Fine Structure of the Ovary in the Red Abalone
*Haliotis rufescens* (Mollusca: Gastropoda)

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**Summary.** The ovary of the red abalone consists of a series of trabeculae or polygonal chambers that extend from the ovarian wall toward the wall of the digestive gland. The ovarian wall consists of smooth muscle cells and bundles of collagen fibrils, that extend into the trabeculae. Presynthetic, synthetic and early postsynthetic oocytes are surrounded by a single layer of follicle cells which bind these oocytes to the trabeculae. The follicle cells contain bundles of microfilaments. Mature oocytes lack a follicle cell layer and are found free in the chambers formed by the trabeculae. Each oocyte is surrounded by a vitelline layer, a chorion, and a thick jelly coat. Stages in the formation of these layers are described. It is suggested that prior to spawning the trabeculae detach from the wall of the digestive gland and retract toward the ovarian wall. Contractions of the ovarian wall may force oocytes out of the ovary into the seawater where fertilization occurs.

**A. Introduction**

Although the reproductive biology of certain gastropods, such as *Aplysia*, has been extensively studied (Thompson and Bebbington 1969; Dudek et al. 1980; Stuart and Strumwasser 1980) little work has been done on more primitive forms, such as the abalones. Several histological studies have outlined the basic structure of the ovary (Booolootian and Farmanfamaian 1962; Newman 1967; Tomita 1967; Young and DeMartini 1970; Brickey 1979; Tutschultz and Connell 1981). However, further morphological information is needed to provide a foundation for studies on the mechanisms involved in natural and induced (Morse et al. 1979) ovulation and spawning. In this paper we present information on the anatomy of the ovary in the red abalone (*Haliotis rufescens*) at the electron microscopical level. We chose to examine the ovary in the red abalone because it is of particular interest.
to aquaculture companies. It is the largest species of abalone (Cox 1960), and unlike other abalones, it is capable of spawning throughout the year (Webber and Geise 1969).

B. Materials and Methods

Large (shell length 20 cm), mature, red abalones (Haliotis rufescens Swainson 1822) were collected subtidally off Fort Ross Beach in Sonoma County, California; small abalones (shell length 8–10 cm) were purchased from California Marine Associates, Cayucos, California. They were maintained in a refrigerated (15° C), 150 gallon aquarium filled with Instant Ocean (Marineland Products) and fed the brown alga Egregia menziesii.

The ovaries were examined by light microscopy (LM) and transmission (TEM) and scanning (SEM) electron microscopy. For LM, tissue was soaked in Bouins fixative for 40 hrs, dehydrated in acetone, cleared in toluene and embedded in paraplast. Sections, 8–10 μm thick, were stained with hematoxylin and eosin, PAS, and Alcian blue (pH 2.5) according to the procedures described in Humason (1972).

Tissue to be examined by TEM was placed in a solution of 3% glutaraldehyde and 0.1 M sodium cacodylate (pH 7.8) at room temperature for 3 hrs. During this time, the tissue was dissected into pieces less than 1 mm on a side. Following a brief rinse in buffer, the tissue was post-fixed in 1% OsO₄ in 0.1 M sodium cacodylate for 1 h at room temperature and dehydrated in a graded series of acetone. It was then infiltrated and embedded in Spurr's low viscosity plastic and sectioned on Porter-Blum MT-1 and MT-2 ultramicrotomes. Thin sections were stained 1 h in uranyl acetate, 5 min in lead citrate and viewed in a Hitachi H 500 TEM.

For SEM, tissue was processed through 100% acetone as described for TEM. It was then dried in a Denton DCP-1 critical point drying apparatus, coated with a gold-palladium mixture in the Technics Hummer II and viewed in a JOEL JSM 35C SEM.

C. Observations

1. General Anatomy of the Ovary

When the shell of an abalone is removed by severing its adductor muscle, a conical appendage is visible wrapping around the right-posterior margin of the adductor muscle (Fig. 1). A transverse section through the conical appendage reveals that it is composed of two organs; an inner, cylindrical digestive gland surrounded by the ovary (Fig. 2). The tissues associated with the ovary, beginning from the outside and moving toward the digestive gland, are as follows: 1) glandular epithelium, 2) ovarian wall, 3) trabeculae extending to the wall of the digestive gland, and 4) oocytes.

