intracellular electrolytes or for the binding of hormones or inhibitors of electrolyte transport is fraught with many uncertainties. The unavoidable problems arise from the fact that slices have a dead space that not only requires measurement, but also retards the binding or the effects of inhibitors or hormones. In large measure, these problems can be circumvented by using isolated tubules or cell preparations. Isolation of cells from the rectal gland of the shark is desirable for the measurement of the changes in intracellular concentration of electrolytes that attend activation of secretion by the gland with cyclic AMP, as well as for the measurement of the binding of ouabain to the Na-K-ATPase present in the membrane which is probably activated as a result of that stimulation. We report here the isolation of cells from the rectal gland of the shark and measurements of their oxygen consumption in vitro.

The rectal glands of two sharks were removed and perfused with 100 ml each of shark Ringer's in vitro as described (Am. J. Physiol. 233:F298-F306, 1977), for over 10-20 minutes to remove all red cells. The glands were then perfused with 10 ml of shark Ringer's containing 0.2% collagenase, 0.2% hyaluronidase and 10% fetal calf serum. Perfusion was then stopped, the glands sectioned longitudinally in half, then minced into 0.5 mm squares using a McIlwain tissue slicer. The minced tissue was placed in oxygenated shark Ringer's containing 0.2% collagenase and 0.2% hyaluronidase and 10% fetal calf serum and digested for variable periods of time at room temperature. The digestion process was followed by examining the tissue suspension under light microscopy at timed intervals. It was found that one hour of digestion yielded the largest proportion of viable isolated cells. The tissue digest was centrifuged at 500 rpm in a refrigerated international centrifuge for 1 minute to remove the undigested tubules; and the supernatant (containing the cells) was then centrifuged at 1500 rpm for 3 minutes to harvest the cells. The cells were washed once with shark Ringer's, then suspended in a final volume of 1 ml of shark Ringer's and stored in an ice bath until used for experiments.

Viability of the cell preparations was assessed by determining the exclusion of dye of cells suspended in a 1% trypan blue solution. The percentage of cells taking up the dye was variable, but was always less than 10%.

Oxygen consumption was measured using a Clark electrode in a chamber containing 2 ml of shark Ringer's of the following composition (in mM): Na 280; K 4; Cl 290; PO_4 1; Ca 2.5; Mg 1; SO_4 0.8; Urea 350; Hepes 40; pH 7.6. Glucose 5 mM, acetate 2.5 mM and pyruvate 10 mM were used as metabolic substrates in all determinations. The chamber was maintained at 20°C using a constant temperature circulator and equilibrated with room air prior to the addition of the cells. The electrode was calibrated using a) the known solubility of oxygen at ambient pO_2 for the "full scale" reading and b) the Radiometer® "Zero pO_2" calibration solution for the baseline. The rate of oxygen consumption was calculated from the change in pO_2 readings taken at timed intervals.

As shown in Table 1, the rate of oxygen consumption of isolated rectal gland cells averaged 17.6 ± 1.7 μM O_2/g/hr.

Table 1.--Oxygen Consumption in Cells Isolated from the Shark Rectal Gland

	Basal	Stimulation		Inhibition
		dbcAMP 2×10^{-3} Theophylline 2×10^{-3}	VIP	
Control	17.6 ± 1.7 (39)	38.9 ± 3.6 (28)**	--	---
Ouabain 10^{-4}M	--	58.1 ± 9.0 (7)	--	32.2 ± 9.8 (7)*
Furosemide 10^{-4}M	--	36.6 ± 4.3 (10)	--	25.2 ± 3.7 (10)*
V.I.P. 5 μg/ml	16.0 ± 2.5 (9)		21.9 ± 4.2 (9)+	

*$p < .05$

**$p < .0005$

+$p < .025$ paired t-test

This value is of similar magnitude to that observed in isolated perfused rectal gland under unstimulated conditions (J. Membrane Biol., 53:215-221, 1980). The addition of cyclic AMP and theophylline to the bath evoked immediate increase in the rate of oxygen consumption as was demonstrated in the isolated perfused gland. VIP, the probable humoral agent that stimulates rectal gland secretion in the live animal, induced a 40% increase in oxygen consumption by these cells. Ouabain 10^{-4}M reduced oxygen consumption by 50%, comparable to the effects of this drug on oxygen consumption in several epithelial tissues, but lower than that observed in the perfused rectal gland preparation. Furosemide, known to inhibit oxygen consumption in the isolated rectal gland, also reduced it here by 30%.

Viable isolated rectal gland cells can thus be prepared that respond in a predictable way to stimulators and inhibitors of chloride transport, and, therefore, can be used for the measurements of the events that attend transcellular stimulation of transport.

EFFECT OF UREA HYPEROSMOLARITY ON ACID SECRETION IN DOGFISH GASTRIC MUCOSA

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The gastric H^+ secretory mechanism uses metabolic energy to transport H^+ from the cell cytoplasm into the secretory tubules; the energy required for this process depends upon the pH and electrical gradients across the membrane,