

Ultrastructural studies of oogenesis in *Bolinus brandaris* (Gastropoda: Muricidae)*

MARÍA JOSÉ AMOR¹, MONTSERRAT RAMÓN² and MERCÈ DURFORT¹

¹ Departamento de Biología Celular, Facultat de Biologia, Diagonal 645, 08028 Barcelona, Spain. E-mail: mamor@ub.edu

² Institut de Ciències del Mar (CSIC), Passeig Marítim de la Barceloneta 37-49, 08003, Barcelona, Spain.

SUMMARY: Ultrastructural studies of oogenesis in *Bolinus brandaris* are described. Although the initial phase of oogenesis is common to most animal species, vitellogenesis can be considered a species-specific characteristic. In the vitellogenesis of *B. brandaris*, mitochondria and endoplasmic reticula play a relevant role in the formation of myelinised membranous systems. Nuclear envelope, Golgi body and the oocyte plasma membrane invaginations are three possible origins for annulate lamellae. The latter can be considered membranous reservoirs. There are two sources for the vitellum: exogenous (from follicular cells) and endogenous (from the endoplasmic reticulum of the same oocyte).

Key words: vitellogenesis, mitochondria, endoplasmic reticulum, annulate lamellae, imposex.

RESUMEN: ESTUDIO ULTRAESTRUCTURAL DE LA OOGÉNESIS DE *BOLINUS BRANDARIS* (GASTROPODA: MURICIDAE). – Aunque las primeras fases de la oogénesis son comunes en la mayoría de las especies animales, la vitelogénesis sin embargo, puede considerarse específica para cada una de ellas. En la vitelogénesis de *Bolinus brandaris*, cabe destacar el papel de las mitocondrias y del retículo endoplasmático en la formación de sistemas membranosos que asemejan figuras mielínicas. La envoltura nuclear, el complejo de Golgi y las invaginaciones de la membrana plasmática oocitaria podrían constituir los posibles orígenes de las láminas anilladas, que pueden considerarse un reservorio membranoso. El vitelo a su vez, puede tener dos orígenes: extrínseco (procedente de las células foliculares) e intrínseco (sintetizado por el retículo endoplasmático del propio oocito).

Palabras clave: vitelogénesis, mitocondria, retículo endoplasmático, láminas anilladas, imposex.

INTRODUCTION

Muricidae comprise more than 2000 Neogastropod species distributed worldwide. Some of these species are commercially exploited in, for instance, Thailand (Nugranad *et al.* 1994) and Chile (Gutiérrez and Gallardo, 1999). *Bolinus brandaris* (Linnaeus, 1758) is common in the Mediterranean and constitutes a locally important resource in Spain, Italy and Turkey (Martín *et al.* 1995). On the other hand, *B. brandaris*, like many other prosobranchia species, is

affected by the imposex phenomenon (i.e. penis and spermduct are superimposed onto the female gonochoristic ducts; Smith, 1971), in response to tributyltin (TBT) pollution in sea water, mainly caused by anti-fouling paints. This phenomenon is caused by the alteration of the steroid metabolism (Oehlmann *et al.* 1993) and has been shown to cause alterations to the genital tract (Oehlmann *et al.*, 1991).

One of the first reports about prosobranch reproduction is by Schitz (1920), who examined gametogenesis with light microscopy in *Hexaplex trunculus*. Further details on the ultrastructure of members of the family Muricidae are provided by Bottke (1972) in

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Viviparus contectus; Durfort (1973a) in *Murex ele-nensis*; Griffon and Gomot (1979) in *Viviparus vivip-arus*; Buckland-Nicks (1973) and Buckland-Nicks and Chia (1976) in *Littorina sitkana*; Buckland-Nicks *et al.* (1982 and 1983) in *Fusitriton oregonensis*; and Romanova (1978) in *Littorina saxatilis*. From 1987 to 1990 we studied the spermatogenesis of *B. brandaris* (Amor, 1987; Amor and Durfort, 1990a,b). In the present study we document the ultrastructural characteristics of the gametogenesis of female *B. brandaris* affected by imposex.

MATERIALS AND METHODS

Thirty female individuals of *Bolinus brandaris* were collected in April 1999 from a coastal Mediter-ranean site (Sant Carles de la Ràpita, Spain) at depths of 15 to 25 m using an artisanal dragged gear (Martín *et al.* 1995). Imposex in this site was moni-tored in a previous study (Ramón and Amor, 2001) and reached 99.7% of the females examined (N=301). The shell was cracked with a vice and the gonads of imposex females were carefully removed. Thin sections were fixed in 10% formalin and stained with hematoxylin-eosin and the PAS tech-nique (i.e. cytochemical stain with periodic acid and Schiff reagent) for study by light microscopy. Thinner sections were processed for transmission elec-tron microscopy following routine double fixation, i.e. glutaraldehyde and 2.5% OsO₄, both buffered using Sørensen's phosphate buffer. Samples were embedded in Spurr's resin after progressive dehy-dration. About 1 mm-thick sections were obtained and stained with methylene-blue borax to select the areas most suitable for the ultrathin sections. These were about 30 nm thick and were cut using a Reichert-Omu ultramicrotome with a diamond knife. Sections were picked up on copper grids and stained with uranyl acetate and lead citrate. Thierry's technique (Thierry 1967) was sometimes used, and then the sections were picked up on gold grids. We used a 301 Philips transmission electron microscope at the Serveis Científic-Tècnics of the University of Barcelona.

RESULTS

Oogenesis of *Bolinus brandaris* followed four main stages: premeiosis, meiosis, vitellogenesis and the formation of the mature oocyte. Premeiosis and

meiosis are characterised by the presence of a round nucleus 6 µm in diameter, in which drops of heterochromatin are spread throughout the nucleoplasm. The cytoplasm is nearly empty, except for some mitochondria and dictyosomes (Fig. 1A). The most developed apparatus is the centriole, shown as a microtubule organising centre (Fig. 2A).

During meiosis, the most outstanding phase is prophase I, in which the nucleus enlarges and chro-mosomes and synaptonemal complexes appear. As is usual at this stage, the cells are undifferentiated and the nucleus/cytoplasm ratio is high.

