On the Structure and Function of the Alimentary Canal of *Tigriopus japonicus* (Copepoda; Harpacticoida)—II Cellular Renewal in the Mid-gut Epithelium

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(Received February 16, 1980)

The cellular dynamics and related morphology in the mid-gut epithelium of *Tigriopus japonicus* were studied. Colchicine administration at concentrations above 2 $\mu\text{g/ml}$ resulted in a marked decrease in number of the epithelial cells, dysfunction in the faecal pellet production, and death of the test animals. At a lowered temperature, the survival time of colchicine-treated animals was markedly prolonged. The estimated turnover time of the glandular and absorptive cells in the metasome at 20°C was 9.6 to 10.2 days and 13.6 to 15.7 days respectively. Electron microscopic examination revealed that the epithelial cells arise from undifferentiated cells scattered on the basal lamina of the epithelium, grow in size and abundance of cellular components as maturation progresses, become smaller with age on account of condensation involving the nucleus and cytoplasm, and finally liberate themselves from the epithelium.

Based on the histological observation, the previous study of this series¹⁾ suggested that *Tigriopus japonicus* has the cellular renewal system in the mid-gut epithelium, but could adduce no experimental evidence. Structural observations on the alimentary canal of copepods have been carried out in various species, but we have yet little information concerning the cellular dynamics in the mid-gut epithelium except for some suggestive speculations. Taking the above situation into account, some attempts were made to elucidate the cellular dynamics in the mid-gut epithelium of *Tigriopus japonicus*.

Material and Methods

All the specimens used, the adult females of *Tigriopus japonicus*, were obtained from a laboratory culture.

The effect of colchicine on the survival and faecal pellet production was first examined. Groups of 11 or 12 animals were reared in 90 ml of colchicine-seawater in 100 ml glass beakers at concentrations of 0, 1, 2, 5, 10, and 100 $\mu\text{g/ml}$, and fed on dried *Chlorella* powder (*Chlorella* Industry Co., Ltd.). The test media were daily renewed by transferring the live animals with the least possible amount of the media into the new media, and the dead animals and faecal pellets remaining in the used media were fixed with formalin and counted under a

disection microscope. Temperature was maintained at 20°C during the experiment.

The temperature effect on the survival of colchicine-treated animals was examined next. At 15 and 25°C, groups of 18 animals were reared in colchicine-seawater at a concentration of 2 $\mu\text{g/ml}$. The mode of rearing was the same as described above.

The effect of colchicine on tissues was examined by light microscopy using intact and colchicine-treated animals. Colchicine concentrations of 2 and 5 $\mu\text{g/ml}$ were adopted, and the mode of rearing was the same as in the first experiment. The test animals were fixed *in toto* in HELLY's fluid, dehydrated in ethanol, and embedded in paraffin. Sagittal sections were cut at a thickness of 5 μm and stained with MAYER's acid hemalum and eosin.

The turnover time of the mid-gut epithelium was determined using the method of STEVENS and LEBLOND²⁾, but simply replacing the rate of renewal estimated from the mitotic counts in the method by the rate of cell loss, on the assumption that the mid-gut epithelium is in a steady state and the rate of cell loss during colchicine treatment is constant. The rate of cell loss was estimated in the glandular epithelium (in the anterior diverticulum) and the absorptive one in the metasome at two different times during the treatment.

For electron microscopic examination, intact animals were fixed in cold MILLONIG's fixative or

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1% osmium tetroxide-seawater, dehydrated in ethanol, and embedded in Epon. Thin sections were cut using a SORVALL MT-1 ultramicrotome, stained with lead citrate and uranyl acetate, and examined with a JEOL T6S electron microscope.

Results

Colchicine administration at concentrations above 2 $\mu\text{g}/\text{ml}$ resulted in the dysfunction in the faecal pellet production and death of the test animals (Fig. 1). To the naked eye, the animals treated at 100 μg colchicine/ml became less active soon after the beginning of the treatment, but those treated at 10 and 5 μg colchicine/ml and 2 μg

Fig. 1. *Tigriopus japonicus*. Survival rate and faecal pellet (FP) production during colchicine treatment at 20°C. Colchicine concentration ($\mu\text{g}/\text{ml}$): A 100, B 10, C 5, D 2, E 1, and F 0.

Fig. 2. *Tigriopus japonicus*. Survival rate during colchicine treatment (2 $\mu\text{g}/\text{ml}$) at 15 and 25°C.

colchicine/ml were vigorous for the first 2 and 3 days respectively.

