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Author(s)	NODA, Kōichi
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Migratory Cells Traversing the Mesoglea in Hydra[1)]

By

Kôichi Noda

Zoological Institute, Hokkaido University

(With 2 Plates)

During the course of investigations on the budding process in hydra, the present writer has occasionally been encountered with the cells that are passing through the mesoglea. Morphologically, these migrating cells can be divided into two types, the first type of cells resemble interstitial cells which are seen during the regeneration to differentiate into mucous cells, and the second type are as large as digestive or epithelio-muscular cells. There has been a report on the first type of migrating cells by Burnett (1962), who demonstrated in regenerating hydra that interstitial cells adjacent to the wound increase in size and then migrate through the mesoglea to the gastrodermis. They finally transform into mucous cells. However, there have been no detailed descriptions of the second type of migrating cells.

This paper deals with the cytological study of the behavior and fate of these migrating cells of the second type in the non-budding, budding, and regenerating hydra.

Materials and Methods

The animals used in the present study were *Hydra magnipapillata*. Since the species identification is not easy for live specimens, all of the animals used in this study have their origin in a single animal obtained from the pond in the Botanical Garden of Hokkaido University in Sapporo. They were cultured by the method of Loomis and Lenhoff (1956). The temperature was maintained at 18°–20°C. In the present study only well fed animals, 24 hours after feeding, were used. In order to fix the hydra in stretched condition, they were transferred to petri dishes with a quantity of the culture solution to permit the animal to extend freely. The dishes were left undisturbed for several minutes, after which an excess of appropriate fixative was added rapidly. Fixation with acetic acid was especially suitable for this purpose. All of the sections were made perpendicular to the long axis of the hydra.

For cytological examination, the extended animals were fixed in Zenker-Helly (equal mixture of Zenker and Helly) for three hours. Serial paraffin sections 5μ thick were stained with modified Azan. In the modified Azan procedure, the section were stained with 1% acid fuchsin prior to the 5% aqueous phosphotungstic acid treatment but the

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others were the same with the usual Heidenhain Azan tennique.

For the demonstration of lipid, hydra fixed in Ciaccio solution were cut in 7μ thickness. Every other section was stained with Sudan black B or modified Azan respectively. Glycogen was demonstrated by means of the periodic acid-Schiff (PAS) reaction. For this purpose, hydra were fixed in Gendre's solution for three hours at 0° to 4°C. Paraffin sections were cut 7μ in thickness. Each cut from a long serial section was mounted on three separate slide glasses successively. The sections on the first slide was treated with Azan stain, the second slide with PAS test, and those on the third slide with PAS technique after salivary digestion.

The number of migrating cells was determined by counting nuclei. To determine their distribution in the body column the number of migrating cells in hypostome, gastric region, budding region (and bud), peduncle, and basal disk were compared.

Results

The migration occurred singly or in group of two or three cells. All of them had granules which were stained with aniline blue of Azan stain. These granules were PAS-positive even after salivary digestion (Figs. 22–23). The size of the granules was almost the same as the mucous granules which were seen in the apical portion of epithelio-muscular cells. In this paper the term, "blue granules", was given to denote these granules in order to distinguish them from the mucous granules. A part of the boundary of the migrating cells showed a strong affinity to azocarmine in Azan stained preparation. It appeared as a thick membranous structure in these preparations.

Two kinds of migrating cells were discernible. In type A cell, the nucleus and most cytoplasm were found in the epidermis and only a small portion of the cell remained in the gastrodermis. It appeared to be amoeboid and blue granules were distributed in its epidermal side. In type B cell, most of the cytoplasm and the nucleus were found in the gastrodermis. It was rather columnar in shape and had blue granules near the mesoglea (Figs. 6 and 8). Type B cell had the membranous structure only at the boundary near the mesoglea. Sometimes it had a spherical body which appeared to be liberating blue granules near the mesoglea (Figs. 5–8). In many cases, type A cells and type B cells occured in the same part of the body column contacting with each other. In some cases type A cells contained food-inclusions (lipid and probably "protein reserve droplets" of Burnett, 1959) in the gastrodermal side, whereas all the type B cells contained three kinds of food-inclusion (glycogen, lipid, and "protein reserve droplets") in the gastrodermal side (Figs. 5–8 and 20). Only one out of the total 256 migrating cells that had been observed contained food-inclusions in the epidermal side. This cell, belonging to type A, was seen in regenerating hydra bearing bud with outopushed tentacles.

