FURTHER STUDIES ON ELASMOBRANCH CO_2 AND HCO_3^- EXCRETION: ROLES OF BRANCHIAL AND ERYTHROCYTE CARBONIC ANHYDRASE IN SQUALUS ACANTHIAS.

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Although we have shown that the elimination of metabolic CO_2 and normalization of plasma HCO_3^- following a HCO_3^- load in the dogfish depends on branchial carbonic anhydrase (CA) (Swenson, et al, Bull MDIBL 22: 72, 1982 and Maren, Comp Biochem Physiol 5:201, 1962), others (Perry et al, J Exp Biol 101: 47, 1982) have claimed that red cell CA is primarily responsible. Since the functions of red cell and gill CA in fish CO_2 exchange are controversial, we examined the effects of two CA inhibitors with differing actions against these enzymes with the aim of 1) quantifying the contributions of gill, rectal gland and intracellular buffering to HCO_3^- clearance following a HCO_3^- load and 2) defining the roles of red cell and gill CA in HCO_3^- excretion and CO_2 exchange in the resting and swimming fish.

Methods: Unanesthetized Squalus acanthias (2 kg) were used, except in experiments involving rectal gland fluid measurements, in which the fish were pithed. On the day prior to experimentation the dorsal artery was cannulated for drug/fluid administration and blood sampling. Free swimming fish were allowed unrestrained movement in a large holding tank. Resting non-swimming fish were transfered to a small darkened box 24 hours before the study. The effects of methazolamide (30 mg/kg) and benzolamide (1 mg/kg) were studied in resting and swimming fish for their effects on both HCO_3^- excretion and CO_2 exchange. These doses of methazolamide and benzolamide are those in which methazolamide totally inhibits both red cell and gill carbonic anhydrase whereas benzolamide results in selective gill enzyme inhibition (Swenson, et al, Bull MDIBL, 22:72, 1982). There were four major groups studied.

Group I: Resting Fish/HCO_3^- Infusion: Seven control fish received 18 mmoles of $NaHCO_3$ over one hour by constant infusion. [Three of these fish were used to measure possible rectal gland fluid contributions to HCO_3^- excretion. The rate of normalization of plasma HCO_3^- in these three fish did not differ from four unanesthetized controls.] Plasma HCO_3^- clearance was measured by hourly determinations of arterial pH, PCO_2, total CO_2 and PO_2. To measure appearance of HCO_3^- into seawater during and following the HCO_3^- infusion, it was necessary to establish a temperature controlled (14-15^oC) aerated recirculating reservoir of seawater (5 liters). Hourly determinations of seawater for temperature, total CO_2, pH, PCO_2, and PO_2 were made to calculate HCO_3^- excretion into seawater and monitor the ambient oxygen content and temperature. The possibility that HCO_3^- excreted by the fish might be lost to the atmosphere as CO_2 (from aeration and recirculation) was examined by following the total CO_2 content of seawater in the same system without a fish present. In over four hours there was no loss of HCO_3^- (<0.2mM) in this open system, when the seawater [HCO_3^-] was varied between 2 to 5 mM. Arterial, seawater and rectal gland fluid pH and PO_2 were measured by a blood gas analyzer (IL-213, Instrumentation Laboratories), maintained and calibrated with standards at 14^oC. Total CO_2 of plasma, seawater and rectal gland fluid were measured with a Kopp-Natelson microgasometer. PCO_2 and HCO_3^- were calculated from the Henderson-Hassalbalch equation, using a pk'_a of 6.07 and α factor of 0.052 for the CO_2/HCO_3^- system at 14^oC and ionic strength of seawater and dogfish plasma (Pleschka and Wittenbrock, Pflugers Arch 329:186, 1971). Four fish were given methazolamide and four fish were given benzolamide 15 minutes before the start of the HCO_3^- infusion.

Group II: Swimming Fish/HCO_3^- Infusion: The protocol and timing of drugs, HCO_3^- infusion and sampling were similar to that described above. Except for the 1 hour period of HCO_3^- infusion, the fish were able to swim freely. Methalozamide or benzolamide were given 15 minutes before the HCO_3^- infusion.

Group III: Resting Fish/Normal Acid-Base Status: These fish were treated in a similar fashion as those in group I, but in this case no HCO_3^- infusion was given. The effects of methazolamide or benzolamide were tested in four fish each. No control subset was included, since it has been shown that no measurable acid base changes occur in fish when give a sulfonamide that lacks activity against carbonic anhydrase (Maren, Exp Eye Res 16: 403, 1973).

