the plasma membranes contain a sodium, potassium, chloride cotransport system. The transport system has sodium, potassium and chloride binding sites, the latter being shared with the loop diuretics.

Thus the model for the rectal gland as proposed by (Silva and coworkers, Am. J. Physiol. 233:F298-306, 1977) can now be modified. Chloride secretion involves first accumulation of chloride inside the cell against its electrochemical potential via a sodium, potassium, chloride cotransport system located in the basal lateral membrane. Sodium extrusion from the cell via Na-K-ATPase results in a favorable gradient for sodium which provides the predominant driving force for the sodium, potassium, chloride cotransport system. Potassium recycling would occur via passive efflux through barium sensitive potassium channels (Silva et al. Bull. MDIBL 21:12-13, 1982) located in the basal lateral membrane. Chloride movement into the lumen would then occur via a sodium independent mechanism which may or may not be linked to potassium.

THE RENAL AND BRANCHIAL HANDLING OF CO_2/HCO_3^- IN THE MARINE ELASMOBRANCH

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CO_2 and HCO_3^- physiology in marine fish differs from that in terrestrial vertebrates. (W.W. Smith, J. Cell. Comp. Physiol. 14:95, 1939) and (Hodler et al (Am. J. Physiol. 183:155, 1955) showed that urine of the marine elasmobranch and teleost has no measurable HCO_3^- and a fixed pH of 5.7-5.8. These species lack renal carbonic anhydrase. Furthermore attempts to alter urinary pH by bicarbonate loading and/or carbonic anhydrase inhibition (Hodler et al., 1955: Boylan et al., Bull MDIBL 13:17, 1973; Murdaugh and Robin, in Sharks, Skates and Rays, 249, 1967 and Swenson et al., this bulletin) or nonbicarbonate base loading (Cohen, J. Cell. Comp. Physiol. 53: 205, 1959 and Swenson et al., this bulletin) have been notably unsuccessful. These fish avidly reabsorb HCO_3^- and maintain an acid urine in order to prevent precipitation of Mg^{++} and Ca^{++} salts (Smith, 1939). The lack of any significant renal acid base regulation in these species thus directed attention to the gills. Hodler et al., 1955 showed that HCO_3^- excretion occurs across the gill and this was confirmed by Murdaugh and Robin (vide supra) in more definitive experiments. This complex organ serves at least four crucial functions: acid-base regulation, nitrogenous waste excretion, NaCl homeostasis and gas exchange. We wished to explore in more detail elements of both the renal and branchial handling of HCO_3^- and CO_2 in the dogfish Squalus acanthias.

METHODS--Male sharks weighing 2 kg were caught by net in Frenchman Bay, Maine and kept in live cars until used. During the experiment they were placed in small boxes with free-flowing seawater (T=15-16°). The urinary papilla and dorsal artery were cannulated with PE-90 polyethylene tubing and the fish restrained in a normal orientation by the use of two wide soft encircling sponge rings that fit snugly in the box and around the fish. Blood pressure was monitored continuously and arterial blood samples drawn aneorobically for measurement of pO_2, pH, pCO_2, inulin and carbonic anhydrase inhibitor concentrations. Only those fish whose blood pressure remained stable (> 30 mm Hg) and arterial pO_2 > 90 mm Hg were used. Urine was collected for measurement of flow rate, pH, inulin, total CO_2 and titratable acid. pO_2 and pH were measured on a standard blood gas analyzer. Plasma and urine total CO_2 were measured manometrically and pCO_2 was calculated from the Henderson-Hassalbalch equation using a pk of 6.1 (Maren, Bull. MDIBL, 11:63, 1971) and a factor of 0.045, for the CO_2/HCO_3^- system. Urine titratable acid was measured by titration of 1 ml of urine to pH 7.8 with 0.1 N NaOH. GFR was measured by use of 2 μci of 14-C-inulin injected at the beginning of the experiment. The experimental design included a two hour period of baseline measurements after the inulin was given, followed by a two hour infusion of 30 m mole of $NaHCO_3$ (15 ml/hr/kg of 1M $NaHCO_3$) and a post infusion period lasting 4 hours. In certain experiments, at the conclusion of the bicarbonate infusion either benzolamide (1 mg/kg) or methazolamide (30 mg/kg) was given.

