feel that there are high and low activity erythrocyte carbonic anhydrases. Certain tissues in the dogfish (which lacks a high activity red cell enzyme) have a high activity carbonic anhydrase, (Maren and Friedland, vide supra). The isolation procedure employed here would not readily separate isoenzymes and the possibility of isoenzymes in teleost red cells was explored in a different manner.

In cells that contain only one isoenzyme it is possible to calculate the intracellular enzyme concentration from an Easson-Stedman analysis (Maren et al., J. Pharmacol. Exp. Therap., 130:389, 1960). The actual molar concentration of the enzyme is obtained by direct titration with benzolamide or another powerful carbonic anhydrase inhibitor. However, should low and high activity forms co-exist, this method will underestimate the true intracellular enzyme concentration because a low activity isozyme contributes little to overall activity while physically binding the inhibitor. Measurement of the total binding of a carbonic anhydrase inhibitor in intact cells provides a direct determination of the intracellular enzyme concentration, independent of the presence or absence of isoenzymes. This method offers no information about absolute or relative activities of the enzymes. By employing both methods, one ascertains the presence of high and low activity enzymes, if the two values are divergent. The results for several teleosts, an elasmobranch and man are shown in Table 2. The wolffish is rarely caught in the waters off Mount Desert Island and I was not able to do erythrocyte ethoxzolamide binding in this fish. Therefore I studied two other teleosts and the pattern is quite clear. The data on ethoxzolamide binding indicates that these teleosts have 8-14 times more enzyme than direct titration shows. One sees the same discrepancy in human red cells, (see references in Table 2), that are known to have low and high activity isozymes. The red cell concentration of the high activity enzyme C is approximately 25 μM, whilst for the low activity enzyme B it is 125-150 μM. The value for direct titration with benzolamide in human blood gives 27 μM, almost identical to the concentration of enzyme C, and the value from red cell ethoxzolamide binding gives the sum of enzymes B and C. The same situation would probably obtain in the turtle erythrocyte, whose low activity enzyme is 8-10 times more concentrated (Hall and Schraer, vide supra). This probably is the case in the wolffish since recovery of enzyme as determined by protein content yields four times as much enzyme as would be calculated from direct titration data of Table 2. In contrast the shark red cell is known to have only a low activity enzyme (Maynard and Coleman, J. Biol. Chem., 246:4455, 1971) and the equivalent results of the two methods (Table 2) support this conclusion.

Further work will be necessary to characterize these isoenzymes in teleost erythrocytes. However the preliminary data strongly suggests that red cell high and low activity isozymes of carbonic anhydrase have existed in the lower vertebrates dating back to the evolution of the teleosts.

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DOGFISH (Squalus acanthias) RENAL AMMONIA RESPONSE TO AN ACID LOAD

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Although acid-base balance and its regulation has been well studied in mammalian systems, little is known concerning the control of acid-base balance in fish. It is generally assumed that ion-exchange mechanisms operating in the gill maintain the normal pH of the body fluids, but definitive evidence for this hypothesis is lacking. The role of the kidney has received relatively little attention. Recently the rainbow trout has been shown to completely compensate for an acid load by renal H^+ excretion (Wood and Caldwell, J. Exp. Zool., 205(2):301-307, 1978).

In mammals, the ability of the kidney to eliminate H^+ and anions as urinary NH_4^+ salt provides one of the major mechanisms for disposing of an acid load. The purpose of the present study was to examine the renal ammonia

response to acid-loading in the dogfish. In addition, dogfish kidney extracts were assayed for the presence of enzymes involved in ammonia production and its regulation in mammals.

In vivo experiments. Female dogfish, Squalus acanthias, weighing 3 to 6 kg were used. The fish were divided into two groups (5 fish per group) with one group receiving saline/HCO_3^- and the other saline/HCl. Blood samples from a caudal vessel and urine samples (collected in a balloon via a urinary catheter) were obtained from each fish during pre and post injection periods. Following catheterization, there was a recovery period of at least 12 hours before sampling began. Control samples were collected during two consecutive 12-hour periods. After slow intravenous injection of either saline/HCl (0.65 mmoles HCl kg, total volume = 40 ml) or saline/HCO_3^- (250 mM NaCl, 5mM $NaHCO_3$, total volume = 40 ml), samples were taken at 2, 6, 12 hours and thereafter at 12-hour intervals for 2 and one-half days. Blood pH, urine volume, urine pH, and urine ammonia and titratable acid were assayed. Titratable acid was measured by titration with .02 N NaOH to pH 7.4; ammonia was measured by the method of Chaney and Marbach (Clin. Chem. 8(2): 130-132, 1962).

