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THE EGG CASE OF THE OVIPAROUS ELASMOBRANCH, *RAJA ERINACEA*, DOES OSMOREGULATE

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Like many other elasmobranchs (Wourms, 1977), the little skate, *Raja erinacea*, lays individual eggs covered with a specialized structure termed the egg case or 'Mermaid's Purse'. Collagen is the major component of the egg case, which is secreted by the shell gland in the anterior region of the oviduct (Wourms, 1977). Smith (1936) and Price & Daiber (1967) suggested that the egg case provided an osmotically isolated environment until the embryo was capable of urea retention and osmoregulation. However, Reed (1968*a*) showed that the encapsulated embryos of the skate, *Raja binoculata*, possess the enzymes of the Krebs ornithine–urea cycle and are able to retain near-adult levels of both urea and trimethylamine oxide, even at very early stages (Reed, 1968*b*). The retention of urea seems to be secondary to a urea-impermeable embryonic membrane since Needham & Needham (1930) found that the egg cases of *Syllium* (= *Scyliorhinus*) *canicula* are permeable to urea, and this has been substantiated by recent work on the egg cases of the same species (Hornsey, 1978; Foulley & Melinger, 1980). Despite the ability to maintain high urea levels, it appears that the full complement of elasmobranch osmoregulatory mechanisms is not present in at least the early stages of encapsulated elasmobranch development since Libby (1959) found that *R. eglanteria* cannot survive in sea water if it is removed from the egg case before day 20 of the 64-day developmental period. In this species, at day 20 a mucous plug in the egg case is dissolved and sea water enters. This opening of the egg case to sea water during the later stages of development is also found in other oviparous elasmobranch species (Wourms, 1977).

Thus, it appears that, despite the ability to produce and retain urea, other (either extraembryonic or egg-case) sites must limit either the rate or effects of osmotic and ionic movements, (i.e. osmoregulation is extraembryonic). Recent studies (Hornsey, 1978; Foulley & Melinger, 1980) have shown that the egg case is extremely permeable to water and various organic molecules, and relatively more permeable to Na than to urea. However, isotopic fluxes of Na and Cl across an intact egg case have not been published and the egg-case fluids have not been chemically analysed to determine the magnitude of the osmotic and ionic gradients facing the developing embryo.

Adult *Raja erinacea* were collected via hook and line in Frenchman's Bay, Maine, and maintained in live cars or aquaria supplied with running sea water. Egg cases were collected from the live cars or aquaria containing the adults or, in a few cases, were removed from gravid females during the months of July and August 1978, 1979 and 1980. No differences were found between egg cases that had been spontaneously

laid and those removed from the female. The cases were maintained in running sea water (12–17 °C) for 2–10 days before experimentation. Examination of the contents of the egg cases at the termination of the experiment (and in other instances where intracapsular fluids were sampled only) indicated that visible embryos were not present. In all cases the circular blastodisc (radius of approximately 5 mm) was visible as a light area on top of the orange yolk. The rate of efflux of Na, Cl or urea was determined by injecting the isotope (^{22}Na, 2 μCi in 10 μl; ^{36}Cl, 0·25 μCi in 10 μl; [^{14}C]urea, 6 μCi in 5 μl, all isotopes carried in distilled water) directly into the case. The site of injection was sealed with silicone grease, but loss of the seal during the course of most experiments did not result in noticeable leakage of the isotope from the egg case. The injected cases were placed in 100–200 ml of aerated sea water in an 800 ml plastic beaker and the temperature of the experimental baths (12–17 °C) was maintained by running sea water surrounding the beakers. At various times post injection a 3 ml sample of the bath was removed and counted using a Packard Autogamma System (^{22}Na) or dissolved in 10 ml of ACS scintillation cocktail (Amersham) and counted using a Packard Tricarb Liquid Scintillation System with automatic external standardization (^{36}Cl and [^{14}C]urea). The rate constant (fraction of exchangeable Na, Cl or urea exchanged per hour) was calculated via the formula: $K = 1/T \ln C_0/C_t$, where T is the experimental time period in hours and C_0 and C_t are the radioactivity in the egg case at the start and end of an experimental time period, respectively. The initial radioactivity injected into the case was determined by counting samples of the injection solution, and radioactivity at any time subsequent to injection was calculated by subtracting the radioactivity that had appeared in the efflux bath from the initial, injected radioactivity. Preliminary experiments indicated that approximately 2 h were needed for equilibration of the ^{22}Na within the fluids in the egg case, so rate constants for this isotope are calculated from samples taken after an initial 3 h equilibration period. Rate constants were calculated between hour 1 and 2 (post injection) in the case of ^{36}Cl and [^{14}C]urea. After most of the radioactivity had been lost from the case it was transferred into a non-radioactive bath for at least 48 h to allow complete washout of the isotope. These cases (and others, freshly collected) were then opened and 100–200 μl of free internal solution removed and stored frozen in microhematocrit tubes. The samples were then shipped frozen to our laboratory in Miami and analysed for Na and K (via flame photometry) Cl (via amperometric titration), osmolality (via vapour pressure osmometry) and urea (Sigma kit).

