Structure and Function of Digestive Diverticula in the Scallop, Patinopecten yessoensis (JAY)

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Structure and Function of Digestive Diverticula in the Scallop, *Patinopecten yessoensis* (JAY)*

Young Jin CHANG,** Yoshio SUGAWARA*** and Tadashi NOMURA

Department of Fishery Science, Faculty of Agriculture, Tohoku University, Sendai 980, Japan

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Summary

The structure of digestive diverticula in the scallop, *Patinopecten yessoensis*, was studied by both light and electron microscopic observations and the function of epithelial cells in them which has already been reported was discussed at length.

The digestive diverticula are composed of several large ducts which lead to the inside of the stomach. The epithelia of the ducts consist of one kind of ciliated cell. Their cilia, about 6 μm in length, project from the distal surface of the ciliated cell to the lumen. So small food particles from the stomach are transported into the lumina of the tubules by these cells. The tubules consist of three kinds of cells, namely, basophil, digestive-secretory and lipid cells.

The basophil cell is characterized by the presence of many microvilli, about 1.5 μm in length, in which a few cilia can be recognized, and rough-surfaced endoplasmic reticula are well-developed in all cytoplasm. The cells are considered to assist in the transportation of food particles into the inner part of the tubule by beating their cilia.

The digestive-secretory cell is well vacuolated in the cytoplasm and closely resembles digestive cells in other species of bivalves. The cell seems to have both roles of intracellular digestion and secretion, because of having only various size of electron-dense granules in its micro-apocrine secretion at the apical portion.

Lipid cell, which has never been found in any other species of bivalves, contains many granules in the cytoplasm and may be said to have the function of accumulating and metabolizing lipid.

Digestion of food materials in bivalve molluscs is generally divided into two processes, extracellular digestion in the stomach, and intracellular digestion in the digestive diverticula (1). The digestive diverticula, having a large number of

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** Present address: Department of Aquaculture, National Fisheries University of Pusan, Namgu, Pusan 608, Korea.

*** Present address: Department of Biotechnology, Faculty of Science and Technology, Senshu University of Ishinomaki, Ishinomaki 986, Japan

blind tubules emptying into several large ducts which lead to the interior of the stomach, are a part of the digestive tract and excrete digestive enzymes, absorb food materials, and intracellularly digest food particles (7). However, it still can not be said that the structure and function of digestive diverticula in bivalve molluscs have been sufficiently clarified.

Recently, the problem of shell poison has often occurred worldwide and has brought about the hindrance in planned production of economically important bivalve species. Considering the fact that shell poison accumulates chiefly in the digestive diverticula (5), this organ may be a very important study material from the viewpoint of affording basic information about shell poison as well as of the physiology of bivalve species.

This organ is also called the hepatopancreas (16), midgut gland (14) and digestive gland (9). The variation in the names of this organ may also mean that the structure and the function of the organ are still not sufficiently explained.

In fresh-water species, *Anodonta anatina*, the structure of epithelial cells in the tubule has been studied by light and electron microscopy (10, 11). In addition, the epithelial cells in tubules were also studied in three marine bivalve species, *Crassostrea virginica* (4, 15) and *Patinopecten yessoensis* (13) by light microscopy and *Crassostrea gigas* by electron microscopy (6).

We felt the necessity to find out the general structure of the digestive diverticula and fine structure of epithelial cells in the tubule and to discuss the function of every structure of the digestive diverticula in the scallop. It was decided to carry out a detailed cytological and histochemical study on the digestive diverticula of the scallop.

Materials and Methods

Specimens of the scallop were collected from Abashiri waters located in Hokkaido, Japan. Their sizes ranged from 8.4 to 12.8 cm in shell length and their ages, 3 to 4 years old.

For histological and histochemical observations, digestive diverticula were excised in Bouin's fixative. After fixation, the specimens were dehydrated in a series of increasing concentrations of ethanol and embedded in paraffin. Sections were cut 6 μm in thickness and stained with Mayer's hemalum and 1% eosin-erythrosin, and acrolein-Schiff reaction (3).

For the detection of lipid in the tissue, the quick-frozen sections made by a cryostat (-25°C) were stained with Sudan III or Nile blue.

To observe the internal surface of digestive diverticula, a piece of tissue including the stomach, ducts and tubules was frozen directly in isopentane dry ice bath at about -80°C and immediately cut into two pieces with a safety razor. Each piece of tissue was fixed overnight in a 20% formalin solution. After fixation, the tissues were washed several times in 0.2 M cacodylate buffer (pH 7.4)

at 4°C and subsequently postfixed for 1 hour in 1% osmium tetraoxide in 0.2M cacodylate buffer. Fixed specimens were dehydrated in ethanol and dried on a critical point drier (Hitachi HCP-1). Then the tissues were mounted on a specimen holder and coated with gold. Observations were performed with a Hitachi S-700 scanning electron microscope at 15 KV.

In order to observe the fine structure of the epithelial cells of tubule, the digestive diverticula were dissected out and cut into small pieces. The specimens were fixed for 1 hour with 5% glutaraldehyde in 0.1M cacodylate buffer solution at pH 7.4. After fixation, the specimens were washed several times in the buffer solution and then postfixed for 1 hour with an ice-cold 1% osmium tetraoxide solution in 0.2M cacodylate buffer solution at pH 7.4. Subsequently fixed specimens were dehydrated in a series of increasing concentrations of ethanol and embedded in Epok 812. Sectioning was done using glass knives on a Porter-Blum MT-2 microtome adjusted to the range of 800-1,000Å. Tissue sections were mounted on neoprene filmed grids. Double staining was usually applied using 1% uranyl acetate solution for twenty minutes followed by alkaline lead citrate for five minutes at room temperature. A JEM-100B electron microscope was used for the observation.

