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# TROPHIC SOURCES AND PATHWAYS TO THE DEVELOPING GAMETES OF *PECTEN MAXIMUS* (BIVALVIA: PECTINIDAE)

MARCEL LE PENNEC, PETER GÉRARD BENINGER*, GERMAINE DORANGE AND YVES-MARIE PAULET

Laboratoire de Biologie Marine, Université de Bretagne Occidentale, 29287 Brest Cédex, France
*Also: Département de Biologie et Centre de Recherches et d'Études sur l'Environnement, Université de Moncton, Moncton, New Brunswick, Canada

Ultrastructural, histological and histochemical studies were performed on the gonad of adult *Pecten maximus* at various intervals during the reproductive cycle in St Brieuc Bay, France, in order to understand better the sources and transfers of energy to developing gametes in scallops. In addition to the well-known pathways of energy acquisition through feeding and transfer of somatic reserves, a number of novel pathways were demonstrated. These were grouped into two categories: atretic recycling and intestinal loop transfer. Evidence is presented for (1) the recovery of lytic material (resulting from gamete atresia) in the gonad acini, gonoducts, and integument; and (2) the direct transfer of metabolites from the gonad intestinal loop to the developing gametes via vesicular cell-haemocyte couples, which appear to follow fibrous pathways within the loose connective tissue extending from the base of the intestinal epithelium to the acini. A schematic diagram summarizes the sources and transfer mechanisms of the energy exchanges involving the developing gametes in *Pecten maximus*.

## INTRODUCTION

Although the reproductive biology of bivalves has been intensively studied for many years (see Giese & Pearse, 1979; Jong-Brink *et al.*, 1983; Maxwell, 1983; Mackie, 1984 for reviews), some fundamental aspects have emerged only recently, while others remain to be elucidated. Several studies have demonstrated or suggested inter-organ transfers of energy substrates to the gonad during gametogenesis (Vassallo, 1973; Ansell, 1974; Gabbott, 1975; Bayne, 1976; Taylor & Venn, 1979; Barber & Blake, 1985; Pipe, 1987a; Epp *et al.*, 1988); however, the mechanisms of transfer remain unknown. Similarly, although gamete atresia has been characterised in several bivalve species, and the recycling of this material has been proposed, the phenomenon and mechanisms of recycling have only been addressed in the marine mussel, *Mytilus edulis* (Pipe, 1987a). The significance of atresia and recycling in the context of reproductive cost and strategy also requires elucidation (Lubet *et al.*, 1987; Pipe, 1987b; Morvan & Ansell, 1988; Dorange & Le Pennec, 1989).

The present study attempts to identify the sources, transfer mechanisms, and modes of incorporation of energy substrates to the developing gametes of the scallop *Pecten maximus* in St Brieuc Bay, France. Particular attention is devoted to the recycling of atretic material and the role of the gonad intestinal loop.

## MATERIALS AND METHODS

Adult *Pecten maximus* were sampled monthly (January-May and August-December) or weekly (June-July) in St Brieuc Bay (France) using a scallop drag in 1986. The animals were transported to the laboratory and dissected. Sections including the gonad and intestinal loop were fixed in aqueous Bouin's solution, processed in an ascending ethanol-xylene series, embedded in paraffin, sectioned at 5 μm, and stained using the Masson trichrome technique (Gabe, 1968). Ultrastructural analysis of the following structures was performed: gonad external epithelium, evacuating ducts, acini and cells contained therein. Small (~1 $mm^3$) samples were fixed at 4°C with 2·5% glutaraldehyde in 0·2M sodium cacodylate buffer (pH 7·3, adjusted to 1300 mOsm), postfixed with osmium tetroxide, dehydrated, and embedded in Spurr resin. Sections were stained with uranyl acetate and lead citrate, mounted on TEM grids and observed using a JEOL 100 CX

