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Ultrastructural alterations on chloride cells of the yellowtail *Seriola quinqueradiata*, following exposure to the red tide species *Chattonella antiqua*

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Abstract

By means of scanning and transmission electron microscopy, the influence of the red tide species *Chattonella antiqua* was examined with respect to the surface ultrastructures of chloride cells of the yellowtail *Seriola quinqueradiata*. Conspicuous ultrastructural alterations occurred on the apical surface of these cells. The majority of chloride cells in the control gills showed an apical surface with numerous cellular extensions, while more than half the chloride cells affected by red tide organisms exhibited an apical surface with fewer and smaller extensions, a wrinkled apical surface, or a protruded apical surface. These ultrastructural alterations of chloride cell surface may be due to the partial disturbance of salinity by *C. antiqua*, and reflect the changes of the ion-transport function in yellowtail gills exposed to red tide water.

Introduction

In recent years, red tide has often appeared in the Seto Inland Sea, Japan, during the summer and caused great damage to the cultured yellowtail *Seriola quinqueradiata* (Yanagida, 1980). *Chattonella antiqua* and *C. marina* have been identified as the causative organisms of this red tide. The effects of these organisms on the fish have been investigated to some extent (Kobayashi, 1978; Seki *et al.*, 1974; Brown *et al.*, 1979; Yanagida, 1980). Although this research has confirmed oxygen deficiency as the cause of death, the exact mechanisms have remained unsolved. In previous studies, we have investigated the influences of *Gymnodinium* sp. and *C. antiqua* on the primary lamellae of gills of young yellowtails from the morphological and histochemical aspects (Doi *et al.*, 1981; Shimada *et al.*, 1982, 1983). Our results indicated a disappearance of the mucous coat on the gill and the partial destruction of mucous goblet cells located on the afferent ridges. Further, edema formation (the expansion of the intercellular spaces) occurred in the primary and secondary lamellae of gills exposed to red tide organisms. On the basis of these findings, we speculate that the edematous gill lamellae may arise from the partial impairment of osmoregulation, which in turn induces fish death due to oxygen deficiency (Shimada *et al.*, 1983).

In teleosts, the primary epithelium that surrounds the primary lamellae is mainly composed of several kinds of highly specialized cells: pavement cells which form the major surface of the gill, mucous goblet cells, chloride cells and non-differentiated supportive cells (Hoar and Randall, 1970; Berridge and Oschman, 1972; Potts, 1977; Dunel-Erb and Laurent, 1980; Hootman and Philpott, 1980; Karnaky, 1980; Laurent and Dunel, 1980). Among them, chloride cells are presumed to be responsible for NaCl transport in teleosts and believed to play a major role in osmotic regulation (Maetz, 1971; Motais and Garcia Romeu, 1972; Maetz and Bornancin, 1975; Dunel-Erb and Laurent, 1980; Hossler, 1980; Karnaky, 1980; Laurent and Dunel, 1980). As our previous studies have been concentrated on the mucous goblet cells, little attention has been paid to the chloride cells. Using scanning and transmission electron microscopic (SEM and TEM) techniques, we therefore investigated the influences of the red tide species *Chattonella antiqua* on the gill lamellae of young yellowtails, with special reference to the morphology of chloride cells located in the basal part of the interlamellar spaces of the primary lamellae.

Materials and methods

Fish and red tide species. The yellowtails, *Seriola quinqueradiata* (body weight 1080–2620 g), were obtained from the Nippon Saibai Center, Takamatsu, Kagawa Prefecture. The red tide organisms, *Chattonella antiqua*, were provided through the Red Tide Research Laboratory, Takamatsu, Kagawa Prefecture. The conditions of seawater containing *C. antiqua* with densities from 890 to

3 140 cells ml^{-1} were as follows: water temperature, 22° to 25 °C; salinity, 31.1 to 31.8‰ S; pH, 7.8 to 8.2; dissolved oxygen, 4.67 to 5.23 ml l^{-1}. Those of the control seawater were: temperature, 22° to 25 °C; salinity, 31.8‰ S; pH, 7.95 to 8.16; dissolved oxygen, 4.95 to 5.11 ml l^{-1}.

Exposure to Chattonella antiqua. 200 l of seawater containing *C. antiqua* were poured into 500-l tanks, and then the fish were introduced. The tanks were aerated throughout the experiments. The fish died within 25 to 90 min. Immediately after death, dissected gill filaments were prepared for electron microscopic observations. Control gill filaments were obtained from fish kept in natural seawater.

