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Ultrastructural Study on the Ovarian Wall and the Oviduct of the *Asellus aquaticus* (Crustacea: Isopoda)

B. Melike ERKAN
Department of Biology, Faculty of Science, İstanbul Üniversity, İstanbul-TURKEY

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Abstract: In this study the ovarium, ovarian wall and oviduct of *Asellus aquaticus* (*A. aquaticus*) (Crustacea: Isopoda) were investigated by stereomicroscopy, phase contrast microscopy and light microscopy. Long, tube-shaped, separate ovaries of *A. aquaticus* are located dorsolateral to the gut. The germinal zone is confined to the ventral region of the ovary as a thin band. The ovarian wall is composed of a few fibrous layers. The oviduct is located in the anterior one–third of the ovary, not in the central part. The sheath of the oviduct was found to possess some muscle cells, a feature first noted in this study.

They appear to be different from the striated muscles of vertebrates, and are characterized by abundant cytoplasm, typical striated muscle bands and the absence of sarcoplasmic reticulum owing to their presence in a confined region of the oviduct sheath. It is suggested that the muscle cells act as a sphincter and provide elasticity which prevents tearing of the oviduct during discharge of eggs from the ovarium.

Key Words: Crustacea, Isopoda, Ovary, Ovarian wall, oviduct.

Asellus aquaticus'da (Crustacea: Isopoda) Ovaryum Duvarı ve Oviduktun İnce Yapısı

Özet: Bu çalışmada *A. aquaticus* (Crustacea: isopoda) ovaryumunda ovaryum duvarı ve oviduct, stereomikroskop, faz kontrast mikroskobu, ışık mikroskobu ve elektron mikroskobu ile incelenmiştir. *A. aquaticus*'un ovaryumları bağırsağın dorsalateralinde, birbirinden bağımsız uzanan iki uzun tüpsü yapı şeklindedir. Germaryum bölgesi, ovaryumun ventralinde ince bir bant şeklindedir. Ovaryum duvarı, birkaç katlı telsi yapıdan oluşmuştur. Oviduct ovaryumun tam ortasında değil, ovarumun ilk üçte birlik kısmının sonunda bulunmaktadır. Oviduct kılıfında kas hücrelerinin bulunduğu ilk defa bu çalışmada tespit edilmiştir. Bu kas hücreleri bol sitoplazmalı, sarkoplazmik retikulumları olmayan, fakat tipik çizgili kas bantlarının bulunuşuyla, omurgalıların bilinen çizgili kas yapısından farklı gözükmektedir. Ovidukt kılıfında sınırlı bir bölgede bulunması sebebiyle, bu kas hücrelerinin bir büzgen gibi görev yaptığı, yumurtaların ovaryumdan boşaltılması esnasında gerekli esnekliği sağladığı ve oviduktun yırtılmasını engellediği düşünülmektedir.

Anahtar Sözcükler: Krustase, izopoda, ovaryum, ovaryum duvarı, ovidukt.

Introduction

As in other arthropods, the ovaries of Crustacea are located dorsal or dorsalateral to the gut. The ovaries are usually paired organs. Sometimes the lobes of the two ovaries are fused either anteriorly as in peracarids and other lower Crustaceans, or in the middle as in macrurans and stomatopodas species of Decapoda (1).

Two types of ovary are seen in Branchiopods. The ovarium is made up of many ovarioles in Notostraca and Conchostraca. The ovarian follicles that contain growing oocytes and nurse cells, protrude from the germarium to the hemocoele. The ovarium is not branched in Anastroca and Cladocera (3). The Branchiura, in *Argulus japanicus*, a single sac–shaped ovarium, lies on the digestive canal in the thorax (15, 16). In bivalved free–living copepods, there is a single ovarium with two oviducts in the anterior part of the thorax, whereas parasitic species have two ovaries (2, 10).

In freshwater ostracods, the ovaries are paired and bilaterally located (11). In stomatopoda, the gonad is located in the abdomen, between the heart and the gut. Two ovaries unite to become a single tube, with sac–like protrusions observed on the ovarium (2). In Mysidaceae, the ovaries consist of two long tubes connected by a bridge. The oviducts arise from a site near the posterior (10).

In decapods, the crabs have two tube–shaped ovaries connected through a central bridge, giving an H–shaped appearance, and in *Emerita asiatiaca* (Anomura), two ovarian lobes unite at the back of a site that connects the ovaries to each other, creating a cricle in the middle (27). In shrimps, the ovaries extend from the anterior part of the stomach to the telson as double, symmetrical, and partly joined structures (18).

In Cumaceae, the ovaries consist of spindle–shaped sacs. Oviducts probably develop only during ovulation. In amphipods, the ovaries are made of two long straight or branched tubes (10). The ovaries are generally tube–shaped in Isopoda. In some, the ovulatory canal may widen to form a spermatic reservoir (10).

Data about the structure of the ovarium wall is limited. In *Penaeus setiferus,* a marine shrimp, it is reported that the ovarian wall has three layers: an outer thin superficial epithelium, a relatively thick connective tissue layer in the middle, and an inner germinal epithelium (18). The ovaries of Brachyura have one or more connective tissue layers. In *Portnus sanguinolentus*, a Brachyura, the thin wall of ovarium is made of two connective tissue layers and all lobes are also surrounded by a thin connective tissue (25).

The outer epithelium of the ovarium is continuous with the outer layer of the oviduct. There is also a cylindrical epithelial layer innermost (2). In various groups of invertebrates, the presence of a muscle layer in the ovaries or in the oviducts has been reported (5, 6, 36). Tennent and Ito (31) have noted their absence in the ovaries of crustaceans.

