Classification of Diverticular Cells of the Midgut Gland in the Prawn *Penaeus japonicus*

Kaworu NAKAMURA*

Abstract

Histological observation was undertaken with the light and electron microscopes for the diverticula of the midgut gland in the prawn *P. japonicus*. Epithelial cells except for the distal portion of the diverticulum were classified morphologically into three typed cells, light, dark and vacuolated. At the end area of each diverticulum, undifferentiated cells were distributed. After 30 days of starvation, the cavity in the midgut gland developed enlarged openings of primary and secondary diverticula which suggesting a drastic change of the construction. Further, a cellular transition from the light cell to the vacuolated cell was indicated in the result of computing estimation concerning the distribution frequency of each typed cell. It was deduced that the light cell was transformed to the vacuolated cell under a critical condition such as long-term starvation.

The prawn like other decapod has an essential organ, named midgut gland or hepatopancreas, especially as the site of nutrimental digestion, absorption and storage[1-3]. Up to this time, many investigations have been treated in Reptantia species for various properties of the midgut gland[4-22]. However, there have been few reports concerning the morphology and physiology of the midgut gland in the Penaeidae prawns as well as other Natantia species[23-26]. In this report, the classification of diverticular cells was undertaken histologically for the prawn *P. japonicus*. And their transitional property was discussed with the results of rearing experiments under starvation.

Material and Methods

The prawn *Penaeus japonicus* used in the present experiments were 12-15 g. For the histological observation, the ventral portion of the midgut gland was fixed in BOUIN solution for 2 h. After dehydration with BuOH series and paraffin-sectioning, the preparations were stained with PAS-hematoxylin or hematoxylin-eosin. For the electron microscopic observation, pieces of the midgut gland were immersed in cold 5% glutaraldehyde, buffered with 0.25M *s*-collidine at pH 7.6. They were fixed for 3 h in the above solution, and following repeated rinsing in the same buffer, they were postfixed in cold 1% osmium

* Laboratory of Propagation Physiology, Faculty of Fisheries, Kagoshima University, 50-20 Shimoarata 4, Kagoshima, 890 Japan.

tetroxide buffered with the same solution for 3 h. After dehydration through an EtOH series, the samples were then embedded in Epon 812. They were stained with uranyl acetate-lead citrate after sectioning. Electron micrographs were taken by a Hitachi 300 electron microscope. For the gross anatomy of the midgut gland, the tissue fixed in BOUIN solution was divided into two parts, dorsal and ventral. The ventral portion was subjected to transcription of the arrangement and distribution of the cavity and openings of diverticula.

Rearings were conducted for each 10 individuals of feeding or starved condition during 30 days at water temperature 21-25°C. Prawn pellet was used for feeding. After the rearing, each midgut gland of individuals was prepared for the anatomical and histological examinations. The latter examination was conducted with computing estimation of the distribution frequency of each diverticular cell classified beforehand. The result was expressed as a percentage of the cell number among all cells.

Results and Discussion

Composition of Diverticular Cells

The midgut gland consists of many diverticula which diverge dendritically and possess a simple columnar epithelium. The epithelium, except a distal part of the diverticulum, is provided with three typed cells of each different cytoplasm as light, dark or vacuolated (Fig. 1). The terminal area of diverticula shows a localization of undifferentiated cells belonging to a cuboidal type (Fig. 1). They have been named as the Embryonale Zelle or E-cell[10-12, 22].

The light cell, named after its eosinophilic cytoplasm or electron microscopical character of low electron density, is superior in number to the dark cell. It possesses microvilli at the apical surface and a circular nucleus provided with a large nucleolus near the basement membrane (Fig. 2). Its mitochondria show a comparatively developed cristae extending to the internal cavity. The Golgi apparatus is limitedly distributed around the nucleus,
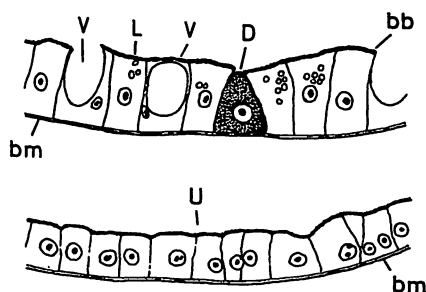

