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A Structural Study of Functional Cells in Hepatopancreas of *Mytilus galloprovincialis* Lamarck, 1819

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Abstract.- In this study, the histological structure of the hepatopancreas of *Mytilus galloprovincialis* Lamarck, 1819 was examined. The samples were collected at Yenikapı shores in the Northern part of the Marmara Sea between June 2004-June 2005. The samples collected were prepared for light microscopic examinations and stained by the histological stains. Lipid as an energy reserve and activity of catalase related with the detoxification function were evaluated seasonally. It was demostrated that under the influence of environmental and biotic factors such as gonadal alterations and food availability, lipid reserves and activity of catalase shows seasonal alterations.

Key words: Hepatopancreas, *Mytilus galloprovincialis,* catalase, lipid.

INTRODUCTION

The hepatopancreas of bivalves consists of a series of blind-endding tubules connected to stomach by a sequence of branching ducts (Morse and Zardus, 1997; Morton, 1983). This sequence of branching ducts consists of primary and secondary ducts distinctive with their different microscopic anatomies. Primary ducts separate from stomach and branch to secondary ducts. Secondary ducts are short and unbranched. Each secondary duct leads to a tubule group (Morse and Zardus, 1997; Owen, 1955,1956). Hepatopancreatic tubules consist of single-layered epithelium that are separated from surrounding connective tissue and muscle cells by a basal membrane (Owen,1966; Morton, 1983; Lobo-da-Cunha, 1999, 2000).

Bivalves have a connective tissue mass which fills the spaces among the parts of digestive system and surrounds it. Tubules and ducts have been packed loosely within the connective tissue (Owen, 1956; Morse and Zardus, 1997). This connective tissue has two kinds of cells; adipogranular connective tissue (ADG) cells and vesicular connective tissue (VCT) cells. ADG cells contain glycogen, protein granulles and lipid inclusions. Vesicular cells are specialized in glycogen storage (Mathieu and Lubet, 1993; Robledo *et al.*, 1997).

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Hepatopancreatic tubules contain two types of cells. These are cylindrical shaped acidophilic digestive cells and pyramide shaped basophilic cells (Morse and Zardus, 1997). Digestive cells that are the most common cells among the tubules are the cylindrical cells which have microvillus on apical surface and contain lipid inclusions that are stained darkly. Basophilic cells are pyramide shaped cells which are located among digestive cells either one by one or in groups of two or three and the apical points of which do not reach lumen (Owen, 1955,1956; Palmer, 1979; Lobo-da-Cunha, 1997, 1999, 2000). Digestive cells in front of hepatopancreatic tubules are separated according to their functions. McQuiston (1969) defined two, Owen (1966), Merdsoy and Farley (1973) three, and Langton (1975) and Morton (1983) four different phases for this functional phase of digestive cells. Generally, these phases are holding phase, absorption phase, digestive phase and disintegrating and reconstituting phase (Langton, 1975; Morton, 1983).

Hepatopancreas, whose histology is defined above, is the organ related with intracellular and extracellular digestion of the nutrients, food absorption, and lipid, gylcogen and mineral storage, and plays an important role in internal defence mechanism, especially in detoxification (Lobo-da-

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Cunha, 1999, 2000). The enzymes of hepatopancreas have been subject to many investigations because of its importance in ecotoxicology and understanding its various functions. The peroxisomes and lysosomes of hepatopancreatic cells can experience functional and morphological changes under the influence of various pollutants, and this is utilized as bioindicator in determining the amount of pollution and pollutive agents. For this purpose, peroxisomal enzymes and especially catalase that is the marker enzyme of peroxisomes are investigated (Cancio *et al.*, 1999; Orbea *et al.*, 1999). The primary function of catalase is transforming hydrogene peroxide, which is toxic, into water and oxygene. Seasonal investigations are performed regarding catalase and other peroxisomal enzymes, especially in studies about sea pollution.

The aim of this study is to reveal the catalase enzyme activity and lipid content of functional cells in hepatopancreas of *Mytilus galloprovincialis*, which is the most commonly consumed mussel in our country and in the world and which is an important source of protein, at the level of light microscope.