The type B migrating cell had blue granules and membranous structure, but its other structural properties resembled those of definite digestive cells. However, it was ascertained that the blue granules were contained in some of the latter,

These cells contained the spherical bodies which included a number of the blue granules (Figs. 3–4). These blue granules were generally smaller than the mucous granules found in epithelio-muscular cells. The spherical bodies varied in size (12–15μ) and showed a weak affinity to acid fuchsin in Azan stain. In some cases, the spherical bodies had the granules which showed red purple in Azan stained preparations (Figs. 1–2). These red purple granules were generally smaller than blue granules.

Often, the ellipsoid or dumb-bell shaped cells were found in the longitudinal muscle layer of the epidermis near the mesoglea (Figs. 10–12). They were surrounded by a membranous structure and had several large vacuoles. The cytoplasm around the vacuoles contained blue granules. Some times, the cells of semispherical shaped cells were also found to occur in the same portion (Figs. 13–14). These cells had the membranous structure only on the surface exposed to the longitudinal muscle layer, and they also contained large vacuole and blue granules. Furthermore, oval shaped and elongated cells containing blue granules were detected in the epidermis near the longitudinal muscle layer (Figs. 15–17). The blue granules contained in these cells were as large as in size as the mucous granules in epithelio-muscular cells, and were found to distribute only in the apical portion of the cells. These cells often had large vacuoles, but contained no membranous structure on the surface.

The number and distribution of the migrating cells were investigated in five non-budding and eleven budding hydra. The migrating cells were detected in the bud and all level of the body column except the basal disk. In the peduncle, one migrating cell was found in each of three budding hydra. Sixteen migrating cells were detected in five non-budding hydra, while 212 such cells were counted in elevn budding hydra. Further, it was found that the number and distribution of the migrating cells changed during the budding process. These migrating cells were also found in regenerating hydra, although their change in the number and distribution would not be treated in this paper.

Discussion

Experimental data indicate that digestive cells make up the spherical bodies in their cytoplasm to form red purple granules which eventually develop into blue granules, and begin to mgirate from the gastrodermis to the epidermis passing through the mesoglea only after the completion of blue granules formation. It was surmised that, as the cells mgirate, spherical body was broken down liberating blue granules. Moreover, these cells contain a membranous structure on the surface of the cytoplasm just before passing through the mesoglea. In view of these facts the present writer came to the conclusion that digestive cells are the predecessors of these migrating cells. All of the migrating cells except one were devoid of food-inclusions at the epidermal side. However, whether these cells liberated or

disintegrated food-inclusions during the migration was not decided in the present study.

It seems reasonable to assume that the migrating cells transform into the ellipsoid or dumb-bell shaped cells containing blue granules inside and a membranous structure on the surface. In addition, oval and elongated cells containing blue granules and no membranous structure were noticed as an intermediate stage of the transformation of the above described ellipsoid cells into epithelio-muscular cells. Occurrence in the gastrodermis of digestive cells containing the blue granules seems to indicate that their transformation into epithelio-muscular cells takes place without accompanying high degree of dedifferentiation. This conclusion is in agreement with the results by other investigations that isolated gastrodermis of a hydra can regenerate its epidermis, mesolgea and gastrodermis (Normandin, 1960; Haynes and Burnett, 1963; Davis *et al.* 1966). According to Davis *et al.* (1966), digestive cells at the periphery of the explant differentiated directly into epithelio-muscular cells.

Burnett (1966, 1967) suggested that an "inhibitor" produced by gastrodermal cells accumulates in the gastrovascular cavity so that digestive cells are prevented from undergoing differentiation into epidermal cells. If his hypothesis is correct, could the migrating cells observed in the present study individually escape from the inhibitor's effect and how ? Experiments are now being undertaken in our laboratory to clarify these problems.