Results

1. *Position and external features of the digestive diverticula*

The digestive diverticula of the scallop occupy about 6% of the weight of soft body part and are located in the internal side of ligament. Near the abdominal side of the digestive diverticula, the foot, gonad, adductor muscle and heart are situated. The colour varies from light green to black. The anterior end of this organ includes the esophagus opening covered with the labial palp.

2. *Internal surface of the digestive diverticula*

The esophagus beginning from the mouth leads to the stomach in the digestive diverticula which has numerous blind tubular structures. The stomach wall consists of the two parts, a gastric shield and a ciliary sorting area. The surface of the gastric shield has several entrances of digestive diverticula ducts (Fig. 1-1). Numerous cilia measuring about 4 μm in length are arranged on the surface of the ciliary sorting area and granular materials averaging 2.5 μm in diameter are found between the cilia (Fig. 1-2). Each duct has several branches and they become narrower as they descend to the deep side of digestive diverticula. Finally, their ends lead to blind-end tubules. A number of tubules in cross section were found with each tubule measuring 100 μm to 150 μm in diameter (Fig. 1-3). Close observation of the duct reveals that single-layered epithelial cells are found to be arranged along the surface (Fig. 1-4). The cells are ciliated columnar epithelial cells, measuring about 35 μm in height and having cilia about 6 μm in length

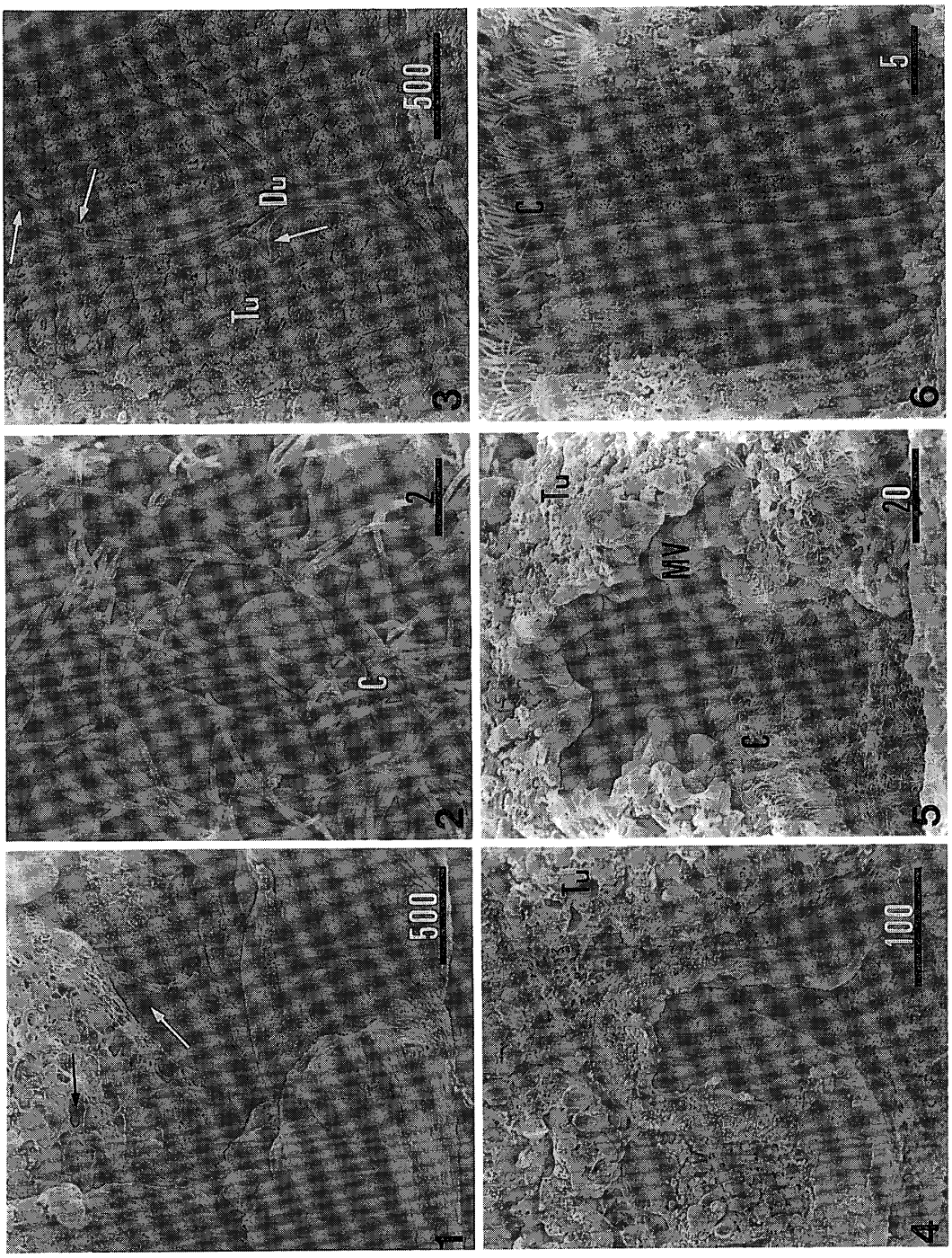

Fig. 1. Structures of internal surface of digestive diverticula.
1. Several entrances (arrows) of digestive diverticula ducts on the stomach wall. 2. Ciliary sorting area of stomach. 3. Structure of duct sectioned longitudinally and numerous tubules cross-sectioned around the duct. Several entrances (arrows) of the tubules are found. 4. Basal part of branch of duct. 5. Structure of part transformed from duct having cilia into tubule having microvilli. 6. Epithelial cells of duct. C; cilia, Du; duct, MV; microvilli, Tu; tubule. The units of each scale are microns.