FIGURE 1

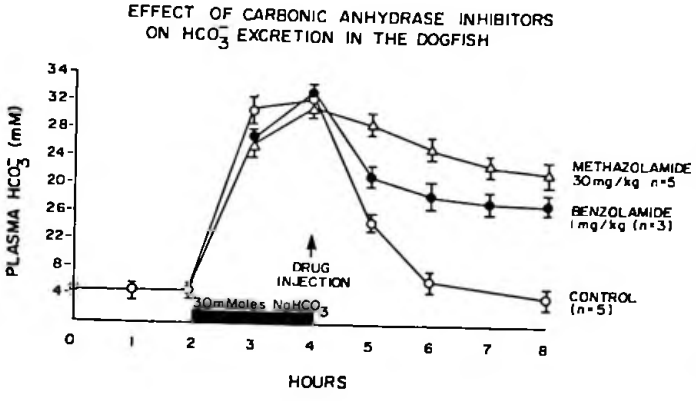


FIGURE 2

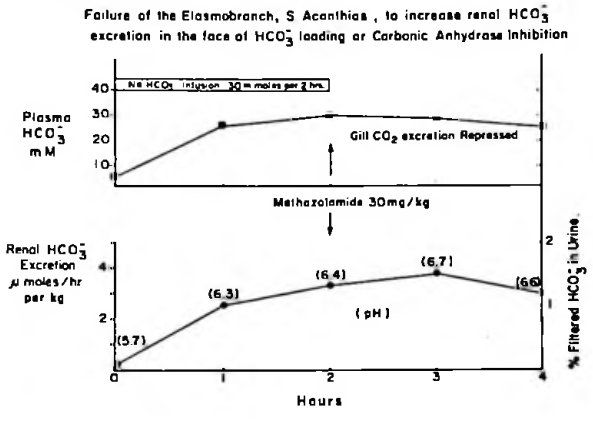
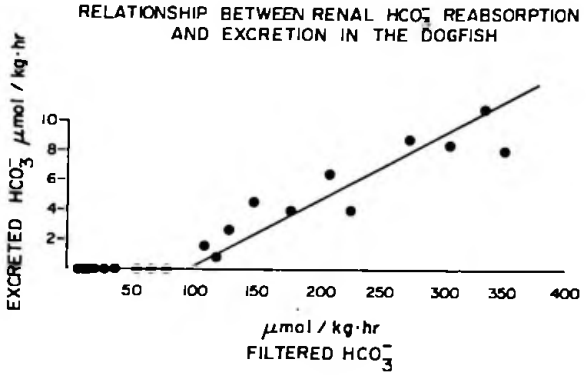


FIGURE 3



In other experiments gill tissue, plasma and erythrocytes were sampled before and 2 hours after benzolamide administration for measurement of drug levels, enzyme activity, and inhibition (Maren, J. Pharmacol. Exp. Therap. 130:26, 1960 and Easson and Stedman, Proc. R. Coc. Lond. Ser B. 121:142, 1936). We tested the effects on urine pH, flow, GFR, CO_2 and titratable acid of 4-pentenoic acid, an inhibitor of renal fatty acid metabolism known to cause bicarbonaturia in mammals (Kleinman et al. Am. J. Physiol. 224: 95, 1973). This was given at three successive increasing doses: 10 mg/kg x 2 hrs, 20 mg/kg x 2hrs, and 100 mg/kg x 2 hrs. Dr. John Boylan has kindly contributed his unpublished data with Dorothy Antkowiak (1974 this laboratory) on the renal effects of sodium maleate, another drug known to cause bicarbonaturia in mammals (Gmaj et al., Am. J. Physiol. 222:1182, 1972). In Boylan's experiments urine flow, GFR, pH, total CO_2, Na, glucose, and K^+ were measured following as much as 1 g/kg/hr x 4 hrs.

RESULTS--The effects of bicarbonate infusion and carbonic anhydrase inhibition are shown in Figure 1. The clearance of HCO_3^- across the gills is quite rapid with a half time of ~50 mins. Our results are similar to those of Murdaugh and Robin (1967). Isotopic data in the normal non-alkalotic dogfish yields a half time of ~ 10-20 mins. (Schooler et al. Comp. Biochem. Physiol. 18:271, 1966). The difference between these values may reflect stress of alkalosis which might alter gill hemodynamics and ventilation-perfusion relationships. Despite this difference, it is clear that the gill is quite effective at CO_2 removal. Methazolamide and benzolamide markedly reduce HCO_3^- clearance. Methazolamide at this dose inhibits fully the enzyme in all tissues. However, benzolamide at low dosage (1 mg/kg) is known to inhibit selectively renal carbonic anhydrase in mammals while not effectively inhibiting red cell enzyme (Maren, Physiol. Rev. 47:595, 1967). We thought benzolamide might provide such selective inhibition in the dogfish (gill vs. red cells) and our prediction held true. We measured intracellular red cell enzyme concentration to be 15 μM and that of gill ~ 0.4 μM. In the experiment of Figure 1, benzolamide levels in the gill are ~ 0.4 μM at two hours and equivalent to the enzyme concentration. With a K_I of 1.5×10^{-8}M and a free plasma level of 1 μM we calculate ~99% inhibition. Red cell drug level (3.3-4.8 μM) is considerably less than the enzyme concentration and thus could yield little inhibition. The initial difference between benzolamide and methazolamide is explained

