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A Light and Electron Microscopic Study on Epitheliocystis Disease in Cultured Fishes

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Epitheliocystis disease was found in red sea bream *Pagrus major*, transported from Hong-Kong, and tiger puffer *Takifugu rubripes* and carp *Cyprinus carpio* cultured in Japan. Histopathological and electron microscopic studies were made on these diseased fishes. Epitheliocystis cysts of these fishes developed in gills. Cysts of red sea bream were found on the capillary under respiratory epithelium of gill lamellae in the early stage of the infection. Heavily infected gills showed many cysts among the hyperplastic epithelium of the interlamellar spaces, followed by clubbing of the gill filaments. The cysts ranged in diameter from 15 μm to 55 μm. The cysts had a cellular capsule and an inclusion body which was revealed to be packed with chlamydia-like organisms ranging from 0.52 μm to 0.71 μm in size, by electron microscopy. Epitheliocystis cysts of tiger puffer developed on the capillary under the respiratory epithelium of gill lamellae. The cysts ranged in size from 25 μm to 43 μm. Epitheliocystis cysts of red sea bream and tiger puffer indicated that they developed from infected cells forming lamellar capillaries. Epitheliocystis cysts of carp developed within the epithelium of the interlamellar spaces on gill filaments and ranged in diameter from 12 μm to 50 μm.

Epitheliocystis disease has been found in the following freshwater fishes: bluegill *Lepomis macrochirus*,[1] tilapia *Tilapia mossambica* and *T. nilotica*,[2] channel catfish *Ictalurus punctatus*[3] and carp *Cyprinus carpio*,[4] and various marine fishes such as Connecticut striped bass *Morone saxatilis*,[5,6] white perch *M. americanus*,[5] gilt head bream *Sparus aurata*,[7–9] grey mullet *Liza ramada*[7,8] and sea bass *Disentrarchus labraz*[9] in the United States and European countries. This disease was characterized by development of epitheliocystis cysts containing chlamydia and rickettsia like organisms in the skin and gills.

In 1984 and 1985, epitheliocystis disease was found among juveniles of red sea bream *Pugrus major* which were imported from Hong-Kong. This disease was also found among cultured tiger puffer *Takifugu rubripes* in 1983 and carp *Cyprinus carpio* in 1984. These fishes were registered as host fish of epitheliocystis organism. This report describes histopathological characteristics of the affected gills of these fishes and ultrastructure of epitheliocystis cysts of red sea bream.

Materials and Methods

Seventeen juveniles of red sea bream *Pugrus major* (3–5 cm body length) were collected from a population suffering from epitheliocystis disease. These fish were imported from Hong-Kong and reared within a net cage in the small bays of Mie Prefecture for about one week in June of 1984 and 1985. Four 1-year old tiger puffer *Takifugu rubripes* (15–17 cm body length) were found to have epitheliocystis disease among fish reared in the bay of Kagoshima Prefecture in the autumn of 1983. Five 1-year old carp *Cyprinus carpio* (9–12 cm body length) were taken from a population showing slow movement which were cultured in freshwater ponds in Mie Prefecture in the autumn of 1984.

Gills and other visceral organs were fixed in 15% formalin, processed routinely, embedded in paraffin wax, and 3–5 μm sections were satined with Mayer's hematoxylin and eosin (H–E), Giemsa, periodic acid-Schiff (PAS), Goodpasture and Macchiavello for light microscopy. Gills were taken from formalin-fixed specimens, post-fixed in osmium tetroxide, and embedded in epoxy

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resin. Thin sections were stained with uranyl acetate.

Results

Gross Signs

Moribund red sea bream suffering from epitheliocystis disease floated under the water surface and died on the bottoms of the net cage. These fish sustained about 20% mortalities in one month after the importation in 1984 and also in 1985. Diseased fish showed pale gills and necrotic lesions due to infection of *Flexibacter marinus*. The liver was pale. The tiger puffer appeared healthy. While the carps swam slowly, they had no visible external and internal sign.