In vitro experiments. Dogfish (mixed sex) were killed by transection of the spinal cord and the kidneys were quickly removed and placed on ice. Ten percent homogenates were prepared in a medium containing 50 mM triethanolamine HCl, 5 mM EDTA, 10 mM $MgCl_2$, and 1 mM dithiothreitol (pH 7.5). The cells were then sonicated (75 watt output, 4 min at 50% duty time) and centrifuged for 15 min at 16,000 x g in a refrigerated centrifuge. The supernatant was removed for the enzyme assays. Transaminase activity was assayed by radiometric and spectrophotometric methods (Sollock et al., J. Comp. Physiol., 129: 129-135, 1979, and Schwartz in Methods in Enzymology Vol. XVII B, 866-871; 1971). Glutamate dehydrogenase was assayed in both the aminating and deaminating directions by spectrophotometric assays (Corman and Arvind in Methods in Enzymology Vol. XVII A, 844-845, 1970; E.A. Newsholme, personal communication). Glucose-6-phosphatase and phosphoenol pyruvate carboxykinase activities were assayed by the radiometric methods of Kitcher et al, (Anal. Biochem., 88:29-36, 1978) and Ballard and Hanson (Biochem. J. 104:866-871, 1967), respectively. Fructose-1,6-diphosphatase was assayed spectrophotometrically (E.A. Newsholme, personal communication). All incubations were performed at 15°C and spectrophotometric assays were followed at 25°C.

Results and discussion. Although each fish responded to the acid load by increasing renal ammonia output, the time course of the response varied among fish. At the time of peak ammonia excretion for each fish (ranging from 6 to 36 hours after saline/HCl injection), the renal ammonia production increased an average of 4.6 (S.E.=0.7) times the control levels. In the group of five fish given only saline, the peak renal ammonia output was only 1.6 times control levels (S.E.=0.4) and occurred at 3 to 12 hours after injection. Figure 1 shows the patterns of renal excretion of ammonia for two representative fish given saline/HCO_3^- or saline/HCl injections; the accompanying data for absolute ammonia and titratable acid excretion are shown in Table 1. In the acid-loaded fish the renal ammonia output increased 5-fold over control levels (at 24 hours) and the titratable acid excretion doubled (at 6 hours). However, it should be noted that the absolute increases in ammonia and titratable acid excretions (above control levels) during the 72 hour post-injection period were 19 μEq and 188 μEq, respectively, and totaled only 7% of the administered acid load (3 mEq HCl).

Blood and urine pH values were measured before and after injection. In fish given saline/HCl, blood pH averaged 7.52 $\pm$ .02 (mean $\pm$ S.E.) during pre-injection periods and dropped to a minimum of 7.30 $\pm$ .03 at 1 to 6 hours after injection; there was significant variation in the time at which this minimum occurred. For saline treated fish pre-injection blood pH was 7.55 $\pm$ .05; values 1 to 6 hours after injection averaged 7.49 $\pm$ .05. Control urine pH's averaged 5.92 $\pm$ .04 for acid-loaded fish and 5.90 $\pm$ .09 for fish given only saline. Post-injection urine pH's were 6.18 $\pm$ .04 and 5.93 $\pm$ .08, respectively.

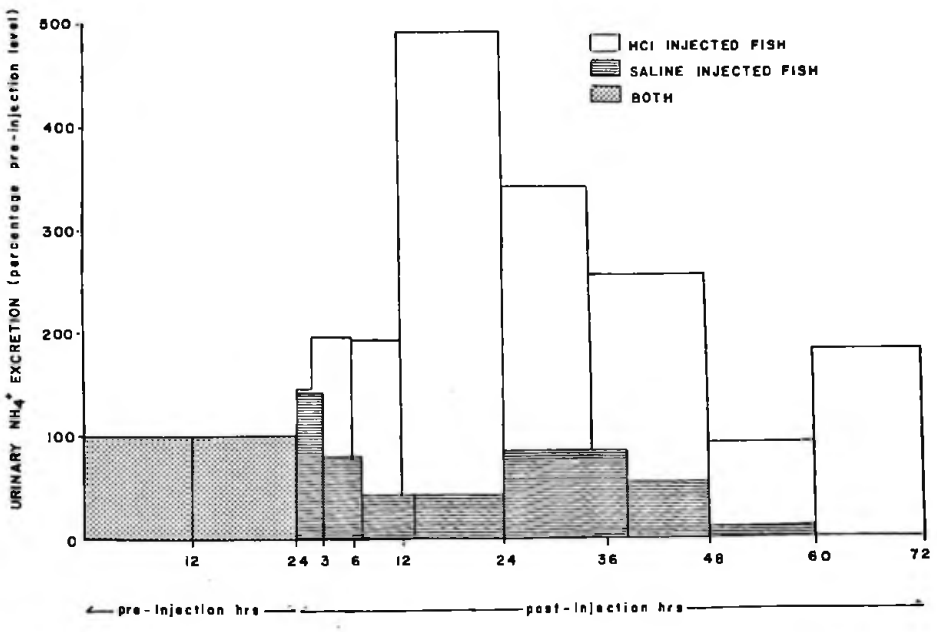

Figure 1. Renal excretion of ammonia for two representative fish given saline/HCO_3^- or saline/HCl (0.65 mmoles HCl/kg). Values are expressed as a percentage of the control (pre-injection) ammonia excretion for each fish.