Vitellogenesis in *B. brandaris* can be divided into three main stages:

In the first stage, the cell is 30 µm in diameter and the nucleus (22 µm) is round or oval. It shows one or two nucleoli (1.6 µm), in which we can often distinguish granular and fibrillate phases (Figs. 1B(a) and 2B). The nuclear envelope shows abun-dant well-developed pores (1 pore per µm² of about 90 nm in diameter). Slight invaginations increase the envelope area and favour the passage of nuclear precursors for vitellogenesis (Fig. 1B(a)). The cyto-plasm increases in volume and its organelles increase in both number and in volume. Thus, we can distinguish vacuoles of various sizes, PAS posi-tive β-glycogen granules and vesicles (4-5 µm) full of material of varying electron density. Mitochondria are well-developed (1.5 µm long) and can be rod-shaped, curved or elongate. The cristae and matrix are well formed. Both are near the nucleus and their number increases progressively, leading to the for-mation of mitochondria clusters. Mitochondria divide by bipartition or gemmation (Fig. 2C). At the end of this stage, several mitochondria loose their cristae and become vesicles, and some are invaded by pre-vitellic material (Figs. 1B(a) and 2C).

In the plasma membrane, intercellular bridges and desmosomes can be seen among young oocytes, as well as microvilli (Fig. 1B). Desmosomes and intercellular spaces showing accumulations of pre-vitellogenic material are also shown among oocytes and follicle cells caused by electron-dense vesicles possibly from follicle cells origin (Figs. 3A(a,b) and 3B). Sometimes, the plasma membrane invaginates to form small vacuoles, which aggregate to create a large, round reticulate of annulate lamellae (Figs. 3A,B). The Golgi body is well developed and is formed by several dictyosomes, with abundant cis-ternae. These produce vesicles of varying electron-density, multivesicular bodies and also annulate lamellae (Fig. 3C(b)). Clusters of annulate lamellae

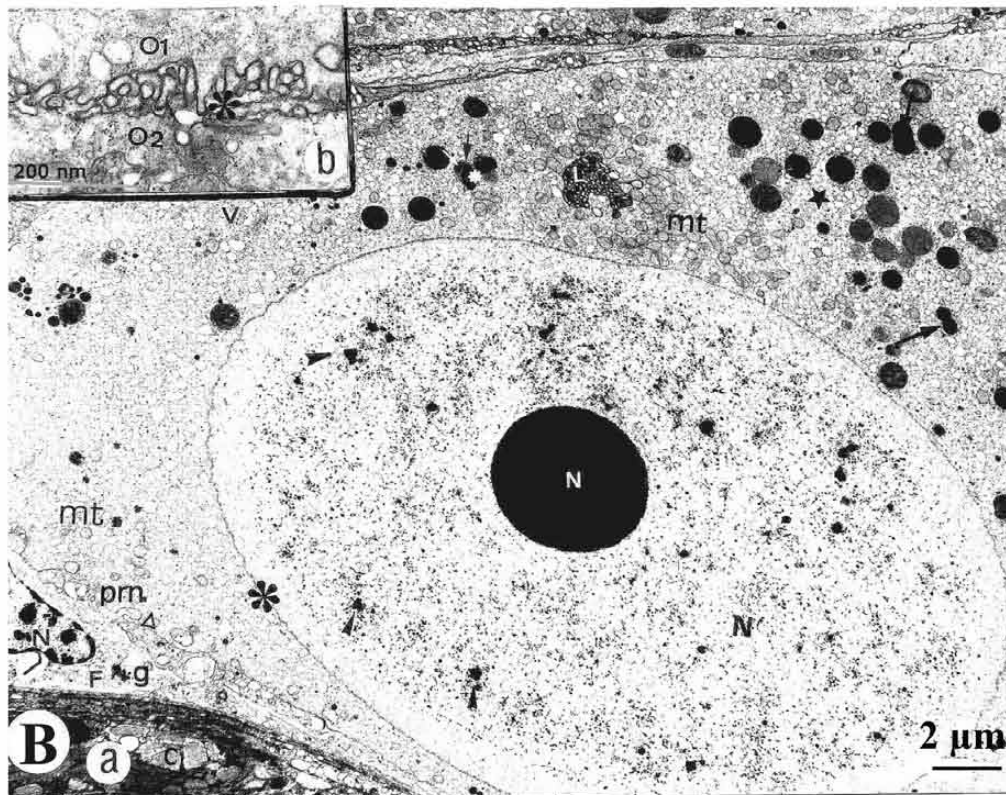
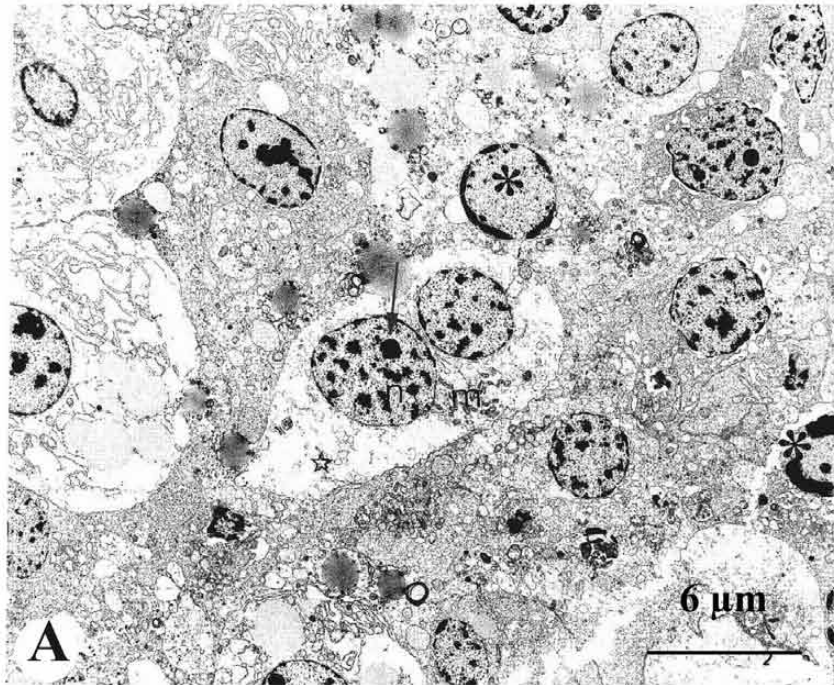