Group IV: Swimming Fish/Normal Acid-Base Status: These fish were treated in an equivalent manner as those in group II, except that there was no infusion of bicarbonate. The effects of methazolamide and benzolamide were tested in three fish each. No control subset was included.

Results: The effects of bicarbonate infusion and carbonic anhydrase inhibition in resting fish (Group I) are shown in Figure 1. In control fish the disappearance of plasma HCO_3^- is rapid with a half time of 50 minutes with almost complete correction in three hours. This loss of plasma HCO_3^- is primarily the consequence of clearance across the gills since our measurements of HCO_3^- appearance in the recirculated seawater matches the plasma loss. Both CA inhibitors markedly and equally reduce the HCO_3^- clearance. In swimming fish the normalization of plasma HCO_3^- was faster than that in resting fish. However the effects of the two inhibitors were equal (Table 1). Despite a rise in plasma HCO_3^- to 38mM at 1 hour and 10mM at 4 hours, rectal gland fluid total CO_2 concentration rises only from 1.5mM to 3.5mM at 1 hour and returns to 1.5mM at 4 hours. Rectal gland bicarbonate excretion totals only 0.05 mEq over the entire 4 hours, approximately 2% of the infused bicarbonate.

Table 1 also shows the acid base data in resting and swimming animals given methazolamide and benzolamide without HCO_3^- infusion. Methazolamide causes an acute respiratory acidosis which is nearly compensated by 4 hours. The surprising finding is that benzolamide causes no alteration in CO_2 exchange or acid base status in resting fish. Methazolamide causes a more severe respiratory acidosis when compared to resting fish. Benzolamide in swimming fish causes only mild respiratory acidosis. We ascribe the greater respiratory acidosis in swimming fish given these drugs to the differences in CO_2 production between rest and swimming. We observed these same differences in PCO_2 between the resting and swimming animals given bicarbonate (Table 1).

Discussion: The chief findings are that benzolamide (1 mg/kg) and methazolamide (30 mg/kg) produce equally profound HCO_3^- retention in S. acanthias following $NaHCO_3$ infusion. However the effects of these drugs differ in regard to CO_2 exchange in the normal and alkalotic fish. In resting fish only methazolamide causes respiratory acidosis. In swimming fish a respiratory acidosis develops with both drugs; quantitatively greater with methazolamide. These findings will be related to the functions of red cell and gill carbonic anhydrase in HCO_3^-/CO_2 excretion.

The gill is the primary and possibly the only site of regulation of acid base balance in elasmobranchs (Heisler, Fish Physiol Volume 10, Academic Press 1984). While previous studies have dealt mainly with respiratory or metabolic

Table 1. ACID BASE STATUS IN ALL GROUPS TWO HOURS AFTER DRUG ADMINISTRATION

	Control			Methazolamide: Gill and RBC Inhibition			Benzolamide: Gill Inhibition Only		
	pH	pCO_2 (mmHg)	HCO_3 (mM)	pH	pCO_2 (mmHg)	HCO_3 (mM)	pH	pCO_2 (mmHg)	HCO_3 (mM)
Group 1									
Resting fish: HCO_3^- infusion	8.08	3.2	17.6	7.94	7.1	28.0	8.29	3.0	26.5
Group 2									
Swimming fish: HCO_3^- infusion	7.84	1.9	6.1	7.78	8.2	21.7	7.97	5.4	22.9
Group 3									
Resting fish: Normal	7.88	2.0	6.3	7.64	4.4	9.0	7.90	2.1	7.7
Group 4									
Swimming fish: Normal	7.83	2.1	6.2	7.33	7.8	8.0	7.62	4.1	7.9