TABLE 1

Showing the failure of a high HCO_3^- load to influence HCO_3^- reabsorption or acid excretion in a marine fish (Squalus acanthias). (n = 4)

Urine						Plasma		
pH	Flow	GFR	Titratable Acid	$[HCO_3]$	$[CO_2]$	pH	pCO_2	$[HCO_3]$
	ml/hr·kg		mEq/L	mM	mM (mmHg)		mmHg	mM
5.8	1.1	3.0	30	0.1	0.2 (4.6)	7.43	4.6	4.4
$NaHCO_3$ infusion 30 m moles in 2 hours								
6.1	1.9	3.5	35	0.6	0.6 (13)	8.16	6.0	30

In terms of mammalian physiology, an extraordinary result:

1. Increasing filtered load of HCO_3^- 7- fold does not affect HCO_3^- excretion.
2. Titratable acid does not change in the face of greatly increased HCO_3^- reabsorption, suggesting that these are independent events.

TABLE 2

EFFECTS OF CARBONIC ANHYDRASE INHIBITORS ON CO_2 EQUILIBRIA IN SQUALUS ACANTHIAS

Hrs	Plasma			Drug Concentrations (μM)		
	pH	pCO_2 mm Hg	HCO_3^-, mM	plasma	rbc	gill
Benzolamide* 1 mg/kg						
0	7.54	6.5	8.2	0	0	0
2	7.20	15.0	9.0	1.0	3.3	0.4
6	7.50	12.1	13.8	0.4	3.6	0.2
24	7.67	10.2	16.2	< 0.2	4.0	< 0.2
Acetazolamide+ 30 mg/kg						
0	7.52	5.6	7.0	0	0	0
2	7.20	13.2	8.2	110	100	--
6	7.45	11.3	12.0	99	--	--
24	7.54	9.3	15.0	76	82	--

Drugs injected at 0 time, intravenously.

*From Maren and Maren. Bull. Mt. Desert Island Biol. Lab. 13:38, 1963.

+From Maren, Comp. Biochem. - Physiol. 5:201, 1962.

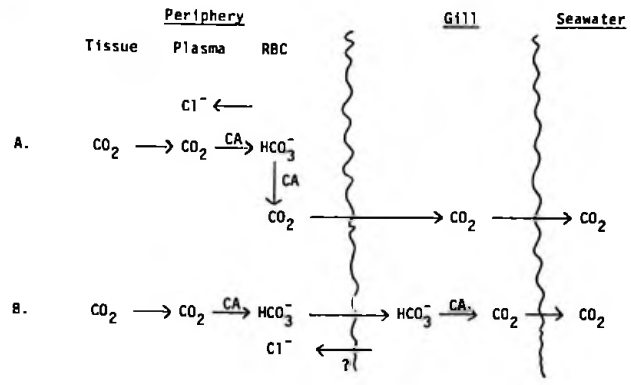
by the slower diffusibility into tissues of benzolamide (Holder and Hayes, Mol. Pharm. 1:266, 1965) which permits an early uninhibited rate of HCO_3^- loss before sufficient enzyme inhibition has occurred.

Figure 2 shows the renal effects of HCO_3^- loading and carbonic anhydrase inhibition. The elevation of plasma HCO_3^- from 4 to 30 mm produces only a trivial degree of bicarbonaturia, less than 0.1% of the HCO_3^- excretion rate of the gill. Furthermore, methazolamide is without effect on HCO_3^- reabsorption, since no further increase in HCO_3^- excretion occurs. Figure 3 plots the relationship between filtered and excreted HCO_3^-. It shows that some bicarbonaturia occurs above a filtration rate of 100 μeq/hr x kg (five to ten-fold the normal filtration of HCO_3^-). There is no TM for HCO_3^- since there appears no upper limit to bicarbonate reabsorption. This is also true in the mammalian kidney (Garg, J. Pharmacol. Exp. Therap. 194:96, 1975). Boylan et al. (1973), Hodler et al. (1955) and Murdaugh and Robin (vide supra) reported no bicarbonaturia when plasma HCO_3^- was < 60 mM. Analysis of their data suggests that either they failed to give enough HCO_3^- in relation to the GFR or the hyperbicarbonatemia was not sustained long enough to generate a filtration of > 100 μmol/hr · kg. Table 1 shows that there is no effect of alkalosis and increased HCO_3^- is reabsorbed proximally in the nephron independent of acid secretory mechanisms. This agrees closely with Deetjen and Maren (Pflugers Arch. 346:25, 1974) who showed that the rate of urinary acidification in the isolated perfused skate nephron was independent of HCO_3^- reabsorption.