Diehl and Burnett (1965a, b) stressed that digestive cells have been found not to differentiate into other cell type in intact and regenerating hydra. In the present study, however, it was found that migrating cells were present in non-budding, budding, and regenerating hydra, and that their number and distribution were changeable during the budding process. Therefore, it is conceivable that there is a relation between migration of digestive cells and the budding process, although no evidence is avaible at present whether the region containing many migrating cells is highly active during the budding process or not.

Detailed studies on the number and distribution of migrating cells during the budding and regneration are now undertaken and will be described elsewhere.

Summary

Migrating cells passing through the mesoglea were found to take place in non-budding, budding, and regenerating hydra. These cells are as large as digestive or epithelio-muscular cells and had granules which were stained with aniline blue of the modified Azan. They often contained food-inclusions within their cytoplasm at the gastrodermal side, and their cell boundary stained well with azocarmine of the Azan stain.

Except for the basal disk, the migrating cells were detected in the bud and all level of the body column of hydra. However, a signifficant difference in the

number of migrating cells was found between the non-budding and the budding individuals.

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Explanation of Plates VIII - IX

Plate VIII

Figs. 1–4. Micrographs showing the digestive cells with spherical bodies (s) which contain red-purple granules (Figs. 1–2) or blue granules (Figs. 3–4). A large vacuole is frequently seen within these bodies. G, gastrodermis; m, mesoglea. Modified Azan stain. × 600.

Figs. 5–6. The successive serial sections showing the migrating cells of type A (A) and type B (B). In type B cell passing through the mesoglea (m), the spherical body (s) is seen to liberate blue granules (b) in the epidermal side of the cell. Note a part of the boundary of the cells showing a strong affinity to azocarmine. Modified Azan stain. × 600.

Figs. 7–9. The successive serial sections showing the migrating cells of type A (Figs. 7–8) and type B (Fig. 9). The type B cells have membranous structure only at the boundary near the mesoglea. One of them has the spherical body (s) liberating blue granules (b) near the mesoglea (m). Type A cell (A) shown in figure 9 has blue granules within its whole cytoplasm in the epidermal side. Modified Azan stain. × 600.

Figs. 10–11. Two consecutive serial sections showing a dumb-bell shaped cell. The boundary of the cell shows a strong affinity to azocarmine. Blue granules are distributed throughout the cytoplasm surrounding the vacuoles. Modified Azan stain. × 600.

Plate IX

Fig. 12. Photograph showing the ellipsoid shaped cell. The boundary of the cell appeared as a thick membranous structure which stained strongly with azocarmine. Blue granules are distributed throughout the cytoplasm surrounding the vacuoles. G, gastrodermis; m, mesoglea. Modified Azan stain. × 600.

Figs. 13–14. Two consecutive serial sections showing semispherical cell. This cell has the membranous structure only on the surface exposed to the longitudinal muscle layer. Blue granules are seen in the cell. Modified Azan stain. × 600.