MATERIALS AND METHODS

In this study, ten mature mussels (anterio-posterior length of shell between 6.5-9.0 cm) were collected monthly from Yenikapı-Istanbul (Marmara Sea, Turkey) between June 2004 and June 2005. Water temperature was measured at the time of collection and recorded.

Histological methods

Hepatopancreas particules examined under light microscobe were fixed for 12 hours in Bouin's solution. 5-6 µm thick sections of the tissues were stained with hematoxylin + Eosin (H+E), Periodic acid-Schiff's reagent + Fast Green (PAS+FG) (Drury *et al.*, 1967) and Bromphenol Blue (Mazia *et al.*, 1953).

Histochemistry of lipids

The tissue samples were fixed for 2 hours at +4°C in paraformaldehyde in phospate buffer (pH 7.4). 10 µm thick sections were cut with cryomicrotome at -24°C. Sections were stained with Sudan III + Hematoxylin (Drury *et al.*, 1967).

Histochemistry of catalase

Hepatopancreatic samples from ten samples taken monthly, were kept in 0.2 M phosphate buffer pH 7.4 contaning 10% sucrose at +4ºC for 10 min. Tissues were frozen in liquid nitrogen after being dried with filter paper. Samples were embedded in Jung and 7-8 µm thick sections were cut with cryomicrotome at -24°C. Sections were fixed in 4% formaldehyde in 0.2 M phosphate buffer pH 7.4 with 6% sucrose and 2.5% NaCl at 4°C for 5 hours. Following fixation, sections were rinsed in the same buffer and incubated in a freshly prepared medium containing 0.2% 3,3'-di-aminobenzidine tetrahydro-chloride (DAB, Sigma D-5637), 0.3% H_2O_2 and 0.01 M imidazole (DAB I-0125) in 0.01 M Teorell-Stenhagen buffer at pH 10.4. Incubation was performed in the dark at 42°C for 40 min. Sections were rinsed with Teorell-Stenhagen buffer after the completion of incubation and subsequently with distilled water; then dehydrated and mounted in Entellane (Cancio *et al.*, 1999; Orbea *et al.*, 1999).

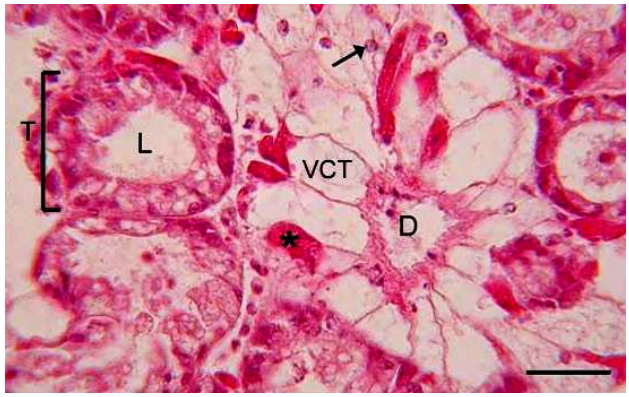

Fig. 1. General view of hepatopancreas. Hepatopancreatic tubule (**T**), tubule lumen (**L**), vesicular connective tissue (**VCT**), adipogranular connective tissue cell (*****), amoebocyte (→) vessel (**D**). Stain: H+E. Bar: 30 µm.

RESULTS

Histological studies

The hepatopancreas of *Mytilus galloprovincialis* is composed of branching duct systems. The surrounding connective tissue is composed of ADG cells and VCT cells (Fig. 1).

ADG cells are long, triangular eosinophilic cells. ADG cells give positive reaction with protein and lipid stains, and negative reaction with Periodic acid Schiff (PAS). VCT cells are polygonal eosinophilic cells. These cells have vacoules filled with glycogen and have a thin cytoplasm. VCT cells give PAS positive reaction, and negative reaction with lipid and protein stains.

