ON THE SPECTRAL PROPERTIES OF RAJA ERINACEA EGG CAPSULES

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At oviposition Raja erinacea egg capsules are a deep greenish-brown. The dorsal body wall is semi-translucent while the ventral wall is transparent allowing a clear view of capsular contents (upper capsule, Figure 1). With incubation in sea water capsular material gradually darkens. By one year the capsule has turned black and the capsular walls no longer transmit light (lower capsule, Figure 1). Neither the chemical bases nor the environmental requisites for this color change have been investigated. However, we have shown that capsule formation involves the enzymatically catalyzed conversion of dihydroxyphenols to quinones (Koob and Cox, The Bulletin 24, 78-80, 1984) and this process may be partially responsible for color development. This report describes an experimental study on the effects of oxidation and reduction on the spectral properties of little skate egg capsules.

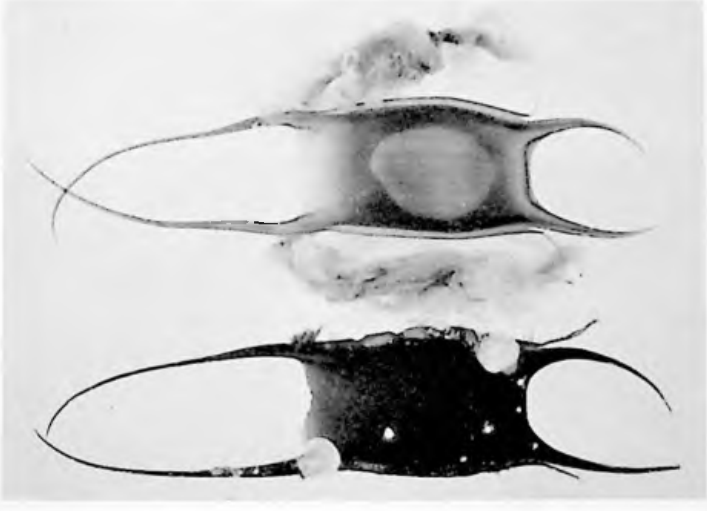

Figure 1. Ventral aspect of Raja erinacea egg capsules incubated 2 days (upper) and 363 days (lower). The lower capsule had been incubated in Frenchman Bay and contained a live embryo with yolk sac.

Materials and Methods

Spawning little skates were maintained in fresh circulating sea water and freshly oviposited egg capsules were collected within two days of oviposition. Capsular contents were removed and the ventral body wall was separated from the rest of the case. Only the ventral wall was used for the studies reported here since its transparency is much greater than the

dorsal wall. Full-thickness strips of ventral wall 5 mm in width were cut perpendicular to the long axis of the capsule. Each strip was bisected along the mid-line producing a matched pair of specimens. One strip was chemically treated while the matching strip served as the control. Reduction of specimens was accomplished by two additions of $NaBH_4$ in 0.1M NaH_2PO_4, pH 7.4, allowing thirty minutes of incubation at ambient temperature after each addition. The incubation fluid was then acidified with CH_3COOH, the strips were washed with distilled water and subsequently equilibrated in sea water. Oxidation of capsular specimens was catalyzed by 0.1M $FeCl_3$ in distilled water for 24 hours at ambient temperature. These strips were likewise washed and equilibrated in sea water. Control samples were incubated in the fluids lacking either $NaBH_4$ or $FeCl_3$. Following chemical treatments spectral properties were assessed with a Beckman DU-40 scanning spectrophotometer by trimming specimens to exactly 4 mm in width and placing them in quartz microcuvettes. The specimens were arranged at right angles to the incident light so that the light path was totally occluded by the specimen. Both absorbance and transmittance were measured at wavelengths from 360 to 900 nm. Spectral scans of the treated specimens were compared to those of the matching control strip in order to generate difference spectra. All measurements were performed in sea water.

Results

Absorbance spectra of control strips revealed that essentially no light at wavelengths between 360 and 470 nm was transmitted through the capsular material. At higher wavelengths absorbance gradually decreased and the amount of transmitted light increased. Approximately 60% of the incident light at 900 nm was transmitted.

Reduction of capsular specimens with $NaBH_4$ resulted in a loss of color intensity and an increase in transmitted light. The strips changed from deep greenish-brown to light green. Figure 2 shows the average difference spectrum between control and treated strips. The peak absorbance lost upon reduction was at 470 nm.

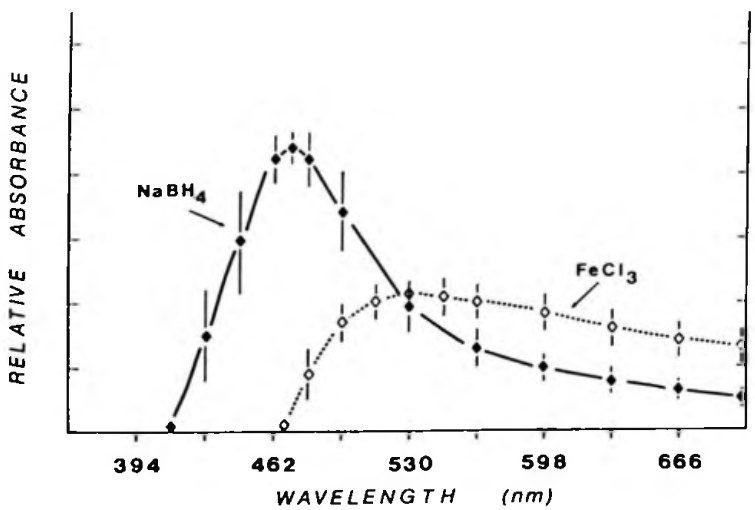

Figure 2. Average difference spectra between control and $NaBH_4$ reduced or $FeCl_3$ oxidized egg capsule material.

$FeCl_3$ catalyzed oxidation of capsular material caused an increase in color intensity with a concomitant decrease in transparency. The material turned a dark brownish-black especially at the cut edges. The average difference spectrum between control and oxidized samples indicated that oxidation caused an increase in absorbance at wavelengths greater than 470 nm (Figure 2). No clear peak of absorbance was found. Following oxidation with $FeCl_3$ little light at any wavelength was transmitted through the capsular material.

Discussion

These observations indicate that *Raja erinacea* egg capsules remain chemically reactive following oviposition and during incubation in sea water. The susceptibility of egg capsules to $NaBH_4$ reduction and $FeCl_3$ oxidation suggests that capsular material possesses inherent redox potential. While the chemical nature of this redox potential has not been identified, the products of catechol oxidase or polyphenol oxidase are known to form reversible oxidation-reduction systems, for example melanin. Further experimentation with less powerful oxidizing and reducing agents will help to identify the basis for capsular redox potential.

The change in capsular color and transparency caused by $FeCl_3$ catalyzed oxidation mimics the natural color change which occurs gradually during the lengthy incubation of skate egg capsules in sea water. The identity of the principle undergoing oxidation is currently unknown; however, preliminary evidence indicates that catechols are present in capsular material at oviposition and these could be oxidized to quinones forming dark pigments. Oxidation of capsular material with $FeCl_3$ reduces catechol content in freshly oviposited capsules (unpublished), indicating this mechanism is possible. Measurements of catechol contents of capsules incubated under natural conditions for varying amounts of time will determine whether catechol oxidation occurs *in situ*. These incubations, as well as others under chemically defined conditions, are currently underway. This work was supported by a grant from the Lucille Markey Charitable Trust and M.D.I.B.L. to T.J.K.