

AMMONIA AND ACID EXCRETION BY THE LITTLE SKATE, *Raja erinacea*

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It is now quite clear that extraction of needed NaCl from the freshwater milieu is carried out via $\text{Na}^+/\text{NH}_4^+$ or H^+ , and $\text{Cl}^-/\text{HCO}_3^-$ or OH^- ionic exchange mechanisms in a wide variety of freshwater organisms (Maetz et al., Persp. in Exp. Biol. 1:77-92, 1976). In addition, it has recently been shown (Evans, Comp. Biochem. Physiol. 51A:491-495, 1975; Evans, J. exp. Biol. 70:213-220, 1977) that at least $\text{Na}^+/\text{NH}_4^+$ exchange takes place across the branchial epithelium of marine teleosts. Concurrent work (Payan, P. and J. Maetz, J. exp. Biol. 58:487-502, 1973; Bentley et al., J. exp. Biol. 64:629-637, 1976) has indicated that the $\text{Na}^+/\text{NH}_4^+$ and possibly Na^+/H^+ exchange systems also exist in the gill of the shark, *Scyliorhinus caricula*. It has been proposed by both laboratories that the presence of these ionically-inappropriate exchange systems is secondary to the overriding needs for nitrogenous waste excretion and acid-base regulation. The present study was initiated to investigate the role of these ionic exchange systems in the excretion of ammonia and acid by the little skate.

Small (500-1000 g) *Raja erinacea* were collected by hook and line and maintained unfed in running seawater (15°-17°C) until transfer into the experimental solutions. All experiments were performed at room temperature which varied between 20-25°C. Experiments consisted of transferring individual skates to 4 liters of seawater for 30 minutes, thence into the same volume of either K^+ - and Na^+ -free artificial seawater (according to the formulation of Evans and Cooper, Nature 259:241-242, 1976, corrected to the salinity of the local seawater) or seawater containing 1×10^{-4} M amiloride for an additional 30 minutes, and finally into 4 liters of seawater again. Fish were "washed" in ion-free solutions for 30 seconds before transfer into the K^+ - and Na^+ -free artificial seawater experimental bath. Triplicate 5 ml samples of each bath were removed after the fish had been removed and either frozen and returned to Miami for analysis of ammonia concentration (modified method of Harwood and Kuhn, Water Res. 4: 805-811, 1970) or titrated to pH 8.20 with a radiometer Autotitrator using 1 mM NaOH as the titrant. In all cases samples were also removed at the start of the 30 minute experimental period and analyzed for either ammonia or acid as a zero-time control. In a few cases animals were implanted with PE 10 bridges filled with 3 M KCl in 2% agar in order to measure transepithelial electrical potentials (TEPs) as described elsewhere (Kormanik and Evans, this bulletin).

TABLE 1

Acid and ammonia efflux from *R. erinacea*; effect of removal of external Na^+ and K^+

	SW ₁	-K ⁺ , -Na ⁺ asw8	SW ₂
Acid efflux**	39.2±7.4 (9)	-0.06±4.7 (9)	55.8±15.1 (9)
Ammonia efflux	6.37±1.44 (5)	10.51±3.58 (5)	17.6±6.29 (5)
TEP***	-1 -3	-3 -3	-1 -2

* K^+ - and Na^+ -free artificial seawater

** $\mu\text{m.}100\text{g}^{-1}.\text{hr}^{-1}, \bar{X} \pm \text{S.E. (No. of animals)}$

*** mV, blood rel. to medium

TABLE 2

Acid and ammonia efflux from *R. erinacea*; effect of external addition of amiloride

	SW ₁	SW+10 ⁻⁴ M Amiloride	SW ₂
Acid efflux*	94.6±15.0 (8)	47.3±17.4 (8)	71.4±19.4 (6)
Ammonia efflux	6.76±1.85 (6)	8.44±2.45 (6)	8.39±2.23 (5)
TEP**	-2 0.3 (4)	-2 0.7 (4)	-3 0.3 (4)

* $\mu\text{m.}100\text{g}^{-1}.\text{hr}^{-1}, \bar{X} \pm \text{S.E. (No. of animals)}$

