**A STUDY OF ORGANELLES ASSOCIATED WITH
VITELLOGENESIS IN *ONCOMELANIA HUPENSIS QUADRASI*,
A MOLLUSCAN HOST OF *SCHISTOSOMA JAPONICUM***

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ABSTRACT

Organelles associated with vitellogenesis in *Oncomelania hupensis quadrasi* (Palo, Leyte strain) were studied with transmission electron microscopy. Follicle cells are closely associated with developing oocytes. Pre-vitellogenic oocytes have two types of yolk nuclei. Type I (=ergastoplasmic) consists of layers of rough endoplasmic reticulum enclosing cytoplasmic organelles, and Type II (=vesicular) exhibits a cluster of densely packed, clear to moderately electron-dense vesicles. Vitellogenic oocytes showed two prominent types of yolk bodies : highly osmiophilic yolk bodies stained densely with Sudan black, and less electron-dense yolk bodies with homogeneous matrix surrounded with a cisterna of endoplasmic reticulum. Mitochondria play a significant role in yolk synthesis as indicated by changes in the ultrastructure of these organelles (=metaplasia) during vitellogenesis. The present study documents for the first time in a prosobranch snail, the formation of yolk bodies from organelles mitochondria and endoplasmic reticulum.

INTRODUCTION

Documented studies on molluscan oogenesis at the ultrastructural level are scarce. Among gastropods in particular, the most extensively studied are planorbisid and lymnaeid pulmonate snails^{1,2,3,4,5,6,7,8} and the prosobranch snails, *Ilyanassa obsoleta*^{9,10} *Bembicium nanum*¹¹ and *Viviparus spp.*^{12,13,14}

While working with female *Oncomelania hupensis quadrasi* infected with *Schistosoma japonicum*, a marked reduction in oviposition, and histologically partial or total atrophy of the ovary were noted. Although, Faust & Meleney (1924) had reported destruction of the hepatopancreas and gonad of *O. h. quadrasi* and *Oncomelania hupensis nosophora* infected with *S. japonicum*, the information

on the gonadal pathology is rather cursory. To date, organelles associated with vitellogenesis has not been studied in any strain of *Oncomelania hupensis*. This present description in mature, uninfected *O. h. quadrasi* at the ultrastructural level may be useful in studies related to ovarian tissue damage in any trematode infected-snail host, particularly in schistosome-castrated oncomelanid snails. Also, this report will augment the scanty information documented on vitellogenesis in prosobranch snails.

MATERIALS AND METHODS

Snail Cultivation and Maintenance

Snail cultivation was adapted essentially from techniques of van der Schalie & Davis (1968), French (1974) and Medina (1984), with added modifications. *Oncomelania hupensis quadrasi* (Leyte strain) breeding age snails were supplied by the Center for Tropical Diseases, University of Lowell, Massachusetts. Aluminium trays (176×277 mm; 40 mm deep) were lined with plastic sheets half-filled with sterile muddy soil (autoclaved at 18–20 lbs pressure for two hrs), compacted and then flooded with sterilized tap water to a depth of about 25 mm. A homogenate of filaments of *Nostoc* sp. was added. Culture trays were covered with transparent plastic sheets and illuminated with two-40W cool white fluorescent lights to enhance the growth of algae as snail food. After 3–5 days, excess water was poured off, and young snails (2–3 weeks old) were added and left to grow until maturity. Trays were exposed to light for at least six hrs per day, to enhance algal growth as snail food. Filaments of *Nostoc* sp. and water were added when necessary. Excessively thick mats of algae were removed by gently placing a paper towel on the water to which excess algae adhered. Eggs laid on the sides of the culture trays were collected using a fine camel's hair brush and gently transferred into a sterilized Petri dish containing a small amount of aerated tap water. Newly hatched young were later transferred into Petri dishes prepared in the same manner as the larger culture trays. To provide for the aquatic nature of very young snails, more aerated tap water was added to culture dishes. After 2–3 weeks snails were transferred to larger culture trays where they reached sexual maturity. Snails on the sides and lids of the trays were returned to water daily to prevent desiccation.

