RENAL ACIDIFICATION AND ALKALINIZATION IN THE MARINE TELEOST, MYOXOCEPHALUS OCTODECIMSPINOSUS

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Urinary pH and its regulation in marine teleosts has been a curiously neglected subject. The first information was tucked into a paper by Willie W. Smith on pH and phosphate of elasmobranch urine. She also cited work on the marine long-horned sculpin, Myoxocephalus octodecimspinosus (J. Cell. Comp. Physiol. 14, 95-102, 1939). In 55 fish "collected by Dr. Homer W. Smith and Mr. J. H. Tarofsky at Salsbury Cove they found the pH of urine to average 5.72 ± S.D. of 0.35." They went on to inject 0.1 to 1.0 ml of 10% Na_2HPO_4; the mean pH was 5.95 (14 fish, 28 determinations). Injection of 0.5 - 4 ml of 10% $NaHCO_3$ (23 fish, 30 determinations) likewise did not change urinary pH, which remained at 5.81. Thus it appeared settled that the sculpin had fixed urinary pH, and this seemed confirmed 16 years later by Hodler, Heinemann, Fishman and H. W. Smith (Am. J. Physiol. 183, 155-162, 1955), who reported pH of 6.25 in 38 fish and no change following carbonic anhydrase inhibition. These data were precisely what was found in the marine elasmobranch. The doctrine emerged that all marine fish had a fixed urinary pH of about 6 and that this could not be altered by acid-base changes or by acetazolamide, since the kidneys lacked carbonic anhydrase. (Reviewed by Maren, Bull, Mount Desert Island Biol. Lab. 27, Supplement, p. 28, 1987.)

These ideas were confirmed many times for the elasmobranch (Swenson and Maren, Am. J. Physiol. 250, F288, 1986), but weaknesses had appeared sporadically in the application to teleosts. Hickman reported urinary pH in the southern flounder, Paralichthys lethostigma, ranging from 5.7 - 8.2, while living in sea water (Can. J. Zool. 46, 439-455, 1968). Perhaps this was ignored because this is a euryhaline species. More serious was the report of Compton-McCullough et al. (Bull. Mount Desert Island Biol. Lab. *29*, 44-45, 1989) that the urine of M. octodecimspinosus, the same species used by the Smith group (vide supra) had a urinary pH ranging from 6.2 - 7.6. We therefore reopened the question of urinary pH control in the same species, the long-horned sculpin.

Fish were caught by trawl and placed in large tanks of running sea water. At the start of each experiment they were put in deep trays of cold sea water containing 1:10,000 MS 222, with gills immersed. Blood was withdrawn and injections made through tail vein or in some cases intraperitoneally. The urinary papilla was tied, and at end of each experiment blood was withdrawn from the tail vein, fish were killed, and urine collected from the bladders. Throughout the experiment the fish were swimming freely in the tank. Chemical analysis for CO_2 was done with the Kopp-Natelson microgasometer, for titratable acid (TA) by titration to pH 7.8 with 0.1 N NaOH, and for phosphate by the colorimetric molybdate method.

We report on four conditions: I. Untreated controls; II. Fish receiving $NaHCO_3$; III. Fish receiving methazolamide to inhibit carbonic anhydrase; IV. Fish receiving imidazole buffer to stimulate urinary acid secretion (A), and the same protocol (B) with added methazolamide.

I. Table 1 gives normal flow and secretory values from the literature, to put our data in perspective. Note the relatively high urine flow and low filtration (GFR) compared to mammals and the very high secretory rate for p-amino hippurate (PAH) compared to GFR. When urine flow is low, U/P for phosphate can rise to about 25. The data given are for 1 - 2 days after capture, during moderate laboratory diuresis (Grafflin, Biol. Bulletin 71, 360-374, 1936; Forster, J. Cell. Comp. Physiol. 42, 487, 1953) and is the phase during which we worked. The lower urine flow for the 24 hour period (Table 2, compare entries in Row A) reflects filling of the bladder, since the normal flow of about 10 ml per day exceeds or is at the limit of bladder capacity. This accounts for the lower values in the early literature.

TABLE 1. FLOW AND CLEARANCE CONSTANTS IN LONG-HORNED SCULPIN

Urine Flow	GFR	PAH	Inulin	PO_4
	$ml/hr \cdot kg^{-1}$		U/P	
1.5 (F)	2.9 (F)	108 (F)	2 (F)	
1.4 (Present)				3-6 (Present)

(F) = Forster, see text.