There have been few studies on the ovaries of Isopoda. Furthermore, they have not included the ovarian wall and the oviduct. Isopoda is an important group for studies on the accumulation of heavy metals in environments contaminated with industrial waste products and also for their role in food chains (8, 9, 33, 34, 35). This study was carried out to elucidate the ovarian morphology of *A. aquaticus*, a fresh water species of Isopoda.

Materials and Methods

Specimens of *A. aquaticus* have been collected from rearing ponds of the Botany Department of İstanbul University (Turkey) since 1991.

Dissected ovaries were fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer at pH 7.2 and post–fixed in 1% OsO4 in the same buffer. After dehydration in alcohol and propylene oxide, the ovaries were embedded in Araldite (12). Semithin sections obtained with a Reichert OM U3 ultramicrotome were stained with toluidine blue (14). Ultrathin sections were placed on 200 mesh Formvar–coated copper girds and stained with uranyl acetate (32) and lead citrate (24). The girds were then examined with a Zeiss EM 9 electron microscope.

In order to show the presence of polysaccharides, semithin sections were stained according to King's method (19). To show the oviduct, some of the ovaries were examined with a Nomarski DIC photomicroscope (Zeiss Axiopod) following the initial fixation, whereas others were examined and photographed with a photomicroscope (Carl Zeiss) following initial fixation and staining with hematoxylin.

Results

The general structure of ovarium

The ovaries of sexually mature *A. aquaticus* were two long tube–shaped structures lying separately from each other dorsolateral to the gut (Figs. 1, 2). When the ovaries reached full maturity, they lay along the entire abdomen as a tube with regular shaped lobes generally containing the mature oocytes. At this stage, a thin band–shaped germarium region and their rather large oviducts could be differentiated at the ventral part of the ovarium (Fig. 2). The oviducts were located at the end of the anterior third of the ovarium, not in the middle (Figs. 1, 2).

After the discharge of mature eggs from the body, the ovaries were converted to empty, flat, clear sacs containing a few oocytes in the germinal region. It was observed that the fertilized eggs that were discharged from the body were later collected in the embryonal sac at the ventral side of the animal and that young embryos developed within this sac. After the discharge of mature eggs from the ovarium, new oocytes began to develop inside and it was possible to observe nearly all developmental stages of the oocytes together.

Ovarian wall

It was not possible to differentiate a sheath or membraneous structure enveloping the ovarian structure with the naked eye. However, phase contrast microscopy revealed the presence of a boundary outside the ovarium (Fig. 2).

Figure 1. Ovarium by stereomicroscope, anterior end (A), posterior end (P), oviduct) (*), oocytes (arrow) stained with hematoxylin, 80 x.

Understanding whether the structure lining the ovarian wall was cellular or fibrous was only possible by examining the semi–thin sections stained for light microscopy and the silver sections prepared for electron microscopy. Semi–thin ovarium sections, one micrometer in length, taken from (araldyte) blocs and stained with toluidin blue were examined with light microscopy and the ovarian wall was observed to be a thin line (Fig. 3). When such sections were treated with PAS and stained with Fast Green, after resin removal, a PAS (+) reaction results in a thin stained line (Fig. 4). The wall structure also continued along the oviduct.

With electron microscopy, the wall structure was observed to be a stratified fibrous structure (Fig. 5) While the wall structure appears smooth in some regions, its surface in other parts was folded (Fig. 6). Such folds were also present in the oviduct region. Round or oval granules were sometimes seen in the fibrous structure (Fig. 7). The number of these granules increased during

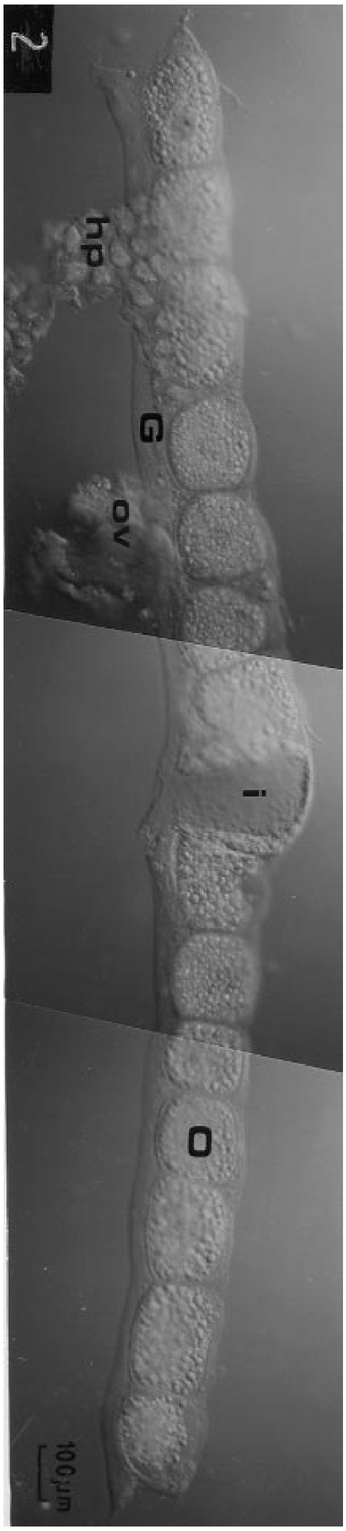

Figure 2. Ovarium by phase contrast microscope, Germarium (G), oocytes (O), oviduct (ov), hepatopancreatic remnant (hp), site where ovarium was held by an instrument (i), scale: 100 µm.