Microscopic Observation

1. Red sea bream

In early stages of the infection many round epitheliocystis cysts developed on the capillary under the respiratory epithelium of gill lamellae (Fig. 1). Some cysts were in the epithelium in the interlamellar spaces of gill filaments. Cysts ranged in diameter from 15 μm to 55 μm. These cysts had a thin cellular capsule with a flattened nucleus and a basophilic inclusion containing many fine granules which were stained positively with Giemsa and Macchiavello but negatively with PAS. With the enlargement of the inclusion of cysts, the surrounding cellular capsule became thin membrane like (Fig. 2). Larger cysts pressed against the capillary and extended the respiratory epithelium of lamellae around the cysts, resulting in deformity and fusion of the affected lamellae. On the other hand, heavily infected, and advancedly infected gills showed extensive hyperplasia of epithelial cells around the cysts in the interlamellar spaces resulting in clubbing of gill filaments (Fig. 3). The most cysts were present within thickly layered epithelial cells in the interlamellar spaces of the gill filament. In this stage, many cysts were degenerated and shrunk resulting in the separation from the surrounding laminal epithelial cells (Fig. 4). Degenerated cysts had the shrunk, marginally fragmented inclusion and thickened membranous matter which contained a nucleus with a visible nucleolus (Fig. 4, 5). Therefore, the membranou matter was judged as a cellular capsule of the host cell. With the degeneration of cysts, the inclusion mostly disappeared and on the contrary the host cell has recuperated the shape in the ordinary cell. Some gill filaments were also infected with *Fiexibacter marinus*, resulting in necrosis.

The liver, spleen, kidney, heart, digestive tracts and skin had no epitheliocystis cyst. Fish with heavily infected gills showed slightly to severely atrophic hepatic cells and hyaline droplet degenerations of renal epithelial cells.

2. Tiger puffer

Epitheliocystis cysts developed with slight infection in gill lamellae. The cysts had the cellular capsule with a visible flattened nucleus and the basophilic inclusion containing fine granules. The cysts were apparently present on the capillary under the respiratory epithelium of lamellae and protruded from the lamellae (Fig. 6). The cysts ranged in size of diameter from 25 μm to 43 μm. No epitheliocystis cyst was found in the skin or

(Fig. 1–8)

Fig. 1. Gill filaments of red sea bream. Epitheliocystis cysts in early stage (arrows) developed on the capillary under the epithelium of lamellae. With the enlargement of cysts, affected lamellae were destroyed, followed by epithelial hyperplasia. H–E stain, ×400.

Fig. 2. Gill filament of red sea bream with epitheliocystis cysts in the slight infection. Affected lamellae showed deformity, hyperplasia of epithelial cells and fusion. H–E stain, ×200.

Fig. 3. Heavily infected gill filaments of red sea bream showed clabbing. H–E stain, ×100.

