ON THE ROLE OF EGG JELLY IN *RAJA ERINACEA* EGG CAPSULE

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Surrounding the fertilized ovum in elasmobranch egg capsules at oviposition is a viscous jelly originating from the proximal region of the shell gland. Little has been reported about the composition, properties, or function of this material in any elasmobranch, oviparous or viviparous. In the little skate egg capsule, as well as those of other skates and oviparous sharks, the egg jelly disappears after approximately one third of development is completed, opening the capsule to embryo-assisted flow of sea water through the slits in the four horns (Long and Koob, *Bull., MDIBL* 36, 117-119, 1997). The jelly begins to liquefy early in development, liquefaction proceeding from nearest the ovum outward. Initially, the small amount of liquefied jelly immediately surrounding the egg provides a liquid medium in which the embryo is suspended and is able to move freely. Eventually, all of the jelly in the capsule lumen liquefies and as a result the embryo with suspended yolk sac can utilize the entire space. The final event is removal of the dense, gel-like jelly in the horns, at which time the embryonic tail appendage begins to pump water through the horn slits. We have initiated studies to delineate the composition, properties, and molecular mechanisms for liquefaction, i.e., the overall function of the egg jelly, in the skate egg capsule. This report focuses on egg jelly carbohydrates since preliminary compositional analyses indicated that they make up a significant proportion of the jelly and polysaccharides form viscous biological materials. Two functions for the egg jelly are posited: it functions solely as a structural support and/or it serves as a nutritive source for development.

The viscosity of the egg jelly differs in the various capsular regions. While the lumen jelly has a relatively low viscosity, the jelly located at the seams and in the hollow horns is a stiff gel. The carbohydrate composition of the jellies from these compartments was measured to determine whether the different properties result from either differing carbohydrate composition or concentration. Jelly from the three compartments was collected from three capsules within 24 hours of oviposition. One sample was hydrolyzed in 2 M trifluoroacetic acid (TFA) for neutral sugar analysis; a matching sample was hydrolyzed in 4 M hydrochloric acid (HCl) for amino sugars; hydrolysis was carried out at 100°C for either 3 hours (TFA) or 5 hours (HCl). Carbohydrates in these hydrolyzates were identified and quantified by HPLC chromatography on a Carbo-Pac PA1 column (Dionex) using pulsed amperometric detection. This method detects neutral and amino sugars, but does not detect acidic carbohydrates, such as glucuronic and sialic acids.

Four carbohydrates, galactosamine, glucosamine, galactose and fucose, were detected in the hydrolyzates of egg jelly by the HPLC method described above. Jelly from all three compartments contained these carbohydrates, but the relative proportions of the four carbohydrates and their concentrations differed significantly (Fig 1). Highest carbohydrate concentrations were found in the horn jelly. Somewhat lower concentrations were measured in the seam jelly. Lowest carbohydrate concentration occurred in the lumen jelly in which it was

11% and 7% that of the seam and horn jellies respectively. While galactosamine and glucosamine predominated in all three samples, the relative concentrations of the four carbohydrates varied among the three jellies (Fig. 1). These results suggest that the difference in the viscosity or stiffness of the jellies from the three regions is in part a result of the differing carbohydrate composition.

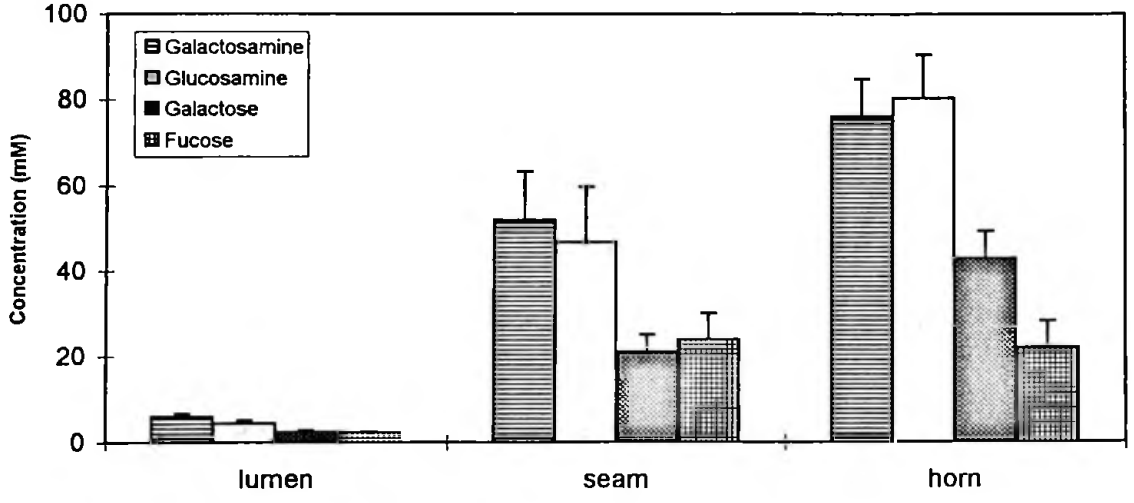

Figure 1. Mean carbohydrate composition in egg jelly from the capsule lumen, hatching seam and horns of egg capsules within 24 hours of oviposition. The values presented are averages from samples isolated from three eggs; error bars represent standard deviations.