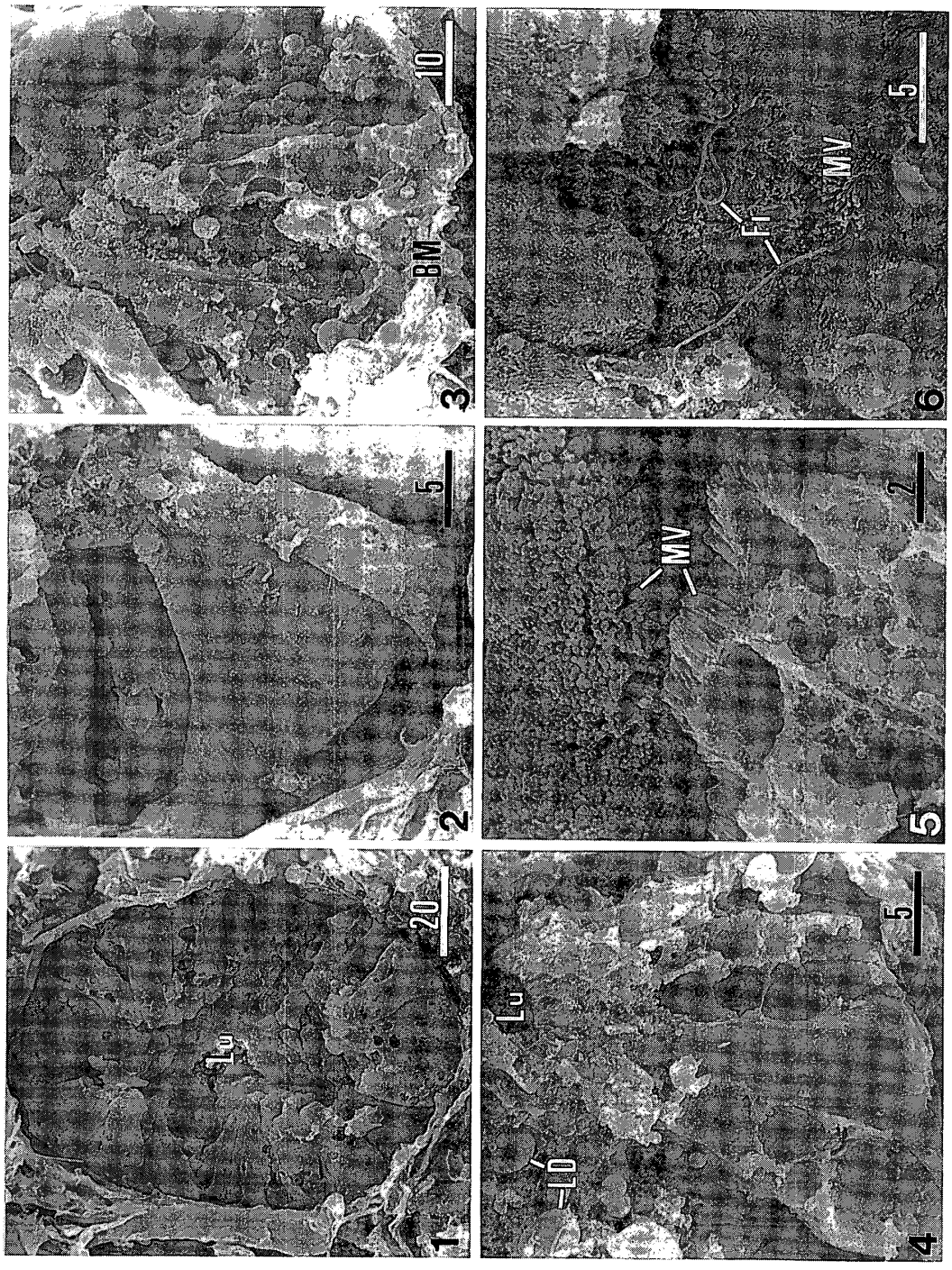

Fig. 2. Structures of internal surface of digestive diverticula tubule.
1. Cross section of a tubule. 2. Surface of epithelial cells in a tubule. 3. Longitudinal section of epithelial cells in a tubule. 4. Intracellular structures of epithelial cells showing presence of several granules. 5. Microvilli of epithelial cells. 6. Numerous microvilli on the epithelial cells. Note the presence of a few cilia among the epithelial cells. BM; basement membrane, Fl; beating cilia, LD; lipid droplet, Lu; lumen, MV; microvilli. The units of each scale are microns.