2. Glandular Epithelium

The epithelium is composed of a single layer of cells. The most obvious cells in this layer are secretory and they are supported by smaller non-secretory epithelial cells. In SEM (Fig. 3), the dome-like apices of the secretory cells are seen to bear numerous short microvilli and 0–5 circular invaginations. The invaginations are 1 to 4 μm in diameter and appear to be the pores through which a mucus-like material is released. In some scanning electron micrographs, strands of electron lucent material were seen extend-
Fig. 1. Schematic diagram of dorsal surface of abalone with shell removed. The ovary is situated in conical appendage CA that wraps around right posterior margin of adductor muscle AM. Cephalic CT, epipodial tentacles ET, ctenidium C, visceral mass VM, mantle MA, foot F.

Fig. 2. Schematic diagram of the abalone ovary showing trabeculae Tr extending from ovarian wall OW and epithelium E toward the wall of digestive gland DG. Attached to trabeculae are germinal epithelial cells GE and oocytes D in various stages of development. In the lumen between trabeculae are mature oocytes O that contain yolk granules (solid bodies) and lipid droplets (empty circles). Each oocyte is surrounded by a thick jelly coat JC.

ing from the invaginations and merging with a sheet of mucus that covers the epithelial cells.

In sectioned material, the epithelial cells are seen to rest on a thin, fibrous basal lamina and are connected to one another by desmosome junctions (Fig. 4, inset). The cells are filled with spherical (0.7 μm diam) electron dense granules that are membrane bound. These granules swell and become
Fig. 3. SEM of epithelial cells covering conical appendage. Pores $P$ at the apices of many cells. $\times 2,600$

Fig. 4. TEM of epithelial cell showing numerous electron dense secretory vesicles $V$. Microvilli $M$, nucleus $N$. $\times 5,300$. Inset: two desmosomes connecting adjacent epithelial cells. $\times 24,000$

Fig. 5. TEM of an epithelial cell cut in cross section to show its nucleus $N$ and the "chimney" $P$ into which the vesicles are secreted. $\times 6,000$

Fig. 6. TEM of smooth muscle cells $SM$ and bundles of collagen fibrils $CF$ in ovarian wall. $\times 9,200$. Inset: transverse section though thick $T$ and thin (arrows) filament. $\times 130,000$
less dense prior to secretion. In paraffin sections they stain with both PAS and Alcian blue. Figure 5 shows a cross section through an epithelial cell. In the center of the cell is a membrane-bound space or “chimney” that has been shown by serial sections to be continuous with the apical invagination seen with SEM. In addition to the mucous granules, these cells contain numerous mitochondria, Golgi bodies and cisternae of rough endoplasmic reticulum (RER).

3. Ovarian Wall

The ovarian wall is 60 μm thick in large abalones and 40 μm thick in small ones. This layer is composed of smooth muscle cells and bundles of collagen fibrils. The smooth muscle cells are long and spindle-shaped with an ovoid, centrally situated nucleus. The cytoplasm is filled with thick (625 Å diam) and thin (60 Å diam) filaments (Fig. 6 and its inset). Mitochondria and RER are present although not abundant. Adjacent smooth muscle cells are usually separated by bundles of collagen fibrils and on the rare occasions that the plasma membranes of smooth muscle cells were in contact, intermediate junctions were observed.

4. Trabeculae and the Lining of the Digestive Gland

The trabeculae are thin sheets of tissue extending from the ovarian wall toward the wall of the digestive gland (Fig. 7) which partition the ovary into compartments. Sections cut parallel to the surface of the ovarian wall show the polygonal outline of the trabeculae (Fig. 8) with an average diameter of 500 μm. In the lumens of the compartments lie the developing oocytes. The trabeculae are lined by germinal epithelial cells that are flat, except in the region where their ovoid nuclei are located. In SEM, these cells are covered by extracellular material that masks the cell surface. The cells rest on a basal lamina that is 0.2 μm thick and electron dense.

Cells previously termed jelly-cells (Brickey 1979) were typically found in clusters (Fig. 9) between the germinal epithelial cells and oocytes that were in their synthetic and early postsynthetic stages of maturation (see Oocytes section). Jelly cells have an irregular shape and their cytoplasmic filaments are filled with large vesicles that contain a fibrillar material of low electron density (Fig. 9). Mitochondria are the only organelles that are consistently observed in these cells, although in some sections RER and Golgi bodies were also seen at times.