Table 1. Renal ammonia and titratable acid excretion for representative fish given saline/HCO_3^- or saline/HCl injections

Treatment	Time (hrs)	Titratable Acid μEq/hr	NH_4^+ μEq/hr	Treatment	Time (hrs)	Titratable Acid μEq/hr	NH_4^+ μEq/hr
saline/HCO_3^- injection*	12	60	.55	saline/HCl injection**	12	39	.20
	24	85	.53		24	30	.13
	injection				injection		
	3	87	.77		2	29	.24
	7	122	.37		6	71	.33
	14	81	.20		12	39	.32
	24	44	.21		24	25	.82
	38	105	.45		34	35	.57
	48	47	.25		48	28	.43
	60	37	.03		60	30	.15
					72	10	.30

*wt of fish = 6.80 kg

**wt of fish = 4.67 kg total acid administered = 3mEq (.65mEq/kg).

In vitro enzyme assays identified significant activities of glutamate dehydrogenase and the gluconeogenic enzymes in dogfish kidney extracts. Glutamate dehydrogenase activity in the aminating direction averaged 41 μmoles/g·min and 3.1 μmoles/g·min in the deaminating direction. Assays or rat kidney extract showed that aminating activity was

26 μmoles/g·min and deaminating activity was 2.3 μmoles/g·min. Activity levels of gluconeogenic enzymes were 1.44 μmoles/g·hr for PEP carboxykinase, 33.6 μmoles/g·hr for fructose-1,6-diphosphatase, and 1.68 μmoles/g·hr for glucose-6-phosphatase. Transaminase activity could not be detected by spectrophotometric or by radiometric methods using a number of amino acids (asp, met, ala, ser, gly, leu, orn, arg, cys) as the amino donor. Other sources of renal ammonia including the glutaminase reaction and purine nucleotide cycle were not assayed.

The identification of glutamate dehydrogenase and the gluconeogenic enzymes in dogfish kidney extracts indicates the potential for renal ammoniagenesis and support the results of *in vivo* experiments showing the ability of the dogfish kidney to increase renal ammonia excretion in response to an acid load. The inability to demonstrate transaminase activity leaves the source of renal ammonia in question. Glutaminase and purine nucleotide cycle enzyme activities need to be investigated. This work was supported by NSF grant PCM 75-14322.

ION TRANSPORT IN ELASMOBRANCH AND MARINE TELEOST LENSES WITH PARTICULAR RESPECT TO HCO_3^- MOVEMENT.

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Elasmobranch and marine teleost lenses, unlike those of "higher" vertebrates, lack carbonic anhydrase (Maren, T.H. and B.R. Friedland, Bull. MDIBL., 18:79, 1978). Ion transport studies in these species may elucidate the relationship between carbonic anhydrase and the movement of bicarbonate and other ions in this tissue. Last year, we reported preliminary initial efflux rate constants for chloride and bicarbonate (Friedland and Maren, Bull. MDIBL 18:82, 1978). This study reports steady state entry and efflux rates for labelled Na^+, $K(Rb)^+$, Cl^-, and HCO_3^- using *in vitro* lens incubation techniques. *Squalus acanthias* lenses were used in most cases; data on ^{86}Rb uptake in the flounder lens is included. Additionally, we report intracellular ion concentrations, electrical potential, and Na-K ATPase activity of the elasmobranch lens.

Table 1 gives the electrolyte composition of freshly dissected lenses of *S. acanthias*. There are several unusual

Table 1. Comparison of lens (intracellular) and aqueous (extracellular) ionic composition in *S. acanthias* with nernst equilibrium potentials. Values are ±s.e.m., number of samples (n)

	Aqueous* (mM)	Lens[@] (meg/kg lens H_2O)	Lens Intracellular[+] (mM)	Calculated Lens Nernst Potential (mV)
Na^+	279±6 (9)	54±1.6 (15)	40±1.6	+50±3
K^+	5±1 (10)	61±1.1 (18)	61±1	-64±0.4
Cl^-	253±5 (9)	77±2 (18)	63±2	-36±1
total CO_2	8.5±0.3	33±0.6	32±0.6	+34± .5
Urea	350	340±26	323±23	

*from Maren, T.H., Comp. Biochem. Physiol., 5:193, 1962.

[@]Lens water content was measured to be 54±2%.

[+][Intracellular] calculated assuming a 5% extracellular space and extracellular concentrations equal to aqueous.