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Metabolite Transfer Between the Intestinal-Loop and the Gonadal Tissue in *Pecten maximus* (Mollusca, Bivalvia)

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Abstrak

Problematik transfer metabolit dari intestin ke acini gonad melalui hemosit makrofag telah ditemukan pada scallop *Pecten maximus*. Sebanyak 1 ml dari 4 mg/ml larutan ferritin tanpa-Cadmium (Sigma) telah diinjeksikan pada lumen bagian proksimal dari descending portion pada intestin. Dua jenis penelitian telah dilakukan yaitu *in vivo* dan *in vitro*. Pada penelitian in vivo, pengamatan dengan menggunakan mikroskop cahaya dan mikroskop elektronik menunjukkan bahwa ferritin ditemukan berada pada lumen dan diantara mikrovili. Pada sel intestin, ferritin ditemukan pada vakuola dan lisosom. Di bagian basal, ferritin ditemukan pada jaringan pengikat, hemosit makrofag, dan dekat acini gonad jantan dan betina. Penelitian *in vitro*, memberikan hasil yang sama dengan penelitian *in vivo*. Berdasarkan hasil tersebut di atas, disimpulkan bahwa intestin berperan dalan nutrisi untuk gametogenesis dan bahwa transfer metabolit dilakukan oleh hemosit makrofag.

Kata kunci : *Pecten maximus*, intestin, acini gonad, trasfer metabolit, ferritin.

Abstract

Problem of metabolite transfers from intestine to gonadal acini by way of macrophagous haemocytes was investigated in scallop *Pecten maximus*. A 1 ml of 4 mg/ml solution of Cadmium-free ferritin (Sigma) was injected into the lumen of the proximal extremity of descending portion of the intestine. Two types of experiments were carried out, *in vivo* and *in vitro*. For the first, observation by light and electron microscopy was showed that ferritin was detected in the lumen and between the microvilli. In the intestinal cells, ferritin was observed in the vacuoles and lysosomal type. Under basement lamina, it is noted the presence of ferritin on underlying connective tissue, macrophagous haemocytes and near the gonadal acini male and female. The *in vitro* experiments gave the same results. Based on these results, it is concluded that intestine play a role in the nutrition for gametogenesis and that the metabolite transfer is by way of macrophagous haemocytes.

Key words: *Pecten maximus*, intestine, gonadal acini, metabolite transfers, ferritin

Introduction

In molluscs, it has been reported an inter-organ transfers of energy substrates to gonad during gametogenesis (Vassallo, 1973; Ansell, 1974; Gabbott, 1975; Bayne, 1976; Taylor and Venn, 1979; Barber and Blake, 1985; Pipe, 1987; Epp *et al.*, 1988). This energy transfer may be passed by a storage organ, as digestive gland for example, before being transferred to the gonad as in *Aequipecten irradians* (Sastry and Blake, 1971). In sea urchin, this transfer is direct from intestine/ gut to gonad by way of perivisceral fluid (Giese, 1959; Farmanfarmaian and Philips, 1962; Barnes *et al.*, 1963; Mauzey, 1966).

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In scallop *Pecten maximus,* after looping several times the intestine leaves the stomach and the digestive gland posteriorly, descends ventrally and enters the gonad at its most antero-dorsal extremity (Beninger and Le Pennec, 1991). The descending portion of the intestine passes through approximately 4/5 of the gonad before it loops upward. It subsequently leaves the gonad and passes through the pericardial cavity.

In *Pecten maximus*, intestine is limited on all of their length by a ciliated simple prismatic epithelium with a microvillosity on their apex. Intestinal epithelium is more or less plicated, the height are variable and depend on the zone. Intestine is lied on the basal membrane and surrounded by a dense underlying connective tissue (Widowati, 1994).

In scallop, although it has long been known that part of the intestine loops through the gonad, no direct assimilation of metabolites from the intestine has yet been established (Le Pennec *et al.,* 1991). Moreover these authors have suggested that during the gametogenesis, the scallops found the energies from two types of sources: atretic recycling and the intestinal-loop transfer.