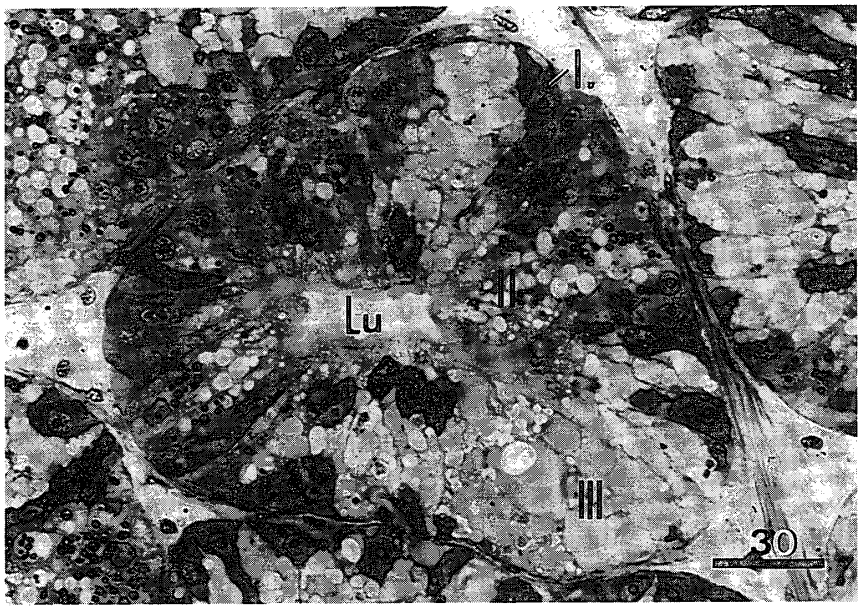

Fig. 3. A micrograph of cross section of digestive diverticula tubule.
I ; Type I cell, II ; Type II cell, III ; Type III cell, Lu ; lumen.
The unit of scale is micron.

(Fig. 1-6). The area of transformation from duct to tubule is distinguishable by its two parts, the epithelia of the duct having cilia and that of the tubule containing microvilli (Fig. 1-5).

On the internal surface of the tubule many epithelial cells are arranged, but the lumen is irregular in shape due to the variations in the height of the epithelial cells. Deeper the lumen becomes narrower and finally ends with the terminal part of the tubule (Fig. 2-1). The epithelial cells of which membranes were well preserved are cylindrical in shape, measuring 25-35 μm in height (Fig. 2-2). However, the epithelial cells of which cytoplasma is exposed reveal various sizes of depressions and granules (Fig. 2-3, 4). The free surface of every epithelial cell bears a number of microvilli. The microvilli are short, averaging about 1.5 μm long, and show the same thickness of proximal and distal parts (Fig. 2-5). Viewed from the lumen of the tubule, it is noted that a few beating cilia measuring about 12 μm long arise from the narrow space between the cells with microvilli bundles (Fig. 2-6).

3. Classification of epithelial cells in the tubule

On the basis of cytological observations by a light and an electron microscope, the epithelial cells of the tubules are differentiated into three kinds of cell as follows ;

Type I

The cell is buried by other kinds of cells because of being the shortest in length among the three types of cell. The cytoplasm of the cell contains large

