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,

and there must be some electrochemical gradient beyond which H^+ secretion cannot occur. Since the contents of the secretory tubules consist largely of HCl isotonic to the cell cytoplasm, created by the osmotic equilibration of H_2O in response to HCl secretion, one cannot readily alter the pH of the depths of the tubules by changing the pH of the bulk mucosal solution, even if acid secretion could be measured under such conditions. However, since for HCl, pH = -log (0sM/2), it should be possible to change the pH of the primary secretion by alterations in the osmolarity of the serosal bathing solution, if a suitable agent without side effects can be found.

Urea seems a likely candidate; since elasmobranch tissues contain both high salts and high urea, the dogfish gastric mucosa seems a suitable tissue. Accordingly, acid secretory rate (J_H) was measured as a function of solution osmolarity in an attempt to determine the limiting primary secretion pH.

The gastric mucosa of Squalus acanthias, stripped of its outer muscles, was mounted as a flat sheet between fluid-filled "Ussing" chambers as previously described (Kidder, Bull. MDIBL, 14, 1974). Before mounting, fine scissors and forceps were used to remove additional connective tissue, hopefully reducing the diffusion limitation of 0_2 access (Kidder, Am. J. Physiol., 231:1240, 1976), and the tissue was maintained at 17 ± 1°C, gassed with 10% $C0_2$/90% 0_2 throughout. The standard serosal solution contains (mEq/l): Na, 250; k, 10; Ca, 5; Mg, 2; Cl, 244; $HC0_3$, 30; Urea, 350; Glucose, 25; Histamine, 0.1; Mecholyl, 0.025. The standard mucosal solution is similar, with 30 mM NaCl replacing $NaHC0_3$, and omission of the secretagogues. Osmolarity was varied by changing the urea content of these solutions, monitoring the osmolarity by freezing-point depression.

In one series of tissues, the urea content of the serosal bathing solution was varied from 0 to 1 M, which alters the total solution osmolarity between 511 and 1500 mOsM. Acid secretory rate was measured by pH-stat during the last half hour of the 2 hour incubation period for each condition; generally, 3 such periods were measured per tissue, one of which was always standard solutions. The mucosal bathing solution remained standard throughout this series. The results are shown as Figure 1. The significant decrease in J_H upon removal of urea is consistant with my previous

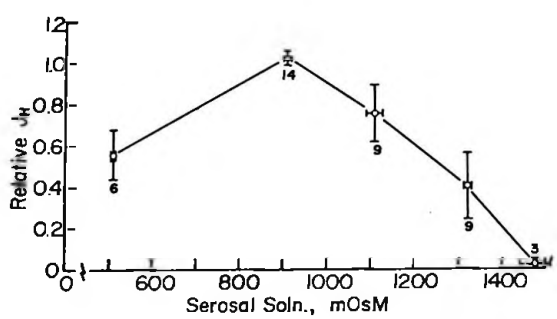

Figure 1

report (Kidder, Bull. MDIBL, 18, 1978), of a urea effect in hyperbaric conditions, but contrary to the observations of Hogben (Science, 129:1224, 1959). The decline in J_H with increasing urea above normal is expected from the considerations outlined above, but other explanations are possible.

In 3 tissues, the mucosal solution osmolarity was changed while maintaining standard serosal solutions, with the results given in Table 1. There is a tendency for these results to parallel those of Figure 1, although none of the means

Table 1.--The Effect of Mucosal Osmolarity on Acid Secretion at Constant (Standard) Serosal Osmolarity

Period*	Mucosal Soln.	mOsM	Rel J_H	N
1	Standard	847	1.00	3
2	Urea-free	505	0.88 ± 0.23	3
3	Standard	847	1.31 ± 0.30	3
4	Hypertonic	1170	0.30 ± 0.43	2

*For each 2-hour period, secretory rate measured for the final half-hour.

is significantly different from 1.0 with these few experiments.