Table 1. *Histochemical tests performed on the epithelia of the gonoducts, integument and intestinal loop of the gonad in* Pecten maximus

| Substance tested | Technique | Control | Results | | |
|---|---|---|---|---|---|
| | | | Gonoducts | Integument | Intestinal loop |
| Glycogen | PAS (McManus, 1946, in Lison, 1960) | amylase digestion | +- (sr)<br>++ (sm) | +- | + |
| Lipase | Gomori (1952, in Gabe, 1968) | heat denaturation | + (cc) | + (cc) | +(ac) |
| Non-specific esterases | Burstone & Folk (1956, in Gabe, 1968) | substrate omission | + | + | ++ |
| Alkaline phosphatase | Gomori (1952, in Gabe, 1968) | substrate omission | + | + | +(ac) |
| Acid phosphatase | Grogg & Pearse (1952, in Gabe, 1968) | substrate omission | + | ++ | ++(ac) |
| Arylsulphatase | Lowe *et al.* (1982) | substrate omission | +- | +- | + |
| N-acetyl-glucos-aminidase | Moore (1976) | substrate omission | + | + | +(ac) |
| β-glucuronidase | Moore (1976) | substrate omission | - | +- | +(ac) |
| Arylamidase | Burstone & Folk (1956) | substrate omission | + | + | +(ac) |
| Amylase | Shear & Pearse (1963) | | +(l) | + | +(l) |
| Glycogen phosphorylase | Chayen *et al.* (1969) | (1) omission of glucose-1-phosphate<br>(2) enzymatic digestion of tissue glycogen | + | - | - |

Abbreviations: ac, apex of cells; cc, certain cells; sm, sexual maturation; sr, sexual resting period; l, lumen; -, not detected; +-, weak reaction; +, positive reaction; ++, strong positive reaction.



electron microscope. Semi-thin sections were obtained using the same technique and stained with toluidine blue.

Several histochemical tests were performed on sections of the gonad intestinal loop and the gonad itself, as indicated in Table 1. Sections of fresh tissue were frozen in liquid-nitrogen-cooled isopentane, stored at -70°C, freeze-sectioned at 9 μm using a cryotome, and processed *en-bloc*.

## RESULTS

The reproductive cycle of *Pecten maximus* in St Brieuc Bay over a three-year interval including the sampling period of the present study has been described by Paulet *et al.* (1988), Dorange (1989), and Paulet (1990). It is characterized by gametogenesis beginning in January, accelerating from April to June-July as the water temperature steadily rises. Two principal gamete emissions occur in June-July, and a minor emission can occur in August. A sexual resting period follows, from September to February.

### *Oocyte atresia and recovery of lysed material*

Oocyte atresia was observed throughout the year, but principally in late vitellogenesis. However, lysed material may invade adjacent acini, bathing young oocytes and auxiliary cells from successive cell cohorts (Figure 1A,B). Developing oocytes present endocytotic vesicles at their apical poles (Figure 1C). In addition, macrophagous haemocytes are numerous amid the lytic debris of degenerating oocytes. The cytoplasm of these haemocytes contains abundant cellular residues in the female portion of the gonad (Figure 1D) and sometimes entire spermatozoa in the male portion.

Lysed material expelled from the acini enters the network of gonoducts (Figure 1D). The ciliated epithelial cells lining the gonoducts present abundant microvilli interspersed between the cilia, and endocytotic-like vacuoles are frequently visible at their apical poles (Figure 1E,F). The periodic acid-Schiff (PAS) reaction shows that these cells contain little glycogen during the sexual resting period, but abundant glycogen during gonad maturation or maturity (Table 1). Positive histochemical reactions were obtained for various lysosome-located hydrolases (Table 1); these enzymes are involved in the catabolism of proteins, lipids, and carbohydrates.

Lysed material escaping from the gonoducts through the gonopore comes into contact with the epithelia of several pallial organs: mantle, gills, and the integument of the gonad itself. Histological and ultrastructural observations reveal the epithelium of the gonad integument to be comprised of three cell types: non-ciliated microvillous cells (dominant cell type), patches of ciliated microvillous cells (Figure 1G), and scattered mucocytes. Numerous, variously-sized vacuoles are found within the cytoplasm of the microvillous cells. With the exception of glycogen phosphorylase, the same histochemical characteristics are found in these cells as in the evacuating duct epithelial cells (Table 1). Lytic debris is also frequently observed in the intestinal lumen (Figure 1H).