Electron microscopic preparations. For SEM studies, the gill filaments were fixed for a minimum of 12 h with 2% paraformaldehyde and 2.5% glutaraldehyde on 0.1 *M* cacodylate-HCl buffer (pH=7.4), and this was followed by immersion in 2% tannic acid for a period of 6 h. After several washes with the buffer for at least 12 h, the specimens were postfixed for 3 h in 2% osmium tetroxide, dehydrated through a graded ethanol series and dried with an Hitachi HCP-2 critical point dryer using iso-amyl-acetate and liquid CO_2, and then glued on stubs covered with silver paint. Chloride cells in the gill filaments are predominantly located in the interlamellar regions of primary lamellae. In order to observe the surface of the chloride cells, the secondary lamellae of the gills were removed by tweezers and Freon spray. The specimens prepared were coated with a thin layer of Pt-Pb, and examined with an Hitachi S-550 scanning electron microscope.

For TEM studies, small pieces of the gill filaments were fixed for more than 12 h with 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 *M* cacodylate-HCl buffer (pH=7.4), postfixed for 2 h with 1% osmium tetroxide in the same buffer. The specimens were dehydrated through a graded ethanol series and embedded with Epon resin. Ultrathin sections cut by a Richert-Jung OMU4 microtome were stained with uranylacetate and lead nitrate, and examined with a JEOL 200-CX transmission electron microscope at 100 kv.

Cell count. Chloride cells are located mainly in the interlamellar spaces and along the afferent ridge. However, a large number of mucous cells also exist on the afferent ridge and it is difficult to distinguish between chloride and mucous cells in this region by their surface morphology. Therefore, we only counted chloride cells located in the interlamellar spaces. The number of fish examined was 20 for control and 14 for exposure experiments. The surface morphology of 200 to 700 chloride cells per individual was examined in order to identify the influence of red tide organisms.

Results

Morphology of chloride cells of control gill filaments

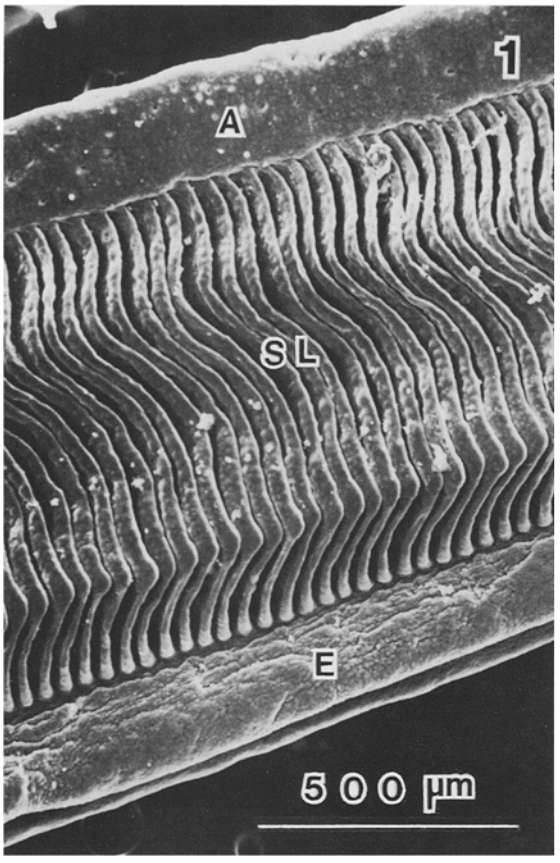
Scanning views of gill filaments obtained from yellowtails in the control seawater were described briefly in a previous paper (Doi *et al.*, 1981). At low magnification, secondary lamellae appeared to be regularly spaced on primary lamellae and the width between secondary lamellae was approximately identical, i.e. 25 μm (Fig. 1). The SEM observations showed that chloride cells with numerous cellular extensions were abundant in the interlamellar regions, either scattered or in clusters among pavement cells forming the outer layer of the interlamellar epithelium (Fig. 3). However, the surface ultrastructures of pavement cells in these regions were different from those of pavement cells on the afferent ridge (Doi *et al.*, 1981). Pavement cells on the afferent ridge showed a surface with microplicae of fingerprint-like patterns (Fig. 2), while those in the interlamellar regions revealed a rather flattish surface appearance with very short and small projections (Fig. 3).