FIG. 1. – **A:** Ultrastructural panoramic view of the ovary. Young oocytes can be seen (star). The nucleus (n) showing the chromatin dispersed in small drops, the nucleolus (arrowhead) and the mitochondria (m) are apparent in the cytoplasm. Note the non-germinal cells, apparently connective cells (asterisk), too. **B:** Detail of an oocyte in early stages of oogenesis. (a): The nucleus (black N) shows a compact and large nucleolus (white N). The chromatin is granulated, although some condensed droplets appear in the caryoplasm (arrowheads). The nucleus envelope shows 'undulations' and nuclear pores (black asterisk). The cytoplasm is limited by the plasma membrane (pm) showing microvilli (white arrowhead). In the cytoplasm, note the clusters of mitochondria (mt). Vesicles of variable electron density (star) are detected, together with lysosomes (L). The white asterisk shows electron dense material embedding degenerate mitochondria, which still remain cristae (black arrow). Other electron dense vesicles with mitochondria morphology are also observed (black arrows). Outside the oocyte, a follicular cell (F) with glycogen granules (g) are shown and below, connective tissue (C) is detected; (b): microvilli (asterisk), present between two oocytes (O1 and O2), are shown.

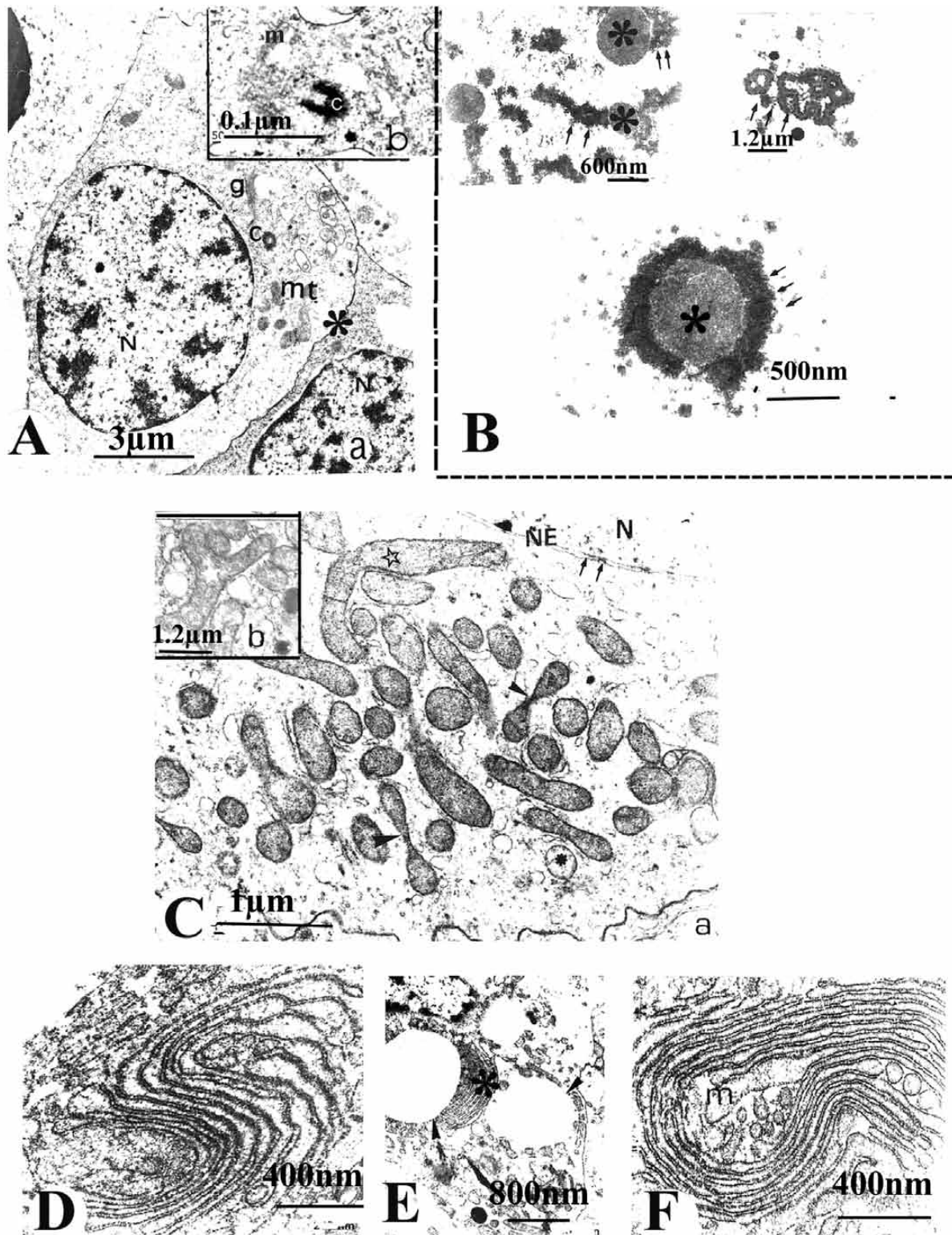


FIG. 2. – **A**: Oocyte undergoes meiosis. (a): The presence of chromosomes in the nucleus (N) is shown. In the cytoplasm, limited by the plasma membrane (asterisk), mitochondria (mt), the Golgi body (g), and the centriole (c) are observed; (b): a longitudinal section of the centriole (C) surrounded by microtubules (m) is shown. **B**: Diverse morphologies of the nucleolus in the phase of ribosomic aggregates (nucleolus-like bodies). The fibrillar (arrows) and the granular (asterisks) phases of the nucleoli are clearly detectable. **C**(a): Typically-shaped mitochondria (star) are surrounded by degenerate-like (asterisk) mitochondria. Some of them seem to begin division (arrowheads). The nucleus (N) is surrounded by the nuclear envelope (NE), which shows nuclear pores (arrows); (b): atypical shaped mitochondria are often seen. **D**, **E** and **F**: Organisation of the rough endoplasmic reticulum (ER) during early vitellogenesis. **D**: ER with arched morphology. **E**: ER (asterisk) surrounds a inside lipid vesicle (arrowheads). **F**: ER appears cup-shaped and some mitochondria (m) are detected.