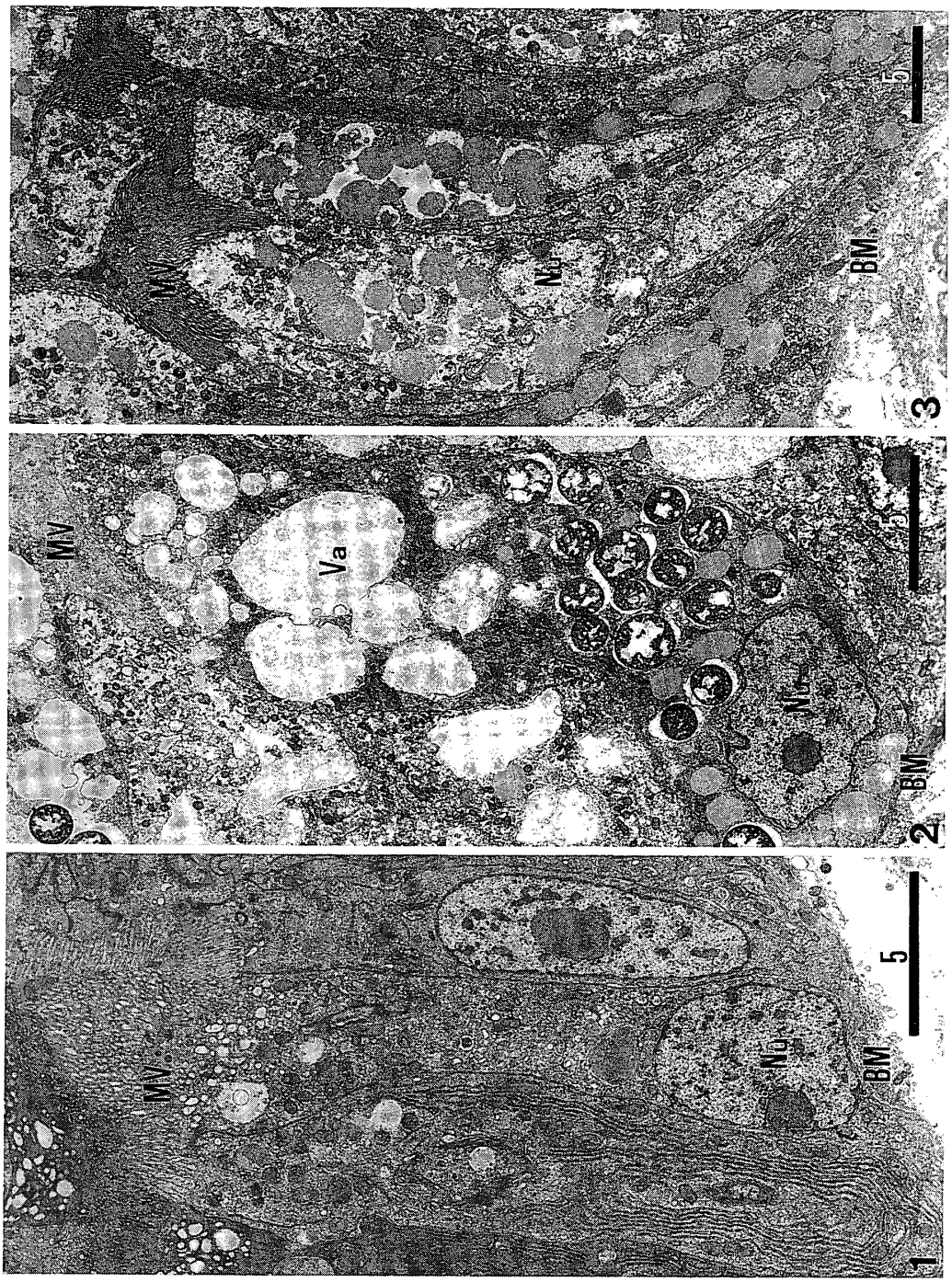

Fig. 4. Classification of epithelial cells showing the nucleus and cytoplasm in digestive diverticula tubule. 1. Type I cell. 2. Type II cell. 3. Type III cell. Nu ; nucleus, BM ; basement membrane, MV ; microvilli, Va ; vacuole. The units of each scale are microns.

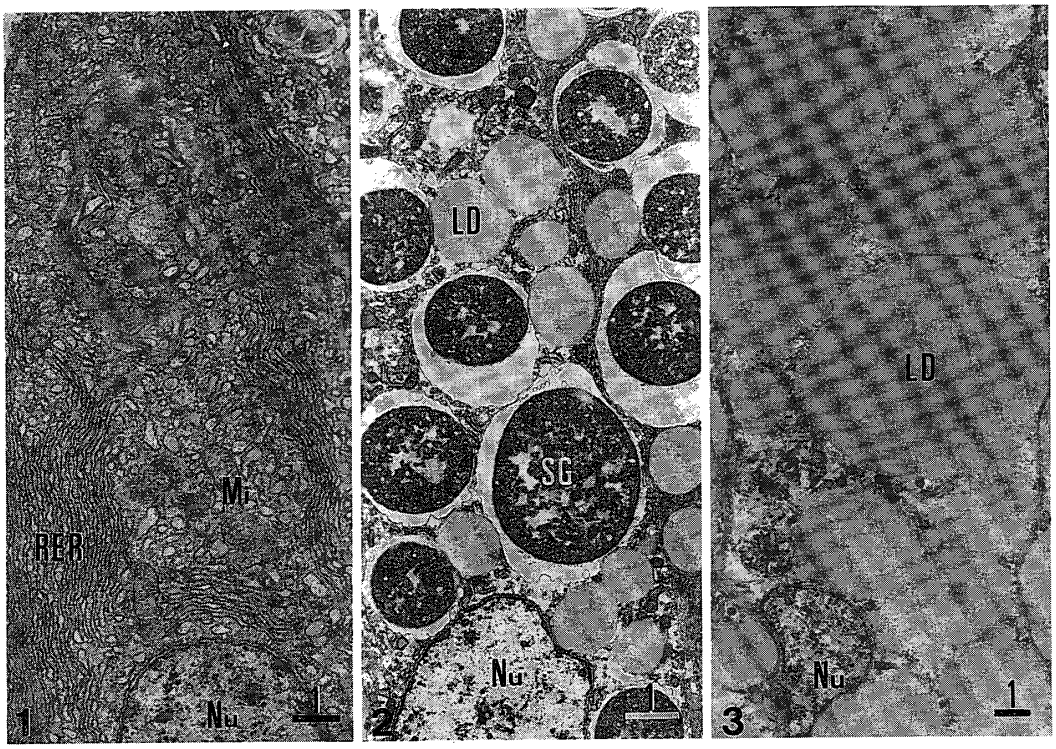

Fig. 4′. Higher magnification of the longitudinal section through the level of epithelial cell supranucleus. 1. Type I cell. 2. Type II cell. 3. Type III cell. LD ; lipid droplet, Mi ; mitochondria, Nu ; nucleus, RER ; rough-surfaced endoplasmic reticulum, SG ; special granule. The units of each scale are microns.

quantities of basophilic materials which are well stained by basic dyes such as haematoxylin and toluidine blue (Fig. 3). The round nucleus is situated near the basement membrane, and the cytoplasm contains lumps of electron-dense materials. Large quantities of rough-surfaced endoplasmic reticula concentrate in the adjacent nucleus, and mitochondria occur throughout the cytoplasm (Fig. 4-1, 4′-1). On the free surface of the cell, microvilli measuring 1.5 μm in length and 0.1 μm in diameter are well developed in large numbers, and, particularly, the presence of a long beating cilium is often observed. The cilium measures about 12 μm in length and is about 0.3 μm in diameter (Fig. 2-6 and Fig. 5).