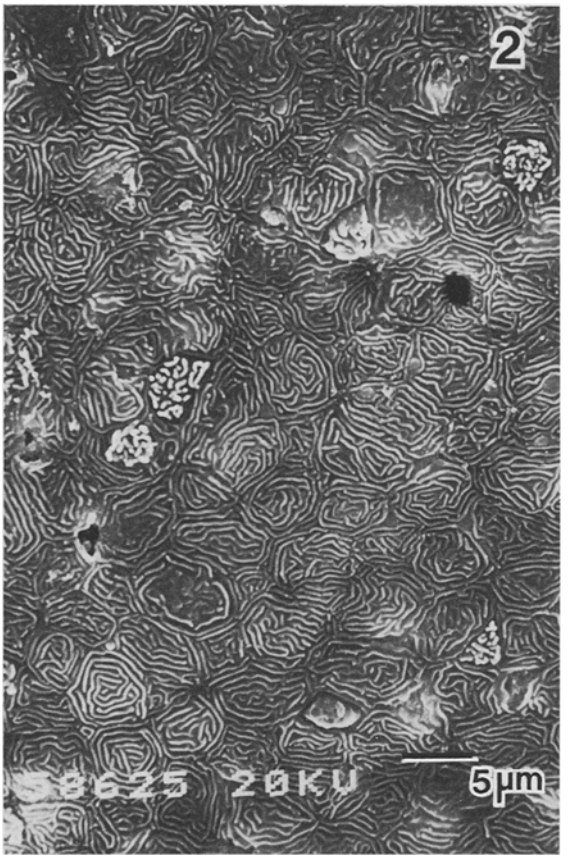
In the thin sections of the interlamellar epithelium, the ultrastructural characteristics of chloride cells were clearly observed. These cells contained a large number of mitochondria which were in close association with a densely branched tubular system opened on the basolateral plasma membrane (Fig. 7 a). The apex of each chloride cell with its numerous cellular extensions was firmly bound to the neighbouring pavement cells by a long and tight junctional apparatus. The mitochondria and tubular reticulum were separated from the apical membrane by an underlying zone particularly rich in microfilaments. A terminal web was anchored by parietal desmosomes. Vesicles of various sizes converged in the apical region (Laurent and Dunel, 1980).

The SEM observations at high magnification revealed that the dimension and number of cellular extensions on the apical surface were rather uniform among the individual chloride cells located in the interlamellar epithelium of control gill filaments (Fig. 7 b). They were about 0.3 to 0.6 μm in diameter and 50 to 200 in number per cell in the majority of chloride cells (Table 1). However, about 4% of chloride cells exhibited smaller (0.2 to 0.4 μm in diameter) and fewer (20 to 100 in number) cellular extensions.

Morphological changes of chloride cells following exposure to artificial *Chattonella antiqua* red tide

Low magnification SEM of gill filaments obtained from the fish, which had died within 25 to 90 min after exposure to *Chattonella antiqua,* showed that the arrangement of secondary lamellae had been disturbed. In the semi-thin section, a severe edema formation, i.e. an expansion of intercellular spaces, was observed both in the secondary





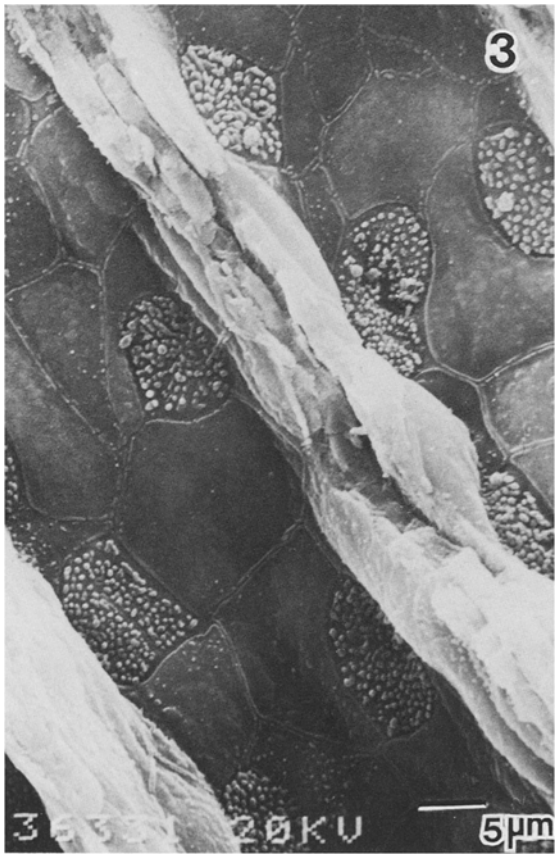


Fig. 1. Scanning electron micrograph of normal gill filament showing the regular arrangement of secondary lamellae at intervals of about 25 μm. The afferent (A) and efferent (E) sides are distinguishable from the shape of the secondary lamellae