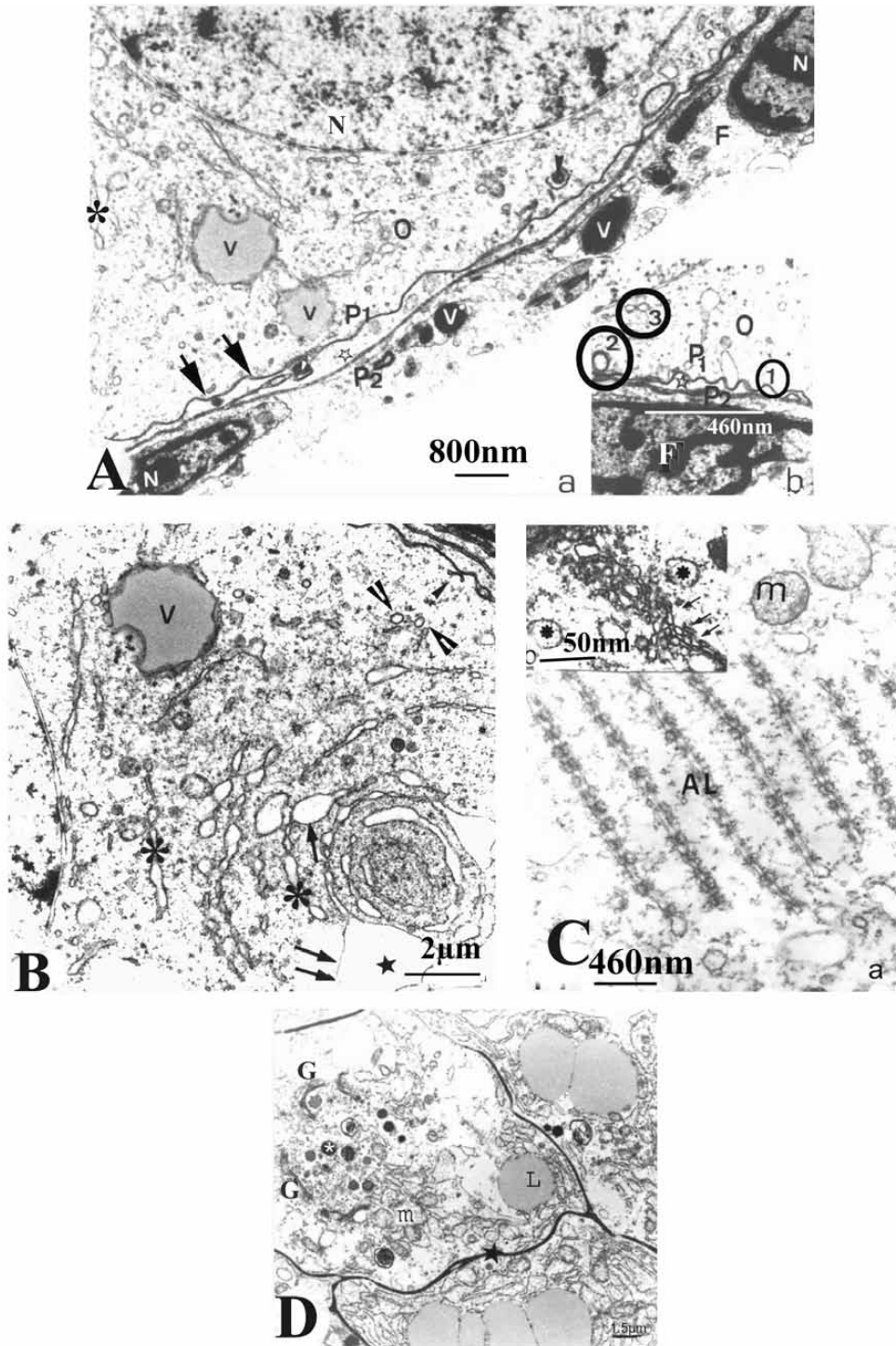


FIG. 3. – **A** and **B**: Three possible origins for annulate lamellae and the Golgi body. Several invaginations in the oocyte (O) plasma membrane (A, P1) can be seen. These invaginations (Aa, arrows) may grow to form empty vesicles (Aa and B, arrowheads). Later, these vesicles seem to join together, generating reticulum-like annulate lamellae (Aa and B, asterisk). The sequence is shown in A(b), i.e. 1: membrane invagination; 2: a vesicle is formed. 3: two vesicles begin to join. **B** shows the swellings in one extreme of reticulum-like annulate lamellae (arrows). These swellings become large vesicles (star). Free vesicles (v) containing vitellum-like material are detected in Aa and B. In the neighbouring, follicular cells (F), the nucleus (white N), the plasma membrane (P2), and some electron-dense vesicles (white v) are also detected in Aa. The space between both kinds of cells is also shown (Aa and b, star). **C**: Two alternative origins for the annulate lamellae. (a) shows annulate lamellae (AL) which are similar to the nuclear envelope, from which they seem to originate; (b) shows detail of the annulate lamellae found in the Golgi body area (arrows). This suggests annulate lamellae may originate in the Golgi body. Degenerated mitochondria (*) are also seen. **D**: Panoramic view of several oocytes showing the intermembranous spaces among them. Note the electron-dense material accumulated inside the oocytes (asterisk), possibly precursors of exogenous vitellum. This material seems to fill the intermembranous space (star). There are also several intracytoplasmic organelles. L: lipid vesicles; G: Golgi body; m: mitochondria; White asterisk: electron-dense vesicles; black asterisk: annulate lamellae.