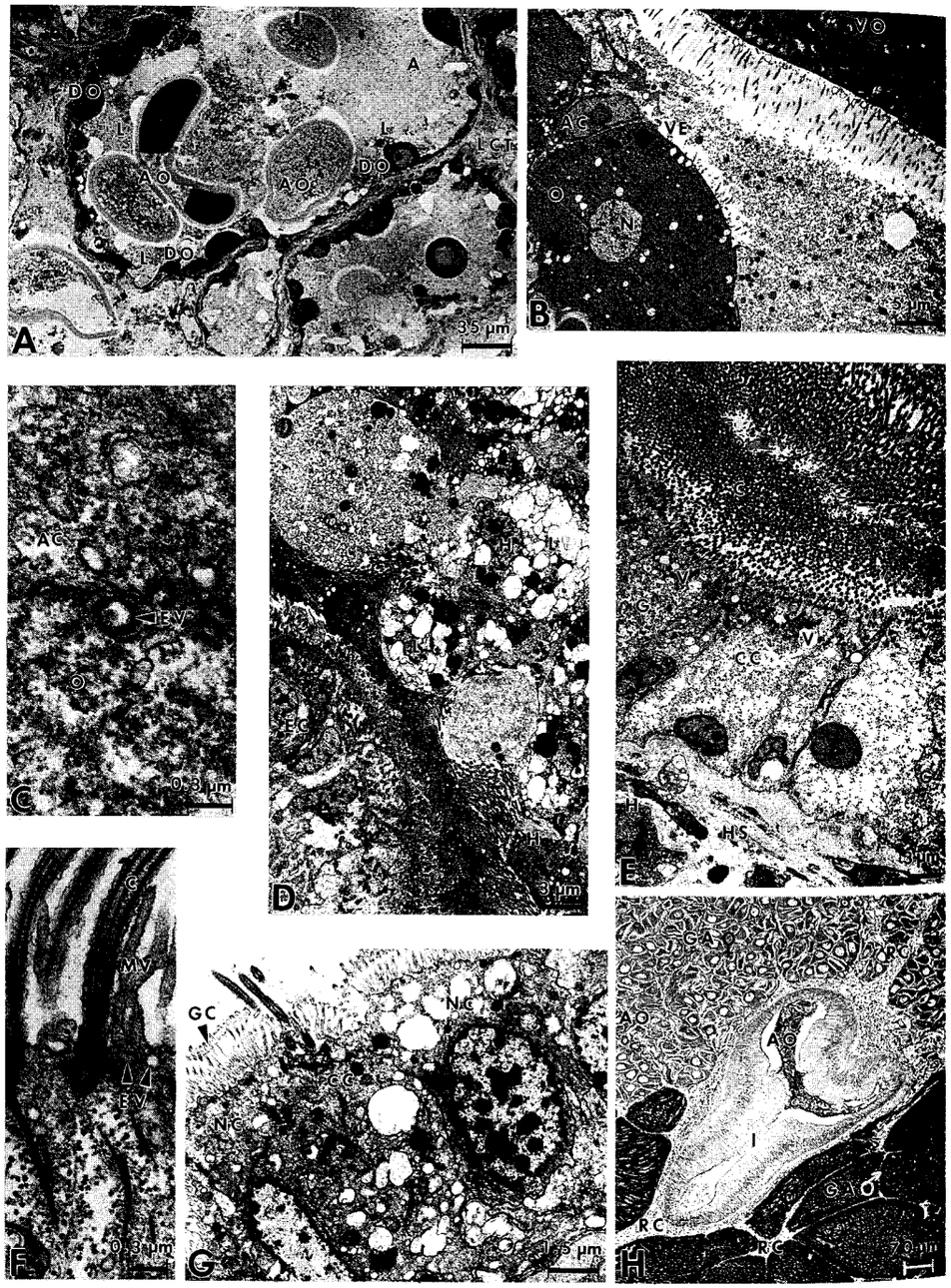


Figure 1. *Pecten maximus* gonad. (A) Semi-thin section showing two acini with oocytes at various stages of maturation. Developing oocytes (DO) are in contact with lytic debris (L) from atretic oocytes (AO). LCT, loose connective tissue. Toluidine blue stain. (B) Transmission electron micrograph (TEM) showing an auxiliary cell (AC) in association with a vitellogenic oocyte (O); both are in contact with lytic debris. N, nucleus; VE, vitelline envelope in formation; VO, mature oocyte at end of vitellogenesis. (C) TEM showing endocytotic vesicle forming in a vitellogenic oocyte (O) at its junction with an auxiliary cell (AC). (D) TEM showing lytic debris (L) in macrophagous haemocytes at the junction of an acinus and a gonoduct. EC, epithelial cell of gonoduct. (E) TEM of transverse section of gonoduct. Note the numerous vacuoles (V) in the apical region of the ciliated epithelial cells (CC), and the abundant glycogen (G) within these cells. The haemolymphatic sinuses (HS) containing haemocytes (H) are in close contact with the basal poles of the epithelial cells. C, cilia. (F) TEM detail of the apical region of a gonoduct epithelial cell, showing endocytotic vesicles (EV), cilia (C), and ramified microvilli (MV). (G) TEM of the external epithelium of the gonad integument. A ciliated cell (CC) is situated between two non-ciliated cells (NC). Note the brush border microvilli configuration and associated glycocalyx (GC). (H) Paraffin transverse section of the intestine (I) at the junction of the male (GA♂) and female (GA♀) portions of the gonad. Note canals (RC) filled with loose connective tissue originating in the peri-intestinal region and ramifying between the acini. Degenerating oocytes (AO) are frequently observed in the gonad intestinal loop of scallops presenting atretic phenomena in their acini. Their origin is uncertain. Masson trichrome technique.



*Structure and histochemistry of the gonad intestinal loop*

