of this species (Bull. Mount Desert Island Biol. Lab. IV, part 3, p. 72, 1959) it seemed worthwhile to re-investigate the problem.

Five flounder were catheterized and urine collected for 24 hours in a balloon sewed to the catheter. Two fish were untreated; three received 25 mg/kg of acetazolamide intramuscularly at zero time. In all five fish the 24-hour urine was pH 6.70-6.95. Total CO_2, however, was 0.9 mM/L in the control fish and 4.7 mM/L in the treated fish. This corresponds to pCO_2 of 7.5 mm. Hg in the control, and 22 mm. Hg in the treated animals. Parrallel changes in pCO_2 were found in the plasma, in accord with the general pattern of respiratory acidosis which is found in all fish following carbonic anhydrase inhibition (Hodler *et al.*, Am. J. Physiol. *183*, p. 155, 1955).

It therefore appears that the urinary changes are accountable to the systemic effects of the drug, and it may be said that no marine fish yet examined has shown a renal response to acetazolamide, or to sodium bicarbonate. The presence of carbonic anhydrase in the kidneys of marine teleosts remains unexplained.

Carbonic Anhydrase Inhibition In The Rectal Gland Of Squalus Acanthias

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Dr Wendell Burger (Science, *131*: 670, 1960) reported on the function of the rectal gland of the spiny dogfish *Squalus acanthias*, showing it to secrete Na^+ and Cl^- in relatively high concentrations. He demonstrated a method of continuous sampling of the secretion practical over 3 - 4 day periods and showed a secretory response of the gland to small intramuscular injections of saline solutions. Previously unreported observations by Dr. Thomas Maren (MDIBL 1959-1960) show the gland to contain carbonic anhydrase (C.A.). Concentration was (mean $\pm$ S.E.) 92 $\pm$ 14 units/gm. of tissue. This is the highest C.A. concentration of any tissue in this species.

The effects of inhibition of this enzyme in relation to rectal gland secretion were studied. Burger's method of collection, and specific inhibition with methazolamide were employed. Methazolamide is a carbonic anhydrase inhibitor with activity and effects similar to those of acetazolamide, but with the advantage of better penetration into cells, and a lower pK, so that its sodium salt, used for injection, is close to neutral.

Two protocols were used. In the first, three fish were prepared according to Burger. A baseline flow of 0.2 ml/kg/hr or more was established; if this did not occur the fish was not used. The response to three successive intravenous injections (approximately 4 hours apart) of Molar NaCl (2 meq/Kg) was then determined. There is no apparent difference between intramuscular and intravenous injections of NaCl. Typically the gland responds with increase in flow in 30-90 minutes. The flow increases to 1.2 - 1.8 ml/Kg/hr, is maintained for 60 - 120 minutes and then falls

abruptly to baseline. The fish were then injected intravenously with methazolamide (25 mg/Kg) and challenged again with NaCl as before, at times varying from 15 minutes to 2 days later. The only change in flow was a slight increase following injection of the drug, which was similar to the flow increase after small amounts (< 2 meq/Kg) of NaCl. It is assumed that this is the glandular response to injection of a salt solution. No decrease in flow, change in time of response to saline or change in ionic composition followed injection of the drug. Determinations of drug concentrations in gland tissue and in secretion showed sufficient amounts for virtually total inhibition of the enzyme.

The second protocol was identical, except that saline stimulation was not used and the baseline flow 18 - 24 hours before and after injection of drug was measured. The only difference in flow was a temporary increase in secretion rate following injection of the drug, as noted before.

The conclusion is that systemic C.A. inhibition does not reduce the secretion of the rectal gland.

It is thought, however, that the effects of inhibition are opposed and thus masked by systemic increase in pH, HCO_3^-, and pCO_2 accompanying use of C.A. inhibitors in this species (Hodler, *et al.*, Am J. Physiol, *183*: 155, 1955). It is well demonstrated in other C.A. systems that changing these entities opposes or mimics the effects of specific inhibition. Specifically, in the rabbit, metabolic alkalosis blocks the reduction of intraocular pressure that is normally elicited by acetazolamide (Am. J. Ophthal, *59*: 291, 1960). It would follow that decreasing pH, HCO_3^- and pCO_2 should decrease secretion of the gland, mimicking the theoretical effect of C.A. inhibition. Preliminary experiments indicate this to be plausible.

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The Chemistry Of CO_2 Accumulation In The Alkaline Gland Of The Skate

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The two earlier reports on this subject in the present *Bulletin* pose the problem of how the alkaline (Marshall's) gland in the skate concentrates CO_2 from plasma through a 50 fold gradient. Table 1 gives typical electrolyte data for *R. erinacea* the species most used in the present studies.

Table 1

	total CO_2	pH	Cl^-	Na^+	K^+	Urea	Osm
Plasma	5.5	7.4	250	280	4	270*	920
Gland Fluid	220	9.3	230	600	7	120	934

data reported as mM/L of fluid

* an additional 100 mM/L is probably Trimethylamine oxide by analogy to the dogfish.