Between the epithelial layers on either side of a trabecular sheet is a layer of connective tissue typically 15–40 μm wide. This layer contains smooth muscle cells (Fig. 10) and collagen fibrils that have the same appearance as those described in the ovarian wall. They are oriented parallel to the long axis of the trabeculae.

Most of the trabeculae in the specimens we examined extended to, and merged with, the wall of the digestive gland. A few of the trabeculae, followed through serial sections, terminated before reaching the digestive
Fig. 7. SEM of an ovary that has been sliced in half showing trabeculae $Tr$ extending from ovarian wall $OW$. All but two mature oocytes $O$ were washed out of ovarian compartments prior to fixation, however numerous developing oocytes $D$ remained attached to trabeculae. $\times 65$

Fig. 8. Light micrograph of a section through the ovary in a plane parallel to the surface of the ovarian wall showing the polygonal outline of the ovarian compartments. Germinal epithelial cells $GE$, mature, postsynthetic oocytes $O$, trabeculae $Tr \times 34$

Fig. 9. TEM showing a jelly cell containing vesicles of lesser $V1$ and greater $V2$ electron densities. $\times 11,400$
Fig. 10. TEM of smooth muscle cells SM and bundles of collagen fibrils CF in the trabeculae. Nucleus N of smooth muscle cell. × 21,600 Inset: SEM showing the elongate nature of smooth muscle cells (arrows) from the trabeculae. × 40

Fig. 11. TEM of a section through a presynthetic oocyte showing “watery” appearance of the cell. Mitochondria m, nucleus of the oocyte N₁, nucleolus n. Nucleus of follicle cell N₂, basal lamina (arrows). × 3,700

Fig. 12. TEM showing nucleus N and extensions (arrows) of a follicle cell that surrounds a presynthetic oocyte D. × 5,300. Inset: a higher magnification (×18,000) of a follicle cell containing a bundle of microfilaments (arrows). × 14,000
gland. The surface of the digestive gland facing the ovary is covered by flat epithelial cells that rest on a thin basal lamina.

5. Oocytes

Interspersed among the germinal epithelial cells are oocytes in various stages of development. We have adopted the nomenclature of Brickey (1979) who recognized the following stages of oocyte maturation: 1) germinal (described in the previous section), 2) presynthetic, 3) synthetic, and 4) postsynthetic.

Presynthetic oocytes are ovoid, 5–20 μm in diameter and are attached to the trabeculae (Figs. 11 and 12). The nucleoplasm and cytoplasm appear watery and contain few structural components. Several modifications of the basic fixatives, including different osmolarities, were used in an attempt to “retain” the cytoplasm of these cells, and all attempts failed. Because more mature oocytes and other ovarian tissue fixed well, we conclude that the empty appearance of presynthetic cells represents their true condition. The nuclear envelope is perforated by numerous pores. The cytoplasm contains ovoid and ring-shaped mitochondria, a few cisternae of RER and several large, spherical vesicles (1.5 μm diam) filled with a homogeneous, electron lucent material. Presynthetic oocytes are surrounded by a single layer of follicle cells (Figs. 11 and 12) that bind the oocyte to the basal lamina of the trabeculae. Each follicle cell is very thin except for a bulge where its irregularly shaped nucleus is situated. The cytoplasm contains Golgi bodies, smooth endoplasmic reticulum, RER, numerous free ribosomes, and occasional bundles of microfilaments (Fig. 12 inset).

Oocytes in the synthetic stage are pear-shaped and about 50 μm long (Fig. 13). The nucleus and cytoplasm appear denser than those in earlier stages, and the nucleus includes a large (4.2 μm diam), spherical, electron dense nucleolus (Fig. 14). The cytoplasm contains numerous Golgi bodies, small mitochondria, free ribosomes and clusters of small (0.3 μm diam) vesicles that appear empty. The follicle cell layer surrounding these oocytes is easy to observe, especially over the part of the oocyte that projects into the lumen of the ovarian compartment. In this region the cells are not so densely packed as they are along the edge of the trabeculae. When viewed

Fig. 13. SEM of pear-shaped oocyte in synthetic stage of maturation surrounded by a single layer of follicle cells. Two of the six radial thickenings on the rough surface of the follicle layer are indicated by the arrows. × 1,450

Fig. 14. TEM of a section through a synthetic oocyte showing the well developed nucleolus n, and the follicle cell layer (arrows). × 4,500