The intestinal epithelium is simple and composed of tall, ciliated cells. Numerous exocytotic vesicles can be observed, detached or in formation, at their apical poles; vacuoles are also abundant in the apical region (Figures 1H, 2A). In animals with atretic acini, degenerating oocytes are frequently observed in the lumen, but their origin is as yet uncertain (Figure 1H). The basal lamella is thick and convoluted, and beneath it is a relatively compact layer of connective tissue containing smooth muscle fibres. The intestine is surrounded by a sheath of very loose, fibrous connective tissue, which extends into the gonad and ramifies among the acini (Figure 1H). Haemolymph fills the spaces of this tissue (Figure 2C).

Beneath the smooth muscle layer, hump-shaped cell masses are frequently observed, and these can be seen extending in a thin pathway to neighbouring acini (Figure 2B,C). Examination of these cell masses (herein referred to as intestine-acinus transfer complexes) shows them to be composed of large (~ 30 μm), greatly-vacuolated cells (herein referred to as vesicular cells), closely associated with smaller (7 μm) haemocytes which present well-developed cytoplasmic extensions. The pathways to the acini appear to have fibrous matter (probably collagenous fibres) at their cores (Figure 2B,C). The large, vesicular cells and their accompanying haemocytes appear to detach from positions beneath the smooth muscle layer, and can be seen along the fibrous pathway and beneath the developing oocytes of the adjacent acini (Figure 2B,C,D).

The results of the histochemical tests on the intestine are presented in Table 1. The epithelium of the gonad intestinal loop shows much the same profile as the gonoducts and gonad external epithelium, with most of the enzymes being located at the apices of the cells (next to the lumen and in the region of the exocytotic vesicles). In addition, amylase activity was detected in the lumen.

## DISCUSSION

In suspension-feeding bivalves, including *Pecten maximus*, ingested particles which enter the diverticula of the digestive gland are subjected to the secretory and absorptive activity of the gland cells (see Purchon, 1977; Morton, 1983 for reviews). The resulting metabolites are presumed to be distributed via the vascular system to the various body