Hepatopancreas duct system consists of braching primary ducts and non-branching secondary ducts. This duct system connects to hepatopancreatic tubules. The internal surfaces of tubules are compared of single-layered epithelium and there is a very thin basal membrane underlying epithelium. Additionally, myoepithelial cells lie on the base of tubule epithelium and collagen fibrils are present in the connective tissue. Single-layered epithelium of tubules is composed of two types of cells. These are digestive and basophilic cells (Fig.2).

The height of basophilic cells within the tubule is 11±1 µm, the basal width 12±1 µm and the diameter of cell nucleus is 5 µm. Basophilic cells have an extensively basophilic cytoplasm. Basophilic cells do not contain lipid granules. They give negative reaction with PAS and strong positive reaction with mercury bromophenol, a protein stain. While the height of digestive cells in the tubule vary with phases that the cells undergo, the height of the cell is 12±1 µm and the width is 4 µm. Apical cytoplasms of digestive cell are positively stained because of several small granules. In the apical cytoplasm of digestive cells, granules of 1-2 µm in diameter are stained with PAS. Digestive cell is less stained with protein stain compared to basophilic cell.

Different phases of digestive cells are observed in hepatopancreatic tubules. There are five different phases that are absorptive phase, digestive phase, disintegrating phase, reconstituting phase and holding phase in digestive cells of the tubules. There are several granules in apical cytoplasm of digestive cells in the absorptive phase and lumen content is similar to those granules. The height of digestive cells at this phase is 25±1 µm (Fig. 2A). In the digestive phase follwing the absorptive phase, the whole cytoplasm is filled with these granules. The height of digestive cells at this phase is 25±1 µm (Fig. 2B). At the disintegrating phase that is the next phase, they start to detach from their apicals and are destroyed. The height of digestive cells at this phase is 15±1 µm (Fig. 2C). In the reconstituting phase following the disintegrating phase, the height of cells within the tubules are 6 µm. At this phase, the shapes of cells range from flat to cubic (Fig.2D). At the holding phase, there are empty vacuoles and cell is a typical digestive cell with 15±1 µm height (Fig. 2E).

Histochemical studies

Catalase exists both in the digestive cells and basophilic cells of the hepatopancreatic tubules. In our investigations, catalase enzyme activity was maximum in February and minimum in September, November and December (Table I, Fig. 3A).

Table I.- Monthly record of water temperature, and staining of hepatpancreas of *Mytilus galloprovincialis* for catalase activity and lipid content.

Months	Water temperature (°C)	Staining for catalase activity	Staining for lipid content
June 2004	20.0	++	++
July	23.5	++	++
August	22.5	++	++
September	22.5	+++	+++
October	22.5	+	++
November	16.5	+	+
December	10.5	+	+
January	10.0	+++	+++
February	9.0	++++	++++
March	8.0	++++	+++
April	12.0	++	+++
May	18.0	++	++
June 2005	20.0	++	+++

+ very weak staining; ++ weak staining; +++ moderate staining; ++++ strong staining; +++++ very strong staining.

Digestive cells store lipids inside hepatopancreas. Lipid storages within the digestive cells increase and decrease in certain times of the year. Maximum lipid storage is observed in February and minimum in November and December (Table I, Fig. 3B).

DISCUSSION

ADG cells gave PAS negative reaction in this study, which is contrary to the findings of Robledo *et al*. (1997) about *Mytilus galloprovincialis*. This shows us that ADG cells do not store carbohydrates.