Fig. 1. Diagrammatic representations of epithelial cells of the proximal and distal diverticula, indicating arrangements of the light, dark, vacuolated and undifferentiated cells. Upper, proximal diverticulum; lower, distal diverticulum. bb, brush border; bm, basement membrane; D, dark cell;, L, light cell; U, undifferentiated cell; V, vacuolated cell.
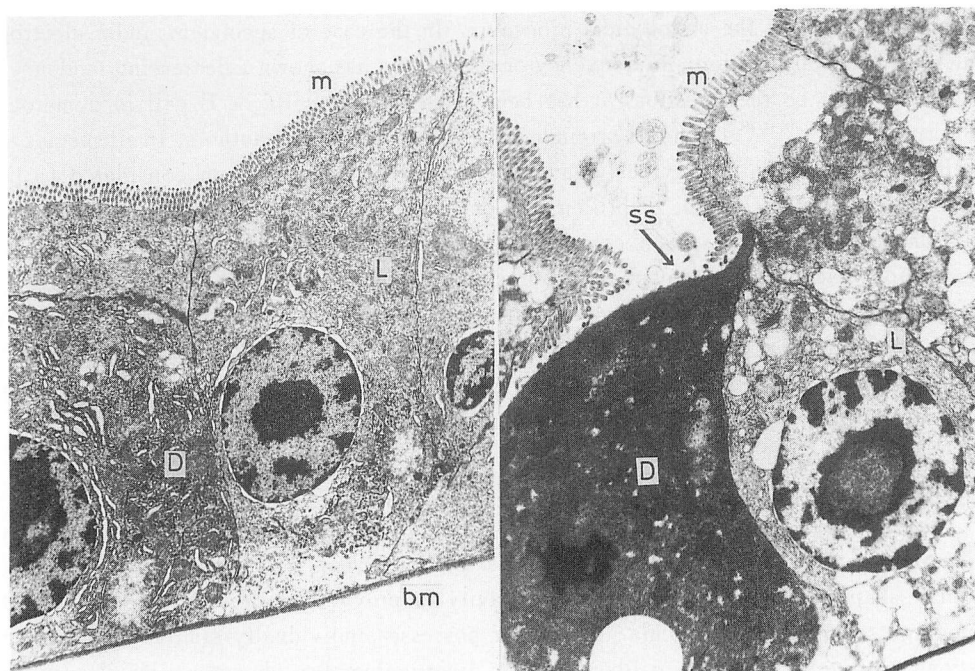
Fig. 2. Electron micrographs of the light and dark cells. The light cell has microvilli at the apical surface, and these structures are unobserved for the dark cell. The dark cell shows a secretory process like a perspiration (arrow in the right figure). bm, basement membrane; D, dark cell; L, light cell; m, microvilli; ss, secretory substance.

likewise the rough surfaced endoplasmic reticulum. The smooth surfaced endoplasmic reticulum does not show a developed condition. In the light cytoplasm, an appearance of unidentified vesicles of low electron density has been recognized with their undetermined size and number. This light cell corresponds to DALL's secretory cell in the prawn *Metapenaeus bennattae*[23] due to similar characters of the cytoplasm.

The dark cell is clearly defined as DALL's storage cell by its basophilic character[23] (Fig. 1). Its cytoplasm shows homogeneously fine and dark on account of exceedingly high electron density (Fig. 2). Shape of the cell is drop-like or triangular, provided with non-brush border (Fig. 1) or non-microvilli (Fig. 2), and shorter than the light cell. Its nucleus is clearly larger than that of the latter cell. It is a distinct feature of the dark cell that the rough surfaced endoplasmic reticulum is well developed.