Type II

The cell has numerous granules varied in size and in staining quality. These granules are divided into two groups, that is, one is well stained by the haematoxylin and toluidine blue, and the other, stained easily by eosin. This cell is relatively long and contains a round nucleus with a diameter from 5 μm to 6 μm located near the basement membrane (Fig. 3). The basophilic granules are 1 μm to 3 μm in diameter and are special, showing high electron-density. The eosinophilic granules vary in size, about 6.5 μm in the biggest diameter, and they are seen as various sizes of vacuole under the electron microscope. Besides these two kinds of granules, there are lipid droplets showing low electron-density in the

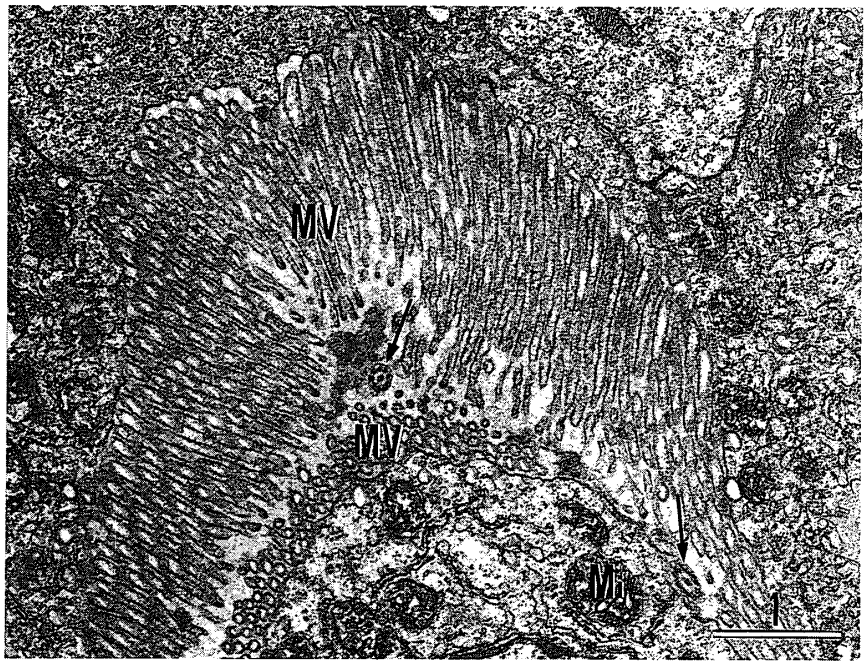

Fig. 5. Free surface of the Type I cell. Note a few cilia (arrows) cross-sectioned among numerous microvilli. Mi ; mitochondria, MV ; microvilli. The unit of scale is micron.

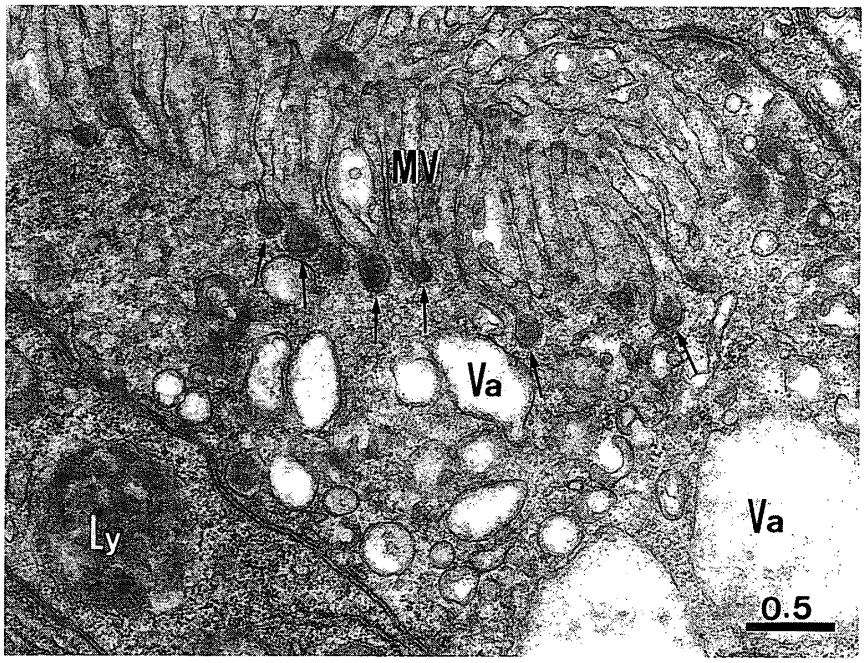

Fig. 6. Free surface of the Type II cell. Note many channels (arrows) containing electron-dense materials in the basal part of microvilli. Ly ; lysosome, MV ; microvilli, Va ; vacuole.
The unit of scale is micron.

