23 μmoles.$100g^{-1}$.hr^{-1}. This represents about 10% of the calculated Na efflux. Since diffusional Na efflux is still probably appreciable in 25% sea water, and also marine teleosts have been shown to have Na uptake mechanisms functioning for both NH_4 and H extrusion (Evans, Am. J. Physiol. 238, R224-R230, 1980; Evans, J. Exp. Biol. 70, 213-220, 1977), it seems unlikely that this urinary loss of Na (although fairly high in its rate) is a significant problem.

In summary, acclimation of two species of sculpin to hyposmotic salinities is followed by a cessation of (substantial) Na reabsorption from the bladder and the production of a nearly blood-isosmotic and-ionic urine, despite the need to excrete excess water and reduce salt loss. This "malfunction" is probably not due to experimental reduction of residence time of the urine in the urinary bladder. However it appears that it does not produce a substantial (and unbalanced) increase in at least Na loss. This research was supported by grants from the Herman Rosenberg Foundation (University of Helsinki) to A.O. and NSF (PCM80-08366) to DHE.

ACID AND AMMONIA EXCRETION BY SQUALUS ACANTHIAS AND MYXINE GLUTINOSA: EFFECT OF HYPERCAPNIA, ACID INJECTION AND NA-FREE SEA WATER

David H. Evans, Department of Biology, University of Miami, Coral Gables, Fl.

In the past few years it has become apparent that marine teleost fish possess a branchial ammonia extrusion system which couples the efflux of NH_4 to the uptake of sea water Na (Evans, Am. J. Physiol. 238, R224-R230, 1980). Preliminary investigations indicated that the marine elasmobranch, Raja erinacea, did not possess this system but did extrude unwanted H in exchange for sea water Na (Evans et al., Bull. MDIBL 18, 64-65, 1978). Since evidence exists for both Na/NH_4 and Na/H ionic exchange in another elasmobranch, Scyliorhinus canicula, (Payan and Maetz, J. Exp. Biol., 58, 487-502, 1973; Bentley et al., J. Exp. Biol. 64, 629-637, 1976) it was appropriate to investigate the presence of these ionic exchange systems in Squalus acanthias. Since these systems are presumably relics of a fresh water ancestory (Evans, Comp. Biochem. Physiol. 51A, 491-495, 1975) it was of interest to test for their presence in the agnathan hagfish Myxine glutinosa, which is a modern representative of a group which has probably never entered the fresh water environment (Hardisty, The Biology of Cyclostomes, Chapman and Hall, 1979).

S. acanthias pups were removed from sacrificed pregnant females and M. glutinosa were kindly supplied by Dr. Bruce Sidell, University of Maine, Orono, (after collection near St. Andrews, N.B.). Both species were kept unfed in running sea water at 12 - 15°C. The rate of acid extrusion was determined by a modification of the technique of Heisler et al. (Bull. Europ. Physiopath. Resp. 12, 77-85, 1978), where 5 ml samples of an efflux bath (200 mls) are equilibrated with 1% CO_2 (in oxygen) while the pH is recorded on a Radiometer Titragraph Recorder (attached to a Radiometer Model PHM 62 pH meter) to the nearest 0.0025 pH units. Since the P_{CO_2} is maintained constant during the analysis, according to the Henderson-Hasselbalch equation the pH is directly proportional to H ion concentration. During the course of the experiment the change in the experimental bath pH is compared to that of a control. The flux (u moles.$100g^{-1}$.hr^{-1}) for a given experimental time period is then calculated by converting the change in pH differential between the control and experimental baths into a H ion concentration and correcting for the buffer capacity of the solutions and the weight of the fish. The buffer capacity for the control and experimental baths are determined directly by titrating 5 ml samples of the solutions before and after the experiment with either HCl or NaOH solutions. In some cases, samples of the experimental bath were removed, frozen, shipped to Miami and analyzed for ammonia via the method of Solorzano (Limn. & Oceanog. 14, 799-801, 1969). Calculations of fluxes were performed on a Univac 1100 computer using programs written by J.B. Claiborne. In most experiments the fish were made hypercapnic by bubbling 5% CO_2 in air (produced with a Wostoff Gas Mixing pump) into the experimental baths. In other experiments, nor-

mocapnic fish were injected with an HCl acid load of 50 u moles/100g. To determine the role of urinary acid and ammonia excretion S. acanthias pups were encased in prophylactics to a point just anterior to the renal/rectal openings. The prophylactics were filled with 10ml of sea water and secured around the body with cotton thread. The acid/ammonia efflux into the prophylactic fluids vs the flux into the experimental sea water was determined for a one hour period before the prophylactic was removed. Three M. glutinosa were cannulated via flared and perforated PE 50 tubing secured into the cloacae with a purse-string suture. Initially, the distal end of the cannulae were led out of the experimental bath to determine extra-renal acid/ammonia efflux. After 2 hours, the cannulae were cut to allow emptying of renal fluids into the experimental bath for an additional two-hour period. To determine the role of Na/NH_4 and Na/H ionic exchange, hypercapnic or acid-loaded fish were transferred to either low Na, K-free solutions (2.5 mM $NaHCO_3$, choline chloride substituting for the rest of the Na and all of the K) or Na-free solutions (2.5 mM $KHCO_3$, 7.5 mM KCl and choline chloride substituting for all of the Na) after an initial period in normal sea water. After one hour in the ion-substituted solutions, the fish were again transferred into normal sea water to test for reversibility of any ion-free effect.