Figure 3. Ovarian wall (ow), oocyte (O), follicle cells (f), stained with toluidine blue, 800 x.

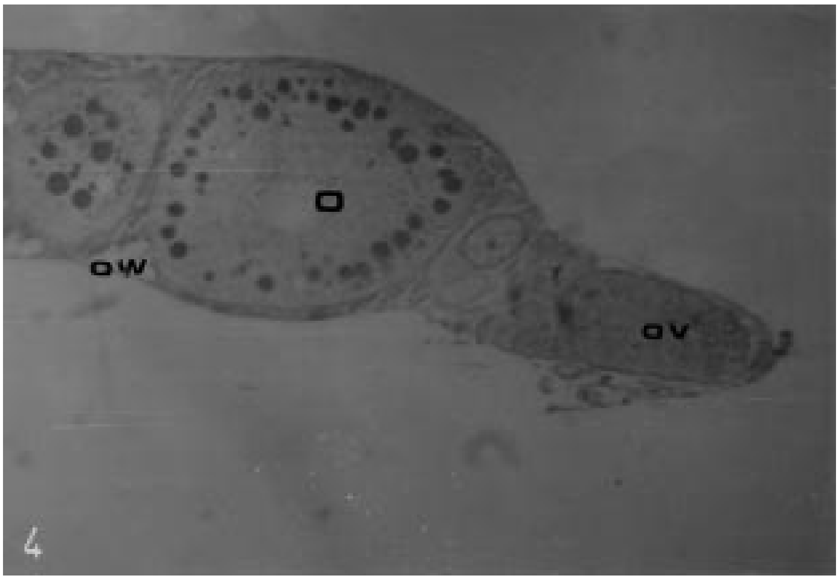

Figure 4. Ovarium and oviduct stained with Fast Green after PAS application ovarian wall (ow), oviduct (ov), oocyte (O), 320 x.

the formation of the vitelline sac as most oocytes became mature (Fig. 8).

Oviduct

The oviduct of *A. aquaticus* which appeared from the front third of each ovarium was a narrow cord lacking a blind lumen until the oocytes became mature (Fig. 10). However, during the maturation stage of most oocytes, the oviduct was seen to become more dilated and a lumen developed (Fig. 9).

The ovarian wall that surrounded the ovarium also covered the oviducts as a thin band, but in contrast to the ovarium, the oviduct had another sheath structure between the oviduct and the wall. There were sheath cells within this structure and also muscle cells with typical obliquely aligned striated muscle bands in a narrow region connecting the oviduct with the germarium part of the ovarium (Figs. 10, 11). Oviduct sheath cells were variably shaped with cytoplasms stained green with toluidine blue. They also had oval or round shaped nuclei, and one or a few granules of various sizes.

In longitudinal sections, the oviduct was seen to have single–layered, regularly ordered cylindrical epithelial cells on the inner surface (Figs. 10, 11). However, in the

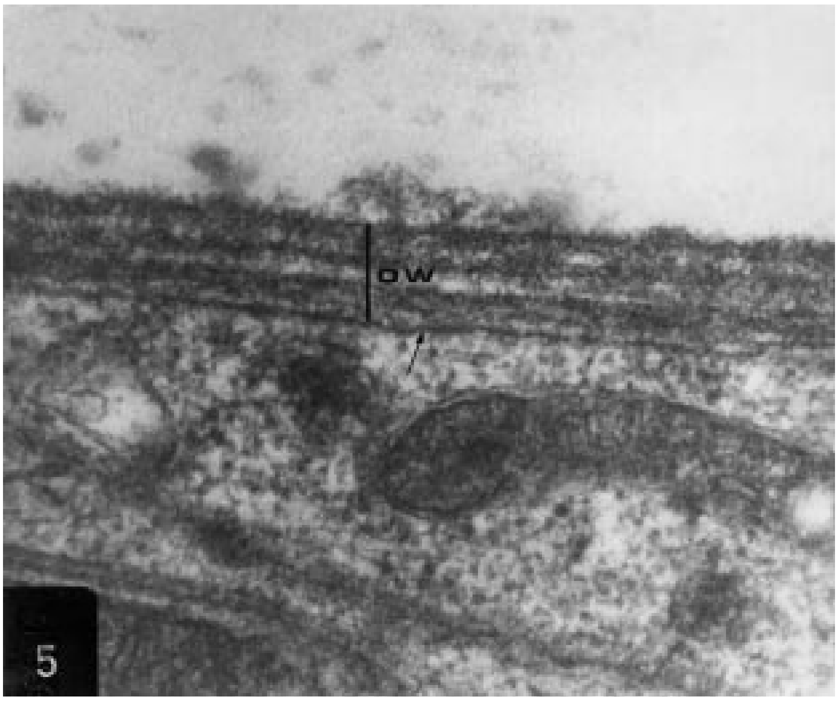

Figure 5. Fibrous structure of ovarian wall. Ovarian wall (ow), follicle cell membrane (arrow), 60.000 x.

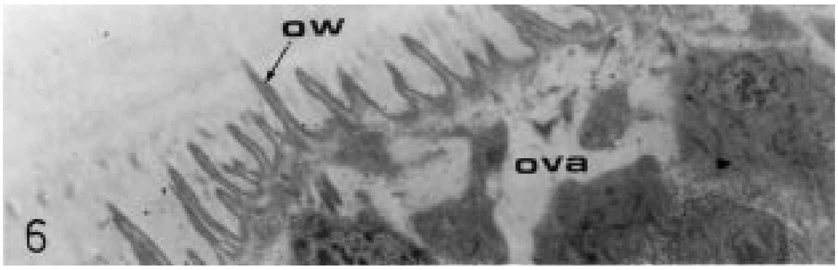

Figure 6. Folds of ovarian wall (ow), ovarium (ova), 1950 x.