In order to determine whether liquefaction of egg jelly resulted from degradation and removal of carbohydrate containing material, carbohydrate analyses were performed as described above on liquefied lumen jelly from eggs at 40 to 70 days of development, the principal period when the lumen jelly is liquefied. Figure 2 shows the concentrations of the four predominant carbohydrates

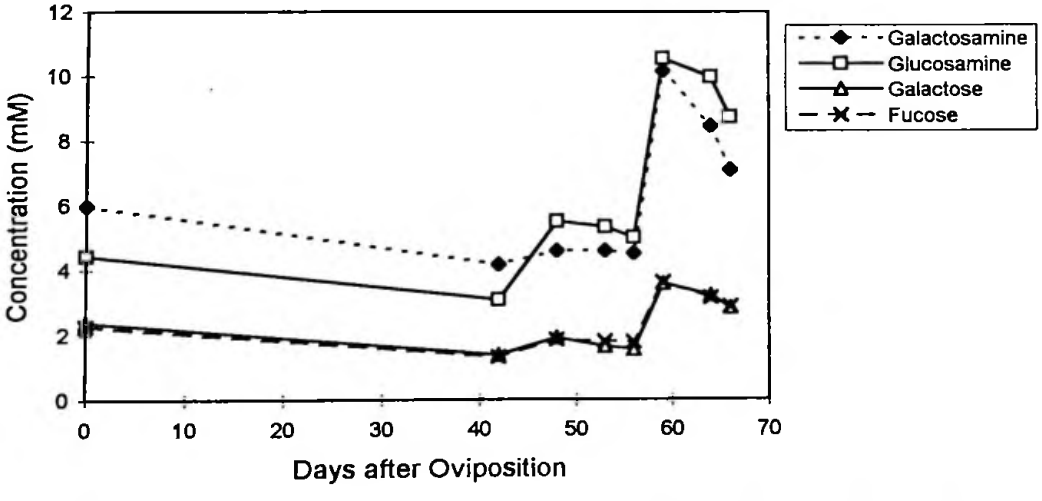

Figure 2. Carbohydrate concentration in liquefied egg jelly with time after oviposition.

identified above with time after oviposition in these eggs. Little change in carbohydrate concentration could be detected in eggs between days 42 and 56, although a slight increase in glucosamine may have occurred between days 42 and 48. The concentration of all four carbohydrates increased between days 56 and 59, then declined until day 66. No loss of carbohydrate was detected, indicating that liquefaction of the egg jelly does not rely on removal of carbohydrate containing material. The simultaneous increase in all four carbohydrates noted between days 56 and 59 is likely the result of liquefaction of the seam and horn jellies.

Results from SDS/PAGE analysis of liquefied lumen jelly from eggs between 40 and 70 days after oviposition are shown in Figure 3. Jelly at oviposition is not solubilized by SDS/PAGE sample buffer and nothing is extracted from this jelly as assessed by SDS/PAGE. At day 42 of development, a wide array of high molecular weight, Alcian blue staining bands was extracted from lumen jelly solubilized in sample buffer and resolved on SDS/PAGE. Extracts of lumen jelly from 48 to 68 day old eggs showed extractable Alcian staining material of smaller sizes. By day 68, the high molecular weight components had disappeared and the three lower molecular weight bands predominated (24 - 66 kDa). None of these bands stained with Coomassie Brilliant Blue.

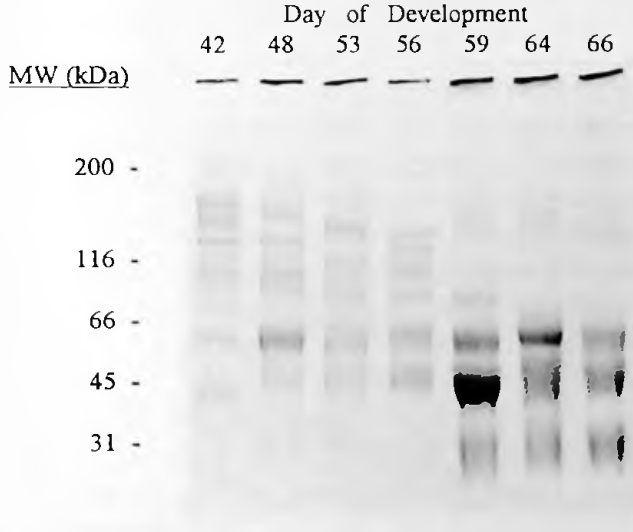

Figure 3. 4-20% linear gradient SDS/PAGE analysis of liquefied lumen egg jelly during the liquefaction process. Egg jelly was diluted 1:1 with gel sample buffer and run directly on the gel. The gel was stained with Alcian blue only.

Based on the observation that there was essentially no loss of the four carbohydrates quantified during the liquefaction process, it appears that the egg jelly does not serve as a substantial source of carbohydrate nutrition for early development. Rather, the egg jelly seems to function as a structural device to support hydrodynamically the egg and developing embryo. The molecular basis for the liquefaction process does not rely on removal of carbohydrate, but instead on depolymerization of the carbohydrate containing materials, as indicated by SDS/PAGE analysis. The nature of the carbohydrate containing material and the exact mechanisms for this depolymerization are under investigation.

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