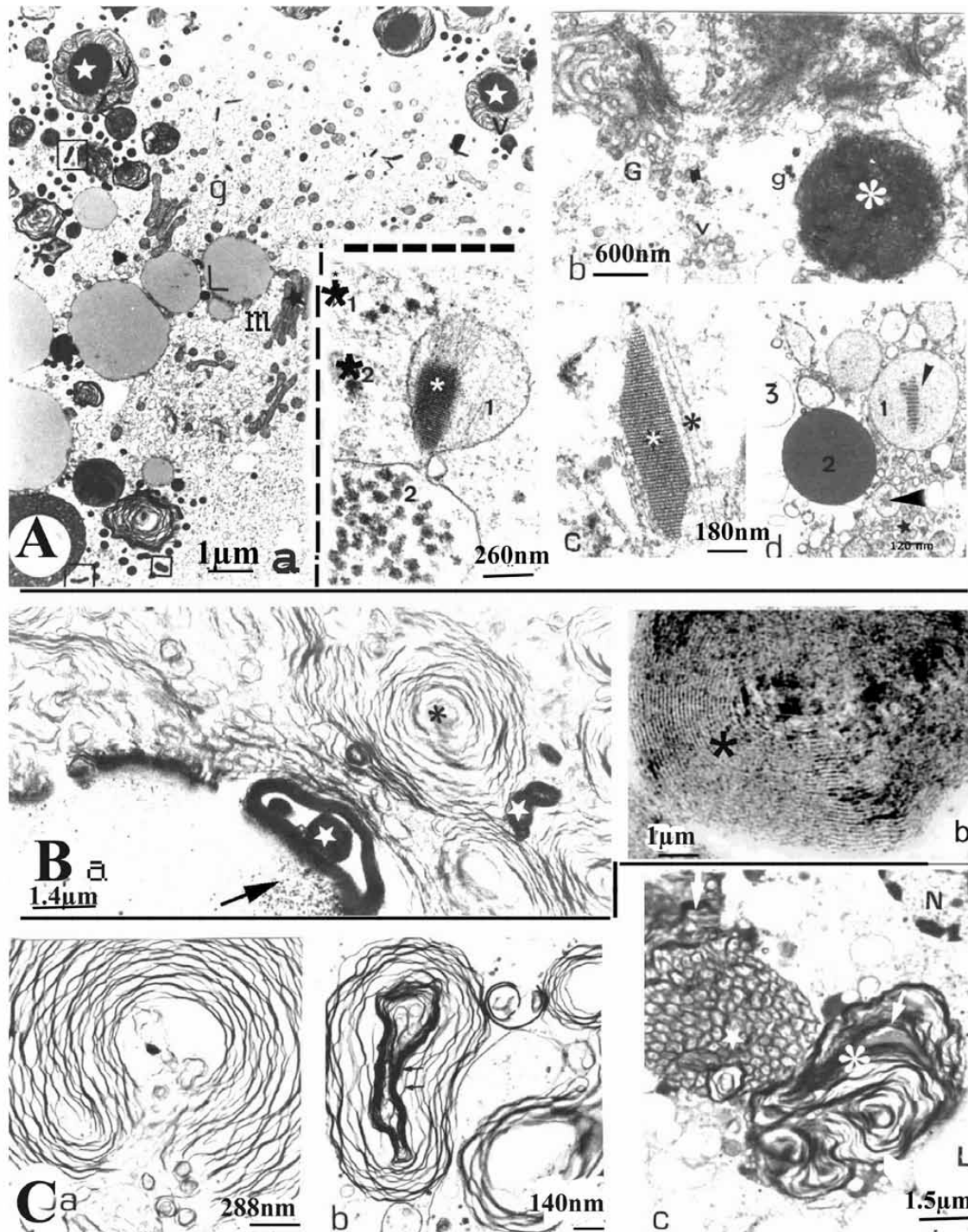
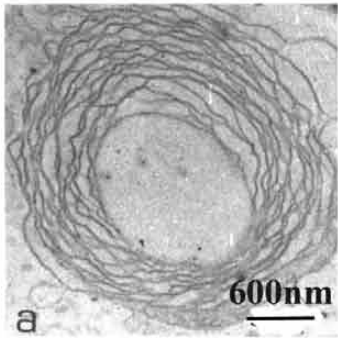
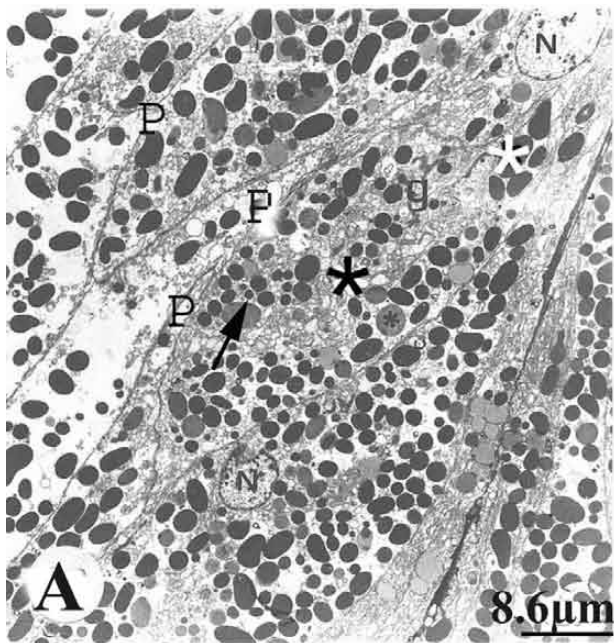
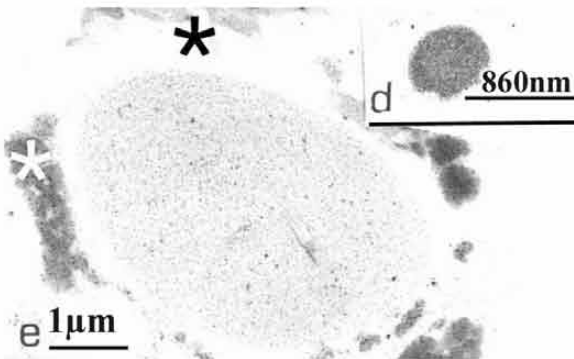
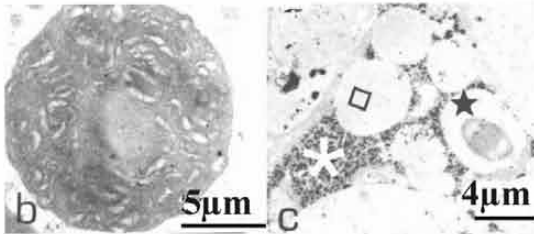