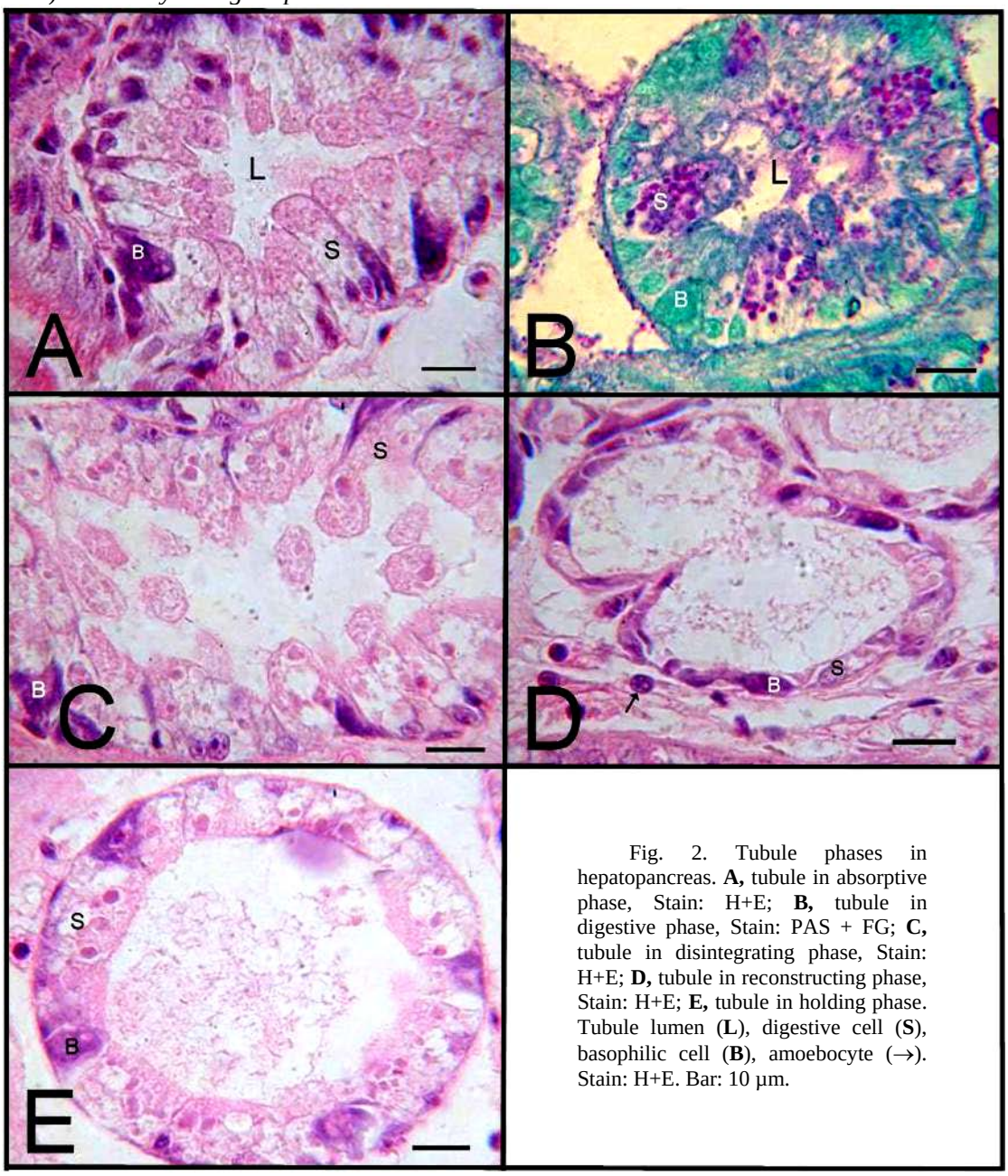

Fig. 2. Tubule phases in hepatopancreas. **A,** tubule in absorptive phase, Stain: H+E; **B,** tubule in digestive phase, Stain: PAS + FG; **C,** tubule in disintegrating phase, Stain: H+E; **D,** tubule in reconstructing phase, Stain: H+E; **E,** tubule in holding phase. Tubule lumen (**L**), digestive cell (**S**), basophilic cell (**B**), amoebocyte (→). Stain: H+E. Bar: 10 µm.

The VCT cells, on the other hand, gave positive reactions both with PAS and glycogen stain, which shows that these cells function as glycogen stores. The presence of myoepithelial cells underlying hepatopancreatic tubule epithelia is suggestive of the idea that secretions and post-digestive waste are transferred to stomach and intestine via contraction of these cells.