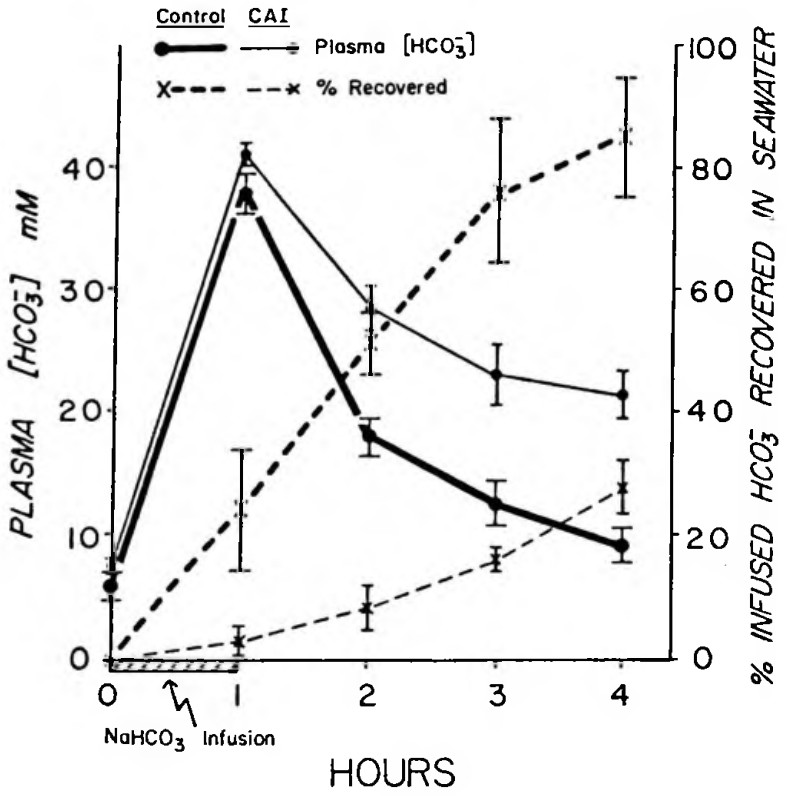

FIGURE 1

Plasma HCO_3^- and HCO_3^- excretion in the dogfish following a 9 mEq/kg $NaHCO_3$ infusion and the effects of carbonic anhydrase inhibition (CAI). Values are means + SE.

acidosis, these studies confirm the primacy of gill in correction of metabolic alkalosis. Previously, we have shown that there is no renal response to alkalosis (Swenson et al Bull MDIBL 22: 75, 1982) and our new results show no rectal gland contribution. Our calculations show that the peak extracellular to intracellular transfer of HCO_3^- in these experiments is only 25% of the infused HCO_3^- load and in the normal animal only 10% of infused bicarbonate remains buffered by the intracellular space at four hours.

The results of carbonic anhydrase inhibition suggest that HCO_3^- excretion involves the catalyzed conversion of HCO_3^- to CO_2 at the gills. Furthermore since these drugs equally inhibit HCO_3^- excretion, and yet differ in their specificity for red cell and gill enzyme, we believe that the important reaction ($HCO_3^- \rightarrow CO_2$) occurs in the gills themselves and not the red cell.

In contrast the results of selective gill and combined red cell and gill enzyme inhibition on CO_2 exchange (excretion of metabolic CO_2) suggest that here both gill and red cell enzyme are involved. Analysis of the blood gas data shows a greater respiratory acidosis with methazolamide than benzolamide in all cases. In swimming fish (Groups II and IV) both drugs cause hypercapnia (greater than can be accounted for by the metabolic alkalosis in Groups I and II), but only methazolamide causes hypercapnia in resting fish (Groups I and III). We interpret these findings to suggest that in a resting animal, red cell carbonic anhydrase activity is sufficient to maintain normal CO_2 exchange, since in the benzolamide treated animals (total gill enzyme inhibition) there are no changes in arterial blood (Table 1). However in the swimming fish whose metabolism and CO_2 production are presumably greater, the gill enzyme becomes necessary; thus inhibition of gill carbonic anhydrase by benzolamide results in a respiratory acidosis (Table 1). Inhibition of both red cell and gill enzyme is additive since methazolamide produced a even greater respiratory acidosis.

Selective red cell carbonic anhydrase inhibition is not possible in the whole animal so that we must infer the role of gill enzyme from differences in gill versus gill and red cell carbonic anhydrase inhibition. However we have shown that CO_2 excretion is abolished with benzolamide in the isolated saline perfused dogfish pup head preparation, where red cells are absent (Swenson and Evans, Bull MDIBL, this volume). These experiments confirm our present findings in the whole animal that gill carbonic anhydrase can function significantly in CO_2 and HCO_3^- exchange. This should not be surprising since all the necessary pathways exist in the gill for CO_2 and HCO_3^- uptake out of plasma and their elimination into seawater.

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