In the previous report, we have produced evidence that most, if not all, of the cAMP-stimulated entry of sodium into the cells of the rectal gland is independent of any simultaneous chloride uptake and is not inhibited by 5×10^{-4} M furosemide. It has been shown above that the level of ouabain binding in the rectal gland is highly dependent on the simultaneous net entry of sodium following inhibition of the sodium pump. It was therefore thought to be of interest to test for any furosemide-sensitivity in the sodium entry process by investigating the effect of furosemide on ouabain binding. As can be seen from Table I, 5×10^{-4} M furosemide produced only a 23% reduction in ouabain binding compared to the 77% reduction in conditions of minimal sodium loading. This further emphasizes the conclusion reached in the previous report, that any furosemide-sensitive mechanism is only of minor significance in the overall sodium entry process in the dogfish rectal gland.

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THE CLEARANCE OF METHAZOLAMIDE BY THE RECTAL GLAND, IN RELATION TO THE FAILURE OF CARBONIC ANHYDRASE INHIBITION TO ALTER SECRETION

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Although the rectal gland has the highest concentration of carbonic anhydrase of any tissue in *Squalus acanthias* (Maren, Physiol. Rev. 47:595, 1967) the function of the enzyme in this tissue has not been discovered. Among secretory organs containing carbonic anhydrase, the situation is unique, in that the sulfonamide inhibitors of the enzyme appear to have no effect upon fluid or NaCl output by rectal gland *in vivo* or *in vitro* (Rawls, Bull. MDIBL 4:58, 1962; Siegel *et al.*, Comp. Biochem. Physiol. 51A:1593, 1975, Silva *et al.*, Amer. J. Physiol. 233:T298, 1977).

We wished to find how methazolamide, a potent inhibitor with good access to mammalian tissue (Maren, *vida supra*) was handled by the rectal gland. The K_I of this drug for the rectal gland carbonic anhydrase is 2×10^{-7} M, very similar to what we found for dogfish red cells (Maren and Friedland, Bull. MDIBL 17:35, 1977). The isolated gland was perfused through its artery at 2 - 5 ml/min with dogfish Ringers solution containing 2×10^{-3} M methazolamide. The gland was stimulated by 0.25 mM theophylline and 0.05 mM dibutyrl cyclic AMP in the perfusion fluid in experiment of Table I and 0.25 mM dibutyrl cyclic AMP and 5 mM theophylline in experiment of Table II. The techniques have been described by Silva *et al.* (*vide supra*).

Tables I and II show the data. It is clear once again that methazolamide did not alter fluid production or chloride output by the gland, during periods in which there was a high concentration of the drug in the gland fluid. The clearance data, taken in the light of the study by Bradley and Georgopoulos (Bull. MDIBL 17:81, 1977) show a high degree of permeability for methazolamide across the rectal gland epithelium. The clearance of methazolamide is about 3 µl/min per g gland (normal gland weighs 3 g) which is 4 times greater than Bradley and Georgopoulos (*vide supra*) found for sucrose, and 10 times greater than their figures for inulin or ferrocyanide. If the drug in ductal fluid (59-270 µM) is in contact with tissue carbonic anhydrase, the concentration is approximately 300-1000 times the K_I. If we calculate on the basis of arterial perfusate or venous effluent (2 mM) the increment is about 10^4.

Further analysis of the experiment of Table II gives an estimate of methazolamide extraction by the gland. The total amount extracted from perfusate is the sum of drug (1) in the glandular fluid

TABLE I

PHARMACOLOGY OF METHAZOLAMIDE IN THE RECTAL GLAND STIMULATED WITH 0.25 mM THEOPHYLLINE AND 0.05 mM DIBUTYRYL CYCLIC AMP

Successive 10 Minute Periods	Outflow Gland	Outflow Vein	Cl^- Output	Concentration of Drug in Gland Fluid	Clearance
	ml/min		µeq/min	µM	µL/min
1	0.066	2.2	33.4	-	
2	0.079	1.9	39.9	-	
3	0.074	1.6	37.3	-	
4	0.100	1.9	46.1	-	
5*	0.111	1.9	50.0	220	12
6	0.100	1.8	42.5	270	13
7	0.097	1.7	40.5	130	6
8+	0.096	1.9	42.8	180	
9	0.089	1.8	42.7	40	
10	0.081	1.7	38.0	5	

*Methazolamide (2 mM) added to perfusate.

+Switched to methazolamide-free perfusate.