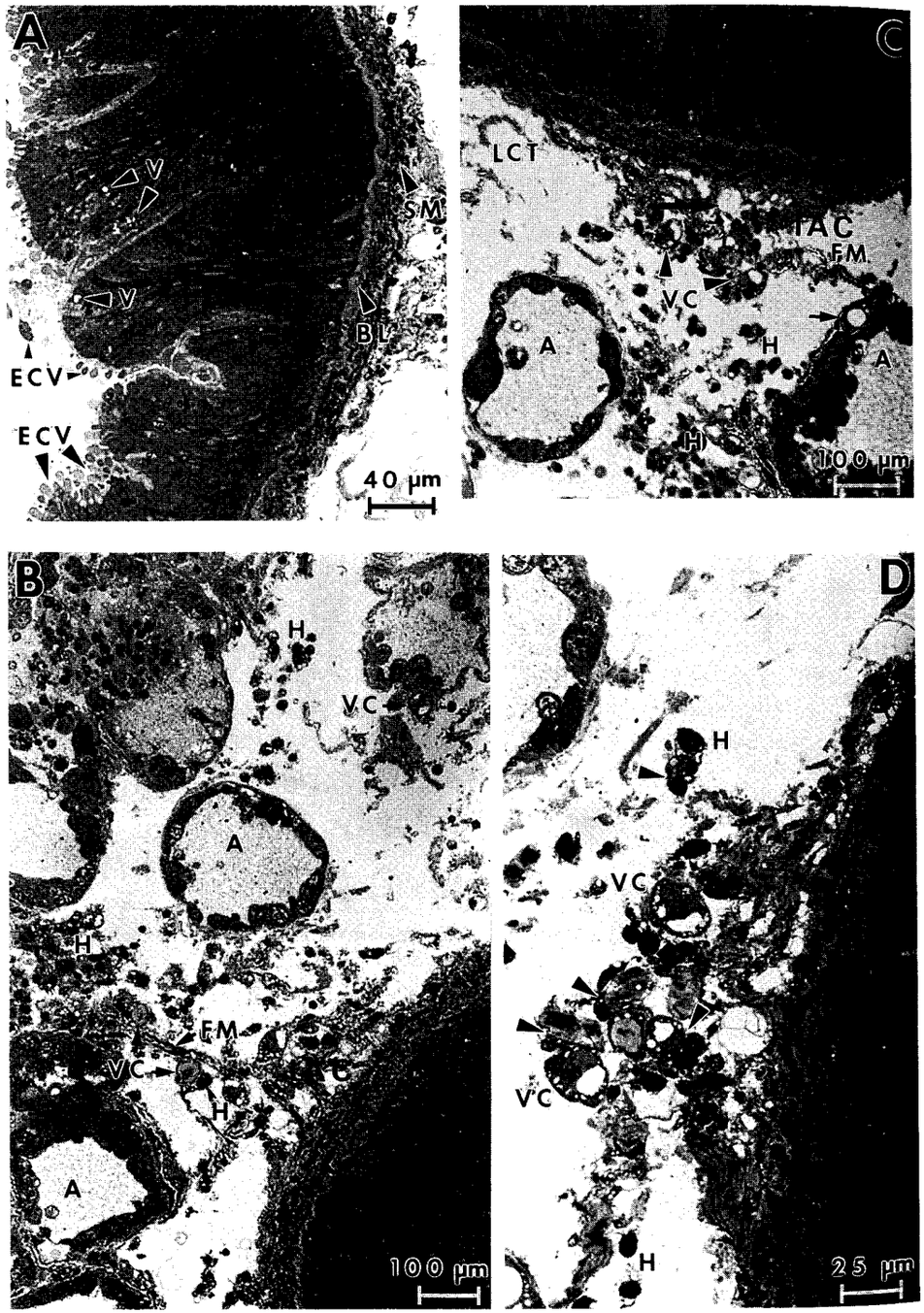


Figure 2. *Pecten maximus*. Semi-thin sections of the epithelium of the gonad intestinal loop. Toluidine blue stain. (A) Low-power micrograph. Note the detached and forming exocytotic vesicles (ECV), the numerous vacuoles (V), especially at the apical poles, and the convoluted basal lamella (BL). SM, smooth muscle fibres. (B) Low-power micrograph of an intestine-acinus transfer complex (IAC), showing haemocyte (H)-vesicular cell (VC) couples attached to fibrous matter (FM) leading to the adjacent acinus (A). (C) Low-power micrograph of another intestine-acinus transfer complex (IAC) and loose connective tissue (LCT) surrounding acini (A). Numerous haemocytes (H) are visible. Note the large, vacuolated vesicular cells (VC) and their accompanying haemocytes, which appear to detach from positions at the base of the epithelium (large arrow) and to follow a pathway to the adjacent acinus. Fibrous material (FM) can be seen at the core of the pathway. (D) Detail of C showing greatly-vacuolated vesicular cells (VC) along pathway to acinus. Smaller haemocytes (H), with extensive filamentous cytoplasmic projections, can be seen in close contact with the vacuolated vesicular cells (arrows). Parts of another pathway may be observed in the upper right quadrant of the micrograph.



