THE RENAL AND BRANCHIAL RESPONSES TO METABOLIC ALKALOSIS AND ACIDOSIS IN THE MARINE TELEOST, MYOXOCEPHALUS OCTODECIMSPINOSUS

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This is a continuation and completion of work reported in 1989 (Bull. Mount Desert Island Biol. Lab. 29, 62-65, 1990). The background and procedures are given there, as well as these findings: The marine teleost, unlike the elasmobranch, can vary urine pH from 5.8 to 8.2, despite lack of renal carbonic anhydrase. Titratable acid can increase in response to a buffer load, through mechanisms which appear quite different from those in the mammal.

In the present work we quantify further these responses, study metabolic acidosis and phosphate excretion, and show the time course of disappearance of acid and alkali load from the long-horn sculpin, Myoxocephalus octodecimspinosus.

I. The Response to Alkalosis. We used 12 meq/kg $NaHCO_3$ (made up fresh as 1 M solution) intravenously. Figure 1, Curve A shows the plasma concentration of HCO_3^- from 1-8 hours after injection. Control HCO_3^- is 5.6 mM, pH 7.5 and pCO_2 5 mm Hg. The 6-fold rise in plasma HCO_3^- at 1 hr is reduced to half at 4 hours. pH at the times 1, 2, 4, 8 hours was 7.94, 7.96, 7.71 and 7.6. The pCO_2 was 9-11 mm Hg throughout. A notable point is that the linear decay from 1-4 hrs is not maintained but slows remarkably as plasma HCO_3^- approaches normal. We presume that HCO_3^- loss in metabolic alkalosis is through carbonic anhydrase catalysis of $HCO_3^- \rightarrow CO_2$ at the gills, as shown for the elasmobranch (Swenson and Maren, Am. J. Physiol. 253, R450, 1987). Comparison of Curves B (8 meq/kg $NaHCO_3$ + methazolamide to inhibit carbonic anhydrase) and Curve C (8 meq/kg $NaHCO_3$ alone) confirms this for the teleost; 2 hours after the $NaHCO_3$ injection, plasma HCO_3^- was twice

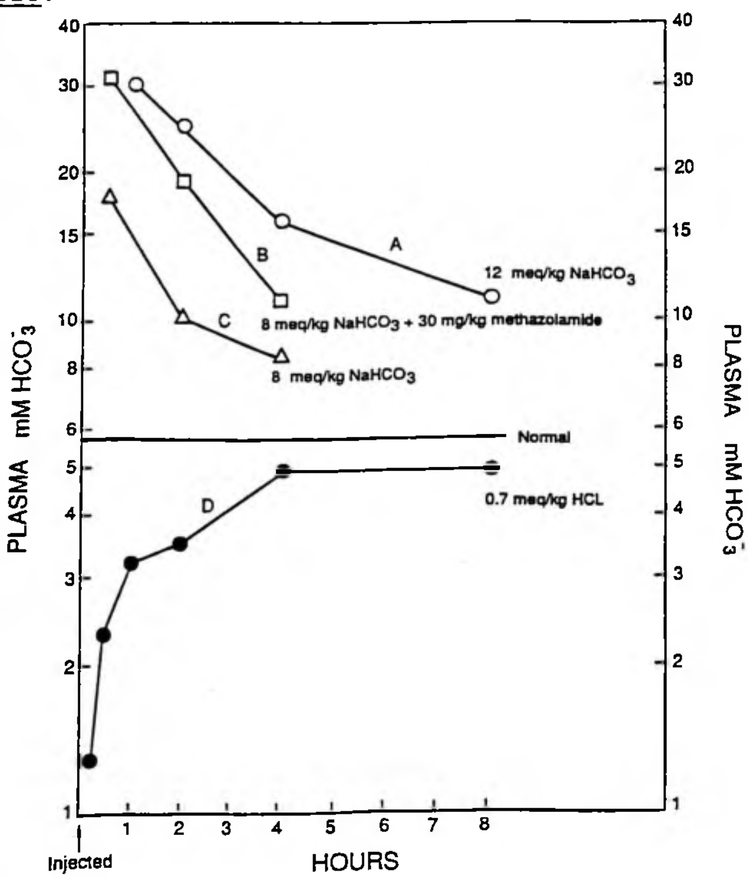

FIG. 1. Plasma HCO_3^- in sculpin following injection of $NaHCO_3$ (A,C) or HCl (D). Effect of carbonic anhydrase inhibition on HCO_3^- disposal (B).

as high in the inhibited (Curve B) fish as in its control (Curve C). In the experiment of Curve B, there was a marked respiratory acidosis due to inhibition of red cell carbonic anhydrase. Plasma pH and pCO_2 at 1/2, 2, and 4 hrs were respectively 7.74, 18; 7.43, 21, and 7.47, 11.

TABLE 1. THE RENAL RESPONSE TO METABOLIC ALKALOSIS AND ACIDOSIS IN THE LONG-HORN SCULPIN

	1	2	3	4	5	6	7	8
	URINE					PLASMA MID-POINT		
	Flow ml/kg·hr	pH	CO_2 millimolar	TA*	PO_4	HCO_3^- mM	pH	pCO_2 mm Hg
A. Control, 0-4 hr (8)	1.4	6.64	3	11	12	5.6	7.5	6
B. $NaHCO_3$ 12 meq/kg (4)								
0-4 hr	1.3	7.8	20	0	21	24	7.96	8
4-8 hr	1.4	6.7	4	12	16	13	7.60	10
C. Methazolamide Series, 0-4 hr								
1. 30 mg/kg alone (4)	0.9	6.64	4	11	17	5.4	7.3	9
2. 8 meq/kg $NaHCO_3$ alone (15)	1.1	7.0	6	9	14	10	7.64	7
3. (1+2) Meth. + $NaHCO_3$ (5)	2.1	6.8	10	--	--	19	7.74	21
D. HCl 0.75 meq/kg (6)								
0-4 hr	1.2	6.13	<1	39	29	3.5	7.28	6
4-8 hr	1.5	6.32	<1	22	39	5.0	7.42	5

* Titratable acid. ( ) gives number of fish.