Figure 7. Folds of ovarian wall (ow), granules (g), ovarium (ova), hemocoel (H), 4900 x.

rather narrow region joining the oviduct and the germinal part of the ovarium, an irregularly shaped epithelial cell type was present. The muscle cells found in the oviduct sheath surrounded this area (Fig. 11). Cylindrical epithelial cells had a thick basal lamina which could be differentiated with light misroscopy and was coiled in some parts (Fig. 11).

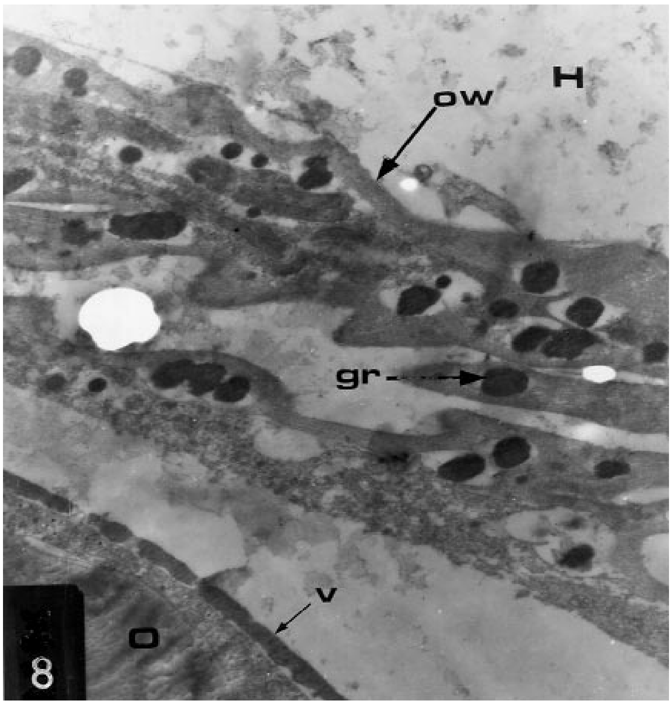

Figure 8. Tangential section of ovarian wall. Ovarian wall (ow), granules (gr), Vitelline membrane (v), oocyte (o), hemocoel (H), 4900 x.

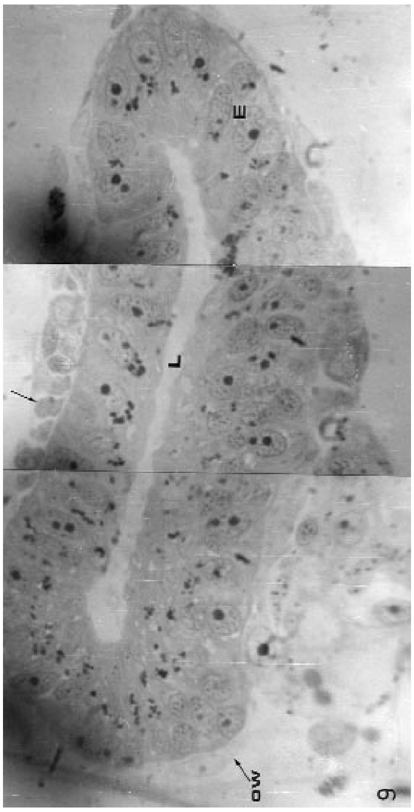

Figure 9. The phase when most oocytes become mature, oviduct with open lumen (L), oviduct wall (ow), cylindrical epithelial cells (E), oviduct sheath cells (arrow), stained with toluidine blue, 800 x.

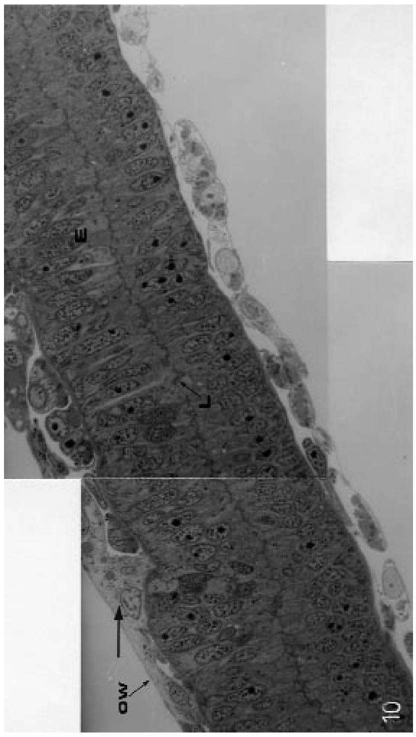

Figure 10. The phase when oocytes have not become mature yet, oviduct with closed lumen (L), ovarian wall (ow), oviduct sheath cells (arrow), cylindrical epithelial cells (E), stained with toluidin blue, 800 x.