** mV, blood rel. to medium

Table 1 presents the results of our determinations of the effect of the removal of external Na^+ and K^+ on the efflux of acid and ammonia. It is clear that, although the ammonia efflux was unaffected, the rate of acidification of the medium was significantly reduced and actually reversed to alkalization in some experiments. Replacement of the animals into normal seawater resulted in a reestablishment of net acidification with an overshoot which was obscured by experimental variability (6 or 7 animals showed a greater acid efflux in SW_2 than in SW_1). One might propose that the overshoot was secondary to a fall in blood pH during the period in Na^+ - and K^+ -free seawater but data on blood pH are lacking. Only two determinations of the TEP were made during these experiments but it is apparent that changes in the TEP cannot account for the substantial alterations of the acid efflux secondary to changes in the external levels of Na^+ and K^+ . The animals were not cannulated but Cohen (J. Cell Comp. Physiol. 53:204-213, 1959) found that *Squalus acanthias* maintained a renal acid excretion of the order of $4 \mu\text{M}\cdot 100\text{g}^{-1}\cdot \text{hr}^{-1}$, which is only 10% of the total acid excretion of the skates in Table 1. In addition, Pierce and Kent (Bull. MDIBL 8:49-53, 1968) have shown that the *S. acanthias* branchial epithelium excreted acid at a rate of approximately $100 \mu\text{M}\cdot 100\text{g}^{-1}\cdot \text{hr}^{-1}$ when perfused with seawater in equilibrium with 2-5% CO_2 in air. It, therefore, appears that the present experiments have determined mainly branchial acid excretion.

Kirschner et al. (Am. J. Physiol. 224:832-837, 1973) have shown that external amiloride inhibited Na^+ influx and both acid and ammonia efflux from the branchially-irrigated freshwater trout so we have tested the amiloride sensitivity of the acid and ammonia excretion from *R. erinacea*. Table 2 shows clearly that 10^{-4} M amiloride significantly inhibited acidification but had no effect on the efflux of ammonia or the TEP. The cause for the much higher control acid efflux in these experiments is unknown.

These experiments indicate clearly that, in *R. erinacea*, ammonia efflux is not coupled to external Na^+ or K^+ and is therefore not via $\text{Na}^+/\text{NH}_4^+$ exchange which has been described for *Scyliohinus canicula* and a wide variety of teleost species (see refs. in introduction). However, it is equally clear that acidification of the medium by this species is sensitive to external Na^+ (and/or K^+) and is, therefore, presumably via Na^+/H^+ (or possibly K^+/H^+) exchange. Since the few determinations of the unidirectional Na^+ fluxes across marine elasmobranchs indicate a rate of from 33-100 $\mu\text{M}\cdot 100\text{g}^{-1}\cdot \text{hr}^{-1}$ (Evans, in "Osmotic and Ionic Regulation in Animals," G.M.O. Maloij editor, Academic Press, 1979) it appears that this ionic exchange system (assuming a 1:1 stoichiometry) may represent a significant Na^+ load to the skate which must be excreted via branchial and rectal gland extrusion mechanisms. Research supported by NSF PCM77-09915.

PRELIMINARY STUDIES OF OSMOREGULATION BY THE PREMATURE "Pup" OF THE DOGFISH, *Squalus acanthias*. AND THE UTERINE LINING OF THE FEMALE

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The dogfish, *Squalus acanthias*, is ovoviviparous with developing young maintained *intra utero* for periods up to two years (Woodhead, Bull. MDIBL 16:103-106, 1976). During the later periods of gestation, the yolk sac bearing "pups" are exposed to seawater secondary to the maternal flushing of the uteri with sea water (Burger, Sharks, Skates and Rays, p. 178, 1967). In addition, we (and other workers) have found that late-term "pups" removed in the summer months during surgery of the female are able to survive in sea water (15° - 17°C) for periods up to two weeks as long as care is taken to avoid rupture of the yolk sac membrane.

Since the highly vascularized uterine walls of the female are also exposed to seawater late in development it is also of interest to investigate the ability of the uterine lining to maintain ionic