The renal response to 12 meq/kg $NaHCO_3$ is shown in Table 1, Row B. The pH rises to 7.8 and total CO_2 (essentially HCO_3^-) in urine increases 7-fold, to 20 mM, or 26 μeq/kg hr^{-1}, about 100 μeq/kg for the 4-hr period of intense alkalosis. It is recognized that this is only about 1% of the injected alkali; nearly all is disposed through the gill and/or by slow intracellular buffering. In the elasmobranch, Squalus acanthias, some 80% of injected $NaHCO_3$ is excreted by gill (Swenson and Maren, ibid); in the freshwater catfish, Ictalaurus punctatus, the amount is 50% (Cameron and Kormanik, J. Expt. Biol. 99, 143, 1982).

Experiments of Row C show 1) That inhibition of carbonic anhydrase has no effect on renal acid-base values but causes a slight respiratory acidosis; 2) That 8 meq/kg $NaHCO_3$ has a relatively small effect on acid-base values, compared to 12 meq/kg (Row B); and 3) That 1 + 2 causes profound retention of HCO_3^-, and when branchial excretion of HCO_3^- is so greatly reduced (see also Fig. 1), its renal excretion increases, from 6.8 to 21 μeq/kg hr^{-1} (Col. 1 x Col. 3). Figure 1 and Table 1 show that a large load of $NaHCO_3$ is "dumped" very rapidly through the catalytic dehydration mechanism. However, when plasma HCO_3^- levels reach about 10 mM, still well above normal, the system is relatively indifferent, just as normal plasma HCO_3^- of 5-6 mM is preserved despite the large gradient from fish to ocean.

II. <u>The Response to Acidosis</u>. The maximum tolerated dose is 0.75 meq/kg HCl, given intravenously. Since the fish has about 5 meq/kg of HCO_3^- in plasma representing total body fluids, there is only about 1-2 meq HCO_3^- in the extracellular fluid of a 1 kg fish. Thus when 0.75 meq/kg is injected, plasma HCO_3^- falls to 1.3 mM in 10 minutes and 2.3 mM in 30 minutes. Fig. 1, Curve D, shows the course of plasma HCO_3^- to recovery in 4 hours. Similar curves have been generated in <u>S. acanthias</u> (Swenson and Claiborne, Bull. Mount Desert Island Biol. Lab. 26, 5, 1986), the freshwater catfish, <u>I. punctatus</u> (Cameron and Kormanik, ibid) and in the marine lemon sole, <u>Parophys vetulus</u> (McDonald et al., J. Exp. Biol. 98, 403, 1982).

Table 1-D shows that the kidneys respond briskly to the acidosis. In the first 4 hours titratable acid in urine increase nearly 4-fold in concentration and output, diminishing to 2-fold over normal in 4-8 hours. During this period plasma acid-base balance reverts to normal (Fig. 1 and Table 1-D). The acid excretion in the 8-hour period may be calculated from the concentration and flow data of Table 1-D as 319 µeq/kg. Subtracting the control value of 123 µeq/kg for the 8-hour period yields 196 µeq/kg or 26% of the injected acid, most of which was excreted in the first 4 hours. The remainder is excreted by the gills, as suggested for other teleost species, the marine <u>P. vetulus</u> (McDonald et al., ibid) and freshwater <u>I. punctatus</u> (Cameron and Kormanik, ibid). More directly in the present context, in long-horn sculpin, given 0.75 meq/kg, J. D. Claiborne has found a large branchial excretion of acid (personal communation).

Our finding of a substantial albeit minor component of renal acid excretion (26%) during metabolic acidosis differs from McDonald et al. (ibid) who found no renal response to acid in <u>P. vetulus</u>. This may have been due to their longer (12 hr) collection periods since our major response was at 0-4 hours. Our data agree with similar experiments in the freshwater catfish (Cameron and Kormanik, ibid) who found that 16% of an acid load was excreted renally, and with King and Goldstein who gave an acid load to <u>S. acanthias</u> (Am. J. Physiol. 245, R581, 1983) and found 15% excreted by the kidney.

The renal response to acid loading is accompanied by an increase in phosphate excretion, from 12 to 29 mM. This represents secreted phosphate, as discussed earlier (Maren et al., Bull. Mount Desert Island Biol. Lab. 29, 62-65, 1990). It is likely that urinary phosphate subserves acid excretion in fish, as the only or chief buffer. Renal NH_4^+ excretion is very small--some 10% or less of titratable acid (McDonald et al., ibid).

In conclusion: The marine teleost can regulate urinary pH, in the absence of renal carbonic anhydrase. In the response to alkalosis, however, the renal response is insignificant because of the very effective branchial response, involving the catalytic reaction $HCO_3^- \rightarrow CO_2 + OH^-$. In acidosis the kidney handles about one quarter of the imposed load, the gill (by analogy to the elasmobranch) probably excretes the remaining acid by mechanisms that do not involve carbonic anhydrase (Claiborne and Swenson, ibid). In phylogenetic terms, marine teleosts are the first to show these renal responses to acid and base, however small. All "higher" vertebrates starting with freshwater fish, have kidney carbonic anhydrase and larger renal responses to acid-base changes.