The present study attempts to demonstrate this direct transfer of metabolites from the intestine into gonadal acini by using the ferritin tracers (*in vivo* and *in vitro* experiments).

Materials and Methods

For *in vivo* experiments, nine scallops were collected from the Brest Bay (Finistere-France). Each scallop received, by injection of 1 ml of the 4 mg/ml of Cadmium-free Ferritin (Sigma) into the proximal extremity of descending portion of the intestine. The scallops were subdivided into three groups of three and were exposed to the ferritin for 10, 15 and 30 minutes respectively. After exposure time, the scallops were dissected for classical histology and electron microscopy.

For classical histology study, the gonad was removed from the scallops, fixed in Bouin solution, dehydrated and embedded in paraffin. The samples were cut in transversal sections at 5 mm thick and stained with Prussian blue (Gabe, 1968). Sections were observed under light microscope.

For electron microscopic study, the intestine were taken off carefully from the gonad. The small pieces of intestine at the different portions: descending, distal extremity of the loop and ascending were taken and then fixed in 2.5 % glutaraldehyde pH 7.3. The samples were rinsed with the sodium cacodylate 0.2 M, and fixed for 1 hour in 1 % osmium tetroxyde. The specimens then rinsed, dehydrated and embedded in Spurr resin (Spurr, 1969). Transversal sections were obtained using a Reichert Ultracut-S ultramicrotome. Sections

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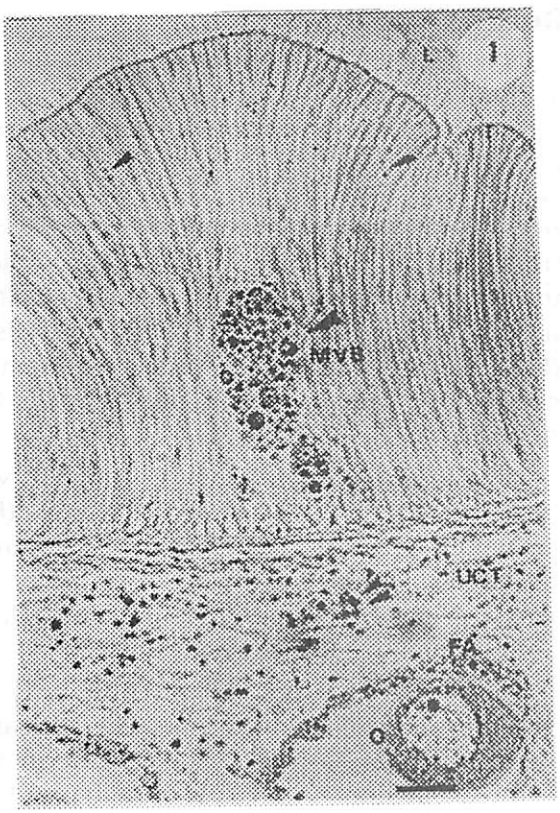
Fig. 1. Light-micrograph, *in vivo* experiments. Ascending portion of intestine, exposed to ferritin solution for 30 minutes. The marker (▼) was observed in the apical part of the intestinal cells, in the multivesicular bodies (MVB) in the basal part and in the underlying connective tissue (UCT) near the female acini (FA). (L) lumen. Scale bar: 25 µm .

Fig. 2. Light-micrograph, *in vivo* experiments. Distal extremity of the intestinal loop, exposed to ferritin solution for 30 minutes. Ferritin (▼) in apex and in the multivesicular bodies (MVB) in the basal part. (L) lumen. Scale bar: 25 µm.

Fig. 3. Light-micrograph, *in vivo* experiments (30 minutes labelling). Ferritin (▼) is seen near the male acini (MA). Scale bar: 40 µm .