Fig. 2. Scanning electron micrograph of afferent ridge of control gill filament. Pavement cells on this ridge showed a flattish apical surface with microplicae of fingerprint-like patterns. It is nearly impossible to distinguish chloride cells from mucous cells in this region by their surface structures

Fig. 3. Interlamellar epithelium of control gill filament. Note uniform cellular extensions on chloride cells. Pavement cells showed a relatively flattish surface appearance with very short and small projections. Mucous cells are few, if any, in this region

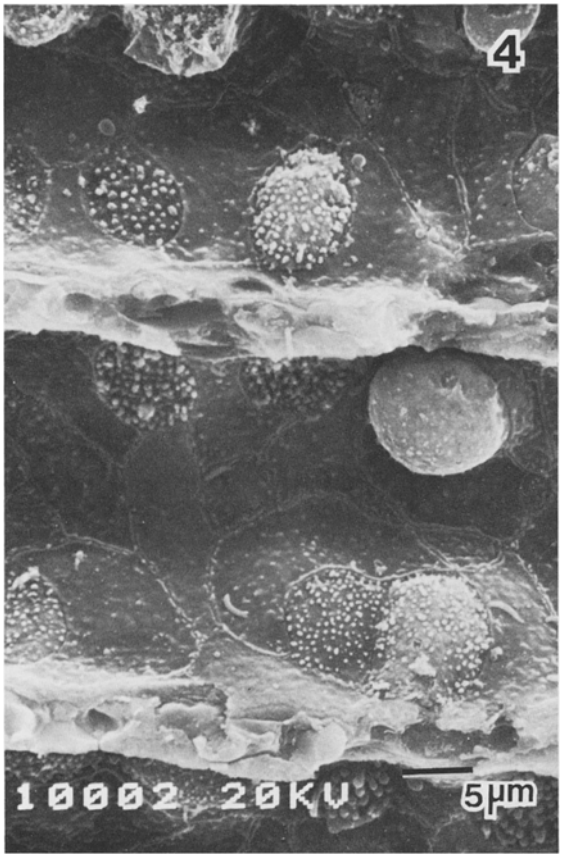


Fig. 4. Interlamellar epithelium of gill filament exposed to artificial *Chattonella antiqua* red tide. It is clear that the chloride cells exhibit an apical surface with a few short cellular extensions or a protruded apical surface with less prominent cellular extensions, instead of uniform cellular extensions

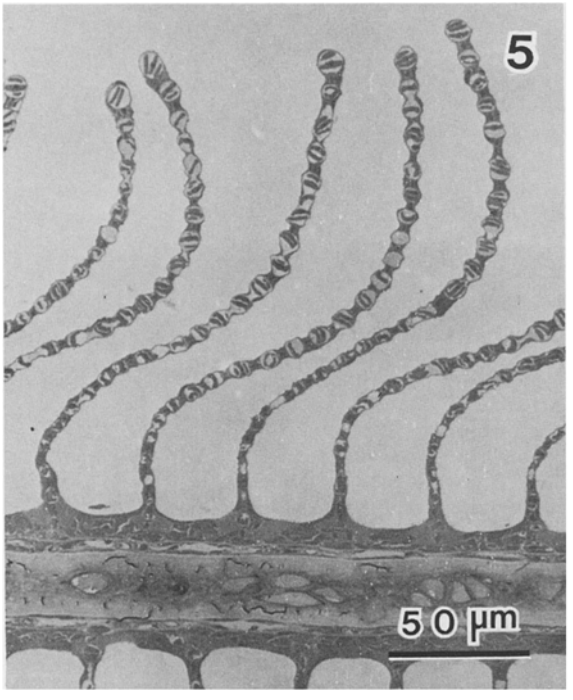
epithelium and the interlamellar epithelium of the primary lamellae (Figs. 5 and 6).