Under light microscopical observation, the vacuolated cell shows a largely expanded vacuole in the light cytoplasm, possessing a nucleus suppressed more or less toward the basement membrane (Fig. 1). It is doubtful, however, whether such a character indicates a normal condition of the cell, as well as its cell number computed in the preparation. The reason is that the cell seems to be very fragile and rapidly transformed from another cell

during treatment of the histological procedure. In the case of specimens under electron microscopical observation, its frequency of appearance has shown a decreasing tendency. Whatever may be the question, it has been named Blasenzelle or B-cell in numerous descriptions[8,10,11,12,15,20,22], and its origin has been discussed among authors. In all species of Reptantia and Natantia previously investigated, the B-cell has been considered to be transformed from a F-cell, Fibrillenzelle or ferment cell, of which cytoplasm shows basophilic. The F-cell seems to correspond to the dark cell in the prawn *P. japonicus* due to its cytoplasmic property except one characteristic, that is microvilli. It is difficult to support the above mentioned theory of cellular transition, because the dark cell in the prawn possesses non-microvilli and the B-cell in the prawn is provided with such structure. Therefore, the B-cell (vacuolated cell) in the prawn would be transformed from the other cell, light cell. The latter has been named Resorptionzelle (R-cell).

Structure Change of the Cavity under Starvation

There has been given little attention to a gross anatomy of the midgut gland. In the prawn *P. japonicus*, a horizontal section of the ventral portion reveals paired cavities which being bobbin-shaped along the mid-line. The cavity is provided with a certain number of openings of primary diverticula. The latter possesses individually similar openings of secondary diverticula. The caliber of each diverticulum has shown a variable ability according to some physiological condition of the prawn. After long-term starvation as in this experiment, the size and depth of the primary and secondary diverticula have become larger and/or deeper than the control (Fig. 3). It seems that such structure changes of the cavity wall resulted from the holocrine secretion of the epithelial cells and successive reconstruction of cellular arrangements.

Computing Analysis of Cellular Transition in Diverticula

The averages of the distribution frequency of the light cell are calculated as 66.4 ± 4.7 and 31.2 ± 1.2 for the control and starvation samples, respectively. The dark cell shows the values of 16.1 ± 1.1 and 26.2 ± 2.6 for these samples, respectively. The vacuolated cell's values for these samples are 16.2 ± 3.1 and 49.0 ± 4.3, respectively (Fig. 4).

By the comparison of the distribution frequency between the control and starvation, the dark cell indicates a rather constant value than the light and vacuolated cells. For the light cell, the value of the control is reduced to about half of that of the starved sample. The vacuolated cell, reversely, shows a remarkably increased value under the starved condition, and its rate of increase corresponds to the rate of decrease of the light cell under the same starvation.