Fig. 4. Light-micrograph, *in vivo* experiments (30 minutes labelling). Ferritin (▼) is observed near the female acini. O: oocyte. Scale bar: 60 µm .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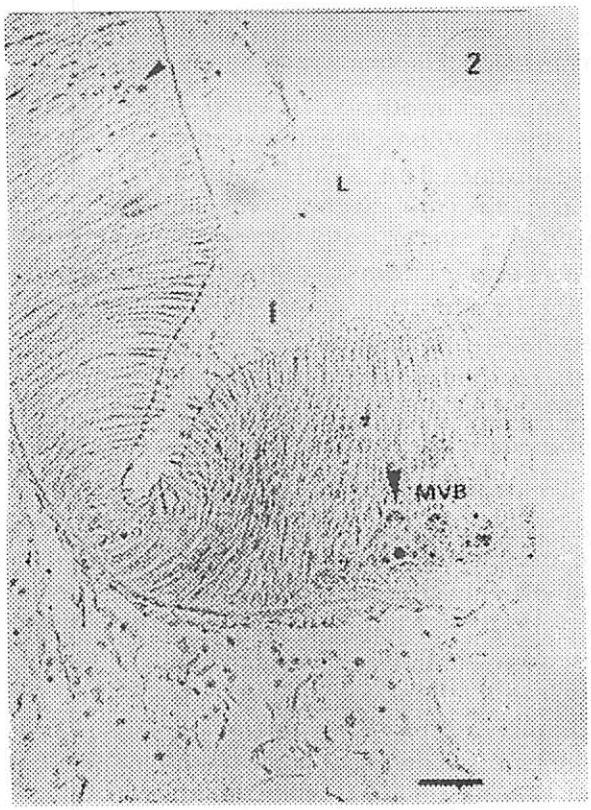
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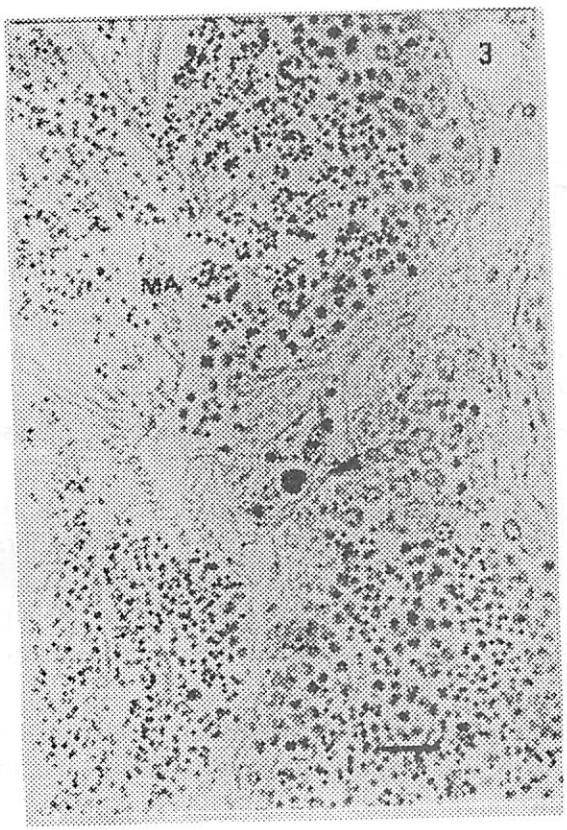
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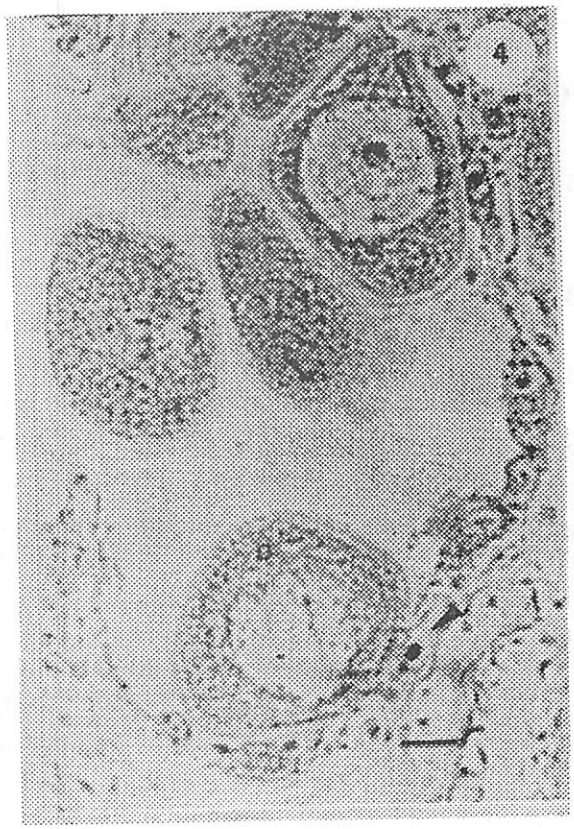
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were observed using a Jeol 100 CX Transmission Electron Microscope (TEM).