The rate constants for Na, Cl and urea efflux from *R. erinacea* egg cases are presented in Table 1. It is clear that the egg-case membranes are quite permeable to these substances, especially when compared with adult elasmobranchs, which display rate constants for Na of approximately 0·001 h^{-1}, Cl of approximately 0·01 h^{-1} and urea of 0·001 h^{-1} (Evans, 1979; Carrier & Evans, 1972). Like all other elasmobranch tissues studied to date (Evans, 1979; Kormanik & Evans, 1978), the egg case displays a substantially greater permeability to Cl than Na; this discrepancy between the rate constants of efflux for Na and Cl is not seen in the teleosts (Evans, 1979). The high rate of Na and urea turnover across the skate egg case corroborates earlier studies

(Needham & Needham, 1930; Hornsey, 1979; Foulley & Melinger, 1980). It is important to note, however, that the rate constants for both Na and Cl efflux from the egg case are of the same order as those described for the Na and Cl efflux from many marine teleost species (see Evans, 1979, for relevant references).

Since the egg-case membranes are relatively permeable to the major solute constituents important in elasmobranch osmoregulation (Evans, 1979), one might suppose that the small volume of fluid contained (along with yolk and albumin) within

Table 1. *Rate constants* of Na, Cl and urea effluxes from skate egg cases*

Na	Cl	Urea
0·24 ± 0·03 (10)	0·60 ± 0·11 (8)	0·23 ± 0·07 (4)

* Rate constant is fraction of exchangeable substance effluxed per hour $\bar{x} \pm$ S.E. (*N*).

Table 2. *Analysis of some constituents of egg-case fluids compared with sea water*

	Na*	Cl	K	Urea	Total
Egg-case fluid	360 ± 18 (25)	403 ± 14 (25)	6·6 ± 0·5 (25)	19 ± 6·5	778 ± 47 (18)
Sea water	446 ± 3 (8)	505 ± 4 (8)	8·8 ± 0·4 (8)	n.d.	944 ± 1 (12)

* Solute concentrations in mM/l; total concentration in m-osmol/kg.
n.d. = not determined. $\bar{x} \pm$ S.E. (*N*).

the egg case would be basically sea water, dictating that membranes surrounding the developing embryo would have to osmoregulate during development. Comparison of the concentration of the major constituents of the egg-case fluids with sea water (Table 2) indicates that the fluids are hypo-osmotic to the surrounding sea water and contain significantly less Na, Cl and K levels than sea water. The egg-case fluids do contain small and variable concentrations of urea. Five of the cases contained urea concentrations below the limits of detection by our method (0·3 mM), while six of the samples contained concentrations above 40 mM. Unfortunately, we have no analysis of the embryonic fluids because the embryos enclosed in the purses in the present study were relatively undifferentiated. However, if we assume that even early embryonic tissues have extracellular osmolalities and ionic concentrations similar to those of the typical adult elasmobranch (i.e. approximately 1000 m-osmol/kg, and Na, Cl, K and urea levels of 230, 220, 3 and 357 mM/l, respectively; Evans, 1979), it is apparent that the developing embryo would face a net influx of both ions and water from the egg-case fluids. These net influxes would facilitate volume expansion during development; however, the relative roles played by this means of water and salt uptake and uptake following metabolism of the yolk are unknown. One might propose that the osmotic gradient has been increased (above what it would be if sea water were enclosed in the egg case) to facilitate osmotic uptake of water into the embryo, while the ionic gradients have been reduced because mechanisms for salt extrusion (branchial and rectal gland) have not been differentiated yet. It is interesting to note that the high egg-case permeability to urea is actually adaptive. Since the embryo is producing urea even during the early stages of development (Needham & Needham, 1980; Reed, 1968*b*), if the egg case were not permeable to urea, its

concentration would increase in the egg-case fluids and eventually the osmotic gradient favouring net influx of water into the embryo would be abolished.

The mode of formation and control of the volume and composition of the egg-case fluids are unknown. One must assume that the initial fluid is deposited as the egg case is formed in the female oviduct and is probably therefore adult extracellular fluid. Because of the high urea and ionic permeability of the egg case, the urea levels in egg-case fluids would fall and the ionic concentrations would rise immediately after the case was laid. However, how the ionic and osmotic gradients between the egg-case fluids and the sea water are eventually established and maintained is unknown and difficult to imagine because of the extremely high permeability of the egg-case membranes. Hornsey (1978) proposed that the membranes may be electrically charged because the permeability of the egg case to Na was greater than to urea, while the reflexion coefficient (ability to generate an osmotic gradient) was also greater for Na. In the present studies, the rate constant of efflux of urea and Na were not significantly different ($P > 0{\cdot}10$); however, there is significantly more Na in the egg-case fluids than urea so the flux (rate constant times concentration) and, presumably, permeability for Na would also be greater than for urea.

These studies indicate that the egg case of *Raja erinacea* is able to maintain significant osmotic and ionic gradients between the surrounding sea water and the egg-case fluids, despite an extremely high permeability to salts, urea, and presumably water. It is clear that the mechanisms involved in maintaining these gradients warrant further study.

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