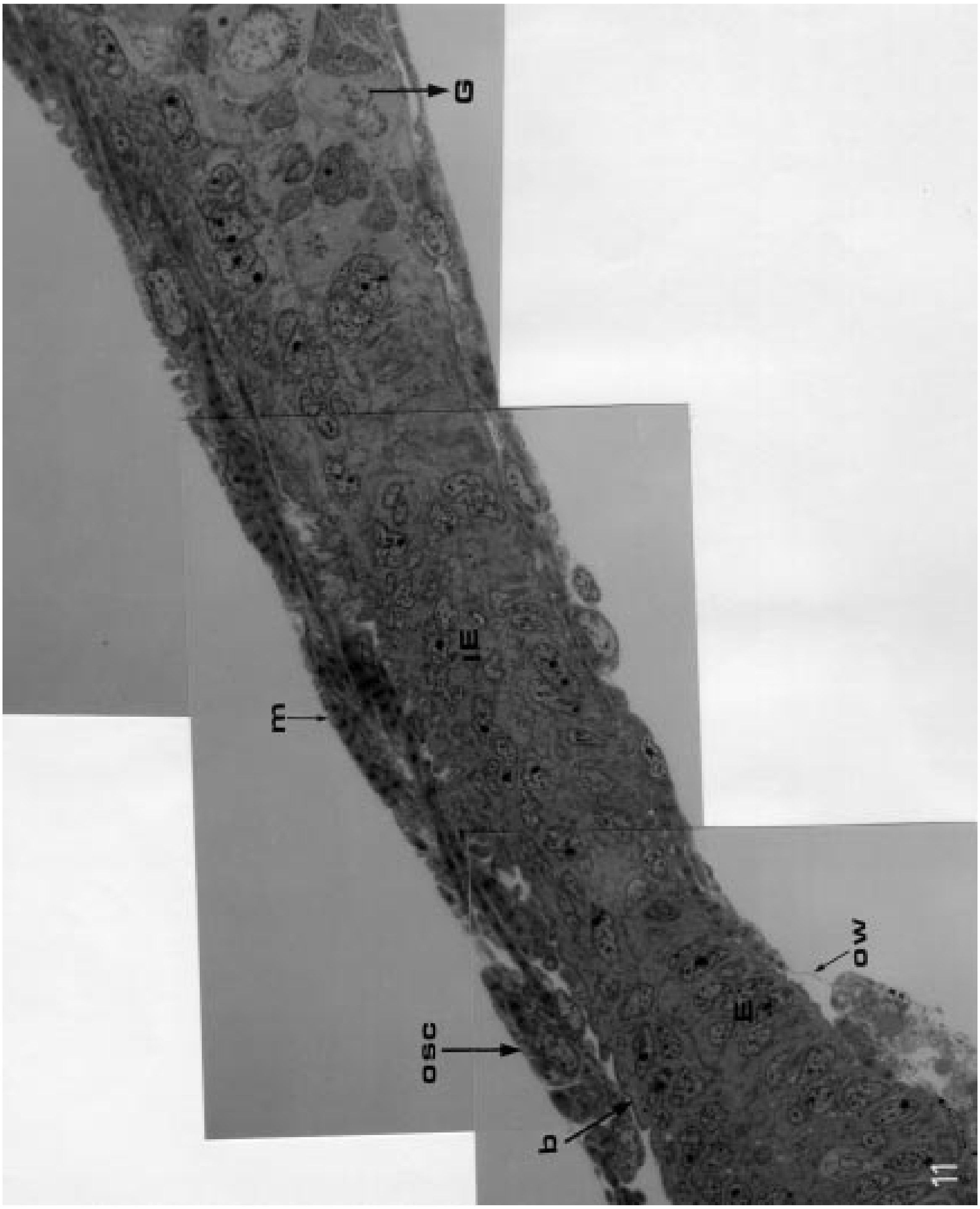

Figure 11. Section of a region of oviduct near the ovarium. Transition of single–layered cylindrical epithelium (E), into irregularly–shaped epithelial cells (IE), oviduct sheath cells (osc), muscle cells (m), part of the ovarian wall (ow), basal lamina (b), beginning of germarium (G), stained with toluidin blue, 800 x.

Oviduct epithelium and oviduct sheath cells showed different staining properties when semi–thin oviuct sections were stained histochemically with PAS and Fast Green dyes (Fig. 12). The nuclei of both cell types were stained green with Fast Green, but their cytoplasm displayed different staining affinities. While the cytoplasm of oviduct sheath cells gave a PAS (+) reaction and were stained dark red, the cytoplasm of oviduct cylindrical epithelia cells were stained pale. Small and lightly stained PAS positive granules were also seen in the cylindrical epithelia (Fig. 12).

With electron microscopy, regular bands of muscle microfilaments, A and I bands were readily distinguished in the cytoplasm of muscle cells of the oviduct sheath. The nuclei of these cells were oval shaped with abundant cytoplasm containing many mitochondria (Fig. 13). In cross sections it was also possible to see the order of myofilaments in muscle cells (Fig. 14). The thick and partly folded basal lamina which separated the oviduct epithelium from the oviduct sheath was easily visible (Figs. 13, 14).