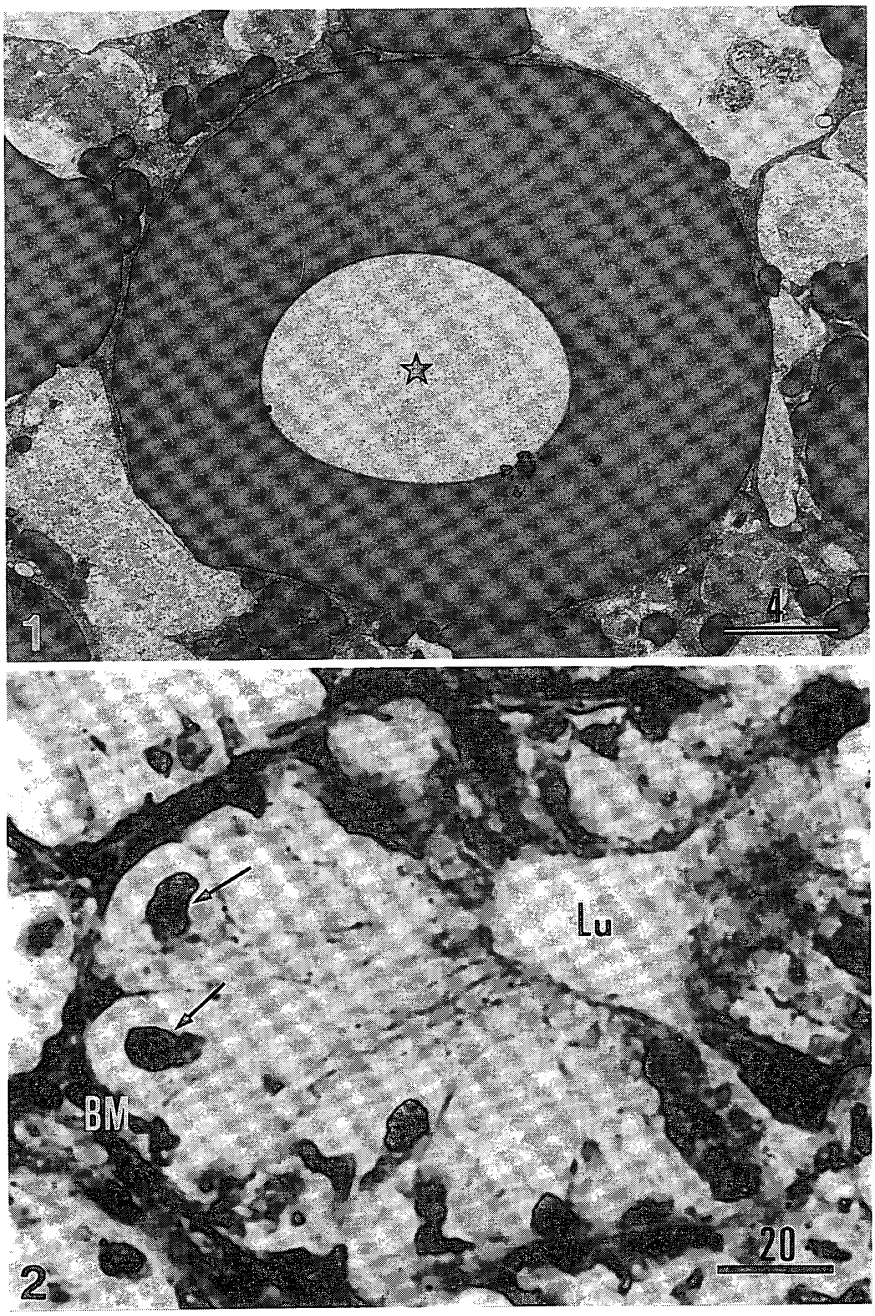

Fig. 7. 1. Cross section of a lipid droplet in Type III cell. A vacuole (asterias) is found in the central area of a lipid droplet.
2. Type III cells have vacuoles (arrows) showing positive reaction in acrolein-Schiff reagent. BM; basement membrane, Lu; lumen. The units of each scale are microns.

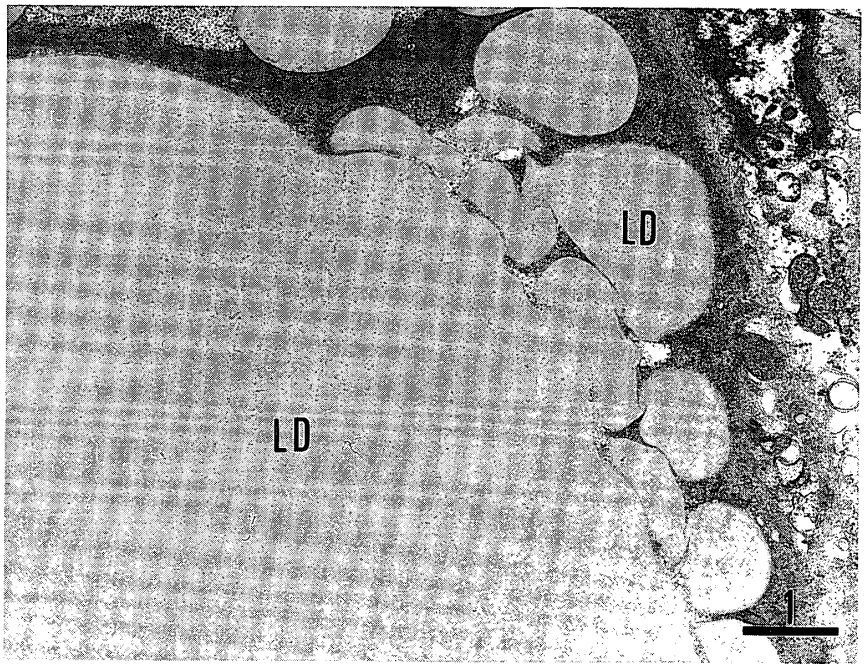

Fig. 8. Mixture of lipid droplets in Type III cell. LD ; lipid droplet. The unit of scale is micron.

cytoplasm of this cell (Fig. 4-2, 4′-2). Numerous microvilli are well developed on the free surface of the cell, and many channels measuring about 0.2 μm in diameter contain electron-dense materials, showing evidence of phagocytotic activity or microapocrine secretion. The channels are in a row at the basal part of the microvilli (Fig. 6).