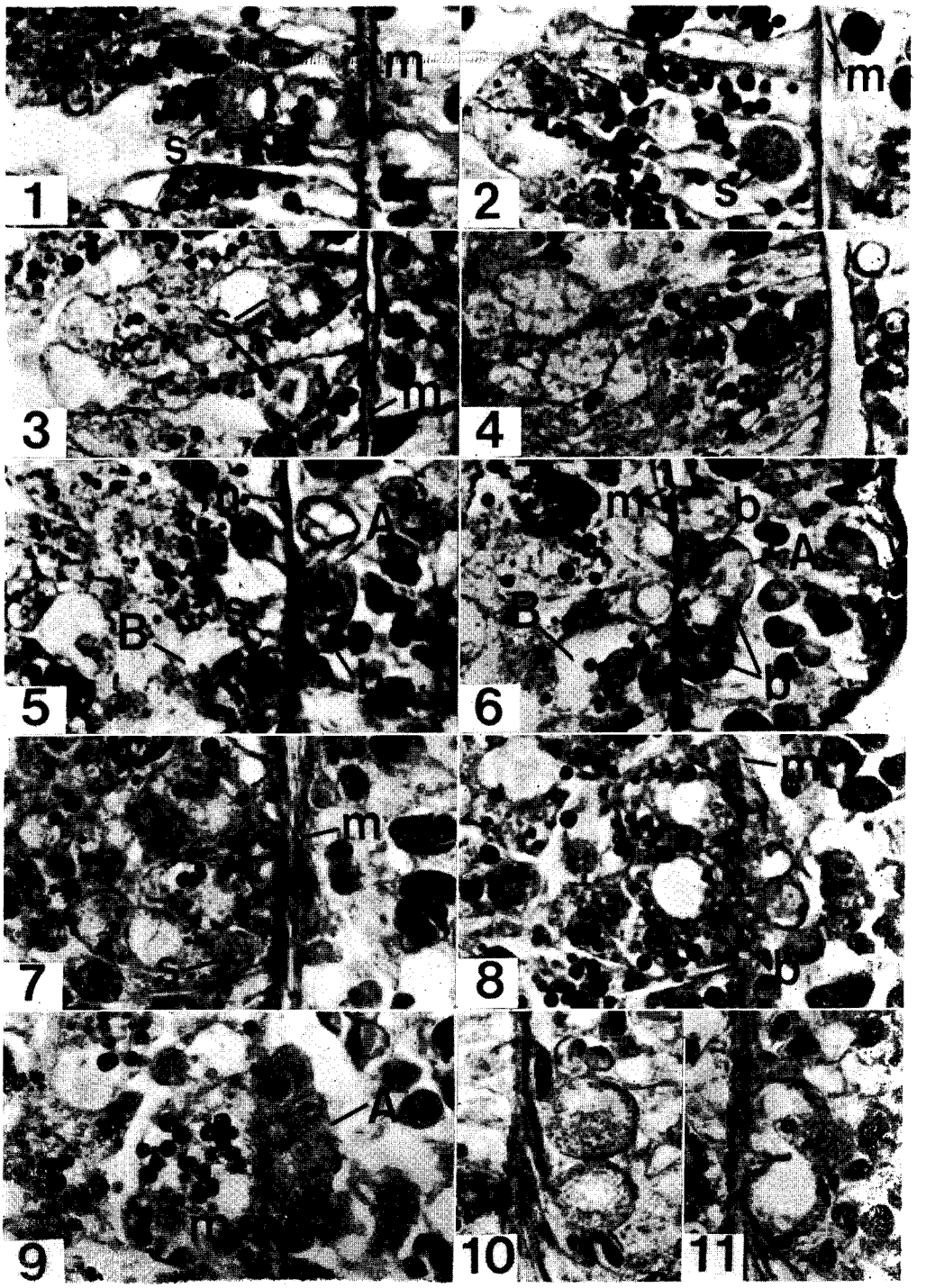
Figs. 15–17. Micrographs showing an oval cell in serial sections (Figs. 15–16) and an elongated cell (Fig. 17). They have no membranous structure. Blue granules are distributed in the apical portion of the cells. Modified Azan stain. × 600.

Figs. 18–19. Two consecutive serial sections from a Ciaccio-fixed animal stained with modified Azan (Fig. 18) and Sudan black B (Fig. 19). These sections show the digestive cell with spherical body (s) which contains red-purple granules. Many lipid droplets are contain throughout the cell except within the spherical body. × 600.

Fig 20 Photograph showing the type A cell (arrow) stained with Sudan black B. The cell has no lipid droplets in the epidermal side, but some lipid dropletes are seen in the cytoplasm remaining at the gastrodermal side. × 1000.

Fig. 21. Section stained by the periodic acid-Schiff (PAS) procedure after salivary digestion showing the type B cell. The cell has spherical body (s) containing PAS-positive granules. A small part of the cytoplasm is seen at the epidermal side (arrow). × 1000.

Figs. 22–23. Sections showing the appearance of the digestive cell (D), the type A cell (A) and type B (B) cell stained by the periodic acid-Schiff (PAS) procedure after salivary digestion. The PAS- positive granules are seen in these cells. G, gastrodermis; m, mesoglea. × 1000.