The data in Table 1 show that normocapnic S. acanthias do not excrete a net amount of acid but do excrete rather

Table 1.--Effect of Hypercapnia on Acid and Ammonia Efflux from the dogfish and the hagfish

	NORMOCAPNIC		HYPERCAPNIC	
Species	Acid	Ammonia	Acid	Ammonia
Squalus acanthias (6)	-5 ± 3	2 ± 1	16 ± 4	4 ± 1
Myxine glutinosa (6)	77 ± 24	20 ± 4	72 ± 11	13 ± 2

Fluxes are in umoles.$100g^{-1}.hr^{-1}$, $\bar{X} \pm$ S.E. (no. of animals)

Animals were maintained normocapnic (bubbled with air) for 2 to 3 hours before hypercapnia was produced by bubbling 5% CO_2 in air into the experimental bath. Fluxes under normocapnic conditions are calculated as means for the entire period, those for hypercapnia as means for the first 2 - 4 hours of hypercapnia.

low levels of ammonia; however, normocapnic M. glutinosa excrete significant quantities of both acid and ammonia. Production of hypercapnia is correlated with a significant ($p < 0.001$) increase in both acid and ammonia excretion by S. acanthias but not stimulation of either acid or ammonia production by hagfish. It is possible that the hagfish were already suffering from a metabolic acid load secondary to stress but the one individual which produced excess mucus (normally associated with stress in this species) did not display an acid or ammonia efflux significantly above the other individuals. Table 2 separates renal/rectal from branchial acid and ammonia efflux from both species during hypercapnia and it is clear that renal excretion of acid by dogfish is negligible but that renal ammonia excretion may be 20% of the total. Only three hagfish were cannulated but these preliminary data indicate that renal excretion of acid is probably minor, while renal excretion of ammonia may be quite significant. However, the sample size is too small to be definite about the role or renal/rectal vs. branchial excretory pathways in hagfish at the present time. The data in Table 3 indicate clearly that hypercapnic dogfish pups excrete all the acid via Na/H exchange but that Na/Nh_4 exchange can only account for about 40% of the ammonia excretion. Acid excretion by hypercapnic hagfish is also totally dependent upon external Na (and possibly K) but the high variability in the data for ammonia efflux preclude a definite statement. (However, in these experiments, 4 of the 6 hagfish did display reductions of from 60 to 90% in the low Na, K-free artificial sea water.) Interestingly, in both the dogfish and hagfish experiments, the reduction

Table 2.--Renal vs. Branchial Excretion of Acid and Ammonia by the dogfish and the hagfish

Species	BRANCHIAL EFFLUX		RENAL EFFLUX	
	Acid	Ammonia	Acid	Ammonia
Squalus acanthias (6)	21 ± 7	5 ± 1	- 3 ± 1	1 ± 0.2
Myxine glutinosa	18	5	4*	14
	29	2	-12	3
	33	12	35	35

Fluxes are in u moles. $100g^{-1}.hr^{-1}$, $\bar{X}$ ± S.E. (no. of animals). All data are on hypercapnic animals (5% CO_2)

*Renal effluxes from M. glutinosa calculated as the difference between the efflux with cannula leading out of experimental bath and the efflux after the cannula was cut to allow renal fluids to enter the bath.

Table 3.--Effect of Removal of External Na and K on Excretion of Acid and Ammonia by the dogfish and the hafgish

Species	Sea Water Control		"Na-and K-free" Sea Water		Sea Water	
	Acid	Ammonia	Acid	Ammonia	Acid	Ammonia
Squalus acanthias (6)	21 ± 7	10 ± 2	- 3 ± 8*	6 ± 1*	47 ± 5	4 ± 1
Myxine glutinosa (6)	79 ± 7	35 ± 4	-15 ± 7	26 ± 14	94 ± 16	18 ± 4

Fluxes are in u moles. $100g^{-1}.hr^{-1}$, $\bar{X}$ ± S.E. (no. of animals). All data are from hypercapnic animals.

"Na- and K-free" sea water contained 2.5 mM $KHCO_3$ and 7.5 KCl in the S. acanthias experiments and 2.5 mM $NaHCO_3$ in the M. glutinosa experiments; in both solutions, choline chloride substitutes for the remaining Na or K.