The vacuolated cell differing from the dark cell has similar properties to the light cell. That is, it is provided with a light cytoplasm and a brush border (Fig. 1). Considering these similarities of cellular characteristic and previously mentioned ability of transformation, the vacuolated cell is concluded to have its origin in the light cell.
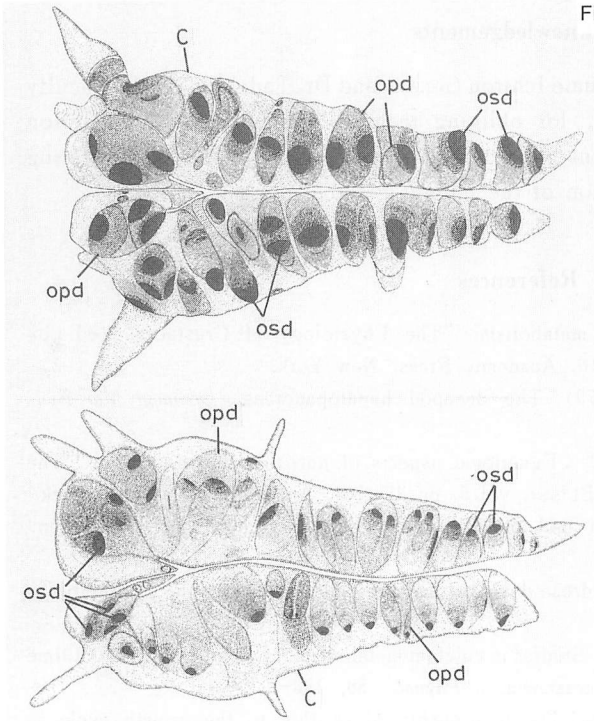
Fig. 3. Horizontally sectioned cavities of the midgut gland under different conditions, 30 days of starvation (upper) and the control (lower). The paired cavity is provided with a certain number of the openings of primary diverticula which have pores of secondary diverticula. Anterior to the left, C, cavity; opd. opening of the primary diverticulum; osd, opening of the secondary diverticulum.
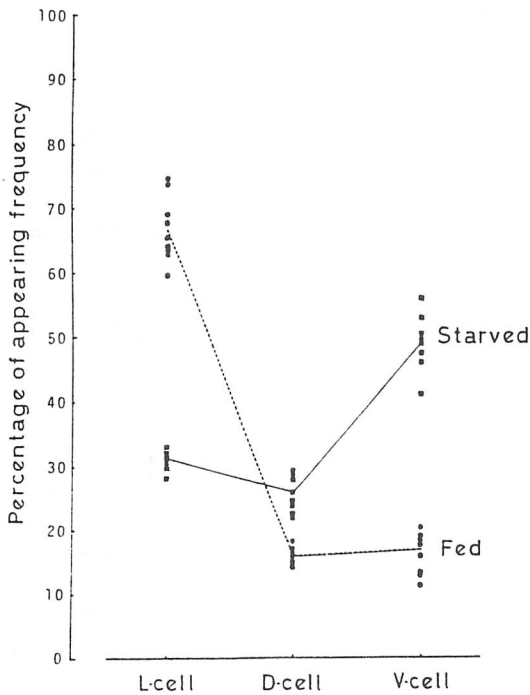

Fig. 4. Percentages of appearing frequencies of the light, dark and vacuolated cells among all cells of diverticular epithelia, under the fed and starved conditions. ●, fed; ■, starved. Each of the light and vacuolated cells (L-and V-cells, respectively) shows alternatively a remarkable change according to the different conditions. The value of the dark cell (D-cell) is rather stable as compared with those of the above cells.

Acknowledgements

References

1) VONK, H. J. (1960) : Digestion and metabolism. "The Physiology of Crustacea" (ed. by T. H. WATERMAN), vol. 1, pp. 291-316, Academic Press, New York.

2) GIBSON, R. and P. L. BARKER (1979) : The decapod hepatopancreas. *Oceanogr. Mar. Biol. Ann. Rev.*, **17**, 285-346.

3) DALL, W. and D. J. MORIARTY (1983) : Functional aspects of nutrition and digestion. "The Biology of Crustacea" (ed. by D. E. BLISS), vol. 5, pp. 215-261, Academic Press, New York.

4) WEBER, M. (1880) : Über den Bau und die Tätigkeit der sog. Leber der Crustaceen. *Arch. mikr. Anat.*, **17**, 385-457.

5) FRENZEL, J. (1893) : Die Mitteldarmdrüse des Flusskrebses und die amitotische Zelltheilung. *Arch. mikr. Anat.*, **41**, 389-451.

6) PAUL, J. H. and J. S. SHARPE (1916) : Studies in calcium metabolism. I. The deposition of lime salts in the integument of decapod crustacea. *J. Physiol.*, **50**, 183-192.

7) PAUL, J. H. and J. S. SHARPE (1919) : The relationship of lecithin to the growth cycle in crustacea. *Biochem. J.*, **13**, 487-490.

8) YONGE, C. M. (1924) : Studies on the comparative physiology of digestion. II. The mechanism of feeding, digestion, and assimilation in *Nephrops norvegicus*. *J. Exp. Biol.*, **1**, 343-389.

9) DORMAN, H. P. (1928) : The morphology and physiology of the hepatopancreas of *Cambarus virilis*. *J. Morph.*, **45**, 505-535.

10) HIRSCH, G. C. and W. JACOBS (1928) : Der Arbeitsrhythmus der Mitteldarmdrüse von *Astacus leptodactylus*. I. Teil : methodik und Technik der Beweis der Periodizität. *Z. vergl. Physiol.*, **8**, 102-144.