Transmission Electron Microscopy

Six mature female *O. h. quadrasi* (25 weeks old) were cleaned of soil particles with a camel's hair brush, lightly crushed and their shells carefully removed under a stereomicroscope. The hepatopancreas (=snail liver) and ovary were cut from the rest of the snail body, fixed in 3% glutaraldehyde in 0.15 M sodium cacodylate buffer, pH 7.4 overnight at 4°C. Tissues were washed in 0.2 M cacodylate buffer four times at 30 min intervals, post-fixed in 1% osmium tetroxide in buffer pH 7.4 for one hr at 10°C, and then stained *en bloc* with 2% uranyl acetate (in 10%

ETOH) for 45 min. Specimens were dehydrated in serially graded ethanol (30, 50, 70, 80, 95%) for 10 min each wash, followed by 100% ethanol and propylene oxide (two changes each) for ten min. Tissue infiltration employing 1:1 mixture of propylene oxide and Spurr resin for six hrs was followed by embedding in 100% Spurr resin in plastic capsules, polymerized at 60°C for 48 hrs.

Sections 80–100 nm thick were cut with a diamond knife using Reichert OM U3 or Sorvall MT 2-B ultramicrotomes and stained with lead citrate for three min. Sections were observed using Zeiss 9S-2 and Phillips 300 electron microscopes.

Histochemistry

Ovaries of mature female *O. h. quadrasi* were fixed in 4% phosphate buffered formalin at 4°C, overnight. Tissues were rinsed in 0.1 M phosphate buffer (pH 7.3) for ten min and placed directly into catalyzed solution A, a formulated water-soluble monomer based on glycol methacrylate (Source: JB-4 Embedding Kit: Polysciences, Inc. USA). After 4–5 hrs, tissues were embedded in plastic capsules with embedding medium, a mixture of one ml JB-4 solution B stirred well into 25 ml of freshly catalyzed JB-4 solution A. Polymerization was allowed to proceed at room temperature for two hrs. Sections (1.5–2 µm thick) were cut with a glass knife using a Sorvall-Porter Blum ultramicrotome. Test for lipids was carried out using Sudan black.

RESULTS

The ovary of a normal, mature female *O. h. quadrasi* is a lobulated organ. Freshly dissected, the ovary is yellowish in color, and is surrounded by the hepatopancreas. A longitudinal section of the ovary viewed with a light microscope shows acini containing cells of various sizes, shapes and cellular constitution, representing oocytes in different stages of vitellogenesis (Figure 1). Close to the acini and hepatopancreas are large and small vesicular connective tissue cells.

Ultrastructurally, the ovarian acinar wall shows follicle cells (Figure 2) with irregularly shaped nuclei and condensed chromatin on the inner surface of the nuclear envelope, and diffuse chromatin throughout the nucleus. Organelles present are mitochondria, endoplasmic reticulum and electron-dense granules (Figures 2,3,4).

Pre-vitellogenic oocytes have round, oval and elongate mitochondria with clear cristae, vesicular smooth endoplasmic reticulum, and electron-dense vesicles. Mitochondria appear in close association with rough endoplasmic reticulum and other cytoplasmic organelles (Figure 5). The endoplasmic reticular network varies in form, with long and concentric cisternae commonly surrounding pre-

Fig. 1 Ovary of mature, uninfected female *O. h. quadrasi*. Pre-vitellogenic oocytes (Pv), vitellogenic oocytes (Vo), vesicular connective tissue cells (Vctc), hepatopancreas (Hp).

Fig. 2 Acinar wall showing inner layer of follicle cells (double long arrows), less electron-dense homogeneous layer (double short arrows), outer germinal epithelium (single arrow). Nucleus (N) of pre-vitellogenic oocyte, vesicular connective tissue cell (Vctc).

Fig. 3 Follicle cell on basal surface of early vitellogenic oocyte (Vo). Note two types of electron-dense bodies (arrows) and pigment cells (double arrows).