tissues (Morton, 1983). During periods of intense gametogenesis, the energetic cost of producing gametes often exceeds the assimilated energy (Barber & Blake, 1981, 1983; Bayne *et al.*, 1983), and metabolic substrates are transferred from somatic tissues to the gonad (Goddard & Martin, 1966; Vassallo, 1973; Ansell, 1974; Comely, 1974; Gabbott, 1975, 1983; Adachi, 1979; Zaba, 1981; Lubet *et al.*, 1987; Besnard, 1988). Such transfers have been demonstrated in various scallop species using radioactive markers (Sastry & Blake, 1971; Vassallo, 1973; Barber & Blake, 1985). Most of the energy supplied to the developing gametes comes from protein and glycogen reserves in the adductor muscle (Comely, 1974; Ansell, 1974; Barber & Blake, 1981, 1985; Lubet *et al.*, 1987; Epp *et al.*, 1988). The main energy reserves of oocytes are lipids and proteins (Holland, 1978); it has long been thought that somatic glycogen is transformed into oocyte lipids (Walne, 1970; Zaba, 1981; Beninger & Lucas, 1984; Lubet *et al.*, 1987; Besnard, 1988), and a probable metabolic pathway has been proposed (Gabbott, 1975).

The results of the present study suggest the existence of a number of additional nutritional pathways to the developing gametes, which can be grouped into two categories: (1) atretic recycling, and (2) intestinal loop transfer.

### *Atretic recycling*

The production of successive gamete cohorts has been viewed as a strategic adaptation to environmental constraints (Morvan & Ansell, 1988; Paulet, 1990). However, mature, non-fertilized gametes have a short life span, and those not emitted enter into atresia (Pipe, 1987b; Dorange & Le Pennec, 1989). Lysis appears to be initiated by putative lysosomes present in the mature oocytes of *Pecten maximus* (Dorange, 1989); lysosomes have also been observed in the oocytes of *Mytilus edulis* (Pipe & Moore, 1985). These organelles would normally be used by the developing larva in the catabolism of its vitelline reserves (Dorange, 1989).

Several authors have proposed that bivalves may recover the products of gamete atresia (Herlin-Houtteville & Lubet, 1975; Motavkine & Varaksine, 1989; Lubet *et al.*, 1987; Dorange & Le Pennec, 1989). Evidence for such recovery within the acini comes from the ultrastructural observations of pinocytosis by young oocytes of *Mytilus edulis* (Albertini, 1985; Pipe, 1987b). The macrophagous haemocytes filled with cellular debris represent another mode of recovery within the acinus.

Both the ultrastructural and histochemical data presented here indicate that the gonoducts are also significant sites of resorption of lytic debris. The dense microvillous

covering of the epithelial cells, as well as the highly vacuolated cytoplasm are characteristic of absorptive cells. The alkaline phosphatase activity, indicative of transmembrane transport, suggests an absorptive role for the gonoduct epithelium. Alkaline phosphatase activity has previously been associated with possible digestive and absorptive function in *Mercenaria* (=*Venus*) *mercenaria* (Zacks, 1955). The lipase, non-specific esterase and arylsulphatase activities are associated with lipid catabolism, while the arylaminidase reaction is indicative of protein catabolism. The products of most of these catabolic activities could be stored as glycogen in the gonoduct epithelial cells, accounting for its observed abundance in these cells in the later stages of gametogenesis. The amylase, glycogen phosphorylase and acid phosphatase activities would thus represent the subsequent utilisation of these reserves by the epithelial tissue. The ultrastructural and histochemical data reported here thus support the hypothesis that bivalve gonoducts can absorb lytic debris from oocyte atresia (Lubet *et al.*, 1987), as previously demonstrated in *Mytilus edulis* (Pipe, 1987a).