Scanning views of the interlamellar epithelium revealed that the conspicuous ultrastructural alterations occurred on the apical membrane of chloride cells following exposure to *Chattonella antiqua* (Fig. 4). Although the features of these alterations were greatly variable among the individual interlamellar regions, the percentage of the occurrence of chloride cells with numerous long cellular extensions, which predominated in control gill filaments, remarkably decreased in the gill filaments of fish exposed to *C. antiqua* (Table 1). Chloride cells with fewer and smaller cellular extensions, which were only occasionally observed in control gill filaments, markedly increased following exposure to *C. antiqua* (Table 1 and Fig. 8). About 26.5% of the chloride cells in the interlamellar

Table 1. Comparative occurrence of chloride cells with various surface ultrastructures in control and affected gill filaments

Surface morphology	Control	Affected
Apical surface with numerous long extensions	94.8%	31.9%
Apical surface with a few short extensions	3.8%	41.5%
Wrinkled apical surface	1.4%	15.1%
Protruded apical surface	0%	11.5%

The number of the chloride cells counted was 200 to 700 per fish for control ($n=20$) and exposed ($n=14$) specimens



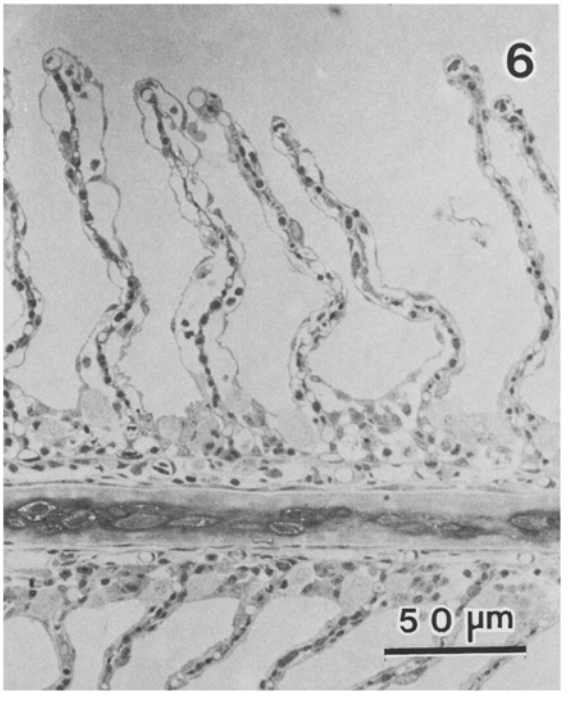


Fig. 5. Semithin section of control gill filament. The capillary system and chloride cells located at the basement of secondary lamellae are visible

Fig. 6. Semithin section of gill filament affected by *Chattonella antiqua.* Note edema formation in the secondary lamellae and interlamellar regions of primary lamellae