Fig. 4 Pre-vitellogenic oocyte (Pv) and follicle cell (Fc). Note eccentric nucleus (N) and short microvilli (arrow) on cell surface of vitellogenic oocyte.

yolk granules and other vesicles and mitochondria (Figure 6).

Two types of yolk nuclei were observed. Type I(=ergastoplasmic) has one to four layers of RER encircling cytoplasmic organelles such as mitochondria, electron-dense and clear vesicles (Figures 5,6,7); and Type II nucleus (= vesicular) consists of clusters of densely packed, clear to moderately electron-dense vesicles (Figure 7). Significantly fewer Golgi bodies compared to mitochondria and RER were found in pre-vitellogenic oocytes. Golgi bodies consist of stacks of saccular membranes which bud off transfer vesicles (Figure 8).

Late pre-vitellogenic oocytes show early signs of yolk synthesis, evidenced by some changes in the ultrastructure of mitochondria, Golgi bodies and the disintegration of yolk nuclei which are very prominent during early pre-vitellogenesis.

Late pre-vitellogenic to early vitellogenic oocytes exhibit changes in mitochondrial shape from elongate to subspherical. Cristae turn inconspicuous and the matrix increases in electron-density. All of these changes signal the beginning of the differentiation of mitochondria into yolk synthesizing organelles (= metaplasia) (Figures 8,9). Metaplasial mitochondria have a cluster of electron-dense crystalline bodies, and electron-dense masses of either concentric lamellae and/or homogeneous materials (Figure 9). As vitellogenesis progresses, yolk bodies become increasingly electron-dense. Some of these yolk bodies are more electron-dense with whorls of concentric lamellae surrounding some matrix substance; they later transform into large, homogeneous yolk bodies of even greater electron density (Type A yolk bodies) (Figure 10). Some Type A yolk bodies retain their fibrous appearance even after completion of vitellogenesis. The increased size of these yolk bodies is apparently due to accretion of immature yolk vesicles, granules and droplets (Figures 10,11). The other type of yolk bodies are less electron-dense with a homogeneous matrix (Type B yolk bodies) (Figure 12), the most striking feature of which is the presence of a single ER cisterna enveloping one or two of this Type B yolk bodies. Type A yolk bodies stained intensely with Sudan black indicative of the presence of fatty yolk; while Type B yolk bodies stained less intensely, suggesting the presence of lipoprotein.

During vitellogenesis the nuclear membrane forms many shallow infoldings, and nucleoli are absent (Figure 13). Wide follicular clefts (Figure 14) form between the follicle cells and the oocyte membrane.

Mature oocytes of *O. h. quadrasi* are classified as telolecithal, with the nucleus displaced by massive amounts of yolk toward the animal pole (Figures 4,13). The apical cell membrane has short microvilli. The cytoplasm has numerous large and small yolk bodies, ribosomes, vesicles and droplets composed of fatty and lipoprotein materials, a few empty vesicles and mitochondria.

Figs. 5 & 6 Pre-vitellogenic oocytes. Fig. 5 Rough endoplasmic reticulum RER (long arrow), and pre-yolk vesicles (double arrows). Fig. 6 Type I yolk nucleus. Note RER encircling some cytoplasm and other electron dense bodies. Mitochondria (Mi).

Fig. 7 Pre-vitellogenic oocyte. Type II yolk nucleus composed of cluster of clear to moderately electron-dense vesicles (arrows).

Fig. 8 Early vitellogenic oocyte. Metaplasial mitochondria (double arrows). Note numerous vesicular endoplasmic reticulum (ER) and immature yolk bodies (short arrows), Golgi bodies (Gb) with transfer vesicles.

Fig. 9 Metaplasial mitochondria with crystalline granules (long arrows) and concentric lamellae (short arrows).

Fig. 10 Yolk bodies (Yb) of different electron densities. Note small yolk vesicles (arrows) around larger yolk bodies.