For urea to be an effective osmotic agent across the mucosal face of the cell, this membrane must be rather impermeable to urea, at least much more impermeable than it is to water. Hogben (Bull. MDIBL, 11m 1971), has reported a passive urea permeability for this tissue of 2.1×10^{-6} cm·sec^{-1}, using ^{14}C-urea in otherwise urea-free solutions. I attempted some different experiments. Three tissues were incubated in standard solutions (both sides) for 2 hours, and then washed out by replacing the solutions on both sides with their urea-free equivalents, changing solutions as often as necessary to maintain urea concentrations below 5 mM, as determined by the diacetyl monoxime-thiosemicarbazide method (Pierce "Rapid Stat"). While the washout from each side can be plotted as the usual sum of exponentials, this is not instructive since the slope of either side reflects a contribution of the other side in reducing cellular urea content. However, since at any time the urea gradient toward the two sides is the same, the ratio of fluxes toward the two sides should be in the ratio of the effective membrane permeabilities.

These results are shown in Table 2, omitting the early periods which consist largely of washout from the tissue

Table 2.--Ratio of Urea Fluxes Toward Serosal and Mucosal Solutions

Time* (min)	Flux Ratio (S/M) 8/19	8/20	8/25	Mean ± S.E.**
22.5	2.94	2.85	2.40	2.73 ± 0.20
45	2.89	4.37	3.05	3.44 ± 0.57
90	2.80	3.02	2.52	2.78 ± 0.18
150	1.78	2.14	2.04	1.99 ± 0.13
210	1.26	1.62	2.06	1.65 ± 0.28
270	-	1.21	-	1.21 -

*Midpoint of period; first recorded period 15 min long, second 30 min, subsequent periods 60 min.

**All values significantly ($P > 0.99$) different from unity except for the value at 210 min, for which $0.95 > P > 0.90$.

surface (mucous coat and serosal connective tissue). The flux ratio peaks around 60 min, and declines toward unity with time, indicating a change in relative urea permeability with time of exposure to zero-urea solutions.

In a further series, steady-state urea flux was determined by maintaining the urea flow from a standard serosal solution into urea-free mucosal solution. The results are shown in Figure 2. Following the initial fall

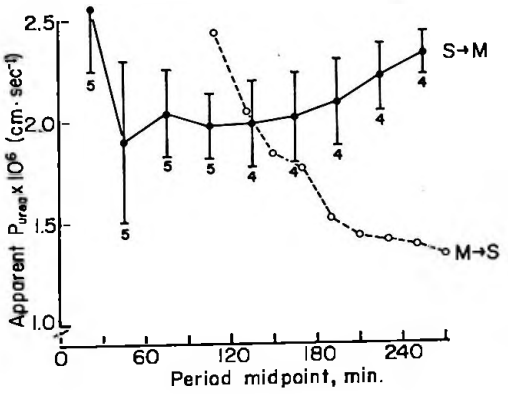

Figure 2

(presumably due to removal of trapped urea) the permeability appears to rise steadily, although the increase is not statistically significant. The values obtained are not far from those reported by Hogben in urea-free solutions. However, the reverse experiment, performed in only one tissue, shows a rapid drop in P_{urea} to rather low values.

One would like to believe that the osmotic properties of the solution bathing the mucosal face of the gastric mucosa were not reflected in cellular osmolarity or changes in membrane properties. The data here presented suggest that this may not be the case, but that the urea permeabilities of both membranes can be altered by gross changes in the urea content of either solution. This question can best be answered by studies with ^{14}C-urea, so that fluxes can be measured into urea-containing solutions.

In light of the permeability data, one cannot be sure that the decrease in J_H seen in Figure 1 represents a thermodynamic loading of the pump by a large reverse H^+ gradient. If it does, the dogfish gastric mucosa is capable of producing a primary secretion of 1475 mOsM, which if it consists entirely of HCl and H_2O, should have a pH of 0.13 and produce a gradient of more than 10^7-fold with respect to cell cytoplasm at pH 7.4. Supported in part by the National Science Foundation Grant PCM77-03336.

THE MDIBL SEAWATER SYSTEM: SOME CHARACTERISTICS AND DATA

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The seawater system consists of pumps which draw water from Eastern Bay, pipes and valves (largely plastic) which distribute the water to laboratories and aquaria, and drains which return the water to the bay. There are three similar pumps and intake lines, two different but interconnectable distribution systems, and many separate drains.

The intake lines are approximately 120 feet long and 3 inch I.D. Both the continuous and intermittent pumps deliver through 3" lines to a central distribution point at the sea water storage tank which is about 150 feet from the pumphouse and 20 feet above the pumps. At this point the systems can be interconnected to supply all lines from a