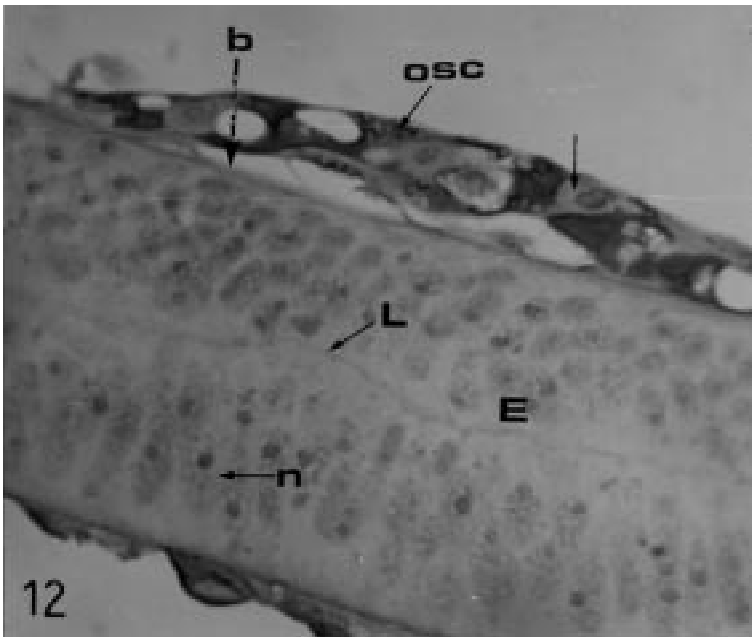

Figure 12. Longitudinal section of oviduct stained with Fast Green after application of PAS. Nuclei that were stained green with Fast Green (n), cylindrical epithelial cells whose cytoplasm was stained pale with PAS (E), oviduct sheath cells that were stained dark (osc), oviduct sheat cell nucleus (arrow), basal lamina (b), closed lumen (L), 800 x.

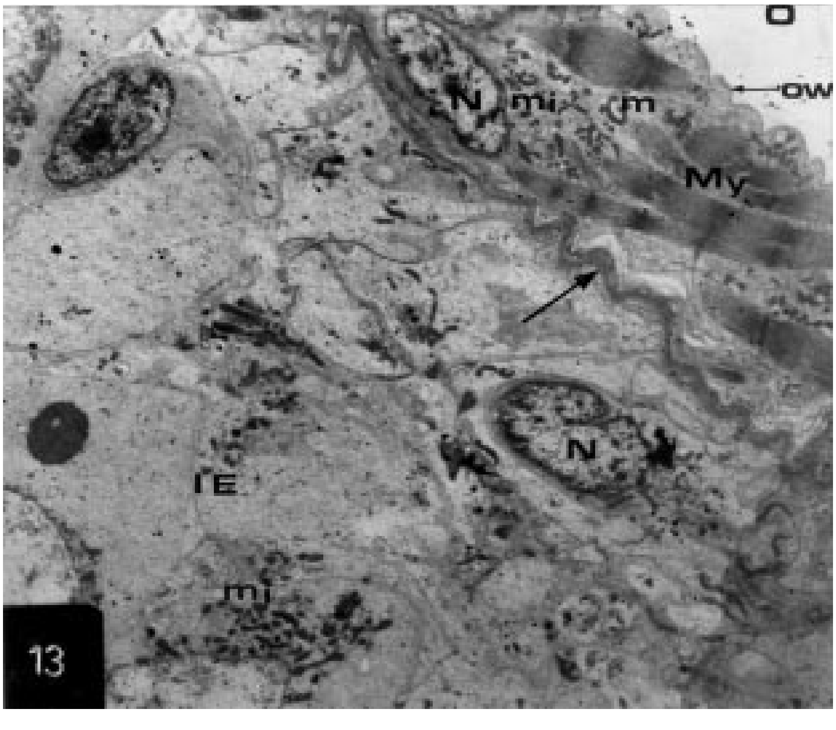

Figure 13. The site of transition of the oviduct into ovarium. Muscle cells in oviduct sheath (m) and the myofilaments inside them (My), irregularly shaped oviduct epithelium (IE), basal lamina (arrow), mitochondria (mi), nucleus (N), oviduct wall (ow), 2500 x.

When the cylindrical epithelial cells were examined with electron miscoscopy, it was observed that the interstitial space was very narrow, the nuclei containing one or more nucleoli lay parallel to the long axis of the cell and occupied most of the cell (Fig. 15). Mitochondria were especially concentrated in the basal part of the cell.

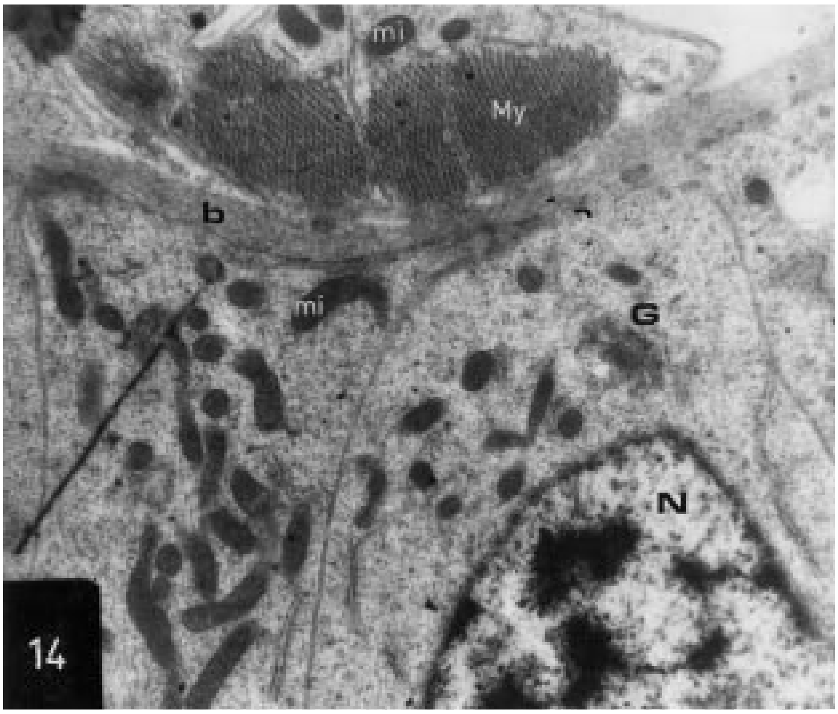

Figure 14. The site of transition of the oviduct into ovarium. The myofilaments of muscle cells in oviduct sheath (my), mitochondria of oviduct epithelium (mi), Golgi complex (G), Nucleus (N), Granular Endoplasmic Reticulum (GER), basal lamina (b), 9800 x.