At the concentration of 2 $\mu\text{g}/\text{ml}$, the animals treated with colchicine at 15°C survived at least several days longer than those treated at 25°C (Fig. 2). The survival rate curve at 25°C was very similar to that at 20°C (Fig. 1), though it slightly shifted to left.

Histological examination revealed that colchicine administration at a reduced concentration (2 $\mu\text{g}/\text{ml}$) resulted in the marked decrease in number of the mid-gut epithelial cells without causing appreciable changes to the other tissues (Figs. 3–5) and that the remaining epithelial cells were much exhausted (Fig. 5). In moribund animals resulting from the colchicine treatment, the proliferation of short rod-shaped bacteria and tissue destruction (Fig. 6) were always found in the mid-gut containing stagnant food and adjacent tissues. These findings suggest that the dysfunction in the mid-gut due to the cell loss is the principal cause of the death of colchicine-treated animals. The bacterial proliferation seems to hasten the death of the test animals.

Table 1. *Tigriopus japonicus*. The rate of cell loss in the mid-gut epithelium during colchicine treatment (2 $\mu\text{g}/\text{ml}$) at 20°C and the turnover time of the epithelium (mean $\pm$ SE)

Type of epithelium	Duration of treatment (h)	Rate of cell loss (%)	Turnover time (h)
Glandular epithelium	78 (6)* ¹	33.85 $\pm$ 5.76	230.4 $\pm$ 39.2* ²
Absorptive epithelium	120 (4)	48.92 $\pm$ 6.00	245.3 $\pm$ 30.1* ²
Glandular epithelium	78 (9)	20.74 $\pm$ 6.41	376.1 $\pm$ 116.2
Absorptive epithelium	120 (4)	36.67 $\pm$ 2.72	327.2 $\pm$ 24.3

*¹ Figure in parentheses is the number of copepods.

*² Significant compared with the absorptive epithelium ($p < 0.05$).

Table 1 gives the rate of cell loss in the mid-gut epithelium estimated at two different times during the colchicine treatment and turnover time of the epithelium calculated from the rate. It is readily seen that the absorptive cells survive a few days longer than the glandular ones. In both the glandular and absorptive epithelia, the difference in the turnover times estimated at two different times was not significant.

Electron microscopic examination casted light on the detailed morphology relating to the cellular renewal in the mid-gut epithelium.

The primitive epithelial cells and undifferentiated cells, though difficult to distinguish in some instances, are scattered on the basal lamina of the

epithelium (Fig. 7). They are small in size and scanty of membranous cytoplasmic organelles except mitochondria. Most of the ribosomes are free in the cytoplasm. Relatively a large nucleus with a prominent nucleolus occupies the central position of the cells.

As maturation progresses, the primitive epithelial cells become larger, grow rich in cellular components, and face on the gut lumen (Figs. 7 and 8).

After the maturation, the epithelial cells reduce their size and become slender with age on account of condensation involving the nucleus and cytoplasm. In an earlier stage of condensation, the contrasts among the cellular components become somewhat indistinct, and accumulated lipid droplets also indicate this stage (Fig. 9). In the glandular epithelium, the sign of condensation is first shown in the strongly vacuolated cells and becomes obvious in the slenderized cells after the discharge of secretory product (Fig. 10). In a later stage of condensation, the epithelial cells considerably reduce their size, so that cellular components except lipid droplets and microvilli are hardly distinguishable (Fig. 11). Finally, the condensed epithelial cells protrude their outer surface membrane into the gut lumen, discharge the cellular components (Fig. 12), and liberate themselves from the epithelium.

Discussion

The results obtained, particularly the loss of the mid-gut epithelial cells caused by colchicine treatment at probably a constant rate and the presence of undifferentiated cells, evidently show the cellular renewal in the mid-gut epithelium of *Tigriopus japonicus*.

In this study, the turnover time of the mid-gut epithelium, which also represents the average time taken for the renewal of the individual cells, was calculated from the rate of cell loss. For the accurate estimation of the turnover time, the mitosis in the tissues must be completely arrested by the colchicine treatment, without causing serious damages to the test animals. It is accordingly desirable to use colchicine at the lowest effective concentration, and, judging from the survival rate curves shown in Fig. 1, 5 μg colchicine/ml seems too high for *Tigriopus japonicus*. But the concentration of 2 $\mu\text{g}/\text{ml}$ may not necessarily be sufficient to arrest mitosis completely. If so, the actual turnover of the mid-gut epithelium requires a little less time than that estimated in this study.

