

EGG CASE PERMEABILITY TO AMMONIA AND UREA
IN TWO SPECIES OF SKATES (RAJA sp.)

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Embryos of oviparous skates are enclosed in egg capsules and bathed by sea water for a gestation period which may last nearly two years. While the tough capsule mechanically protects the embryo, its role as an osmotic barrier has been less clear. Smith (Biol. Rev. 11:49-82, 1936) proposed that the "cleidoic" egg of elasmobranchs was an adaptation for retention of urea. An assumedly impermeable egg capsule is cited by several authors in recent discussions of the evolution of viviparity and urea retention (see Kormanik, Am. Zool., in press). To clarify the role of the egg capsule in osmoregulation, we measured the permeability of the capsule wall to ammonia and urea.

Female skates (Raja erinacea and one other species, see below) were collected by commercial fishermen from Frenchman Bay, ME., and maintained in running sea water. Fresh eggs in capsules were collected daily, and used in subsequent experiments. For the measurements of permeability, sections (2 cm²) were removed from the capsule, mounted in a lucite Ussing-type chamber, filled with the appropriate solution and bubbled with air. Artificial sea water (Na⁺, 450; Cl⁻, 525; Ca⁺⁺, 10; Mg⁺⁺, 56; K⁺, 9; SO₄⁻⁻, 32; HCO₃⁻, 2.5 mM; buffered with TRIS, 5 mM, in the pH experiments) was placed on the outside and inside. A stock solution (1 M) of either urea or ammonia was added to the inside to achieve final concentrations of 100 mM and 10 mM respectively. The sea water concentration was previously adjusted to minimize any osmotic gradient across the capsule wall. In a second series of measurements of ammonia diffusion, the pH of the ASW was adjusted to modify separately the gradients for NH₃ and NH₄⁺ (calculated after Cameron and Heisler, J. Exp. Biol. 105:107-125, 1983). The appearance of urea or ammonia on the outside of the capsule wall was measured at 30 min intervals using the diacetyl monoxime method (Sigma Kit # 535) for urea or the Solorozano method (Limnol. Oceanogr. 14:799-801, 1969) for ammonia. The permeability (Px) was calculated from the equation $J_{net} = P_x \cdot A \cdot \Delta C$ where J_{net} is the net flux, A is the area and ΔC , the concentration gradient. During the course of the experiment (2 hrs.) the gradients changed less than 5%, but were nevertheless corrected for; the net fluxes were linear. Results are expressed as mean \pm 1 S.E.M.

The results of the first series of experiments are presented in Table 1. The permeability we measured for urea, while lower, is comparable to that measured by Hornsey for Scyliorhinus canicula (4.7×10^{-5} cm sec⁻¹; Experientia 34:1597, 1978). The egg case is also quite permeable to ammonia.

Elevating pH had only a small effect on the NH₄⁺ gradient while the NH₃ gradient was enhanced 20-fold (Table 2). There was no significant difference in total ammonia diffusion. Either ammonia diffuses predominantly as the charged form, NH₄⁺, or the capsule wall does not distinguish between these two species. Permeabilities calculated from these data (from Table 2 using two equations for two unknowns) were 2.2 and 2.5 cm sec⁻¹, for NH₃ and NH₄⁺, respectively. Hornsey (ibid.) characterized the capsule wall of S. canicula as having pore radii on the order of 1.4 nm,

Table 1. Permeability of the egg capsule wall of skates to ammonia and urea. T_{amm} is total ammonia. Permeability (P_x) is expressed in $cm\ sec^{-1}$, all values $\times 10^5$, n = number of capsules.

| | P_{urea} | P_{Tamm} | Thickness (cm) |
|----------------------------|--------------------|--------------------|----------------------|
| <u>R. erinacea</u> (8) | 2.00 ± 0.15 | 3.09 ± 0.16 | 0.033 ± 0.002 |
| <u>R. ocellata*</u> (1) | 1.30 | ---- | 0.066 |

* -mother unidentified, tentative classification is based on egg morphology (Bigelow and Schroeder, Fishes of the Western North Atlantic, Memoire I, Pt. II., p.247, Sears Fnd. Mar. Res., New Haven, 1953)

Table 2. Total ammonia fluxes across the egg capsule of R. erinacea when the gradients for NH_4^+ and NH_3 are independently varied. Outside pH was always 7.0, inside pH is given below. Gradients are "inside" - "outside", ($n = 5-6$).

| Expt. | ΔNH_4^+ (mM) | ΔNH_3 (mM) | J_{net} ($\times 10^{-10}\ mol\ cm^{-2}\ sec^{-1}$) |
|-----------------------|-------------------------|-----------------------|--|
| Control (pH = 7.0) | 9.97 \pm 0 | 0.021 \pm 0.001 | 2.49 \pm 0.65 |
| Expt1. (pH = 8.3) | 9.58 \pm 0.04 | 0.42 \pm 0.04 | 2.52 \pm 0.34 |
| (Expt1/cntrl) | 0.96 | 20. | 1.01 |
| Signif. (p<) | 0.001 | 0.001 | n.s. (p>0.2) |

much larger than the hydrated radii for both of these highly water soluble forms, as well as urea and water molecules. The egg capsule wall is quite unlike elasmobranch branchial membranes, where $P_{NH_3}/P_{NH_4^+}$ is quite large (Evans & More J. Exp. Biol. 138:375-397, 1988).

These data confirm our previous observations, and help to explain why neither ammonia nor urea accumulates in capsular fluids (Kormanik, Bull. MDIBL 28;12-13, 1989). Since large gradients exist for the nitrogenous compounds urea and TMAO across the egg membrane (see Kormanik et al., this issue), embryonic membranes appear to be the main barriers to diffusion and not the capsule wall. While only a few oviparous species have been examined, all have shown high capsular permeabilities to small molecules and ions. The relatively high permeability of the capsule should be recognized in discussions of the evolution of urea (and TMAO) retention in the elasmobranchs (Supported by NSF DCB-8904429 to GAK and a Hearst Foundation, Inc. scholarship to NOL.)