*In these experiments the "Na-and K- free" sea water was Na free but contained 2.5 mM $KHCO_3$ and 7.5 mM KCl, with choline chloride replacing the Na.

in acid efflux was totally reversible, actually with an overshoot, but the reduction in ammonia efflux was not. The total dependency of acid efflux from both species on external Na (and possibly K) supports the data already published for R. erinacea (Evans et al., J. Exp. Zool. 208, 413-437, 1979), while the partial dependency of ammonia efflux on external Na (and possibly K) supports the data for teleosts (Evans, J. Exp. Biol. 70, 2p3-220) but is in contrast to that for R. erinacea (Evans et al., op. cit.).

Injection of a mineral acid load into S. acanthias is followed (after a 30 minute lag period) by stimulation of acid efflux which lasts 3 - 5 hours (Table 4). In fact, the rate of stimulated acid efflux is sufficiently high to excrete the injected acid load of 50 umoles/100g within 2 - 3 hours. The fact that significant acid excretion continues past this point indicates that a metabolic acid load may have been produced by the injection of the mineral acid load. This is supported by the finding that injection of saline in control experiments also stimulated acidification of the sea water

Table 4.--Effect of Acid Injection on the excretion of Acid by the Dogfish

HOURS POST INJECTION					
0-0.5	0.5-1	1-2	2-3	3-4	4-5
6.6 ± 8.9	44 ± 5	18 ± 7	15 ± 2.9	4.2 ± 1.7	6.0 ± 3.9

Acid load was 50 umoles/100g, injected IP under MS 222 anesthesia (0.01%).

by S. acanthias (unpublished results). The rate of ammonia efflux was extremely variable (but less than 10 umoles.$100g^{-1}$.hr^{-1}) in these acid-lead experiments, presumably due to the metabolism of the MS 222 used to anesthetize the fish during the injection of the acid load. The rate of renal/rectal acid efflux from fish fitted with prophylactics was essentially zero (0.2 ± 0.4 umoles.$100g^{-1}$.hr^{-1}) during the first hour after acid injection, while the branchial efflux was 57 ± 10 umoles.$100g^{-1}$.hr^{-1} during the second 30 minutes after acid injection. After removal of the prophylactics, these fish were transferred for one hour to Na-free artificial sea water and thence into normal sea water for another hour. The rates of acid efflux during these periods was -10 ± 8.2 and +23 ± 4.0, respectively. Thus, we can conclude that a mineral acid load is excreted by Na/H exchange in the branchial epithelium of S. acanthias, which explains why a recent study of the renal extrusion of acid and ammonia found that only 7% of the injected load appeared in the urine during 72 hours post injection (King and Goldstein, Bull. MDIBL 19, 77-80, 1979). Our data contrast with those on the rainbow trout (Wood and Caldwell, J. Exp. Zool. 205, 301-307, 1978) which indicate that a mineral acid load is excreted renally in this species. The fact that acid extrusion by both species usually becomes negative when Na (and possibly K) are removed from the sea water (Table 3 and text) implies that base (presumably HCO_3) extrusion may also be taking place in sea water, but obscured by a dominant extrusion of acid. This interesting idea should be pursued further.

The present data supports the following conclusions: 1. Hypercapnia and acid loading stimulates acid (and ammonia, in the case of hypercapnia) extrusion by S. acanthias pups but acid efflux via direct proton extrusion is more prevalent coupling of the proton to ammonia, 2. Hypercapnia does not appear to affect either acid or ammonia extrusion from hagfish, 3. Excretion of both acid and ammonia by dogfish is predominantly branchial, 4. Renal pathways for both acid and ammonia efflux from hagfish may be appreciable, 5. Acid extrusion by dogfish and hagfish is entirely via Na/H (and possibly K/H) exchange, 6. Ammonia excretion by both species is only partially coupled to Na (and possibly) K influx.

Of major importance in the present study is the finding that the hagfish possess branchial mechanisms for Na/H and possibly Na/NH_4 ionic exchange. Since this group has apparently never entered freshwater, one must propose that the mechanisms for extraction of at least Na from low salinities (a prerequisite for life in brackish or freshwater) arose in very early marine vertebrates as a means for acid and ammonia extrusion, rather than for Na balance in hypo-osmotic salinities. In the past (Evans, Comp. Biochem. Physiol. 51A, 491-495, 1975) we had proposed that the presence of Na/NH_4 and Na/H exchange in marine teleosts and elasmobranchs was secondary to a previous evolutionary existence in freshwater. Their presence in the totally marine hagfish line of vertebrate evolution indicates a much earlier origin of this system, in sea water, rather than freshwater. This research was supported by NSF PCM80-08366 to DHE.