FIG. 4. – **A**: Detail of the behaviour of cytoplasm during vitellogenesis. (a): Panoramic view of the cytoplasm of the oocyte during this phase. Note the high development of the diverse organelles. g: glycogen granules; L: lipidic vesicles; V: vesicles; m: mitochondria. Young vitelline platelets appear (white stars), as well as degenerate mitochondria full of electrondense material (squares). Inset: detail of striated material filling a vesicle (1) while granules of electrondense material (*1 and *2) seem to enter another vesicle (2), striated material is also seen in a vesicle (white asterisk); (b) behaviour of the Golgi body (G) during the vitellogenic phase. Note its high development and the presence of vesicles (v) and a contiguous multivesicular body (asterisk). Glycogen granules (g) are also seen; (c) striated material (white asterisk) seems to be secreted by the endoplasmic reticulum (black asterisk) and (d) striated material (arrowheads) is also incorporated into a membranous vesicle (1). Other, smaller vesicles seem to incorporate this material, too (star). A vesicle containing electrondense material is also detected (2). Note the numerous vesicles surrounding them (3). **B**: General view of the vitelline platelet “envelope” formation. (a): membranes are arranged in myelin-like bodies (asterisk), while in the core, electrondense, granulate material enters (arrowhead). See the peculiar disposition of the membranes (white stars); (b): shows the above mentioned formation at higher magnification. See the concentric thin bands, which remind of chromatin condensation in male spermatozoa (*). **C**: Detail of the disposition of the membranes around the core: (a): membrane twisting has not yet begun; (b) shows different stages of twisting; (c): note the increasing areas of compaction (asterisk) and the curling shape they acquire as the twisting progresses (star). The presence of the nucleolus (N), a lipidic vesicle (L) and electrondense bands are also note.



B



similar to the nuclear envelope are also often found (Fig. 3C(a)).

The rough endoplasmic reticulum (RER) can appear in different shapes and is highly developed. It usually surrounds the nucleus, although it can be found elsewhere. In the latter case, it can be arched, round or cupulate (Figs. 2D,E,F). The rough ER can be found next to lipidic vesicles (Fig. 2E), as well as surrounding mitochondria (Fig. 2F).

The advanced stage of vitellogenesis is characterised by the formation of vitelline platelets. The nucleus is still active, although the number of nuclear pores decreases. On the other hand, the amount of cytoplasm and the number of organelles both increase. Thus, during this phase, mitochondria, endoplasmic reticulum and the Golgi body are all well developed. Also, various granules can also be seen entering empty vesicles (Fig. 4A(a) inset, 4A(b)). Glycogen granules, lipidic vesicles and granulated vesicles with striated formations are also detected (Fig. 4A(a) inset, 4A(c,d)). The mature vitelline platelets (Figs. 5B(c,e) and 6(h)) typically show a central, electrondense core surrounded by a clear envelope. The core is formed by dark vitelline material surrounded by a membrane; the external envelope of the vitelline platelet is also membranous.

The most important feature of this phase is the vitelline platelet formation. Previtellin material enters empty vesicles, becoming the core (Fig. 4A(a)). At this time, numerous membranes surround it (Figs. 4A(a), 4C(a) and 5B(a)). These membranes, which are Thiery positive, twist around themselves becoming curly-shaped and resemble myelin structures (Figs. 4B(b), 4C(b,c), 5B(b) and 6). The twist increases the contact between membranes and indeed points of fusion among them are detected (Figs. 4C(b), and 6(d,e,f)). Membrane fusion increases until membranous structures disappear and a clear and homogeneous envelope surrounds the core (Figs. 5B(c,e), 6(g,h)). A membrane then covers the vitelline platelet. The completion of

FIG. 5. – **A:** Longitudinal section of mature oocytes limited by the young vitelline membranes (P). A decreasing nucleus (N)/cytoplasm ratio is apparent, caused by the numerous organelles incorporated into the cytoplasm. The most remarkable are the characteristic vitelline platelets (arrow). There are also other vesicles, different in both size and electrondensity (asterisks). **B:** Different performances of the evolution of the vitellin platelets using the Thiery's technique. (a): shows non-twisted membranes around the core; (b): membranes around the core begin to twist. See the positive reaction of their membranes to Thiery's technique; mature vitellin platelets are present (asterisks in c and e). The homogeneous envelope around the core is shown. On the other hand, note the contrast between the reaction to Thiery's of glycogen granules (white asterisk in c and e) and the poor reaction shown by the core. Lipidic platelets are also seen in (c) (square); (d): highly Thiery-positive vesicles are seen.