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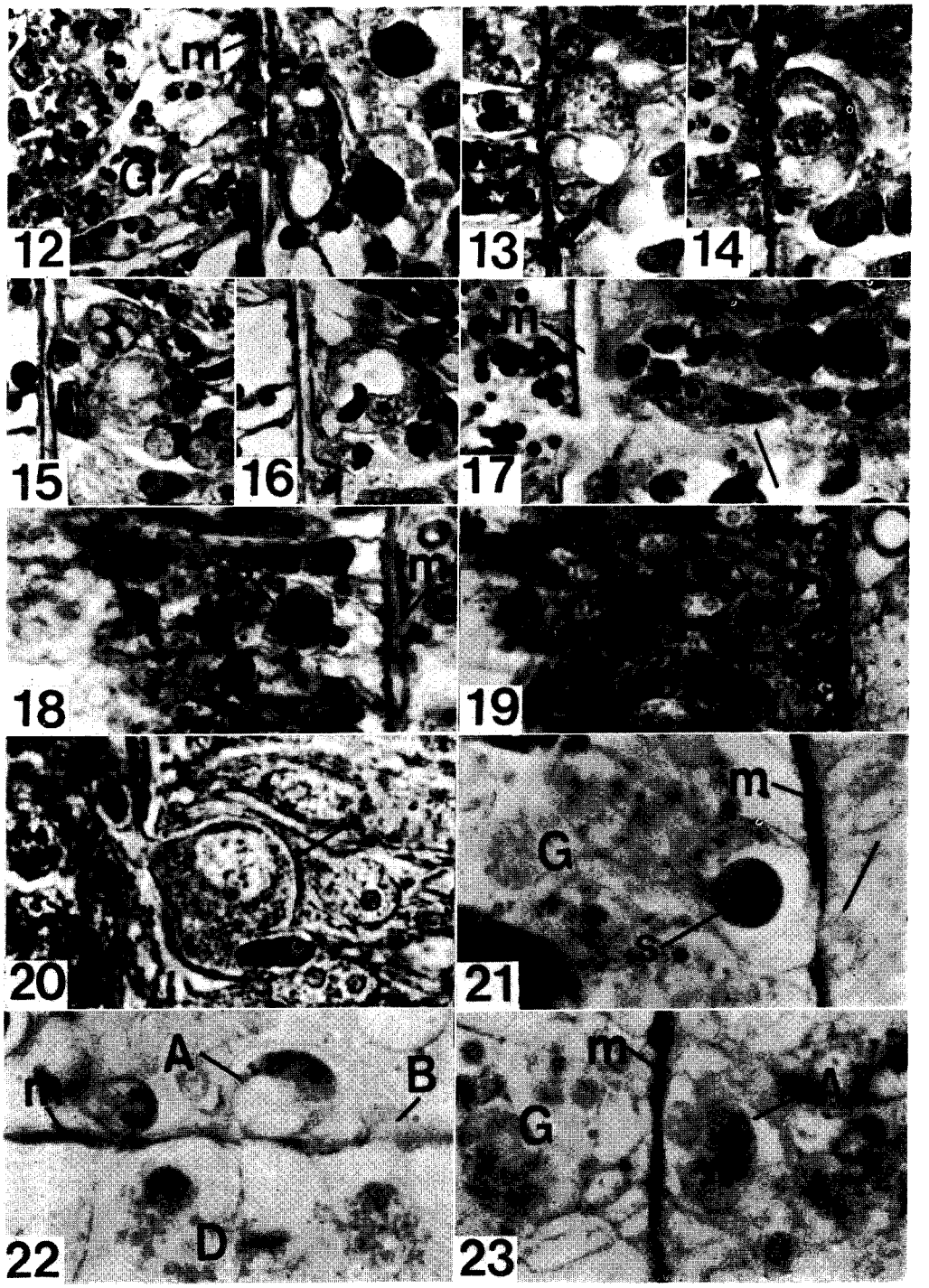

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