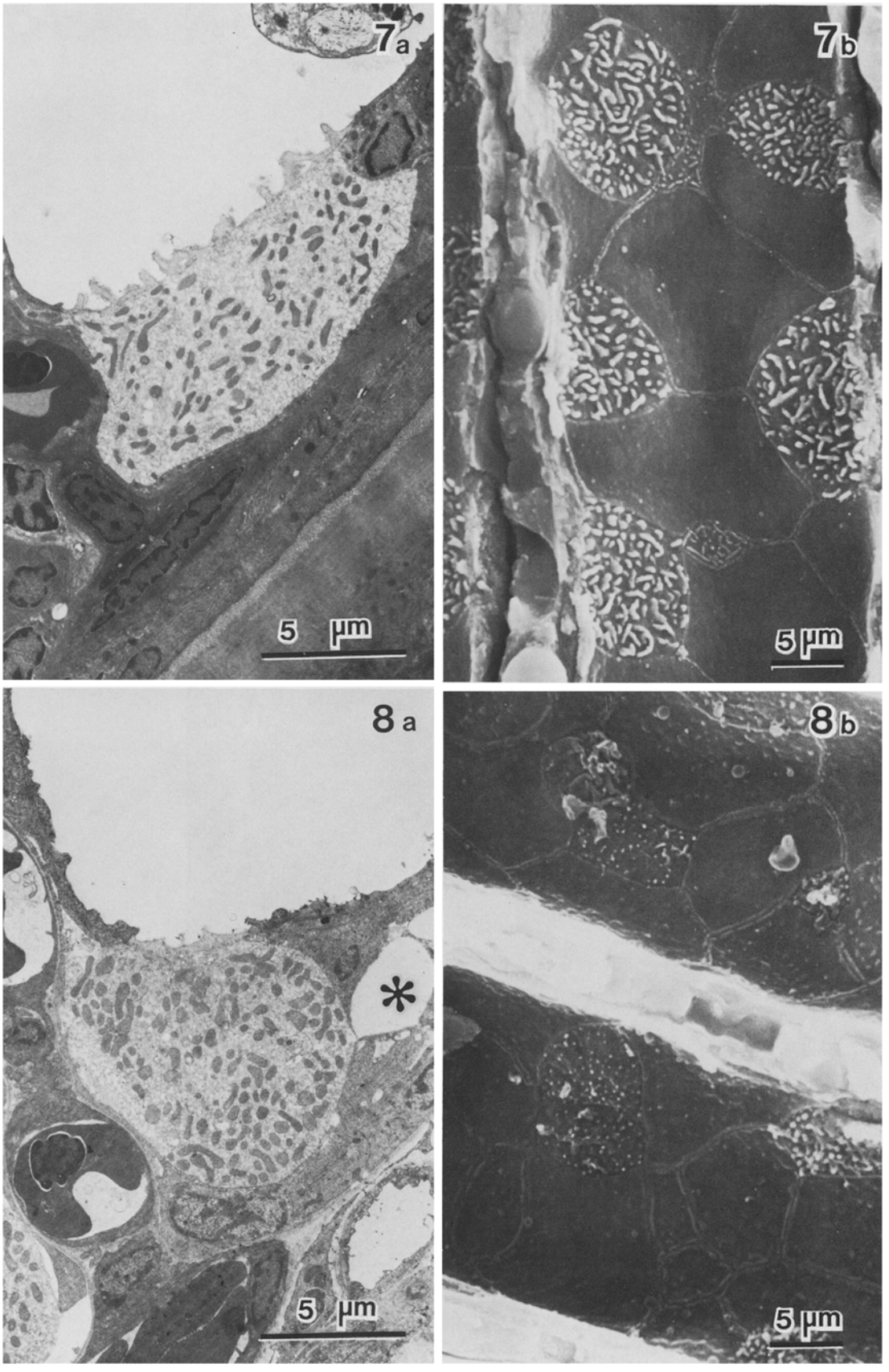


Fig. 7. Transmission (a) and scanning (b) electron micrographs of chloride cells with uniform cellular extensions; about 0.3 to 0.6 μm in diameter and 50 to 200 in number per cell. The interior of these cells is filled with many mitochondria and branched tubular systems. Note underlying zone which separates mitochondria and tubular systems from the apical surface

Fig. 8. Transmission (a) and scanning (b) electron micrographs of chloride cells with a few short cellular extensions. These cells predominated in gill filaments exposed to *Chattonella antiqua.* Note edema (*) formation around the chloride cells