Figs. 11 & 12 Vitellogenic oocytes. Fig. 11 Immature yolk bodies (Yb) with whorls of fibrous lamellae. Small yolk vesicles (short arrows) fused with yolk bodies. Fig. 12 Yolk bodies of less electron density. Note cisternae of endoplasmic reticulum (arrows) around yolk bodies.

Fig. 13 Vitellogenic oocyte with nuclear membrane infoldings (long arrow).
Nucleus (N).

Fig. 14 Follicular clefts (long arrow) between follicle cell (Fc) and vitellogenic
oocyte (Vo). Pre-vitellogenic oocyte (Pv).

DISCUSSION

The Type-I yolk nucleus in pre-vitellogenic oocytes of *O. h. quadrasi* which encloses a portion of cytoplasm with mitochondria and electron-dense bodies is consistent with findings in other prosobranch snails, such as *Ilyanassa obsoleta*^{9,10}, *Bembicium nanum*¹¹ and *Viviparus contectus*¹². Similar ergastoplasmic vitelline bodies have also been described in the mussel, *Mytilus* sp.¹⁵, in the spiders, *Tegenaria parietina*¹⁶, and *Plexippus paykulli*¹⁷, and in cats, mice and hamsters¹⁸. However, while Type I yolk nuclei in young oocytes of *O. h. quadrasi* have one to four stacks of concentric RER lamellae, those of the amphineurans *Mopalia mucosa* and *Chaetopleura apiculata*, *Mytilus* sp., *I. obsoleta*, and spiders are more elaborate, composed of numerous lamellae compactly stacked one over the other.^{9,10,15,16,17,19}

The Type II yolk nucleus noted in *O. h. quadrasi* which consists of a cluster of compactly arranged clear and electron-dense vesicles (= vesicular type), does not seem to fit any of the three categories of yolk nuclei²⁰. Whether this type of vitelline body actually contributes to yolk synthesis or simply disintegrates to form clear empty vacuoles dispersed throughout the cytoplasm, and then simply take up yolk substances from the cytoplasm is not clear. However, the filling of some vacuoles with granular material suggests the uptake of yolky substance from the cytoplasm.

Ergastoplasmic and vesicular yolk nuclei are absent during vitellogenesis in *O. h. quadrasi*. The persistence of mitochondrial and Golgi types of yolk nuclei in 4-16 cell stage embryos had been reported in *Nassarius reticulatus*²¹. Similarly, yolk nuclei had been reported in cleavage cells or spiders¹⁷, and in newly fertilized eggs of *Mytilus* sp.¹⁵, which begin to show signs of degeneration. Schmekel and Fioroni hypothesized that the occurrence of yolk nuclei during segmentation in *Nassarius reticulatus*²¹ is functionally related to the resorption of extra-embryonic albumen more than their participation in yolk synthesis.

In the present study, the structural changes undergone by some mitochondria strongly suggest their participation in the formation of Type A yolk bodies, characterized as highly osmiophilic and exhibit a strong staining reaction with Sudan black. Metaplasial mitochondria have inconspicuous cristae, possess a cluster of electron-dense crystalline granules and one to two concentric whorls of lamellae-like structure, and other homogeneous electron-dense granules. These crystalline granules resemble B-1 and beta-granules in *L. stagnalis*^{1,6} and Type I yolk body granules in *B. glabrata*⁷. In late vitellogenesis, more bundles of concentric or fibrous material appear within mitochondrial derived yolk bodies, concurrent with the loss of crystalline granules and increased yolk body size. Ultrastructurally, the whorls of concentric lamellae resemble yolk granules within multivesicular

bodies as reported in *Physa acuta*²², and *Lymnaea stagnalis*⁶. In *L. stagnalis*, the B-2 yolk body crystalline materials transform into membranes encircling part of the granular matrix. In *O. h. quadrasi*, the disappearance of crystalline granules and increase in bundles of fibrous complexes suggest the probable origin of this yolk body component from crystalline granules. Similarly, the close association of metaplasial mitochondrial with vesicular endoplasmic reticulum (see Figures 8,9) suggests the participation of this organelle in yolk synthesis.

