STUDIES ON SKATE (RAJA ERINACEA) EGG CAPSULE FORMATION II. INTRODUCTION OF CATECHOLS OCCURS IN UTERO

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During spawning in oviparous elasmobranchs secretory cells in nidamental glands contain abundant cytoplasmic granules filled with the precursors of egg capsules. Certain secretory granules located in specialized regions of the glands appear rich in phenolic proteins and phenoloxidase. Histochemical tests have demonstrated their presence in nidamental glands of Raja sp. (Brown, Quart. J. micr. Sci. 96, 483-488, 1955), Chiloscyllium griseum (Krishnan, Biol. Bull. 117, 298-307, 1959) and Scyliorhinus canicula (Threadgold, J. Histochem. Cytochem. 5, 159-166, 1957; Rusaouën, J. exp. mar. Biol. Ecol. 23, 267-283, 1976). These observations are responsible for the view that formation of elasmobranch egg capsules involves a form of phenolic or quinone tanning which evolves after secretion of granules from glandular lamellae. In Raja erinacea, as well as other oviparous elasmobranchs, capsular precursors are white when secreted from the nidamental gland but then gradually develop color as the formed capsule moves out of the gland and into the uterine portion of the oviduct. Tanning continues while the capsule is held in the oviduct, eventually resulting in the deep greenish brown characteristic of skate egg capsules at oviposition.

In the first paper of this series we reported the identification of a catechol oxidase isolated from capsules tanning in utero (Koob & Cox, The Bulletin 24, 78-80, 1984). The enzyme was capable of oxidizing several catechols to their corresponding quinones producing dark pigments. The presence of this enzyme in tanning capsules suggests that catechols are present in the capsular material. We postulated that the catecholic substrate upon which the enzyme acts could be incorporated in granule bound phenolic precursors or alternatively that catechols could be added to the capsular matrix following secretion and assembly of capsular precursors while the formed capsule moves through the uterus. The present report provides evidence that catechols are incorporated into the capsular material in utero.

Materials and Methods

Spawning female skates (Raja erinacea) were palpated several times daily for the presence of capsules forming in the nidamental glands. When nearly completed capsules were detected, they were excised and placed in fresh sea water. The spectral properties of the capsular walls were assessed as previously described (Koob, The Bulletin 25, 123-125, 1985). Dorsal and ventral walls were separated from the capsule and cleaned of adhering albumen. Each wall was dissected into eight successive 5mm-wide strips cut perpendicular to the long axis of the capsule and then carefully trimmed to 4mm x 2cm. All sample preparation was performed with the capsular material in sea water. Specimens were then placed in quartz microcuvettes at right angle to the incident light so the specimen totally occluded the light path. The absorbance spectrum between 220nm and 900nm wavelength was measured with a Beckman DU-40 scanning spectrophotometer. Spectral measurements were made with the specimens immersed in sea water. Following spectral scans specimen thickness was measured with a dial caliper. Specimens were then thoroughly washed with distilled water,

weighed and hydrolyzed under nitrogen in 6N HCl at 108°C for twenty-four hours. Catechol content in diluted aliquots of the resulting hydrolyzates was quantified by the colorimetric procedure of Arnow (J. Biol. Chem. 118, 531-537, 1937) using 3,4-dihydroxyphenylalanine as standard. This method specifically detects o-diphenols which are un- or monosubstituted with the side chain meta or para to the first hydroxyl group; di- and polysubstituted catechols are largely unreactive (Waite & Tanzer, Analyt. Biochem. 111, 131-136, 1981).

Results

Partially formed capsules were found which lacked only the anterior horns. The anterior third of each capsule was well formed, the anterior seam being fully sealed, but still within the lumen of the nidamental gland. The posterior horns and lower two thirds of the capsule body extended into the uterine lumen. A gradation in color was evident in the capsular walls ranging from light greenish brown at the posterior end to pure white at the newly secreted anterior end (Figure 1).

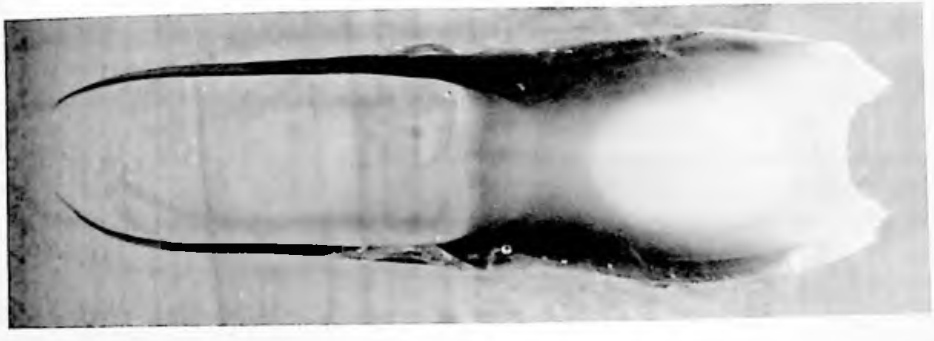

Figure 1. Partially formed capsule analyzed for spectral properties and catechol content.