Lytic debris not absorbed within the acini or by the gonoducts would be expelled through the gonopore and into the pallial cavity. Further recovery of this material may occur on the pallial organs. The bivalve mantle is capable of absorbing fine particulate matter (Bevelander & Nakahara, 1966; Nakahara & Bevelander, 1967; Machin, 1977). The ultrastructural and histochemical data of the present study indicate that the gonad external epithelium itself is also capable of such absorption, as it presents many of the same characteristics as the gonoducts. While the resorption of lytic debris may furnish energy substrates for the epithelium, several features also suggest that metabolites could be transported to the underlying gonad tissue: the presence of amoebocyte transit cells distributed among the epithelial cells, the highly indented basal membrane, beneath which are found hydrolase-containing macrophage cells, and the proximity to numerous haemolymph sinuses (Dorange, 1989). The gonad thus appears to be capable of absorbing and 'recycling' its own lytic debris in the acini, in the gonoducts, and across its external epithelium.

Assuming the resorption via the various pathways described herein to be dependent on anatomical and histological parameters which are constant for most of the life of a sexually mature scallop, the proportion of the original energy invested which could be recovered from lysing gametes will vary according to the extent of atresia (being very efficient at low levels and less efficient at high levels).

### *Intestinal loop transfer*

Although it has long been known that part of the scallop intestine loops through the gonad, no direct assimilation of metabolites from the intestine has yet been established. Based on histoenzymatic tests and observations of enzymatic activity, several authors have suggested that intracellular and/or intracellular digestion may take place in the intestine (Zacks, 1955; Reid, 1966; Payne *et al.*, 1972; Mathers, 1973), and a re-examination of the role of the bivalve intestine has been proposed (Purchon, 1971). The structural and histochemical observations of the present study support 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. Based on the evidence presented here, the following sequence of events is proposed: both extracellular and intracellular digestion are performed by the intestinal epithelial cells; metabolites are transported to the bases of these cells and are transferred to the large vesicular cells