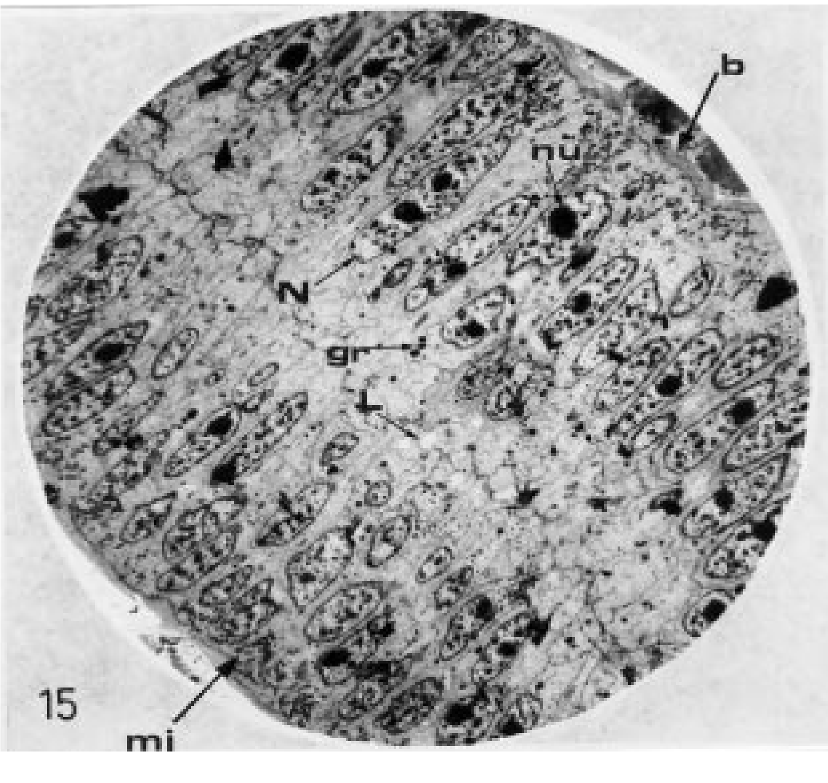

Figure 15. Longitudinal section of oviduct. Nucleus (N), Nucleolus (nu), basal lamina (b), closed lumen (L), mitochondria that were concentrated in the basal part (mi), small granules (gr), 1050 x.

It was possible to see a little granular endoplasmic reticulum and Golgi complex. There were small granules, small pouches within sacs surrounded by membranes, and lysosome–like structures in the cytoplasm (Fig. 16). In some sections, the cytoplasm had short bundles of microtubules and aggregations of small granules. Moreover, it was also possible to see many free ribosomes which were surrounded by a membrane and contained electron dense material (Fig. 17).

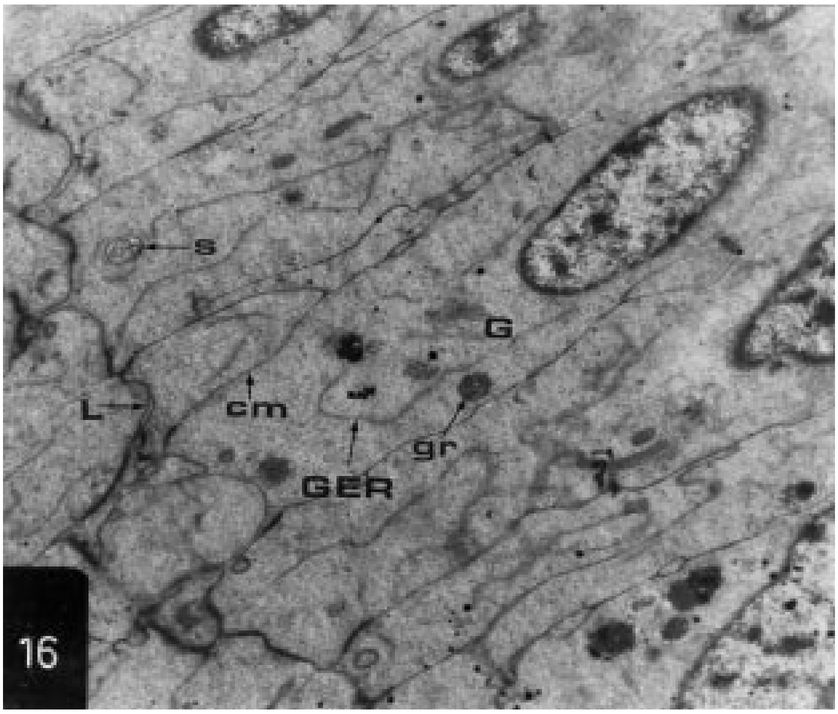

Figure 16. Longitudinal section of oviduct closed lumen (L), cell membrane (cm), Golgi complex (G), Granular Endoplasmic Reticulum (GER), granules (g), membranous sac (s), 5600 x.