Spectral analyses of intact capsular specimens confirmed this graduated color intensity. The most posterior specimen (#1) absorbed the greatest amount of light at wavelengths between 280nm and 560nm, while the anterior-most specimen (#8) absorbed the least light. Specimens two through seven exhibited intermediate levels of absorbance which gradually decreased from posterior to anterior (Figure 2). These results show that the tanning of capsular material occurs after secretion from the nidamental gland and that the extent of color development is directly proportional to the post-secretory interval.

Catechol concentration in the walls of these capsules varied from less than 1ug to 26.6ug per mg wet weight. Specimen #1 from the most tanned, posterior region of the ventral wall contained highest catechol content at 26.6ug per mg wet weight. Catechol content decreased in each successive specimen (Figure 2). The newly secreted white material at the anterior end of dorsal and ventral walls had lowest catechol contents. Specimen #8 of the ventral wall contained 1.4ug per mg wet weight. These measurements indicate that the

catechol content of tanning capsular material increases with time after secretion and relies on events occurring while the capsule moves through the uterus.

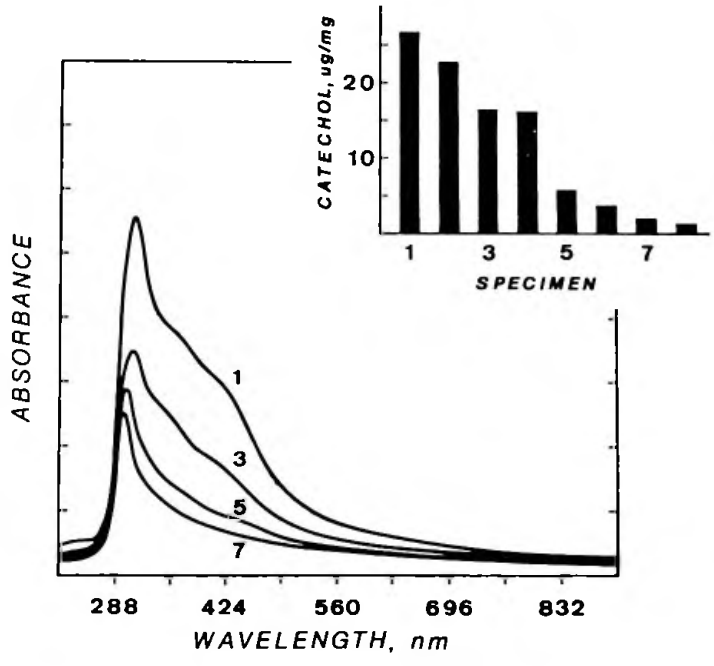

Figure 2. Absorbance spectra of specimens 1, 3, 5 and 7 from the ventral wall normalized to specimen thickness. Inset at upper right shows catechol contents of specimens 1 through 8 from the ventral wall normalized to specimen wet weight. The eight specimens were numbered 1 through 8 from posterior (most tanned) to anterior (least tanned).

Discussion

The parallel increases in color, absorbance and catechol suggest that the introduction of catechols into the capsular matrix is intimately involved in the tanning process. While catechols have little color, their oxidation products, because they are highly conjugated, exhibit rich colors. Catechol oxidation occurs *in utero* as active catechol oxidase is present on tanning capsules (Koob & Cox, *op. cit.*). We believe the introduction and oxidation of catechols in the capsular matrix account for the development of color while the capsule is held *in utero*. This conclusion highlights the importance of egg retention in the uterus for generating the physicochemical properties of capsular material. The extent to which variations in these properties influence hatching success is not known, however, we have found that the duration capsules are held *in utero* can vary from 1½ to 8 days (Koob & Callard, The Bulletin 25, 138-139, 1985).

The source and nature of the catechols which accumulate in the capsular matrix are not known. We speculate that two distinct mechanisms would account for their apparent introduction *in utero*. The phenolic constituents of capsule precursors could be converted to catechols after secretion from the nidamental gland, possibly by hydroxylation of phenolic groups or derivatization of phenols with other reactive capsular constituents. The second possible mechanism invokes the supply of catecholic substances from non-nidamental sources. For example, the uterus could secrete a coating of compounds containing catechols. Further detailed biochemical analyses will be necessary in order to delineate which mechanism operates during the formation of skate egg capsules.