Table 2, Row A, shows the normal urinary pH of 6.64, considerably higher than that reported by the Smith group but well in line with Compton-McCullough et al. (vide supra) in the same species and by Hickman in southern flounder. It also agrees with our data on the winter flounder, <u>Pseudopleuronectes americanus</u> (pH 6.7 - 6.9) from many years ago (Bull. Mount Desert Island Biol. Lab. 4, #4, 57, 1962). Note the close agreement between phosphate concentration and titratable acid (T.A.) showing that phosphate is the sole urinary buffer. The plasma acid-base equilibrium using pK_a = 6.2 (reflecting T° = 17° and osmolarity of 300 mM) shows a slight respiratory acidosis, HCO_3^- = 5.5 mM, pCO_2 = 5 mm Hg, pH 7.5, probably the result of handling.

II. Table 2, Row B, shows that 8 millimole/kg $NaHCO_3$ provoked a modest rise in urinary pH and total CO_2 and decrease in titratable acid. Fig. 1 shows the very large rise in plasma HCO_3^- in the first hours after injection, with rapid return to normal. This illustrates the great capacity of the gill to unload HCO_3^-. By analogy to the elasmobranch, this is through the catalytic dehydration to CO_2 (Swenson and Maren, Am. J. Physiol. 253, R450, 1987). Histochemical analysis by Dr. Per Wistrand (personal communication) shows high concentrations of carbonic anhydrase in gill membranes of both sculpin and dogfish. The HCO_3^- excreted in urine is less than 1% of that injected. Table 2, Row C, shows that 16 millimole/kg of $NaHCO_3$ produces a sharp alkalinization of the urine and disappearance of T.A. This dose is quite toxic, and some fish did not survive. Thus we find, in contrast to the experiment of H. W.Smith and Tarofsky cited by W. W. Smith (vide supra), that the sculpin can alkalinize its urine. It had been thought that the low fixed urinary pH of marine fish was significant in protecting them against precipitates of calcium and magnesium phosphates in the bladder. This may be true of the elasmobranch (pH 5.8), and no precipitates are ever seen. But both Grafflin (vide supra) and Pitts (J. Cell. Comp. Physiol. 4, p. 389, 1934) observed precipitates in the sculpin bladder. Grafflin emphasizes however that in 456 sculpins examined, calculi were never encountered. The question naturally arises whether teleosts have a substance that prevent fine precipitates from becoming concretions at high pH.

III. Table 2, Rows D-F, shows three series of experiments in which methazolamide was injected, at differing times, routes and dosages. No effect of the drug was observed. Following 50 mg/kg intravenously, plasma concentrations were approximately 200 μM at 4 hours and 140 μM at 48 hours. At 4 hrs less than 0.1% of the administered drug appeared in the urine. Since the K_I of methazolamide against this enzyme is 10^{-8} M and plasma concentration is $> 10^{-4}$ M, the inhibition in any tissue with enzyme is > 99.99%. The highest dose (500 mg/ kg) is 100 x greater than required to alkalinize the urine in bird or mammal or fresh water fish (Maren, Physiol. Rev. *47*, 595 1967). Here inhibition is so great that only 1 part enzyme in 10^6 is free.

TABLE 2. URINARY ACID-BASE STUDIES IN THE LONG-HORNED SCULPIN

	Urine						Plasma Initial and Final	
	Flow	pH	TA	PO_4	CO_2	TA Output	CO_2	pH
	ml/kg·h^{-1}		mM			μeq/ hr·kg^{-1}	mM	
A. Control, 4 hr (8)	1.4	6.64	11	12	2.9	15	5.7,5.6	7.46,7.54
" 24 hr (6)	0.35	6.61	21	17	1.2	7	-	-
B. $NaHCO_3$, 4 hr 8 mmol/kg i.v. (15)	1.1	7.00^{**}	9	14	6.2^{**}	10^{**}	5.0,6.4	7.41,7.64
C. $NaHCO_3$, 5 hr 16 mmol/kg i.p. (2)	0.4	8.2^{*}	0	10	18^{*}	0^{*}	-	-
Methazolamide								
D. 4 hr, 50 mg/kg i.v. (4)	0.9	6.64	11	17	4	11	6.1,4.8	$7.50,7.25^{*}$
E. 24 hr, 50 mg/kg i.p. (6)	0.4	6.80	12	15	1.9	6	$5.2,9.1^{*}$	$7.48,7.32^{*}$
F. 24 hr, 500 mg/kg i.p. (4)	0.15	6.85	27	12	2.8	4	$4.3,2.9^{t}$	$7.40,7.15^{*}$
Imidazole								
G. 4 hr, 3 meq/kg (9)	0.28	6.18^{*}	123^{*}	26	$<1^{*}$	34^{*}	5.3,5.3	7.53,7.42
Imidazole (as in G) + Methazolamide								
H. 50 mg/kg i.v. $(6)^{\dagger}$	0.20	6.39	133	25	<1	27	4.8,3.7	$7.54,7.28^{*}$

Significantly ($^{*}p < 0.02$ or $^{**}p < 0.05$) different from control of the same time interval. † Data in H not different from G except for final plasma pH. ^{t} Reflects toxicity.