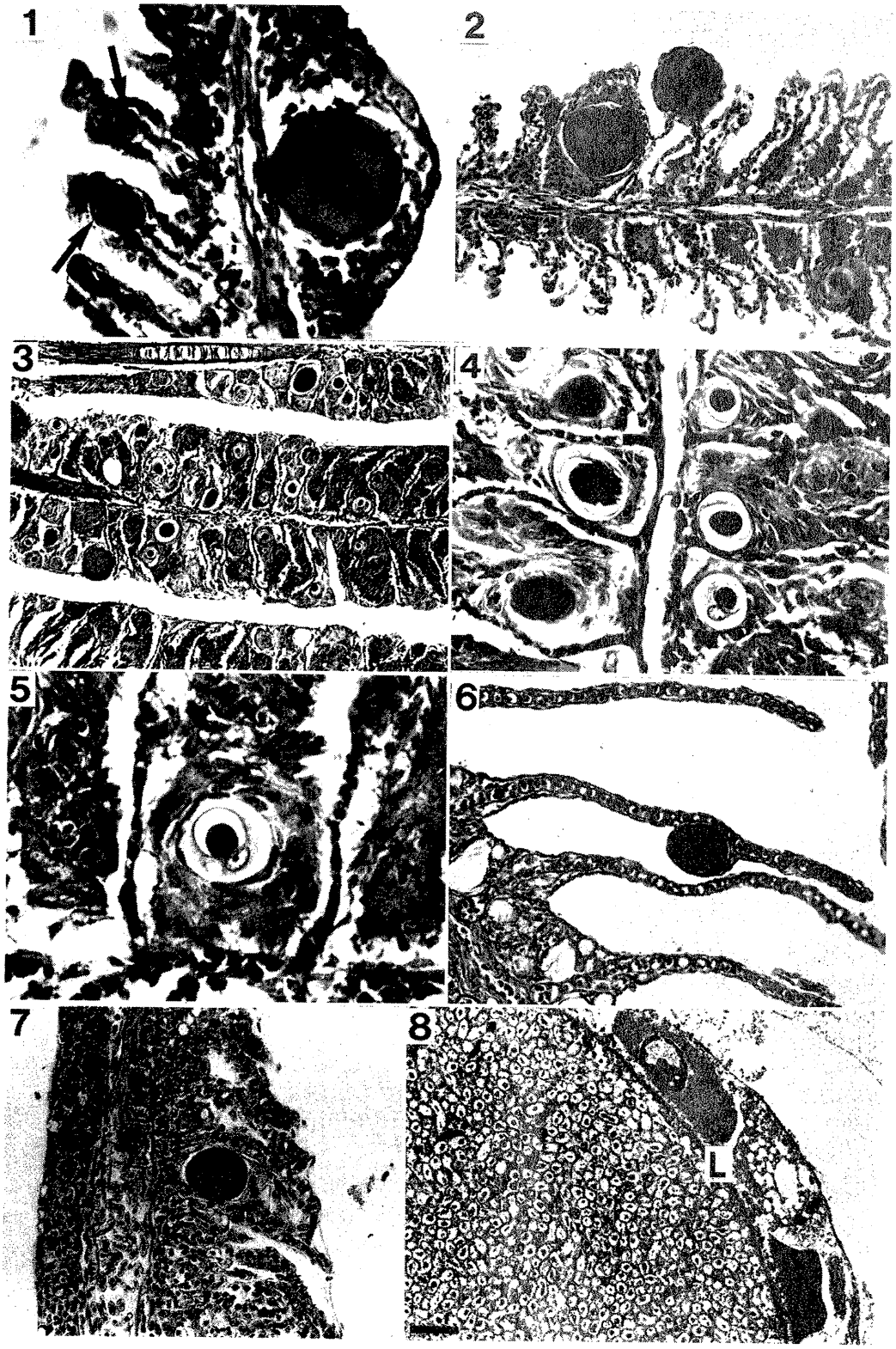
Fig. 4. A detail of Fig. 3. All cysts degenerated showing shrunk, marginally fragmented inclusions among hyperplastic epithelial cells. H–E stain, ×400.

Fig. 5. A detail of Fig. 3. The degenerated cysts displayed visible cellular capsule with a nucleus and a nucleolus. H–E stain, ×600.

Fig. 6. Epitheliocystis cyst in gill lamella of tiger puffer. The cyst developed on the capillary under the epithelium of the lamella. H–E stain, ×200.

Fig. 7. Epitheliocystis cyst in interlamellar space of gill filament of carp. H–E stain, ×200.

Fig. 8. Electron micrograph of epitheliocystis cyst of red sea bream. The inclusion was packed by round particles and separated with electron dense layer (L) from cellular capsule. Bar: 2 μm.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other visceral organs.