11) JACOBS, W. (1928) : Untersuchungen über die Cytologie der Sekretbildung in der Mitteldarmdrüse von *Astacus leptodactylus*. *Z. Zellforsch.*, **8**, 1-62.

12) HIRSCH, G. C. and W. JACOBS (1930) : Der Arbeitsrhythmus der Mitteldarmdrüse von *Astacus leptodactylus*. II. Teil : Wachstum als primärer Faktor des Rhythmus eines polyphasischen organigen Sekretionssystems. *Z. vergel. Physiol.*, **12**, 524-558.

13) RENAUD, L. (1949) : Le cycle des réserves organiques chez les crustacés décapodes. *Ann. Inst. Océanogr. Monaco*, **24**, 260-332.

14) TRAVIS, D. F. (1955) : The molting cycle of the spiny lobster, *Panulirus argus* LATREILLE. II. Pre-ecdysial histological and histochemical changes in the hepatopancreas and integumental tissue. *Biol. Bull.*, **108**, 88-112.

15) VAN WEEL, P. B. (1955) : Processes of secretion, restitution and resorption in gland of midgut of *Atya spinipes* Newport. *Physiol. Zool.*, **28**, 40-54.

16) TRAVIS, D. F. (1958) : The molting cycle of the spiny lobster, *Panulirus argus* LATREILLE. IV. Post-ecdysial histological and histochemical changes in the hepatopancreas and integumental tissue. *Biol. Bull.*, **113**, 451-479.

17) BAUCHAU, A. G. and J. C. MENGEOT (1965) : Protéases et amylases de l'hépatopancréas des crabes au cours du cycle de mue et d'intermue. *Ann. Soc. R. Belg.* , **95**, 29-37.

18) BUNT, A. H. (1968) : An ultrastructural study of the hepatopancreas of *Procambarus clarkii* (Girard) (Decapoda, Astacidea). *Crustaceana*, **15**, 282-288.

19) STANIER, J. E. , M. A. WOODHOUSE and R. L. GRIFFIN (1968) : The fine structure of the hepato- pancreas of *Carcinus maenas* (L.) (Decapoda Brachyura). *Crustaceana*, **14**, 56-66.

20) LOIZZI, R. F. (1971) : Interpretation of crayfish hepatopancreas function based on fine structural analysis of epithelial cell lines and muscle network. *Z. Zellforsch.* , **113**, 420-440.

21) HOPKIN, S. P. and J. A. NOTT (1979) : Some observations on concentrically structured, in- tracellular granules in the hepatopancreas of the shore crab *Carcinus maenas* L. *J. Mar. Biol. Assoc. U. K.* , **59**, 867-877.

22) HOPKIN, S. P. and J. A. NOTT (1980) : Studies on the digestive cycle of the shore crab *Carcinus maenas* (L.) with special reference to the B cells in the hepatopancreas. *J. Mar. Biol. Assoc. U. K.* , **60**, 891-907.

23) DALL, W. (1967) : The functional anatomy of the digestive tract of a shrimp, *Metapenaeus bennettae* RACER and DALL (Crustacea : Decapoda : Penaeidae). *Austr. J. Zool.* , **15**, 699-714.

24) NAKAMURA, K. and K. YONEKURA (1980) : Anatomy of the mid-gut gland of the prawn, *Penaeus japonicus* BATE. *Mem. Fac. Fish. Kagoshima Univ.* , **29**, 259-266.

25) DESHIMARU, O. (1976) : Studies on a purified diet for prawn-Ⅷ. Changes in free amino acid contents in muscle, hepatopancreas and blood of prawn after feeding. *Nippon Suisan Gakkaishi.* , **42**, 655-660.

26) VAN WORMHOUDT, A. , H. J. CECCALDI and Y. L. GAL (1972) : Activité des protéases et amylases chez *Penaeus kerathurus* : existence d'un rhythm circadien. *C. R. Acad. Sci.* , **277** D, 369-372.