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.

In addition to its alkalinity, the gland fluid shows a second remarkable property: the electrolyte data indicate that 1/3-1/2 of the sodium must be "bound" to a high molecular weight anion. Nothing is known at present about the identity of such a substance.

Analysis of the CO_2 equilibria suggests that transport or formation of HCO_3^- is not alone adequate to explain the data. If gaseous CO_2 has the concentration in gland fluid demanded by the equilibrium for plasma - 0.26 mM/L the pH for the equilibrium between HCO_3^- and gaseous CO_2 in the fluid would invariably be lower than that observed. It appears unlikely that the pCO_2 in gland fluid is lower than that of plasma. The tentative explanation for the high pH values is that the primary process is secretion of OH^-, and the total CO_2 measured is a mixture of HCO_3^- and $CO_3^=$. The role of carbonic anhydrase in *R. erinacea* and also in *R. ocellata* would be of speeding the reaction $OH^- + CO_2 \; HCO_3^-$ (1) which occurs on the secretory border of the cell, the H^+ moving to the blood side and ultimately buffered in the circulation. The data suggest that hydroxyl ion is secreted at such rate that a second reaction $OH^- + HCO_3^- \; CO_3^= + H_2O$ (2) also takes place to some extent. Bimolecular rate constants are available for reaction (1), and it is known that carbonic anhydrase is an effective catalyst even at pH 10. Reaction (2) is instantaneous. It is thus not surprising that reactions (1) and (2) are effective in the absence of enzyme, as in *R. stabuloforis* (1959 report).

Carbonic anhydrase inhibition (methazolamide, 50 mg/kg[i.v.]) was ineffective, in a variety of experimental techniques, in reducing the accumulation of total CO_2 in the gland fluid. The probable reason for this is that such treatment increases plasma and tissue total and gaseous CO_2, thus driving reaction 1 faster. Thus the increase of the uncatalyzed rate offsets the abolition of the enzymic rate. In some experiments, carbonic anhydrase inhibition actually increased gland fluid total CO_2.

Attempts were made to quantify secretion in terms of rate and electrical properties. The isolated gland, which is a sac about 3-5 cm. in diameter, was immersed in a flask of elasmobranch-ringers solution and gassed with 1-5% CO_2. The neck of the sac was tied around a cannula, so that periodic samples could be drawn off. CO_2 was accumulated at the net rate of 0.25$\mu M/cm^2/hr$. In separate experiments it was found that back diffusion from the normally high concentration interior was 0.12 $\mu M/cm^2/hr$. In co-operation with Dr. Daniel Tosteson, experiments with the isolated sac using labelled Cl^- were started, since it appeared possible that Cl^- diffusion or transport oppositely might be a limiting factor in the HCO_3^- accumulation process.

Under the tutelage of Dr. Adrian Hogben, segments of the gland were mounted in a flux chamber, and the electrical characteristics measured. The potential difference was -7.8 mV (negative at secretory surface) and the conductance 8.1 millimhos. This is consistent with active anion transport inward. The electrical data indicate a theoretical potential for active ion transport of 0.57 $\mu M/cm^2/hr$.; the actual ion studies in the isolated sac indicate accumulation of HCO_3^- at a rate approaching this.

It was possible to reproduce the potential measurements using the isolated sac; in this procedure the catheter (filled with bathing solution)

functioned as one bridge, and an agar bridge into the solution bathing the exterior of the gland completed the circuit.

It is felt that this system is conducive to further work on the problems of Na^+ binding and sequestration, OH^- secretion, and HCO_3^- - Cl^- exchange.

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The Structure Of The Nephron Of The Aglomerular Kidney Of The Goosefish (Lophius piscatorius am.).

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A combined light and electron microscopic investigation of the aglomerular tubules of the goosefish kidney established several new facts concerning their structure and possibly gave some clues as to the functional interpretation of these findings in the light of earlier extensive studies of the fine structure of the mammalian glomerular nephron.

By serial sectioning for phase contrast microscopy, three main portions of the aglomerular terminal tubule were identified as having quite different structural appearances: the blind ending (or rather beginning of the tubule), the middle long portion, and the connecting portion which opens up into the collecting duct. Of the various parts of the tubule, the blind beginning and the middle portion were submitted to an electron microscopic analysis. The cells, characteristic of the middle long portion of the tubule, are of two categories: light and dark. In the light cells, the nucleus is easily seen together with basally located abundant mitochondria. A vacuole is usually present in the top part of the cell with a lipid granule inside. The dark cells are rather evenly distributed among the light ones, but there is a predominance for light ones over dark cells. The width of the dark cells varies considerably. In tangential sections, it can be seen that the dark cells are stellate shaped with long and narrow cytoplasmic extensions penetrating inbetween the light cells.

The surface of either cell type is provided with a primitive brush border consisting of short and widely scattered microvilli. The basal part of the cells of the middle portion of the tubule displays a seekingly rudimentary system of membranes, reminiscent of the multiple cell membrane infoldings of the mammalian kidney. The cells of the blind beginning of the tubule lack totally even the rudimentary membranes. From a functional point of view this seems to support the hypothesis that true brush border extensions and a well developed system of infolded basal plasma membranes are necessary requirements in nephrons concerned mainly with resorbtive and concentrating processes like in the glomerular ones, whereas the aglomerular nephrons, in which resorption does not occur, more or less lack both a true brush border and an infolded basal plasma membrane.

The dark cell type which was identified in the middle portion of the