We could show no effect of 4-pentenoic acid on urine flow, pH, titratable acid or GFR. Dr. Boylan also failed to demonstrate any effects of sodium maleate on urine flow, pH, Na^+, K^+, glucose pyruvate and GFR. The experiments with sodium maleate and 4-pentenoic acid were in normal nonalkalotic animals; it would be of interest to retest them in the HCO_3^- loaded animal.

Our results with benzolamide and HCO_3^- loading (Figure 1) are qualitatively similar to those of Maren and Maren (Bull MDIBL 5(1):38, 1963) who studied the effects of benzolamide (1 mg/kg) on CO_2 metabolism in the normal animal. Table 2

Figure 4. Paths of Excretion of HCO_3^-/CO_2 in S. acanthias



CA = carbonic anhydrase

Plasma interface at gill surfaces not shown.

Only primary reactions are shown.

If only A were operating (as in the mammal, with lung in place of gill, and air for sea), benzolamide should not have any effect. Since the locus of benzolamide effect appears to be the gill, a scheme akin to B must be considered.

shows these and similar data for acetazolamide (Maren, Comp. Biochem. Physiol. 5:193, 1962). The effects of acetazolamide and benzolamide in the normal fish are equivalent. Both produce an acute respiratory acidosis within two hours that persists approximately 48 hours. As discussed in connection with Figure 1, however (vide supra), the benzolamide data localizes the effective enzyme inhibition to the gill.

Our results unexpectedly demonstrate that the elimination of administered HCO_3^- or metabolic CO_2 in this species species primarily involves gill carbonic anhydrase, not the red cell enzyme. Haswell et al (Am. J. Physiol. 238:R240, 1980) showed that CO_2 exchange across the in situ isolated perfused gill of the rainbow trout was reduced by carbonic anhydrase inhibition, and proposed that only the gill enzyme functions in CO_2 exchange. However, Perry et al (J. Exp. Biol., 1982, in press) have reached the opposite conclusion, based on experiments in the isolated saline perfused trout holobranch preparations; these were unable to excrete HCO_3^-/CO_2. Thus the overall picture in fish is not clear. Our results in the salt water elasmobranch could certainly be different from those in the fresh water teleost, but are still surprising since normal CO_2 exchange in the lung is clearly dependent on red cell carbonic anhydrase (Swenson and Maren, Resp. Physiol. 35:129, 1978). We have previously shown that red cell enzyme in S. acanthias is necessary for rapid attainment of the Bohr effect (Maren and Swenson, J. Physiol. 303:535, 1980).

Figure 4 depicts an outline of the situation and may explain our results following benzolamide, namely the increase in blood pCO_2 in the normal fish and the failure of the fish to excrete a HCO_3^- load rapidly. For this effect to be localized at the gill, scheme B must be a dominant means of excretion of HCO_3^-/CO_2. When this is blocked in the normal fish, the reaction $HCO_3^- \xrightarrow[CA]{gill} CO_2$ is no longer primary, and loss of CO_2 proceeds by diffusion of molecular CO_2, across newly enlarged gradients (Scheme A). Thus pCO_2 is elevated. This type of adjustment is analagous to what occurs in the mammal when red cell carbonic anhydrase is inhibited (Swenson and Maren, vide supra). In the case of the HCO_3^- loaded fish, the same reaction at the gill is critical for excretion of the ion, and when it is blocked, plasma HCO_3^- remains elevated.

In conclusion we have confirmed an avid renal HCO_3^- reabsorptive mechanism in the elasmobranch, independent of carbonic anhydrase, and capable of reabsorbing 10 times the normal filtered HCO_3^-. There is no upper limit to absolute HCO_3^- reabsorption despite a small degree of bicarbonaturia when filtration is greater than 100 μEq/hr · kg. Titratable acid excretion is unaffected by large increases in HCO_3^- reabsorption. Fixed renal mechanisms exist to ensure a constant acid urine even during systemic alkalosis. The gill in these species carries out the catalytic dissimilation of $HCO_3^- \longrightarrow CO_2$ for normal metabolic purposes and for acid base regulation. Our results with general and selective carbonic anhydrase inhibition suggest that red cell enzyme in the elasmobranch is not critical for normal CO_2 exchange, a striking departure from the physiology of CO_2 exchange in lungs. This work was supported by NIH Grant HL-22258.