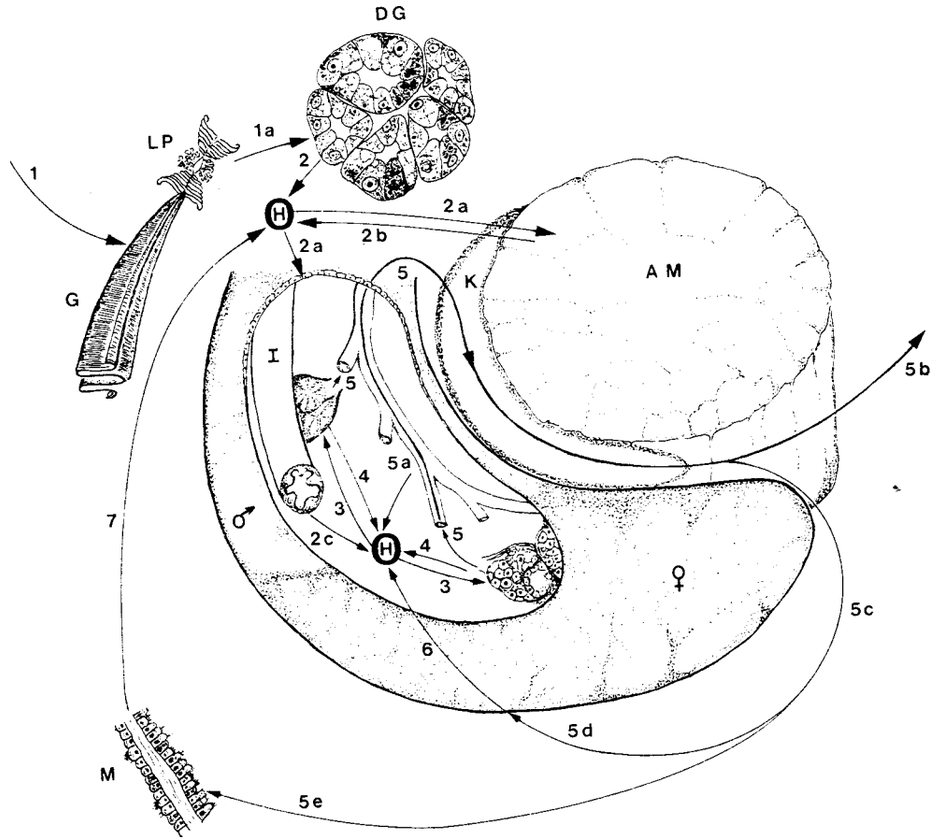


Figure 3. Schematic summary of energy substrate sources and transfer mechanisms to the developing gametes of *Pecten maximus*. 1=ingestion of particulate matter captured and transported by the feeding epithelia (gills, G; labial palp-lip complex, LP). 1a=entry of particles into diverticula of digestive gland (GD). 2=diffusion of metabolites resulting from digestion into the haemolymph and its haemocytes (H). 2a=distribution of these metabolites to various organs, including adductor muscle (AM) and gonad (♂♀) 2b=haemolymphatic transfer of metabolites from the adductor muscle to the developing gametes (2a). 2c=transfer of products of digestion within the gonad intestinal loop to the vesicular cell-haemocyte couples in intestine-acinus transfer complexes. 3=transfer of metabolites from haemolymph (surrounding intestine, via vesicular cell-haemocyte couples and in sub-integumental haemolymphatic sinuses) to acini. 4, 5, 5a, 5b, 5c, 5d, 5e = lytic material resulting from gamete atresia (when this occurs) is incorporated into macrophagic haemocytes (4), or is expelled into the gonoducts and partially absorbed by the gonoduct epithelium (5). This material is also absorbed by macrophagous haemocytes within the gonoducts (5a=these may be expelled from the acini at the same time as the lytic debris). Any remaining lytic material is expelled through the gonopore and into the pallial cavity, whence it may be lost to the external medium (5b), or directed toward the gonad integument (5d) or the mantle epithelium (5e) by the circulation of the pallial fluid. 6, 7 = absorption of lytic material by the gonad epithelium and transfer to subjacent haemocytes (6), or by the mantle epithelium and transfer to subjacent haemocytes (7).

within the intestine-acinus transfer complex. The vesicular cells, accompanied by haemocytes, migrate along the fibrous pathways extending from the loose connective tissue surrounding the intestine and ramifying throughout the gonad to the numerous acini, where their contents are made available to the developing gametes. The role of the haemocytes coupled to the vesicular cells is not yet clear; however, some haemocytes are known to be highly motile (Ebble *et al.*, 1990), and so they may contribute to the movement of the vesicular cells. Alternatively, metabolites could be transferred to the haemocytes which then return to the haemolymph circulation within the gonad. Further research is required to elucidate this point.

Based on observations of variations in the amount of interfollicular (=inter-acinal) connective tissue present in the scallop gonad throughout the sexual cycle, it has long been proposed that reserves are transferred from this tissue to the developing gametes (Coe, 1943; Beninger, 1987). Recently, Pipe (1987a) has demonstrated similar variations in the volume fractions of the adipo-granular and vesicular connective tissue cells in the mantle of *Mytilus edulis*. The results of the present study suggest that the relationship is in fact much more dynamic for the developing gametes of *Pecten maximus*, involving transfer from the gonad intestinal loop to the acini through the fibrous network of the inter-follicular connective tissue. Detailed ultrastructural and autoradiographic studies are now in progress in order to verify this proposed sequence of events (Le Pennec *et al.*, in preparation).

The foregoing description of gonad trophic pathways is summarized in Figure 3. For the pathways involving haemolymph, future investigations should focus on the exact vectors, from the digestive gland to the adductor muscle and gonad (as has been done in the present study for the intestinal loop). In species in which gametogenesis is greatly influenced by external factors such as temperature, it appears that gamete atresia is common, but that the accompanying energy loss is minimized by the resorption of at least some of the lytic debris. A similar hypothesis has been advanced for the bivalve *Bathymodiolus thermophilus*, which inhabits the highly unstable environment of hydrothermal vents (Herry & Le Pennec, 1987).

In conclusion, the results reported herein support the hypothesis that the considerable energy requirement of successive cohorts of developing gametes in *Pecten maximus* is supplied through several trophic pathways to the gonad as depicted in Figure 3. Of these, evidence is presented for two novel pathways: the resorption of atretic material by the various epithelial surfaces which it encounters, and direct transfer of metabolites from the gonad intestinal loop to the gonad acini.

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