Type III

The cell has the biggest cytoplasm volume among the three cell types and contains many granules weak-stained with haematoxylin and toluidine blue (Fig. 3). The granules vary in size, ranging from 1 μm to 20 μm in diameter, and are distributed in every part of the cytoplasm except for the nucleus (Fig. 4-3, 4′-3). They are revealed to be neutral lipid droplets showing the colours orange by Sudan III and pink by Nile blue in the lipid staining. By electron microscopic observations, however, a vacuole measuring about 10 μm in diameter was found in a big lipid droplet which was cut centrally and contained filiform materials (Fig. 7-1). This vacuole shows a positive reaction in acrolein-Schiff reagent and so can be identified as a protein granule (Fig. 7-2). Towards the basal part of the cell, lipid droplets above 10 μm in diameter are mixed with several small lipid droplets, ranging 1 μm to 2 μm in diameter around it, and it gradually becomes bigger (Fig. 8).

Discussion

Recently the structure and function of digestive diverticula, an organ sur-

rounding the stomach, in bivalve molluscs have been studied in several aspects and proved to be a organ different from the liver or pancreas in higher animals such as fish and mammals. Moreover the digestive diverticula are known to be an organ containing numerous, well developed blind tubules which secrete digestive enzymes, absorb digested materials and function in the intracellular digestion of food particles. The organ, however, has several different names, according to the viewpoint of investigators and the property of study materials. It is suggested that, at least in bivalve molluscs, the organ would more suitably be named one name. In this study with scallop specimens, we think that the digestive diverticula is an appropriate name for this organ, a name which was suggested for the oyster by Yonge (15), depending on the function in digestion and in the structure of blind tubules. In this study, the presence of ciliated columnar epithelial cells on the surface of the digestive diverticula duct in the scallop indicates that the small food particles from the stomach can be transported to the tubules by the cilial movement of the duct cells. The ducts resemble the main duct and the lateral duct of *Cyclina sinensis* (8) in shape, but in the size of epithelial cell all the cells are about 35 μm in length in this study, while in *C. sinensis* they vary in cell length ranging 16 μm to 73 μm.

Suzuki *et al.* (13) who studied the digestive diverticula with light microscopic observation reported that there are three kinds of epithelial cells of the tubule: basophil cells stained by basic dyes, secretory cells containing various sizes of granules, and fat cells with massive fat in its cytoplasm. The results in this study are broadly in agreement with those they described. Therefore, we can identify our Type I cell as a basophil cell and Type III cell as a lipid cell, but our Type II cell is better called digestive-secretory cell, because it shows evidence of phagocytotic activity of microapocrine secretion in this study. Basophil cells and digestive-secretory cells in this study resemble dark cells and light cells in *Crassostrea gigas* (7) and basophil cells and digestive cells in *Anodonta anatina* (11). The lipid cell, however, has never been found in any bivalve molluscs yet. Judged from the big lipid droplet mixed with many small lipid droplets around it in this cell, the lipid cell seems to be involved in the accumulation and metabolism of lipid. Considering recent report that the accumulation of shell poison was at its highest concentration in the digestive diverticula among all of the tissues and organs in the scallop (5), the shell poison, especially, if it is fat-soluble poison, can be accumulated at high concentrations in the lipid cell. Hence, we will have to clarify the relationship of the seasonal variation between the shell poison and the lipid cell.

The presence of cilium on the free surface of the basophil cell in this study is very similar to that of *C. sinensis* (8). Sumner (11) also described the presence of cells containing the cilium in *Sphaerium corneum*, but he called this cell a ciliated cell distinct from basophil cells and digestive cells. Moreover, he stated

that this type of cell has cytoplasm which stains strongly with acid fuchsin in Metzner's method, but has no mitochondria. In this study, however, because we could not find any epithelial cells which had cilium but no mitochondria, the ciliated cell can not be identified as the basophil cell found in the scallop in this study. Up to now there is no clear evidence as to what the function of the basophil cell might be. However, considering the presence of a long, beating cilium which has a role of motility, the function of this cell might be to carry materials such as food particles to the digestive-secretory cells.

Digestive cell has been found in the digestive diverticula of various species of bivalve molluscs (4, 8～10). Suzuki *et al.* (13) reported the presence of secretory cell in the scallop as opposed to digestive cell found in other bivalve species. The secretory cells secrete apocrine. The secretory cell seems to be the same cell as the digestive-secretory cell found under the light microscopic observation in this study, but our further observations by electron microscope confirm that there are not only microvilli on the free surface but also many channels containing electron-dense materials, which show evidence of phagocytotic activity or microapocrine secretion, at the basal part of the microvilli in the digestive-secretory cell. Therefore, this cell is supposed to be involved in the function of both intracellular digestion and secretion. However, we could not identify what the electron-dense materials were. As far as we know, there is no report reference to the channels of digestive cell of bivalves yet, although the channels have been found in the digestive cell of *Arion hortensis*, a species of gastropod (2).

The digestive-secretory cell of the scallop has many special granules in its cytoplasm. The many special granules containing electron-dense materials throughout the cytoplasm in the digestive-secretory cell of the scallop in this study resemble the cytoplasmic granules which are believed to be primary lysosomes showing positive reaction to acid phosphatase in the histochemical study on *Mytilus edulis* (12). Although there is no clear evidence as to whether the special granules contain the lysosomal enzymes or not, we do not deny the possibility that the granules having electron-dense materials will be primary lysosomes.

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