Another method, *in vitro* experiment, was done to complete the results of *in vivo* experiment. The same concentration of ferritin (4mg/ml, Sigma) was added into a sterile sea water, into which the intestinal tissue sampled from 2 scallops was immersed during 10 and 30 minutes. The samples were then fixed one part for a classical histology and electronic microscopy. As a control, the other part of samples was immersed into the sterile sea water without ferritin solution.

For the first scallop which the intestinal tissue was labelled for 10 minutes, then put into the culture for 50 minutes. The second, with 30 minutes labelling, the culture was carried out for 2 hours 30 minutes. The culture was done at 20øC. After tissue culture, the specimens then were rinsed for 1 minute with the sterile sea water then fixed for a classical histology and electronic microscopy. This technique was adapted and modified according to Blok *et al.* (1981).

Results and Discussion

Observation on the light microscopy showed that ferritin penetrates rapidly in the intestinal cells from apex to the basal part after 10 minutes of exposure times (Fig. 1). Ferritin was frequently revealed in the cells that are filled with lysosomal type inclusions (Figs. 1 & 2). The marker was found rapidly under the intestinal epithelium: in the haemocytes presents in the connective tissue (Fig. 1), in the inter-acini male and female connective tissue, and also near the gonoducts.

The presence of ferritin was observed near the male (Fig. 3) and female acini (Fig. 4) which are not closed to the intestine. Moreover, ferritin was detected on all of the length and the height of intestine after 10, 15 and 30 minutes of exposure times.

TEM observations reveal that only 10 minutes after injection, ferritin was appear in the intestinal lumen (Fig. 5) and in the cytoplasmic depression just at the base of the microvilli (Fig. 6). In the intestinal cells,

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Fig. 5. Transmission electron micrograph, *in vivo* experiments. Apical part of the intestine labelled for 30 minutes in ferritin solution. Ferritin (▼) was observed in the lumen (L) and an apical vesicle (V). Scale bar: 1 µm.

Fig. 6. Transmission electron micrograph, *in vivo* experiments (10 minutes labelling). Ferritin (▼) in the cytoplasmic depression of apical part. L: lumen. Scale bar: 1 µm .

