ACCUMULATION OF CALCIUM AND MAGNESIUM IN RAJA ERINACEA EGG CAPSULE DURING FORMATION AND AFTER OVIPOSITION

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Eggshells of many terrestrial oviparous vertebrates contain minerals associated with a protein matrix. Minerals are deposited in the shell while the egg resides in the reproductive tract. We examined the possibility that oviparous elasmobranchs too might deposit minerals in their egg capsules during assembly of capsule precursors in the shell gland or during tanning of the capsule *in utero*. Moreover, once oviposited, these egg capsules are exposed to high concentrations of calcium and magnesium in sea water, so we hypothesized that these ions bind to capsular material during incubation. This seemed likely because the capsule is stabilized by a form of quinone tanning and capsules at oviposition contain catechols which avidly bind multivalent cations (Koob & Cox, *The Bulletin 26:* 109-112, 1986; Koob, *The Bulletin 25:* 123-125, 1985). This report presents data on calcium and magnesium contents in *Raja erinacea* egg capsules during formation and tanning *in utero*, and following exposure to sea water.

Capsule specimens were excised from dorsal and ventral body walls, washed exhaustively with water, dried at 65°C and weighed. Bound calcium and magnesium were eluted with 1N HCl and quantified in diluted aliquots of the eluate by atomic absorption spectroscopy. Preliminary experiments established that both calcium and magnesium were firmly bound to the capsule and that complete elution of these minerals was accomplished with 1N HCl. Each value presented below is the mean of 27 specimens or more from a minimum of three capsules (Table 1).

METAL BODY WALL	untanned *in utero* (3)	tanned *in utero* (3)	urogenital sinus (3)	at oviposition (3)	at 1 month (5)
CALCIUM					
DORSAL	0.56±0.13	1.22±0.13	1.68±0.07	1.79±0.03	2.18±0.03
VENTRAL	0.69±0.15	1.17±0.12	1.53±0.05	1.71±0.02	2.14±0.02
MAGNESIUM					
DORSAL	0.09±0.03	0.32±0.02	1.82±0.10	2.07±0.01	2.72±0.03
VENTRAL	0.13±0.04	0.32±0.02	1.82±0.09	2.06±0.04	2.58±0.04

Table 1. Calcium and magnesium contents (μg metal/mg dry weight) in partially tanned capsules from *in utero*, in fully formed capsules from *in utero*, in capsules from the urogenital sinus, in capsules collected within 24 hours of oviposition, and in capsules which had incubated in sea water for one month. Nine specimens from each body wall were analyzed and the calcium and magnesium contents were averaged to yield a mean value for each capsule. The values presented above are the mean ± SEM of the indicated number (n) of capsules.

Partially tanned capsule contained 0.56μg Ca and 0.11μg Mg per mg dry weight. Contents of both calcium and magnesium increased with further time *in utero* reaching levels of 1.20 μg Ca and 0.32μg Mg per mg dry weight in fully formed, tanned capsule. This accumulation of bound calcium and magnesium during formation and in the uterus was coincident with the introduction and oxidation of catechols. Capsules removed from the urogenital sinus, where they are carried for several hours and first experience sea water, contained 1.60μg Ca and 1.82μg Mg per mg dry weight. After incubation in sea water for one day, the capsules contained 1.75μg Ca and 2.06μg Mg per mg dry weight. Capsules continued to accumulate these minerals during incubation in sea water so that by one month calcium and magnesium levels increased to 2.16μg Ca and 2.66μg Mg per mg dry weight.

These measurements show that calcium and magnesium accumulate in capsular material during formation and tanning *in utero*. The bulk of the calcium which is deposited in the capsule appears to originate from maternal sources. Some magnesium also derives from maternal sources while the capsule is in the reproductive tract, but the majority of magnesium is acquired from sea water while the capsule resides in the urogenital sinus. Since calcium and, to a lesser extent, magnesium are introduced into capsular material while the capsule is in the shell gland and uterus, these ions may be involved in the tanning and stabilization of capsular precursors. Since both minerals accumulate rapidly when the capsule is exposed to sea water, they may impart specific physicochemical properties on the capsule. Future studies will be necessary to delineate the function of bound calcium and magnesium in these capsules. These observations establish that calcium and magnesium are integral components of *Raja erinacea* egg capsules, and that oviparous elasmobranchs, like oviparous terrestrial vertebrates, deposit minerals in their eggshells.

These studies were funded by the investigators.