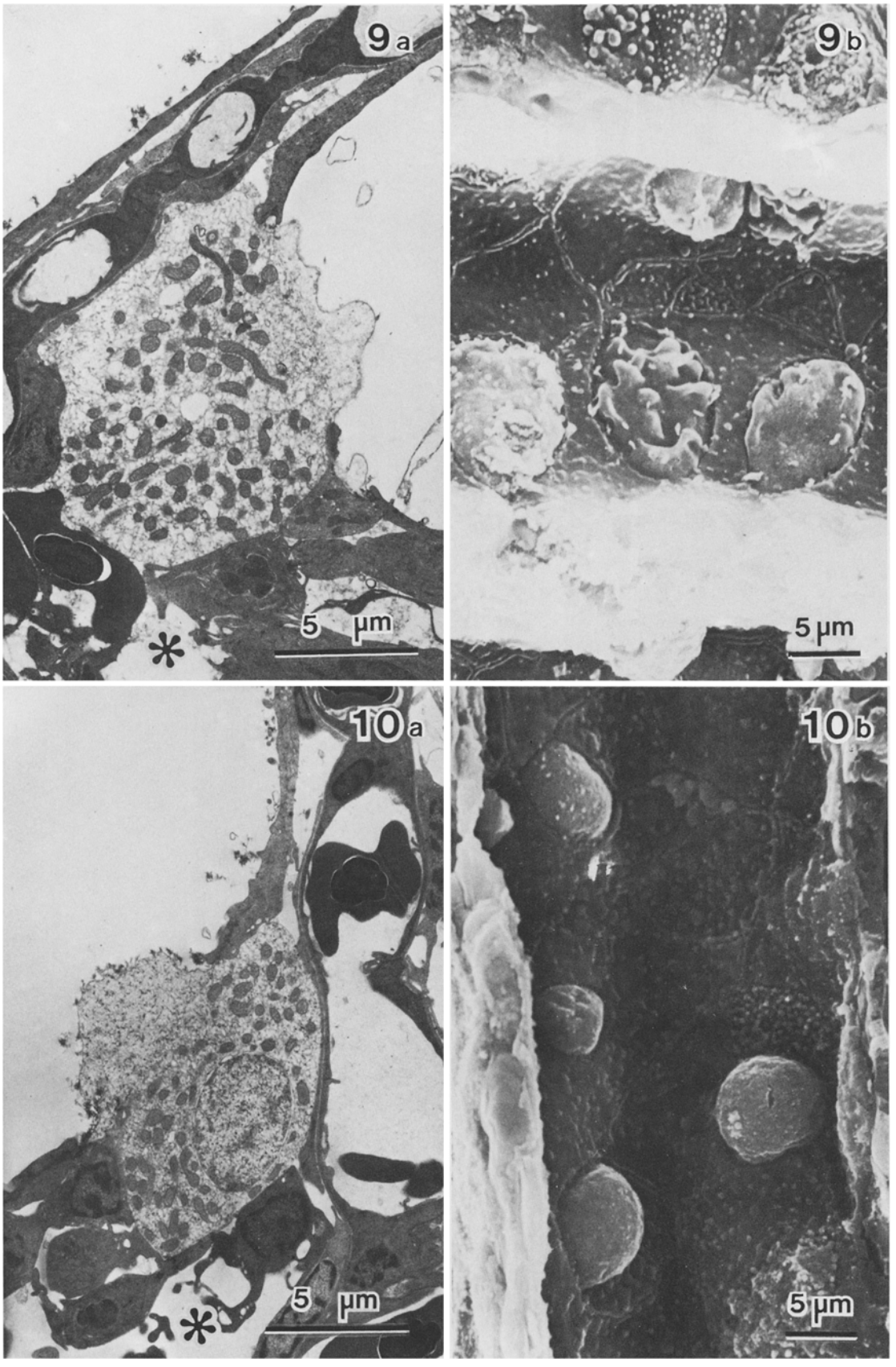


Fig. 9. Transmission (a) and scanning (b) electron micrographs of a wrinkled apical surface on chloride cells exposed to *Chattonella antiqua*. Note the expansion of the underlying zone which separates mitochondria and tubular systems from the apical surface. The occurrence of edema (*) can be seen

Fig. 10. Transmission (a) and scanning (b) electron micrographs of a protruded apical surface on chloride cells exposed to *Chattonella antiqua*. A protruded apical surface was endowed with less prominent cellular extensions. Note the marked expansion of the underlying zone separating mitochondria and tubular systems from the apical surface. The occurrence of edema (*) can be seen

regions of the gill filaments exhibited a protruded apical surface with less prominent cellular extensions or a wrinkled apical surface with a few microplicae, both of which rarely occurred in normal gill filaments (Table 1 and Figs. 9 and 10).

These ultrastructural alterations of apical membrane of the chloride cells following exposure to *Chattonella antiqua* were then confirmed by TEM (Figs. 8 a, 9 a and 10 a). The micrographs of the TEM preparations show that the microfilament-rich region underlying an apical membrane was expanded in the chloride cells with a wrinkled apical surface or with a protruded apical surface (Figs. 9 a and 10 a).

Discussion and conclusions

The present work is presumably the first detailed report concerning the influences of the red tid species *Chattonella antiqua* on the morphology of chloride cells located in the interlamellar regions of the gills. Scanning and transmission electron microscopical observations revealed that conspicuous ultrastructural alterations occurred on the apical surface of the chloride cells following exposure to *C. antiqua*. The majority of chloride cells in normal gills exhibited an apical surface with numerous long cellular extensions, while more than half those affected by *C. antiqua* showed a flattish surface with a few short cellular extensions, a wrinkled surface with plicae, or a protruded surface with less prominent extensions.

Recently, Hossler (1980) reported that the ultrastructural modifications, including the number of cellular extensions from the apical membrane of chloride cells, occur during the adaptation to severe salinity changes. In the present study, the salinity change of the seawater containing cells of *Chattonella antiqua* was very small and the salinity was kept within the acceptable range for yellowtails. However, since the occurrence of the chloride cells with altered surface ultrastructures was highly variable among the individual interlamellar spaces, it may be possible that *C. antiqua* produces the local changes of salinity.

Chloride cells are now generally known to engage in active ion transport and are favored as a primary site of osmotic regulation at gills (Maetz, 1971; Motais and Garcia Romeu, 1972; Maetz and Bornancin, 1975; Sardet *et al.*, 1979; Epstein *et al.*, 1980). Since histochemical and biochemical analyses for the ion-transporting enzyme, Na-K-ATPase, were not carried out in the present study, an ion-transport function in the chloride cells with surface ultrastructures altered by red tide organisms can hardly be discussed. However, it has recently been reported that ultrastructural modifications of chloride cells including the number of the cellular extensions very intimately reflect the changes in Na-K-ATPase content of gill filaments (Hossler, 1980). Therefore, our results may indicate that the conspicuous ultrastructural alterations in the apical surface of chloride cells represent changes in the ion-transport function in gill filaments exposed to red tide. Although it has not yet been confirmed, changes in the ion-transporting function in gill filaments exposed to *Chattonella antiqua* seem to some extent to be involved with edema formation, which in turn is responsible for inhibiting gas exchange in the gills. Oxygen deficiency will then cause the death of fish (Shimada *et al.*, 1982, 1983).