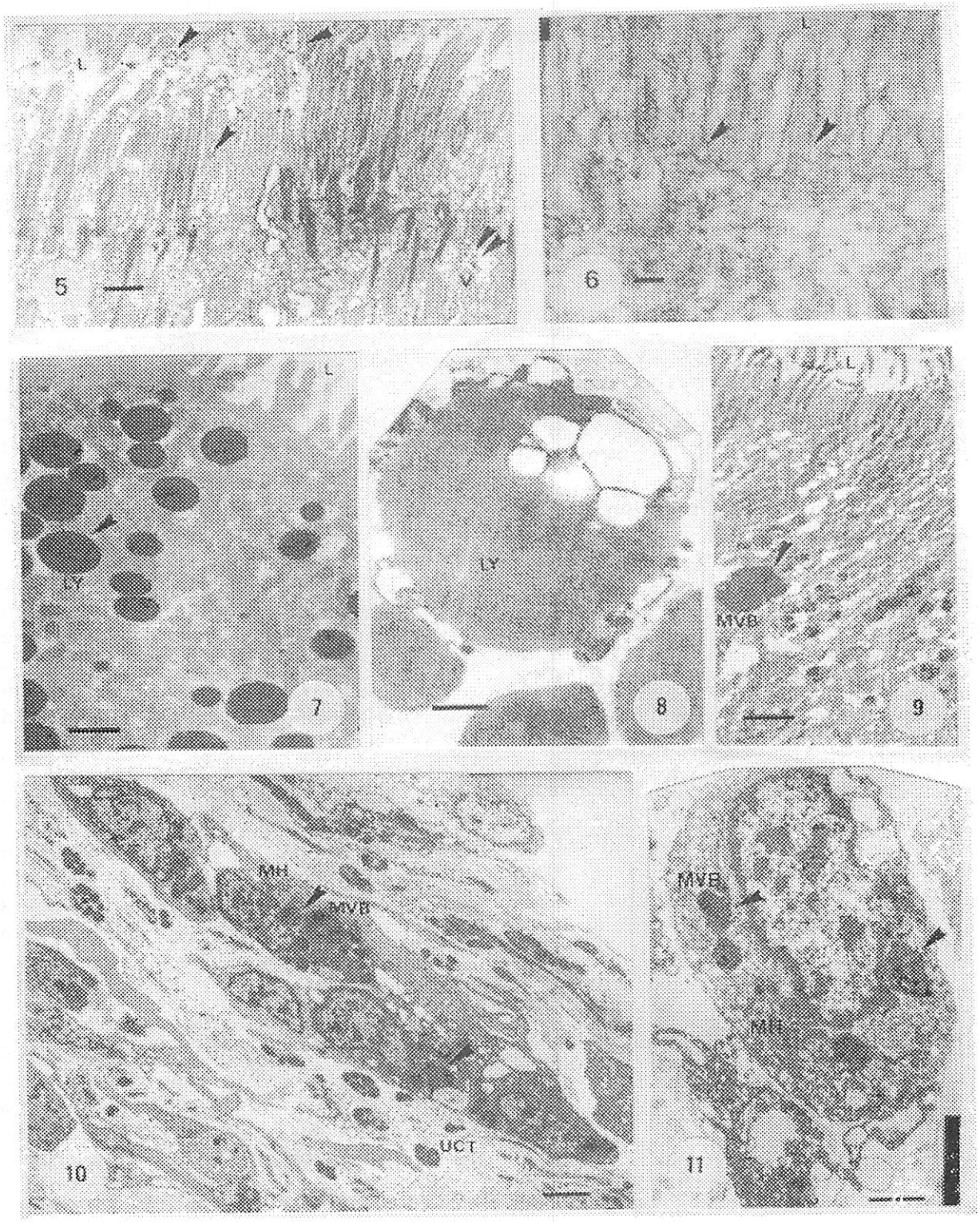
Fig. 7. Transmission electron micrograph, *in vivo* experiments (30 minutes labelling). Apical part of the intestine with ferritin (▼) in the lysosome (LY). L: lumen. Scale bar: 2 µm .

Fig. 8. Transmission electron micrograph, in vivo experiments (15 minutes labelling). Lysosome (LY) with ferritin. Scale bar: 1 µm .

Fig. 9. Transmission electron micrograph, *in vivo* experiments (10 minutes labelling). Apical part of intestine with ferritin (▼) in the multivesicular bodies (MVB). L: lumen. Scale bar: 0,5 µm .