TABLE II

PHARMACOLOGY OF METHAZOLAMIDE IN RECTAL GLAND STIMULATED WITH 0.25 mM DIBUTYRYL CYCLIC AMP + 5 mM THEOPHYLLINE

Successive 10 Minute Periods	Outflow Gland	Outflow Vein	Cl^- Output	Concentration of Drug in Gland	Concentration of Drug in Venous	Clearance
	ml/min		µeq/min	µM		µL/min
1	0.25	5.1	104.4			
2	0.28	5.2	139.6			
3	0.27	5.0	139.8			
4*	0.26	5.2	145.9	69	1900	8.9
5	0.25	4.9	142.3	59	2100	7.5
6	0.24	4.7	121.4	59	2000	7.1
7+	0.25	4.8	124.2	47		
8	0.24	4.5	119.6	23		
9	0.24	4.7	121.8	10		
10	0.19	4.0	96.4	6	39‡	

*Methazolamide (2 mM) added to perfusate.

+Methazolamide free.

‡Concentration in pooled effluent of 40 min post drug period. At this time concentration of drug in gland was 1 µM. Gland weight, 3.0 grams.

during and after drug administration, (2) in the venous effluent following cessation of drug perfusion and (3) in the gland at the termination of the experiment. The value is 7.65 µmoles. This may be compared to the calculation of drug extraction given by A-V difference X perfusion rate. However, because of the high perfusion rates there was no detectable A-V difference in our assay system. If there were an A-V difference of 0.05 mM methasolamide, then the total drug extraction during 30 minutes of perfusion is 7.5 µmoles, in close agreement with our recovery. The gland in the experiment of Table II had a

methazolamide concentration of 1 μM after forty minutes of drug free perfusion. Maren and Friedland (Bull. MDIBL, 1978) showed that the concentration of carbonic anhydrase in the rectal gland is 9.8 μM; thus our value of 1 μM is consistent with drug binding to gland enzyme and subsequent dissociation during control periods.

It is therefore reasonable to suppose that in these and similar experiments the enzyme is fully inhibited, certainly as much as in mammalian systems (i.e., kidney, aqueous humor, CSF, pancreas) in which a clear-cut response is elicited. A failure of response in dogfish rectal gland to carbonic anhydrase inhibitors does not appear due to lack of sensitivity of the enzyme or lack of access of drug.

It may be noted, however, that the absolute rates of sodium turnover in the rectal gland are rather low compared with rates in organs that are affected by carbonic anhydrase inhibition. Rectal gland in the basal state secretes about 5 μeq Na/min per gram of gland; this is increased some 6-fold by stimulation with theophylline + dibutyryl cyclic AMP (Silva *et al.*, *vide supra*) or by vasoactive intestinal peptide (Stoff *et al.*, Am. J. Physiol. 237:F138, 1979). Even these highest rates, however, are considerably lower than renal sodium reabsorption (about 150 μeq/min per gram kidney) or avian salt gland secretion (about 300 μeq/min per gram gland). Such comparisons invite the suggestion that catalysis is unnecessary in the rectal gland because of the low rate. On the other hand the enzyme *is* present, and there may be a buried or potential role of carbonic anhydrase still not revealed by present experiments. Reasons for continuing this assumption include the facts that high pCO_2 (Siegel *et al.*, *vide supra*) and HCO_3 removal (F. H. Epstein, personal communication) both lower secretion. Additionally, cytoplasmic and membrane associated carbonic anhydrase in the rectal gland (Maren and Friedland, *vide supra*) are highly resistant to inhibition by chloride, $I_{50} \sim 1.6$ M, while for dogfish red cell carbonic anhydrase the $I_{50} = 70$ mM. Thus the rectal gland enzyme displays a striking and unusual characteristic necessary for a role in the secretion of a highly saline fluid. It is still tenable that within the rectal gland a relatively high HCO_3 or alkaline milieu involving carbonic anhydrase subserves NaCl secretion, as it does in the avian salt gland and certain other secretory systems (Maren, Physiol. Rev. 47:595, 1967). Failure of response to inhibition of the enzyme, however, remains unexplained.

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TRANSPORT OF 2-DEOXY-D-GLUCOSE AND D-MANNITOL IN THE WINTER FLOUNDER INTESTINE

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The bidirectional transepitnelial fluxes of 2-deoxy-D-glucose and D-mannitol were studied in a continuation of previous work. The fluxes were measured as previously described (Naftalin, R., D. E. Pew, and A. Kleinzeller, Bull. MDIBL 18:107, 1978). The surface area of each port of system was 0.94 cm^2. The serosal-mucosal flux (J_{sm}), the mucosal-serosal flux (J_{ms}), and the net flux (J_{net}) were determined. The unidirectional entry and exit fluxes across both faces of the epithelium (R) were also determined (Naftalin, R. and Curran, P.E., J. Mem. Biol. 16:257-258, 1974).

The flux of 2-deoxy-D-glucose was not affected by 0.1 mM ouabain, 0.1 mM phlorizin, or the addition of glucose (5 mM) to either the serosal side or to both sides of the intestine. The addition of ouabain did however cause a slight decrease in the mucosal uptake as indicated by the R value of 0.261 ± 0.023 as compared to the control value of 0.460 ± 0.009 which may indicate a slight sodium dependence of the transport. Ouabain also caused a small decrease in the phosphorylation of the sugar.