Basophilic cells within the hepatopancreatic tubules are located irregularly in small groups

(Owen, 1956, 1966; Morton, 1983). In most species, for example *Venerupis pullastra*, basophilic cells have cilia on their apical surfaces (Owen, 1955). The basophilic cell has a single flagellum in Nuculidae (Owen, 1956). The *Pecten maximus* has no flagellum in basophilic cells (Le Pennec *et al.*, 2001). In the present study, no flagellum was observed in basophilic cells of *Mytilus galloprovincialis.*

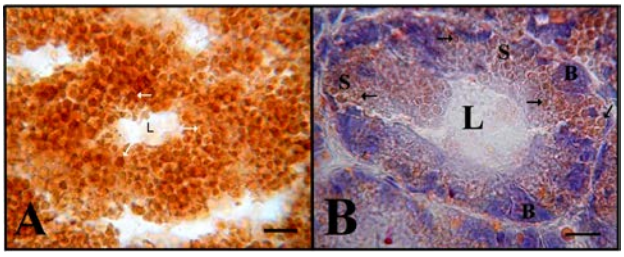

Fig. 3. Marked catalase enzyme and lipid droplets in hepatopancreas; **A,** histochemically marked catalase enzyme (→) in hepatopancreatic tubules in February. Bar:10 µm; **B,** lipids in hepatopancreatic tubules in February. Basophilic cell that does not contain lipid (**B**), tubule lumen (**L**), digestive cell containing lipid (**S**) and lipid droplets (→). Stain: Sudan III + Hematoxylin. Bar: 10 µm.

Apical surfaces of digestive cells have a microvillus lining. Digestive cells are characterized by the presence of several vacuoles and light coloured cylindrical cells. They generally have lipid droplets and glycogen granules in their cytoplasm (Morton, 1983; Lobo-da-Cunha, 1997, 1999). Lipid droplets stored in these cells are mainly located in spaces among food vacoules (Lobo-da-Cunha, 1997). PAS positive granules inside *Mytilus galloprovincialis* is suggestive of carbohydrate digestion and storage in these cells.

Digestive cells have structural differences according to their various functions such as intracellular digestion, absorption, secretion, fagocytosis and food storage. Therefore, they are separated into functional stages according to McQuiston (1969), Owen (1966), Merdsoy ve Farley (1973), Langton (1975) and Morton (1983). Langton (1975), McQuiston (1969) and Morton (1983) have suggested relationship of digestive cells with the tide. According to McQuiston (1969) different digestive cells are observed in tubules in different stages with tide. Since our study was performed in the Marmara Sea, apart from McQuiston, the observation of all stages at the same time is related to the existence and non-existence of nutritional substances, rather than big tides in the oceans.

In bivalves, carbohydrate and protein are especially important in reproductive stages (Cancio *et al.*, 1999; Le Pennec *et al.*, 2001). Increase and decrease in lipid storages are related to efficiency of gonads. Lipid droplets present in hepatopancreas are transferred to gonads via amoebocytes and hemocytes during the development of ovaries (Mathieu and Lubet, 1993; Le Pennec *et al.*, 2001; Beninger *et al.*, 2003). This shows that lipid storage in hepatopancreas are an important source for the continuity of gonadal activities in winter when food sources are restricted. Increase in lipid content in September is suggestive of the storage of lipid in order to get prepared for winter. Additionally, in November and December, decrease in lipids to minimum level is suggestive of an ovulation period right before these months. Maximum amount of vitellogenic oocyte in November in the ovary of *Mytilus galloprovincialis* (Kunduz, 2006) suggests that lipids stored in whole summer is used in winter. Also, the increase in the amount of lipids from January indicates that lipids begin to be stored in order to be used by gonads when needed.

In conclusion, our findings demonstrate that mussels show seasonal metabolic and enzymatic activity changes. Seasonal changes in catalase activity are affected by changes in environmental factors (temperature, food, pollution level) and biotic factors (hormonal condition and reproductive stage) (Cancio *et al.*, 1999; Orbea *et al.*, 1999). The parallel occurrence of catalase activity of *Mytilus galloprovincialis* to seasonal change of lipids shows that seasonal change of catalase is related to gonadal change and food density.

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