Fig. 10. Transmission electron micrograph, *in vivo* experiments (10 minutes labelling). Macrophagous haemocytes(MH), containing ferritin (▼) in their multivesicular bodies (MVB), in the underlying connective tissue (UCT). Scale bar: 0,25 µm .

Fig. 11. Transmission electron micrograph, *in vitro* experiments (10 minutes labelling). Ferritin (▼) in the multivesicular bodies (MVB) in the macrophagous haemocytes (MH) in the underlying connective tissue. Scale bar: 0,5 µm .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ferritin was observed in the vesicles (Fig. 5), the lysosomes (Figs. 7 & 8) and the multivesicular bodies (MVB) (Figs. 9). Under the basal membrane, in the connective tissue, macrophages also contain the marking multivesicular bodies (Fig. 10).

The results of *in vitro* experiments were the same with that of *in vivo* experiments. Observation on the light microscope, after 10 minutes immersion in the ferritin medium, showed that the marker is present in the lumen, apex, basal, underlying connective tissue, near female and male acini. The same results .was given after 30 minutes immersion, 50 minutes and 2 hours 30 minutes of tissue culture.

Under the TEM, the marker was observed in the multivesicular bodies: just under the cytoplasmic depression and far from that and in the haemocytes of the underlying connective tissue (Fig. 11).

The studies showing the absorption using ferritin tracers have been investigated in various organism such as: hamsters *Mesocricetus auratus* (Bockman and Winborn, 1966), snails Biomphalaria glabrata (Heneine *et al.*, 1969), pulmonate snail: *Planorbarius corneus* (Bottke and Sinha, 1979; Bottke *et al.*, 1982) and *Lymnaea stagnalis* (Bottke *et al.*, 1982), cuttlefish *Loligo vulgaris* and *L. forbesi* (Boucher-Rodoni and Boucaud-Camou, 1987), female Holstein Frisian calves (Paar *et al.*, 1992), male Sprague-Dawley rats (Ito *et al.* 1992) and human small-intestinal tissue (Blok et al., 1981).

In hamsters *M. auratus*, it was observed that a non sensitized animals with intraluminal ferritin have a heavy reaction in lumen (Bockman and Winborn, 1966). The ferritin particles were observed within and without intestinal villus, appear above and between microvilli, within surface invagination, in cytoplasmic vesicles and in multivesicular bodies.The results of present study confirm these findings. In the follicle associated epithelium of small large intestine of calves, Paar *et al.* (1992) found that the uptake of macromolecules (ferritin) is done by a membranous cells (M cells). Moreover, ferritin was observed in apical invagination, apical vesicles, multivesicular bodies, basal vesicles and adjacent intercellular spaces. The present study showed that the ferritin marker were observed in the basal region of intestinal epithelium and we have also found it in macrophagous haemocytes (in the underlying connective tissue). Ito *et al.* (1992), have found ferritin in the macrophages of alveolar epithelium of the male sprague-Dawley rats after 60 minutes of exposure time.

In scallop, it is observed that the *in vitro* experiments given nearly the same results with those of *in vivo* experiments. The present study showed that ferritin is absorbed by the intestinal tissue of the scallop by way of endocytosis. This findings is in agreement with those of Cardell *et al.* (1967), Casley-Smith (1967) and Worthington and Syrotuck (1976). However, Blok *et al.* (1981), who worked on in vitro experiments in human small-intestinal tissue, did not obtained the same results as they did not observed ferritin in apical vesicles and tubules. Indeed, ferritin is seen occasionally in the cytoplasm of an absorptive cells cultured 6 hours with ferritin. Moreover, these authors give a hypothesis that the entry of such particles (i.e. ferritin) is not only by way of endocytosis. And the lysosome-like bodies have a function in regulation of cell-coat glycoprotein transport via crinophagic mechanism (fusion of apical vesicles and tubules with lysosome-like bodies) rather than via an exocytotic-endocytotic mechanism.