Clearly there is no functioning carbonic anhydrase in the kidneys of this species, agreeing with Hodler et al. (vide supra) and Rawls and Maren in the flounder (Bull. Mount Desert Island Biol. Lab. 4, p. 57, 1962). There is a marked respiratory acidosis, reflecting the inhibition of red cell carbonic anhydrase.

IV. Table 2, Row G, shows the effect of injecting a proton acceptor (imidazole, pK_a 7.1) on the renal acid output of the sculpin. There is an 11-fold increase in T.A. concentration (compare Row A) and a reduction of urinary pH of 0.5. The T.A. output increases only 2-fold because of a 5-fold decrease in urine flow and attendant signs of toxicity. These changes, though substantial, are less than we observed in similar experiments in the dogfish (Swenson and Maren, ibid.) where a higher dose of imidazole was well tolerated. The mechanism of H^+ formation in fish with no carbonic anhydrase and low pCO_2 is not known; of interest is the high activity of the renal H^+-Na^+ exchanger (Bevan et al., J. Comp. Physiol. B, 159, 1989, In Press).

Row H of Table 2 shows that methazolamide has no effect on the imidazole-induced H^+ secretion. This is a very sensitive test for carbonic anhydrase activity in the mammal, where inhibition lowers T.A. output to 15% of normal (Maren, Physiol. Rev. *47*, 595, 1967, see Table 20).

Histochemical examination of the kidneys of Squalus acanthias and M. octodecimspinosis by Dr. Per Wistrand revealed that there is no evidence of carbonic anhydrase staining in any tubular structures. However, outside the tubules in S. acanthias there was positive staining, probably originating from hematopoietic tissue, blood cells or capillaries. This had also been observed in S. acanthias by Lonnerholm (Acta Physiol. Scand. Suppl. 418, 1, 1974).

In conclusion, we show that the marine teleost, unlike the elasmobranch, can vary urinary pH from 5.8 - 8.2. Neither class of fish has renal carbonic anhydrase, but both can produce H^+ in response to a buffer load, even though pCO_2 is very low. Table 3 shows these relations. In all fish so far examined by us and others, the gill is overwhelmingly the route for excretion of acid or base.

TABLE 3. URINARY ACID-BASE EXCRETION IN MARINE FISH AND MAMMAL

	Teleost	Elasmobranch	Mammal
Fixity of pH	No	Yes	No
pH Response to $NaHCO_3$ ↑	Slight	No	Yes
T.A. Response to H^+ ↑	Slight*	Slight†	Moderate
T.A. Reponse to Buffer ↑	Yes	Yes	Yes
Renal carbonic anhydrase	No	No	Yes

* McDonald et al., J. Exp. Biol. 98, p. 403 (1982). Not thought to be significant.

† King and Goldstein, Am. J. Physiol. 245, p. R581 (1983). Barely significant.

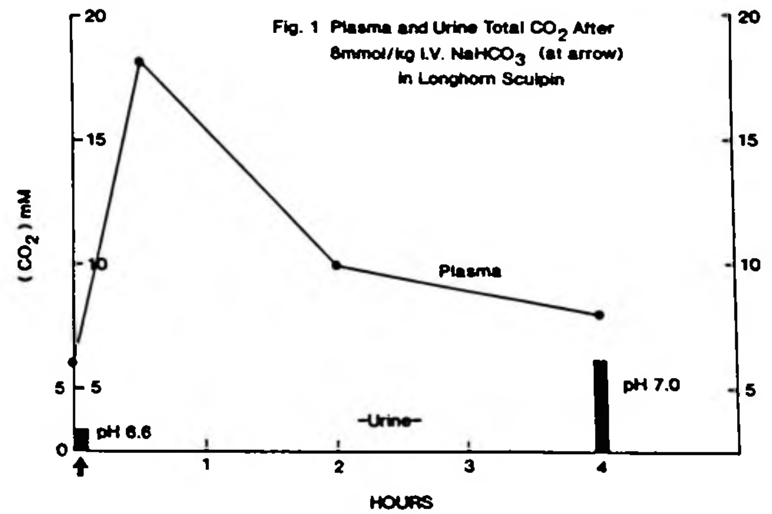

Fig. 1 Plasma and Urine Total CO_2 After 8mmol/kg I.V. $NaHCO_3$ (at arrow) in Longhorn Sculpin