In young vitellogenic oocytes of *O. h. quadrasi*, concentric RER are closely associated with clear and electron-dense vesicles and even with a few mitochondria. During late vitellogenesis, however, less electron-dense yolk bodies with homogeneous appearance become enveloped by a single lamella of ER (Figure 12). This assemblage indicates the role of the endoplasmic reticular network in the formation of Type B yolk bodies in addition to other cytoplasmic components. Type B yolk body formation parallels earlier reports of yolk formation within concentric RER with mitochondria compressed between membrane layers in *I. obsoleta*¹⁰, in a marine snail *Acmaea digitalis*^{23,24} and among spiders¹⁷.

There are four basic modes of yolk synthesis in molluscan oocytes⁹: 1) yolk granule formation from mitochondria; 2) from Golgi vesicles; 3) granule formation in close association with ER and 4) yolk granule formation from more complex structures with the participation of mitochondria, cytoplasm and smooth endoplasmic reticulum. In *O. h. quadrasi*, the transformation of mitochondria into yolk synthesizing vesicles closely associated with vesicular ER, and yolk formation in close association with ER are predominant. This observation is with precedence among snails. Yolk granules primarily of mitochondrial origin have also been shown in the snail *Planorbis corneus*^{2,3} and in the slug *Agriolimax reticulatus*²⁵.

Mitochondrial origin of yolk granules in *B. glabrata*⁷ is doubtful because of the absence of transitional stages between mitochondrial and yolk granules. Also, the yolk granules were negative for the mitochondrial enzyme cytochrome oxidase. While no cytochemical tests were conducted to trace the origin of yolk granules in *O. h. quadrasi*, the structural changes observed in metaplasial mitochondrial and the intense reaction of yolk bodies with Sudan black are a sufficient basis for now, to suggest they may play a role in fatty yolk synthesis.

Yolk synthesis in close association with small and large vesicular and concentric ER, and in some instances with mitochondria, observed in the present study agrees with reports in *Acmaea digitalis*²³, and *Mytilus* sp.¹⁵

Among molluscs whose process of oogenesis had been studied to date, Golgi bodies and RER appear to be the primary organelles involved in yolk for-

mation.^{1,6,7,9,13,14,19,22} Among prosobranch snails, the present study is the first report of yolk body formation from mitochondria and ER. The very few Golgi bodies in developing oocytes of *O. h. quadrasi* are unlikely to produce a large amount of yolk. Transfer vesicles that bud off from the saccular membranes of Golgi bodies presumably are sources of additional yolk material that either fuse with yolk bodies and/or remain in the cytoplasm as small yolk vesicles.

Follicle cells of *O. h. quadrasi* serve mainly for nutrition of developing oocytes evidenced by the presence intimate association with developing oocytes. The presence of various shapes and sizes of electron dense vesicles, mitochondria and ER within follicle cells, suggest their involvement in synthesis of yolk precursors and/or yolk substances. A similar function was reported in *I. obsoleta*⁹ and in *Aplysia* sp.²⁶ Also, the occurrence of microvesicular-like structure beneath the oocyte plasma membrane in late vitellogenic oocytes suggests a nutritive role for follicle cells in *O. h. quadrasi*.

CONCLUSION

Pre-vitellogenic oocytes show two types of yolk nuclei, namely: Type I (=ergastoplasmic) and Type II (=vesicular). Vitellogenic oocytes exhibit two prominent types of yolk bodies, the highly osmiophilic yolk body (=Type A) stained intensely with Sudan black, and the less electron-dense yolk (=Type B) with homogeneous matrix enclosed by a single cisterna of endoplasmic reticulum. Direct transformation of mitochondria into yolk synthesizing vesicles closely associated with vesicular endoplasmic reticulum, and yolk formation in close association with endoplasmic reticulum predominate.

Among prosobranch snails studied to date, this is the first report of yolk body formation primarily from mitochondria and endoplasmic reticulum.

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