3. Carp

Epitheliocystis cysts developed in the epithelium of the interlamellar spaces of the gill filament. The cysts in the early development were slightly enlarged epithelial cell containing basophilic fine granules inside the cytoplasm. With the development of cysts (from 12 μm to 50 μm in diameter), the inclusion became large and the cellular capsule became thin and membranous like (Fig. 7). The gill filaments showed hyperplasia of epithelial cells and fusion of lamellae around the cysts. Neither epitheliocystis cyst nor pathological change was found in the skin and the other visceral organs.

Electron Microscopic Observation

Electron microscopic observation was made on epitheliocystis cysts of red sea bream. Developing cysts had cellular capsules including a flattened nucleus and the inclusion packed with round particles (Fig. 8). The round particles had a limiting membrane and a central mass of electron dense material, and were uniform in the shape. They ranged in size from 0.52 μm to 0.71 μm. In the cysts, the packed particles were separated with electron dense, inner layer of the cytoplasm of host cell. The cysts were covered by the single layer of epithelial cells.

Discussion

Microscopic observation on epitheliocystis cysts of carp revealed the cysts resembled those previously described in carp,[4] bluegill[1] and striped bass.[6] Moreover, cysts of carp found in this study resembled those of carp mucophilosis.[10] And carp mucophilosis was revealed to be epitheliocystis disease by Paperna and Alaves.[4] On the contrary, epitheliocystis cysts of red sea bream and tiger puffer showed a different process of the development. The cysts of these fishes, in the early stage, appeared on the lamellar capillary under the respiratory epithelium but not in the epithelium. Electron microscopy revealed the cysts were packed by round particles resembling those causing epitheliocystis cysts previously described by Zachary and Paperna,[6] Paperna *et al.*[8] and Zimmer *et al.*[3] This indicates the cysts found in red sea bream were exactly epitheliocystis cysts. Gill lamellae have cells forming capillaries between the respiratory epithelium and pilaster cells supporting the capillary lumen.[11] Results on light and electron microscopic observation on infected gills of red sea bream and tiger puffer strongly indicate capillary-forming cells of gill lamellae were infected and developed into epitheliocystis cysts. Paperna and Alaves[4] confirmed epitheliocystis organisms infected not only epithelial cells but also mucous cells and assumed that other cells consisting gill tissues harboured epitheliocystis infection. The present study showed capillary-forming cells of gill lamellae as a new host cell of epitheliocystis. As to red sea bream imported from Hong-Kong, this disease was found every year since 1984. This indicated epitheliocystis organism was native in Hong-Kong.

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References

1) G. L. Hofman, C. E. Dumbar, K. Wolf, and L. O. Zwillenberg: *Antonie van Leeuwenhoek J. Microbiol. Serol.*, **35**, 146–148 (1969).
2) I. Paperna, I. Sabunai, and A. Zachary: *J. Fish Diseas.*, **4**, 459–472 (1981).
3) L. A. Zimmer, M. S. Ewing, and K. M. Kocan: *J. Fish Diseas.*, **7**, 407–410 (1984).
4) I. Paperna and A. P. Alaves De Matos: *J. Fish Diseas.*, **7**, 137–147 (1984).
5) R. E. Wolke, D. S. Wyand, and L. H. Khairallah: *J. Compara. Pathol.* **80**, 559–563 (1970).
6) A. Zachary and I. Paperna: *Can. J. Microbiol.*, **23**, 1404–1414 (1977).
7) I. Paperna: *Aquacul.*, **10**, 169–176 (1977).
8) I. Paperna, I. Sabnai, and M. Castel: *J. Fish Diseas.*, **1**, 181–189 (1978).
9) I. Paperna and F. B. Laurencin: *Aquacul.*, **16**, 173–175 (1979).
10) K. Molnar and G. Boros: *J. Fish Diseas.*, **4**, 325–334 (1981).
11) T. Hibiya: Monograph of Fish Histol., Kodansha, Tokyo, Japan, 1982, pp. 151.