The anterior studies showing the absorption in the intestine did not always give satisfactory results. In the snail *B. glabrata*, for example, it was observed a low Fe59 and a low chemical ion contents in their intestinal tissue (Heneine *et al.*, 1969). Moreover, Trier *et al.* (1987) have failed to show an active uptake of ferritin from the luminal side of the intestinal brush cells in adult Balb/cJ mice, Sprague Dawley rats and Hartley-Stain guinea pigs. In these cells, the microvillus

membranes have few Pface intramembrane particles, little alkaline phosphatase activity and highly enriched cholesterol. Boucher-Rodoni and Boucaud-Camou (1987) observed that in the cuttlefishes *L. vulgaris* and *L. forbesi*, for reason of the non absorptive organ, there was no ferritin absorption in their proximal intestine.

Observations using the light microscope showed that ferritin was found in the proximity of female acini and male acini. It has been observed that ferritin is found as main yolk component in Pulmonate snails *P. corneus* (Bottke and Sinha, 1979). Moreover, Bottke *et al.* (1982) have found that ferritin is an exogenous protein with a small scale autosynthesis probability. According to these authors, ferritin is synthesized in midgut gland (hepatopancreas). Moreover, in early gametogenesis the vitellogenic ferritin is transported across the basal membrane, taken up by adsorptive endocytosis via coated pits and vesicles, termed coated vesicles mediated transport. Concerning the transport of ferritin from the site of synthesized into the oocytes, however, there is not yet clear. In scallop *P. maximus*, regarding the presence of macrophagous haemocytes which some have a motil characteristic (Ebble *et al.*, 1990), in the underlying connective tissue near the basal lamina of intestinal tissue and the gonadal acini, these haemocytes may be a metabolite transport agents from the intestine to the gonadal acini. This results would be in agreement with the suggestion of Le Pennec *et al.* (1991). Bottke and Sinha (1979) who worked on the presence of ferritin in the oocytes of snails *P. corneus*, found the ferritin in the basement lamina, under vitellogenic oocytes and attached to oocytes surface membrane. Moreover, these authors found a local accumulation of ferritin molecules in small pits of membrane and the vesicle inside the oocyte.

There are severals investigations concerning the transfer of energy for gametogenesis. In barnacles and starfish, nutrient reserves are transferred from the storage organ (Sastry and Blake, 1971). In purple sea urchin, the nutrient reserves are transferred directly from gut to perivisceral fluid and finally to gonad (without significant storage) (Giese, 1959; Farmanfarmaian and Philips, 1962; Barnes *et al.*, 1963; Mauzey, 1966). In the Bay scallop *Aequipecten irradians* L, oocytes entering the cytoplasmic growth phase may begin protein synthesis by utilizing the nutrients taken up from the digestive gland (Sastry and Blake, 1971).

There were been long discussion about the role of intestine in absorption of nutrients. Zacks (1955) found that the intestine of *Venus mercenaria* is active in digestion and absorption of nutrients. Other regions of intestine, notably the midgut, may have role in the digestive process (Morton, 1983). Reid (1966 and 1968), Payne *et al.* (1972), Mathers (1973) have recorded the presence of variety of extracellular hydrolases from the bivalve midgut. Owen (1974) suggested that absorption of soluble nutrients might also occur in intestine. Yoshikoshi and Koi (1992) found the digestive enzymes (non specific esterase, á-N-acetyl glucosaminidase and α-glucuronidase) in midgut of 6 species of parasitic copepods. It appears that the digestive role of the intestine may be greater than that previously realized. Le Pennec *et al.* (1991) who worked on structural and histochemical of intestine of *P. maximus*, were supported the concept of a digestive function in the gonad intestinal-loop, as well as the direct transfer of metabolites across the intestinal epithelium to the gonad and its developing gametes. The results of this study confirm proposition of these authors concerning the pathway of transfer metabolites from the intestinal epithelium to the gonad.

Conclusion

Metabolite transfers between the intestinal-loop and the gonadal tissue in *P. maximus* could be done by the way of macrophagous haemocytes.

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