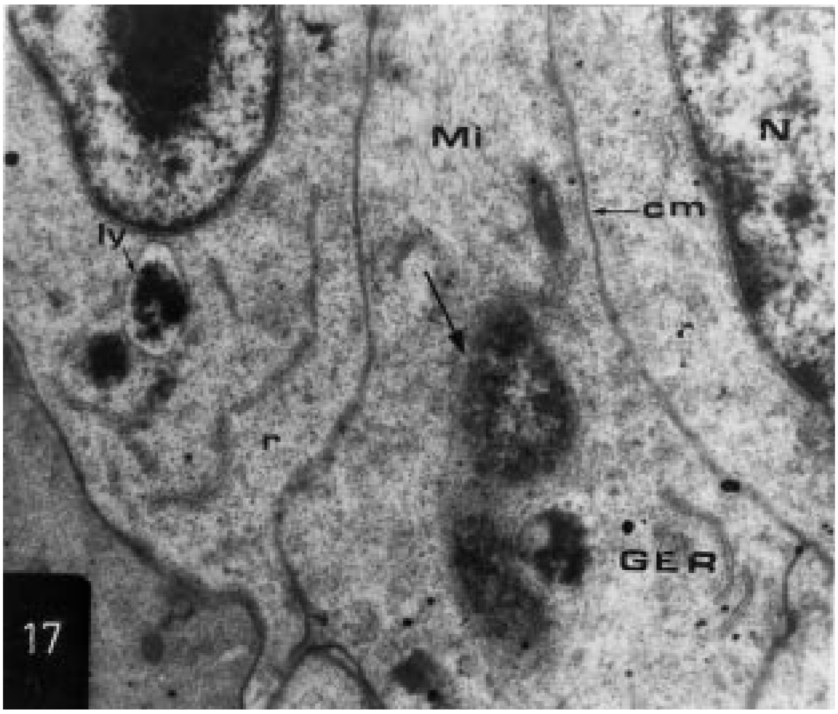

Figure 17. A section of oviduct epithelium. Bundles of microtubules (Mi), the region in which small granules were concentrated (arrow), Granular Endoplasmic Reticulum (GER), ribosomes (r), lysosomes (ly), cell membrane (cm), Nucleus (N), 9800 x.

Discussion

The ovarium of *A aquaticus* is composed of two long, tube–shaped structures with separate oviducts lying dorsolateral to the gut with no relation to each other, as in other Isopods and in Amphipoda (10, 30). Therefore it differs from the ovarian sturctures of other groups of Crustacea, such as Branchiopoda, free–living Copepoda, Stomatopoda, Mysidaceae and Decapoda, which have variously joined single or double ovaries (2, 3, 10, 15, 16, 18, 25).

The ovarioles that are seen in ovarian structures of Branchiopods of Crustaceae, species of Notocostraca and Concostraca and many insect groups, are not present in the ovarium of *A. aquaticus* (3, 7, 13, 20, 21, 29, 37).

In this study, the ovarium of *A. aquaticus* was surrounded at its outermost layer by a socalled ovarian wall which appeared to be a thin line at low magnifications but as a stratified, fibrous structure at high magnifications with an electron microscope. This structure also covered the outer surface of the oviduct. The configuration of the wall was different from the three–layered ovarian wall structure of *Penaeus setiferus* (18) or from the wall structure containing two connective tissues in *Portunus sanguinolentus* (25). However, the wall structure of *A. aquaticus* was similar to that of many insects (5, 22, 23, 25, 28).

The ovarian wall of *A. aquaticus* gave a PAS (+) reaction and some granulated structures were observed in its fibrous structure especially during stages of oocytes maturation. According to Bonhag and Arnold (5) the wall structure 'tunica' in an insect type, *Periplaneta americana* gives a PAS (+) reaction and shows a fibrous structure at high magnifications. According to these researchers, the fibrous structure of the tunica provides the required elasticity for the ovarium which is enlarged by the growing oocytes and it provides a selectively permeable structure between the ovarian cells and the hemolymph. Ramamurty (23) using intravital staining with tripan blue and autoradiographic labelling, has pointed out that in the insect, *Panorpha communis* (Mecoptera), the substances that will form the protein vitellus reach the oocytes by passing through this sheath from the hemolymph. The PAS reaction indicates the presence of polysaccharides and protein carbohydrate complexes (4, 5, 26). In the present study, the PAS (+) reaction of the ovarian wall and the granules seen in the oocytes in their later stages of maturation, suggest that the wall permits the passage of substances from the hemolymph to the oocyte in *A. aquaticus* also. Moreover, the foldings that occur in some parts of the wall structure provide the required elasticity for the enlarged ovarium due to the growing oocytes just as it occurs in the ovaria of insects. The oviducts of *A. aquaticus* open as different channels. Their lumens which are closed before oocyte maturation open after the majority of oocytes grow. Oviducts are seen to have an oviduct sheath structure different from the ovarium. It contains obliquely aligned striated muscle cells in a narrow region that connects the oviduct and ovarium. No data about this sheath structure and muscle cells are found in the literature about Crustacea. These muscle cells have abundant cytoplasm with no sarcoplasmic reticulum but they are different from the usual striated muscle structure of vertebrates because of the presence of typical striated muscle bands. Due to its limited presence in a certain region of oviduct sheath, it can be thought that it works as a sphincter providing the elasticity during the discharge of eggs from the ovarium by working like the theca externa in the grafian follicles of mammals (17) and preventing the rupture of the oviduct.

The difference in the PAS reaction to the cylindrical epithelial cytoplasm lining the oviduct channel and the sheath cell cytoplasm indicates a difference in the substances they synthesize. The absence of structures such as the Golgi complex and secretion granules in cylindrical epithelia suggest that there is no secretory function of the oviduct channel during the discharge of eggs.

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