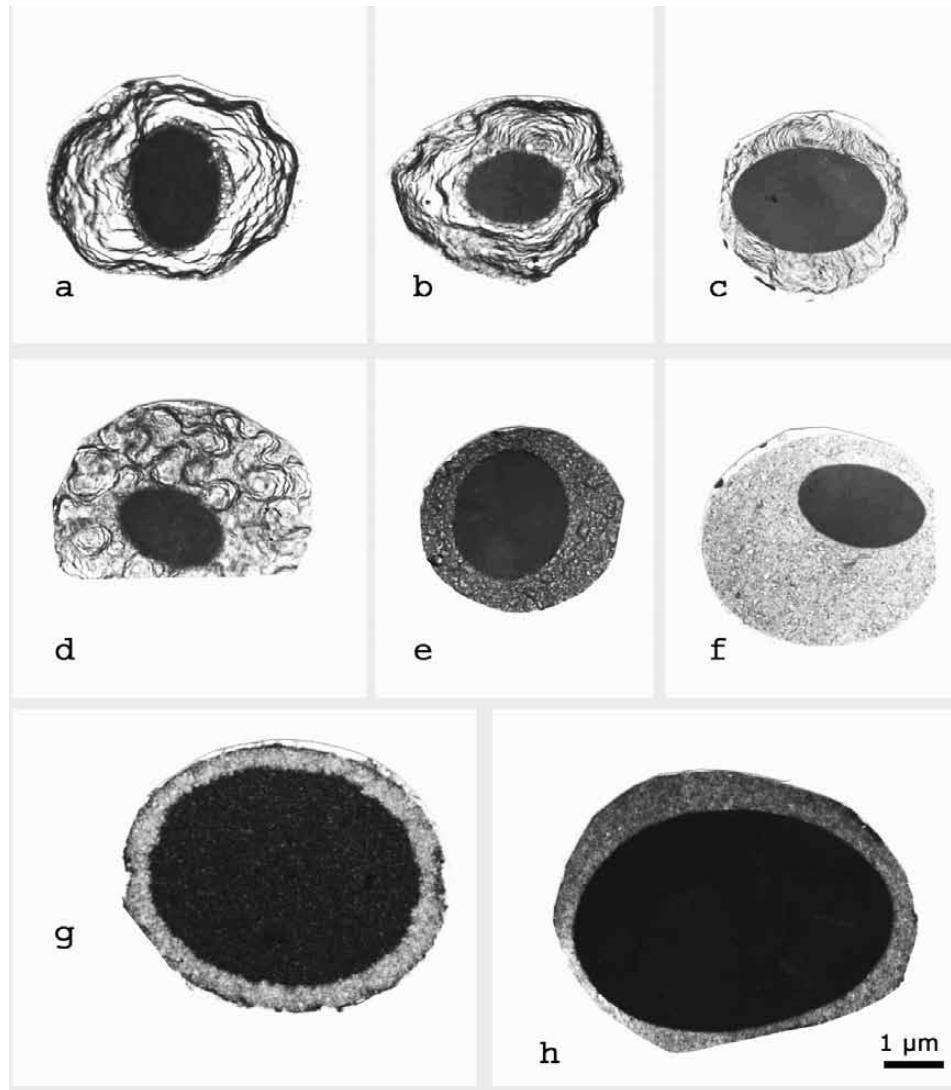


FIG. 6. – Sequence of the progressive organisation of vitelline platelets. (a): membranes are still non-twisted; (b): membranes begin to curve; (c): the twisting of membranes continues; (d): membranes become curly-shaped; (e): curling increases; (f): Membranes fuse as curling process and the vitelline platelet periphery seems homogeneous; (g): the vitelline platelet is almost formed; (h): mature vitelline platelet. The core and the periphery are homogeneous.

vitelline platelet formation and the consolidation of the organelles' morphology characterise the last stage of vitellogenesis. Vitelline platelets fill the cytoplasm, hindering the observation of other organelles. The nucleus is hardly visible under light microscopy. It is round ($50\ \mu\text{m}$ in diameter) and surrounded by a band of heterochromatin, which is spread in dense drops throughout the cytoplasm (Fig. 5A). Dytiosomes are also found throughout the cytoplasm. The thickening of the plasma membrane increases from $2\ \text{nm}$ at the beginning of oogenesis to $3\ \mu\text{m}$ at this stage. Some mitochondria are also observed. The most developed organelles are the vitelline platelets. Their morphology corresponds to the characteristic vitelline platelets mentioned above. They are PAS positive and scarcely

Thiery positive. They are oval and variable in size (about $20\text{-}30\ \mu\text{m}$ by $5\text{-}7.5\ \mu\text{m}$). Other, more uniform platelets are also found without these two components. Some of these platelets are highly Thiery positive, which indicates a high glycogen content, and they can show diverse degrees of electron density. Vesicles containing paracrystalline material are also detected. Lipidic platelets of variable size are observed, as well as glycogen granules, shown by Thiery's technique (Fig. 5B). The mature oocyte is elongate, and about $250\ \mu\text{m}$ long and $60\ \mu\text{m}$ wide.

Although all the females examined were affected by the imposex syndrome in degree 4 (Ramón and Amor, 2001), oogenesis alterations at ultrastructural level were not found.

DISCUSSION

The most remarkable features of the oogenesis process in *Bolinus brandaris* were observed during vitellogenesis. The first stages were characterised by a large nucleus, non-condensed chromatin and developed nucleolus and the existence of nuclear pores which allowed transport of ribonucleoproteins to the cytoplasm, as reported in Mollusca by Davenport and Davenport (1965), Durfort (1973a), Popham (1975), Jong-Brink *et al.* (1983), and Swenson *et al.* (1987), as well as in other invertebrates by Coimbra and Azevedo (1984), Larkman (1984), Ribes (1986), and Sciscioli *et al.* (1991). Although in some Muricidae species a nucleolus vacuolisation was described (Bolognari *et al.* (1981) in *Hexaplex trunculus*), this phenomenon has been not detected in *B. brandaris*.

Some cytoplasmic organelles were very developed, and associated with high synthetic activity. Mitochondria were also conspicuous, and various descriptions can be found in Bruslé (1972), Hill and Bowen (1976), Pfanestiel and Grünig (1982), Larkmann (1984), Sciscioli *et al.* (1991), and Sukhomlinova *et al.* (1998) for several invertebrate groups and in Weakley (1976) for vertebrates. However, no "mitochondria cloud" was detected, as described for *Actinia* sp. by Larkman (1984). Mitochondria can divided in several ways, as shown for other molluscs in Taylor and Anderson (1969), and in Jong Brink *et al.* (1976). Mitochondria were often associated to the rough ER, as reported for *Mytilus edulis* by Durfort (1976a,b). The progressive degeneration of their cristae means that they became empty vesicles, which later appeared to be invaded by vitellogenic material, as has already been described in other molluscs (e.g. Durfort, Durfort 1973a,b; Amor and Ribes, 1995) and invertebrates (Pfanestiel and Grünig, 1982; Larkman, 1984). Despite the similarities, we did not observe the transference of mitochondria from nurse cells to oocytes, as described in some insects (e.g. Tourmente *et al.*, 1990; Stebbings, 1997).