The survival rate curves shown in Fig. 2 indicate that the turnover time of the mid-gut epithelium is prolonged with lowering temperature, though there is no denying the possibility that temperature affects the effects of colchicine.

The mid-gut of *Tigriopus japonicus* is wide and sack-like in the metasome and lined by a single layer of glandular and absorptive epithelia¹⁾, which directly face the gut lumen where the digestion of food probably takes place. In this region of the mid-gut, accordingly, the epithelial cells exist probably in almost identical surroundings, both chemically and mechanically. It is accordingly conceivable that the difference in turnover time between the two types of epithelia is due mainly to the functional difference between the two.

Based on light microscopic observation, the previous study¹⁾ suggested that the glandular cells are merocrine in nature and reconstitute cyclically. The present study adduced a conclusive evidence to the former but a conflicting one to the latter. The fully matured glandular cells discharge their secretory product without involving appreciable loss of part of the apical cytoplasm. In this sense they are certainly merocrine in nature. On the other hand, the glandular cells are strongly vacuolated in the course of maturation and much exhausted, and probably die after a single secretory activity, even though they exist for some time after the discharge of the secretory product. In this sense they are holocrine-like in nature. It is noteworthy in connection with this characteristic mode of secretion that the glandular cells may be somewhat undifferentiated secretory cells, as suggested by the fact that they are similar to the absorptive cells in some aspects of their structure and function, e.g., the well developed microvilli, the formation of peritrophic membrane, and the senile degenerative change in aged cells.

Tigriopus japonicus is frequently encountered in large numbers in tide pools where environmental factors often and extremely fluctuate³⁾, and generally thought to be omnivorous in feeding habit. KOGA⁴⁾ assumed that a period of adult stage is 1 to 2 months, and HANAOKA⁵⁾ observed in the laboratory rearing an instance of the survival for 53 days during which 14 broods were produced. It is evident that the presence of the cellular renewal system in the mid-gut epithelium is advantageous to the tissue repair in the epithelium when injured and is one of the physiological factors effecting the prolonged adult life in this species. In view of this, it will be inferred that the cellular renewal

system in the mid-gut epithelium is in many ways related to various aspects of the mode of life of *Tigriopus japonicus*.

by a grant from the Ministry of Education.

Acknowledgement

I wish to express my sincere gratitude to Professor Yoshio Kô, Faculty of Fisheries, Nagasaki University and Emeritus Professor Takashi HIRIYA, the University of Tokyo, who is also a Professor, College of Agriculture and Veterinary Medicine, Nihon University, for their constant interest and encouragement. This study was partly supported

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Explanation of Plates

Figs. 3-6. *Tigriopus japonicus*. Anterior region of the mid-gut. $\times 365$

Fig. 3. Intact animal.

Fig. 4. Colchicine-treated animal (2 $\mu\text{g/ml}$, 78 h).

Fig. 5. Colchicine-treated animal (2 $\mu\text{g/ml}$, 120 h).

Fig. 6. Colchicine-treated animal (5 $\mu\text{g/ml}$, 72 h). This animal was dying at the fixation. Note the stagnant gut contents, bacterial proliferation, and tissue destruction.

Figs. 7-12. *Tigriopus japonicus*. Electron micrographs of the mid-gut epithelial cells.

Fig. 7. Undifferentiated or primitive epithelial cells (pc) on the basal lamina (bl) of the epithelium. m mitochondria, n nucleus, nu nucleolus. $\times 10,600$

Fig. 8. Mature absorptive cells. er rough-surfaced endoplasmic reticulum, mv microvilli. $\times 10,600$

Fig. 9. Absorptive cells in an earlier stage of condensation. ld lipid droplets. $\times 8,500$

Fig. 10. Glandular cells in varying stages of condensation. sv secretory vacuoles. $\times 6,800$

Fig. 11. Strongly condensed absorptive cell. ad adjacent ordinary absorptive cells. $\times 8,500$

Fig. 12. Extrusion of cellular contents in the strongly condensed absorptive cell. $\times 8,500$

Figs. 7-9 and 11: Millonig's fixation.

Figs. 10 and 12: Osmium tetroxide-seawater fixation.

Plate I.

Plate II.