Fig. 15. TEM early postsynthetic oocyte surrounded by a developing jelly coat JC. The jelly coat extends from the oocyte surface OS to the follicle cells. × 9,000

Fig. 16. TEM of an early postsynthetic oocyte filled with vesicles V and large irregularly shaped spaces S. Above the oolemma lies an electron lucent zone (arrows) followed by the jelly coat JC. × 4,100
Fig. 17. TEM of an early postsynthetic oocyte that is more developed than the one in Fig. 16. The electron lucent layer (arrows) has lifted from the oolemma and large, electron dense granules G are present in the resulting space. Jelly coat JC. ×6,000

Fig. 18. TEM of postsynthetic oocyte showing breakdown of granules G in extracellular space beneath jelly coat JC and electron lucent layer (arrows). ×10,000
Fig. 19. TEM of a mature oocyte showing yolk granules Y and lipid droplets L in the cytoplasm. The oocyte is surrounded by the following three extra-cellular coats: vitelline layer VT, chorion CH and jelly coat JC. Indentations in the jelly coat (arrow) are where the section has intersected a pore. Above the jelly coat are remnants of the follicle cell FC. ×8,000

Fig. 20. SEM of a postsynthetic oocyte showing abundance of pores and four of the six radial grooves (arrows) on the surface of the jelly coat. ×750. Inset: higher magnification (×1,500) of jelly coat surface
by SEM the follicle cells appear as sheets with rough surfaces. Six narrow, thread-like thickenings are commonly seen on the follicle surface (Fig. 13).

Early postsynthetic oocytes are still surrounded by follicle cells that attach them to the walls of the trabeculae. Their cytoplasms are crowded with vesicles (Figs. 15 and 16) that contain a homogenous material with a central electron lucent area surrounded by a zone of higher electron density. Large, irregularly shaped membrane-bound vesicles (presumably lipid bodies) are also present. Oocytes at this stage are surrounded by a developing jelly coat. In the first stage we can identify, the space between the follicle cells and the oolemma is filled with small fuzzy granules embedded in an electron lucent material (Fig. 15). The granules are densely packed along the oocyte surface and become less dense toward the follicle cells. Next in time, a distinct extracellular boundary forms separating the jelly coat from a zone closer to the oocyte surface (Fig. 16). Later in maturation, large electron dense masses of fibrillar material are seen between the oolemma and the lower boundary of the jelly coat (Fig. 17). These masses break up and appear to give rise to the chorion and vitelline layers (Fig. 18).

Fully developed postsynthetic oocytes are easily identified by the following features: 1) large size (up to 200 μm), 2) spherical shape, 3) presence of ovoid (1.8 μm diam), electron dense yolk granules and spherical (6.0 μm diam), electron lucent lipid droplets in the cytoplasm, and 4) the presence of three extracellular coats surrounding the oocyte (Fig. 19). The oocytes lie free in the ovarian compartments and tend to accumulate in the part of the ovary adjacent to the wall of the digestive gland. Only remnants of the follicle cells are seen attached to their surfaces (Fig. 19).

Postsynthetic oocytes that are spawned or surgically removed from the ovary prior to fixation do not stick together. When examined by SEM, these oocytes are covered by a jelly coat (Fig. 20 and its inset) that has a distinctive pattern of pores (1–2 μm diam; average interpore distance 3–4 μm). In addition to the pores, there are six slight grooves that radiate out from a spot. This spot contains some irregularly shaped material that is presumably cellular debris. The six grooves correlate with the six thickenings of the follicle cell layer that are observed in synthetic oocytes. In thin section, the jelly coat is 5.2 μm thick and is composed of an electron lucent, fibrillar material (see Fig. 19). It has distinct upper and lower boundaries, and remnants of cellular processes may be seen inside the pores that pass through this layer (not shown). In paraffin sections, the jelly coat gives a positive PAS reaction and stains with Alcian blue.

Beneath the jelly coat lies a 0.4 μm thick chorion layer (see Fig. 19). In thin sections it is composed of 1–2 layers of ovoid bodies that contain densely packed fibers floating in an electron lucent material. Between the chorion and the oolemma is a 0.2 μm thick space filled with a fibrous, moderately electron dense material that comprises the vitelline layer. Microvilli from the oocyte surface penetrate into this layer (not shown).