As is common in this phase, annulate lamellae were also observed (Dhainaut and Richard, 1976; Durfort 1973a, b; Durfort, 1976; Hill and Bowen, 1976; Pfanestiel and Grünig, 1982; Kessel *et al.*, 1986). The morphology and localisation of annulate lamellae suggest three possible origins. One is the nuclear envelope, as proposed by Pfanestiel and Grünig (1982), Kessel *et al.* (1986), and Ribes (1986). A second possible origin is the Golgi body,

suggested by Jong-Brink *et al.* (1976). A third possibility derives from the observation of invaginations of the plasma membrane to form large reticulate-like annulate lamellae. Similar features were reported by Kessel *et al.* (1986), leading him to propose a plasma membrane origin to annulate lamellae. Our observation of vesicles that later fill in with electron-dense material agrees with this hypothesis. We believe that an endoplasmic reticulum origin for annulate lamellae, as in *Mytilus edulis* and *Trachidermon cinereus* (Durfort, 1973a,b; Durfort, 1976a,b), is less probable. The annulate lamellae could represent a reservoir for membranes needed by the cell to coat the previtellic material.

The rough ER and Golgi body were very active, as is usual in these cells (Taylor and Anderson, 1969; Durfort, 1973a; Jong-Brink *et al.*, 1976; Durfort *et al.* 1982). Their morphology and location also varied considerably, as occurs in other invertebrates (Durfort 1973a; Larkman, 1984) and vertebrates (Wilch-Brauninger *et al.* 1997).

The presence of intercellular open bridges among oocytes in the first stages of oogenesis could indicate a synchronisation role as in the eupyrene spermatogenesis (Amor and Durfort, 1990a). The observation of microvilli-like interdigitations among oocytes connected to the plasma membrane of the follicle cells could indicate an enhanced capture of vitelline material. This material is often accumulated in the intermembranous spaces between oocytes and follicle cells. Similar features have also been described by Durfort (1973a), Popham (1975), and Jong-Brink *et al.* (1976). Griffond and Gomot (1979) also reported this irregular distribution of the oocyte plasma membrane in the prosobranchia *Viviparus viviparus*.

On the other hand, vitelline material has two possible origins: exogenous, (i.e. produced by follicular cells), and endogenous, (i.e. produced by the oocyte). The exogenous origin means stocks of material originated in follicle cells and accumulated in the spaces between both plasma membranes (i.e. those of follicle cells and of oocytes). The presence of microvilli in the oocyte plasma membrane suggested that this material enters the cytoplasm of the oocyte by endocytosis, as described by Pfanestiel and Grünig (1982) in a polychaete. However, this entrance was apparently not by direct contact, as reported in other species (Harrison and Huebner 1997). The endogenous origin of vitellin material means that it is synthesised by the endoplasmic reticulum and later processed by the Golgi body.

Both organelles were highly developed, which supports this hypothesis. Similar observations have been reported from the mollusc *Mytilus edulis* (Durfort 1976a,b). These two possible mechanisms for vitelline material formation were also described for *Aplysia depilans* (Bolognari and Licata 1981), *Planorbis corneus* and *Lymnaea stagnalis* (Bottke, 1972) and *Helix aspersa* (Barre *et al.* 1991 and Bride *et al.* 1992). In the advanced stages of vitellogenesis, nuclear activity decreases, as is shown by the reduction in the number of nuclear pores, the higher density of chromatin and the decreasing nucleolus activity. Mitochondria and dyctiosomes were spread throughout the cytoplasm, together with vesicles of different sizes and electron density, glycogen granules, lipidic droplets and vitelline platelets in formation.

A central electron dense core surrounded by a clear envelope, as described by Durfort (1973a), forms the characteristic vitelline platelet. The core is formed by vitelline material surrounded by a membrane. There are three possible origins for the core membrane. The first is that it is a degenerate mitochondrion as reported Ribes (1986) and Amor and Ribes (1995). The second is that it originates directly from the Golgi body. Hill and Bowen (1976) described this fact, and the high development of this organelle in the oocytes of *B. brandaris* agrees with this hypothesis. A further hypothesis suggests that the core membrane comes from the annulate lamellae. The latter idea is in agreement with our hypothesis about the role of the annulate lamellae as a membrane reservoir. A group of Thiery positive membranes surrounded the previtellogenic material as it entered the vesicles. These membranes twisted around the vesicles, thus becoming curly-shaped. This twisting progressively increased, giving the membranes a resemblance to myelin structures. As the twist increased, gaps between membranes closed and it began to fuse, in a way similar to chromatin condensation in male spermatogenesis (Amor and Durfort, 1990a). Vitelline platelets showed different behaviours in response to two cytochemical carbohydrate reactions. There were highly PAS positive (under light microscopy) but non-positive for Thiery's reaction (under electron microscopy). This could have been due to the presence of carbohydrates mixed with other non-positive material, such as the proteins often found in vitellin platelets. We also observed oval striated bodies and vesicles containing striated formations, similarly to what has been observed in

the gastropod *Murex elenensis* (Durfort, 1973a) and in the sponge *Stelletta grubii* (Sciscioli *et al.*, 1991). Moreover, other vesicles, slightly dyed using Thiery's technique, were also present. Lipidic vesicles were abundant in the cytoplasm, as reported by Popham (1975), Durfort (1976a), and Hill and Bowen (1976) for molluscs, and Pfannestiel and Grünig (1982) for a polychaete. Two origins have been described for the formation of lipid vesicles, i.e. the synthesis by the endoplasmic reticulum (Durfort, 1976b) and the capture from the blood by endocytosis (Ritcher, 1976; Durfort *et al.* 1982 and Jong-Brink *et al.* 1983). However, in *B. brandaris* we only found ER vesicles associated with the formation of lipid vesicles. Therefore, the ER seems to be the only way of forming lipid vesicles.

Glycogen granules from the Golgi body were also detected by Thiery's technique. The oocyte plasma membrane increased in thickness. This, and the presence of abundant dyctiosomes, lead us to think that the latter originated in the Golgi body.

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