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Literature cited

Berridge, M. J. and J. L. Oschman: Transporting Epithelia, pp 32–75. New York: Academic Press 1972

Brown, P. C., L. Hutching and D. Horstman: A red-water outbreak and associated fish mortality at Gordons Bay near Cape Town. Fish. Bull. S. Afr. *11*, 46–52 (1979)

Doi, A., O. Hatase, M. Shimada, T. H. Murakami and T. Okaichi: Ultrastructural changes in gill epithelia of a yellowtail, *Seriola quinqueradiata*, exposed to sea bloom. Cell Str. Func. *6*, 375–383 (1981)

Dunel-Erb, S. and P. Laurent: Ultrastructures of marine teleost gill epithelia, SEM and TEM study of chloride cell apical membrane. J. Morphol. *165*, 175–186 (1980)

Epstein, F. H., P. Silva and G. Kormanic: Role of Na-K-ATPase in chloride cell function. Am. J. Physiol. *7*, 246–250 (1980)

Hoar, W. S. and D. J. Randall: Fish physiology, pp 253–292. New York: Academic Press, 1970

Hootman, S. R. and G. W. Philpott: Accessory cells in teleost branchial epithelium. Am. J. Physiol. *7*, 199–206 (1980)

Hossler, F. E.: Gill arch of the mullet, *Mugi cephalus* III. Rate of response to salinity change. Am. J. Physiol. *7*, 160–170 (1980)

Karnaky, K. J. Jr.: Ion-secreting epithelia; chloride cells in the head region of *Fundulus heteroclitus*. Am. J. Physiol. *7*, 185–198 (1980)

Kobayashi, H.: Sakana no Kokyu to Junkan. *In:* Respiration and circulation in fish, pp 111–124. Ed. by Japan Soc. Sci. Fish., Suisangaku series *24* Tokyo: Koseisha-Koseikaku 1978 (in Japanese)

Laurent, P., and S. Dunel: Morphology of gill epithelia in fish. Am. J. Physiol. *7*, 149–159 (1980)

Maetz, J.: Fish gills: mechanisms of salt transfer in fresh water and sea water. Phil. Trans. R. Soc. Lond. B*262*, 209–249 (1971)

Maetz, J. and M. Bornanchin: Biochemical and biophysical aspects of salt excretion by chloride cells in teleosts. Forsch. Zool. *23*, 323–363 (1975)

Motais, R. and F. Garcia Romeu.: Transport mechanisms in the teleostean gill and amphibian skin. A. Rev. Physiol. *34*, 141–176 (1972)

Potts, W. T. W.: Fish gills. *In:* Transport of ion and water in animals, pp 453–480. Ed. by B. L. Gupta, R. B. Moreton, J. L. Oschman and B. J. Wall. New York: Academic Press 1977

Sardet, C., M. Pisam and J. Maetz: The surface epithelium of teleostean fish gills. Cellular and junctional adaptations of the chloride cell in relation to salt adaptation. J. Cell Biol. *80*, 96–117 (1979)

Seki, H., T. Tsuji and A. Hattori: Effect of zooplankton grazing on the formation of the anoxic layer in Tokyo Bay. Estuar. cstl mar. Sci. *2*, 145 (1974)

Shimada, M., T. H. Murakami, A. Doi, S. Abe, T. Okaichi and M. Watanabe: A morphological and histochemical study on gill primary lamellae of the teleost, *Seriola quinqueradiata,* exposed to sea bloom. Acta Histochem. Cytochem. *15,* 497–507 (1982)

Shimada, M., T. H. Murakami, T. Imahayashi, H. S. Ozaki, T. Toyoshima and T. Okaichi: Effects of sea bloom, *Chattonella antiqua,* on gill primary lamellae of the young yellowtail, *Seriola quinqueradiata,* Acta Histochem. Cytochem. *16,* 232–244 (1983)

Yanagida, T.: The red tide, pp 50–175 Tokyo: Kodansha Scientific 1980 (in Japanese)

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