The cytoplasm of a postsynthetic oocyte is filled with yolk granules and lipid droplets (see Fig. 19). Between these larger organelles are mitochondria, ribosomes and Golgi bodies. The nucleus is large and ovoid (30 × 60 μm) and contains a spherical nucleolus.
D. Discussion

Our findings confirm many suggestions that were based on light microscopical examinations of the ovary and present new information on the structure of the abalone gonad as seen by electron microscopy.

First, the covering of the “conical appendage” is a mucus-secreting epithelium and not a cuticle as was suggested by Young and DeMartini (1970). In the secretory cells, mucous granules are released into a pore from which the mucus is subsequently released.

Second, we have shown that the ovarian wall is composed primarily of smooth muscle cells. The presence of contractile cells in the ovarian wall in *Haliotis rufescens* had been previously determined by applying electric shocks to the conical appendage and noting its slight contraction (Young and DeMartini 1970). Histological studies on the ovarian wall of other species of abalone, however, reported the presence of only a non-contractile connective tissue (Crofts 1929; Bolognari 1953). The composition of the ovarian wall in these species of abalone needs to be reexamined, because we believe that contraction of the ovarian wall plays an important role in expelling mature oocytes from the ovary. Newman (1967) observed abalones spawning and reported that some animals raised and lowered their shells by contracting and relaxing their adductor muscles. Presumably, this increased pressure on the conical appendage and assisted the release of oocytes. However, shell pumping activity has not been observed in other spawning abalones and considering the abundance of smooth muscle in the ovarian wall, action of the shell adductor muscle seems unnecessary for spawning to occur.

Third, we observed the trabeculae in the red abalone extending perpendicularly from the ovarian wall toward the wall of the digestive gland and, in most instances, fusing with that wall. Our findings do not support the suggestion of Brickey (1979) that the trabeculae spiral around the digestive gland toward the right nephridiopore through which the oocytes are released. Instead, our observations agree with those of Newman (1967) who suggested that the connection between the trabeculae and the digestive gland is degraded prior to spawning, thereby forming a channel between the ovary and the digestive gland for the escape of the oocytes. If this suggestion is correct, one would expect fewer trabeculae to be found attached to the digestive gland wall as the time of spawning approaches. Our finding smooth muscle cells are present in the trabeculae suggests, moreover, that once the trabeculae have detached from the wall of the digestive gland, the trabeculae may shorten to facilitate the evacuation of all mature oocytes from the ovary.

Fourth, we have identified a follicle cell layer that surrounds all developing oocytes and binds them to the trabeculae. Previous researchers (Young and DeMartini 1970; Brickey 1979) believed that unlike most molluscan oocytes (Raven 1966), abalone eggs lacked a follicle cell layer. Young and DeMartini (1970) may have observed these cells because they described a thin membrane, which they interpreted as a fixation artifact, covering
immature oocytes. The follicle cells are extremely thin and do not appear
to synthesize any secretory material used during development of the oocyte.
SEM has shown six radial thickenings of the follicle cell layer that leave
their impressions as grooves on the surface of the jelly coat surrounding
fully developed oocytes. In TEM, the follicle cells are difficult to study
because they are so thin. However, small bundles of microfilaments were
seen in these cells. These microfilaments may play a contractile role in
retraction of the follicle cell processes that pass through pores in the jelly
coat, and in ovulation, the retraction of the follicle layer from the oocyte.
Comparable, microfilament-containing follicle cells have been described in
many other species including chitons (Anderson 1969), clams (Keck et al.
1975), lobsters (Talbot 1981), and star fish (Schroeder et al. 1979).

Sixth, we have new information on oocyte coverings. Brickey (1979)
described the formation of the three extracellular coats around fully devel-
oped oocytes and he suggested that the jelly coat of mature oocytes was
produced by exocytosis of material from the jelly cells. Although we cannot
exclude this possibility, it seems unlikely in view of the new observation
that a follicle cell layer is present, at least during the initial stages of jelly
coat formation. We suggest that the jelly coat, like the chorion and vitelline
layer, is produced from materials secreted by the developing oocyte itself.
The secretions of the jelly cells may be responsible for the matrix, first
identified by Newman (1967), in which the eggs are embedded. This material
may prevent the jelly coats of adjacent oocytes from fusing. Many questions
remain concerning the structure and function of the various cell types in
the abalone ovary and of the oocyte coverings, but we hope this initial
work will be useful to future studies on the reproductive biology of abalones.

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