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Scanning Electron Microscopic Studies on Bacterial Gill Disease in Rainbow Trout Fingerlings*

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Abstract Gill epithelia of rainbow trout fingerlings suffering bacterial gill disease were examined by scanning electron microscopy. Surface ultrastructure of the outermost layer of epithelial cells in normal lamellar and filament epithelia was characterized by branching and anastomosing microridges approximately 275 nm in width, with average spacing of about 390 nm and considerably varying lengths. In hyperplastic lesions, little typical microridge structure was observed on the surface of the epithelial cells, instead shortened and thickened contours averaging 520 nm in width were seen along the longitudinal axis with many constrictions. Further, fine granular elements averaging 550 nm in size often appeared on the epithelial cell surface, piled one upon another or in bead-like chains which were frequently connected with short irregular microridges.

The free surface of normal chloride cells was generally characterized by a granular texture studded with short microvillus-like or isometric granular projections averaging 460 nm in thickness. Among these projections a small number of extremely short microridge structures frequently appeared. In bacterial gill disease, the microvillus-like projections were more numerous, denser and thinner than in healthy fingerlings; they averaged a thickness of 310 nm and exhibited several gourd-like constrictions implying plasma membrane vesiculation at their tips. Domed chloride cells were covered with either a number of small verruciform projections irregular in size and shape or a few thick and short projections. The latter surface was difficult to discriminate from the surface in the early stage of the globoid structure appearing on the gill lamellae.

The free surface of mucous cells in healthy fingerlings was rather smooth and often more prominent than other types of cells, but in diseased fingerlings many of mucous cells were domed and their surface was either smooth or had several small prominences which might have been caused by secretory granules underneath.

In a previous paper (Kudo and Kimura, 1983a), transmission electron microscopic (TEM) observations were reported to reveal morphological alterations of gill epithelia in bacterial gill disease: hypertrophy of the lamellar epithelium, transfiguration of the epithelial and chloride cells, the process of formation of a globoid structure identified as "plaque" (see Rucker et al., 1952; Wood and Yasutake, 1957; Kimura et al., 1978), plasma membrane vesiculation suggesting cell injury, the process of fusion of adjacent gill lamellae and a profuse proliferation of epithelial cells coming mainly from the gill filament epithelium. These alterations are involved in hyperplasia of the gill epithelia in bacterial gill disease. Thus, some change in the organization of the surface ultrastructure in gill epithelia following bacterial infection is supposed. The purpose of this paper is to define the surface ultrastructure of gill epithelia in bacterial gill disease.

Materials and methods

Thirty rainbow trout fingerlings, *Salmo gairdneri* Richardson, suffering from the bacterial gill disease and twenty healthy ones were used for the present investigation. Their average body length and body mass were approximately 39 mm and 1 g, respectively. After fixation for 0.5 to 1 h in cacodylate-buffered 2.5% glutaraldehyde the gill tissues were briefly subjected to supersonic treatment for removal of the mucous substance coating the gill epithelium surface and of the bacterial cells, followed by immersion in the same fresh fixative for 1 to 2 h. After washing overnight with 0.1 M cacodylate buffer (pH 7.3) containing 5% sucrose, the gill tissues were immersed for 2 h more in a solution made of an equal volume of 2% solutions of sucrose,

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sodium glutamate, glycine and arginine hydrochloride, and then for 2 to 18 h in a 2% solution of tannic acid after washing well for 30 min in distilled water (Murakami, 1974). After washing in distilled water, postosmication and dehydration, the gill tissues were transferred to amyl acetate, and then dried at a critical point from liquid CO_2. The dried specimens were mounted on stubs, coated with platinum by ion sputtering and examined by a Hitachi S-700 scanning electron microscope (SEM).

Results

Normal surface ultrastructure. At low magnification, a row of leaf-like gill lamellae could be seen on the upper and lower surfaces of each filament, alternately spaced on the two filament sides (Fig. 1). Since the epithelia of both filaments and lamellae consist of the same types of cells (Kimura and Kudo, 1979), the surface ultrastructure can be understood through the characteristic features of each cell type in TEM observations. Epithelial cells in the outermost layer were characterized by a roughly squamous or polygonal outline and branching and anastomosing microridges on the cell surface. These were about 275 nm in width, spaced about 390 nm apart and varied considerably in length without particular orientation (Fig. 2); they often exhibited a typical labyrinth-like structure. The free surface of chloride cells was studded with short microvillus-like, or isometric granular projections of an average of 460 nm thick and also a very few short microridge-like patterns (Fig. 2). The entire surface of the chloride cells often domed slightly and was nearly polygonal in outline. The mucous cell free surface was rather smooth, round or oval in outline, and frequently more prominent than other cell types. On this free surface there often appeared small holes and less electron dense ball-like bodies which were suggestive of secretory granule openings (Fig. 2).

Diseased surface ultrastructure. At low magnification, each filament exhibited a club-like or cudgel-like contour, and fusion between the filaments was sometimes observed at their tips. Hyperplastic lesions were most serious at or near the tips and mild towards the gill arch. Since topographical variations brought out marked differences in the surface appearance of the epithelia, filaments were roughly divided into three regions, A, B and C, for the sake of description (Fig. 3).

In region C, the epithelia of gill filaments and lamellae were similar in surface ultrastructure to that of normal epithelia, except for some of the epithelial cells located near region B, of which lacked the typical labyrinth-like microridge structure.

In region B, the lesions were richest in morphological variations of the epithelia and each cell type. Near neighboring region C the epithelial surfaces presented various appearances, and fusion and hypertrophy of gill lamellae (Fig. 4) as well as formation of globoid structures were frequently observed. The fusion occurred at the lamellar tips, and the hypertrophy was characterized by the rugged appearances of a jutting-out (dome formation) of each cell comprising the gill lamellae (Fig. 5). In hypertrophic lesions, there appeared furrows in various depths and widths between the domed cells. Surface ultrastructure of epithelial cells varied and showed no typical microridge structure except for a few cases of distorted microridges or sparsely granular, roughly broken or irregularly pebbled appearance (Figs. 6, 7). These varied even in the same cell type. The surface ultrastructure of globoid structures also varied, being ruffled, covered with fine granules or smooth (Fig. 8). Around the globoid structures microridges often transformed into bead-like chains (Fig. 8). In morphologically more advanced lesions of region B, traces of the boundaries between the lamellae (Fig. 9) or lamella tips (Fig. 10) were discerned. The traces of lamella tips suggest the process of fusion of the lamellae due to proliferation of epithelial cells in the interlamellar (filament) epithelium. The region showed remarkable variations in microridge structure on epithelial cells; there were signs of several transitional forms between a striking microridge disorganization and microridge fusion (Fig. 11). The disordered microridges were about 520 nm wide, shorter and thicker than in normal cells. Another remarkable finding was the appearance of fine granular elements and relatively thick, short cytoplasmic projections. The granular elements (550 nm in average size) were scattered

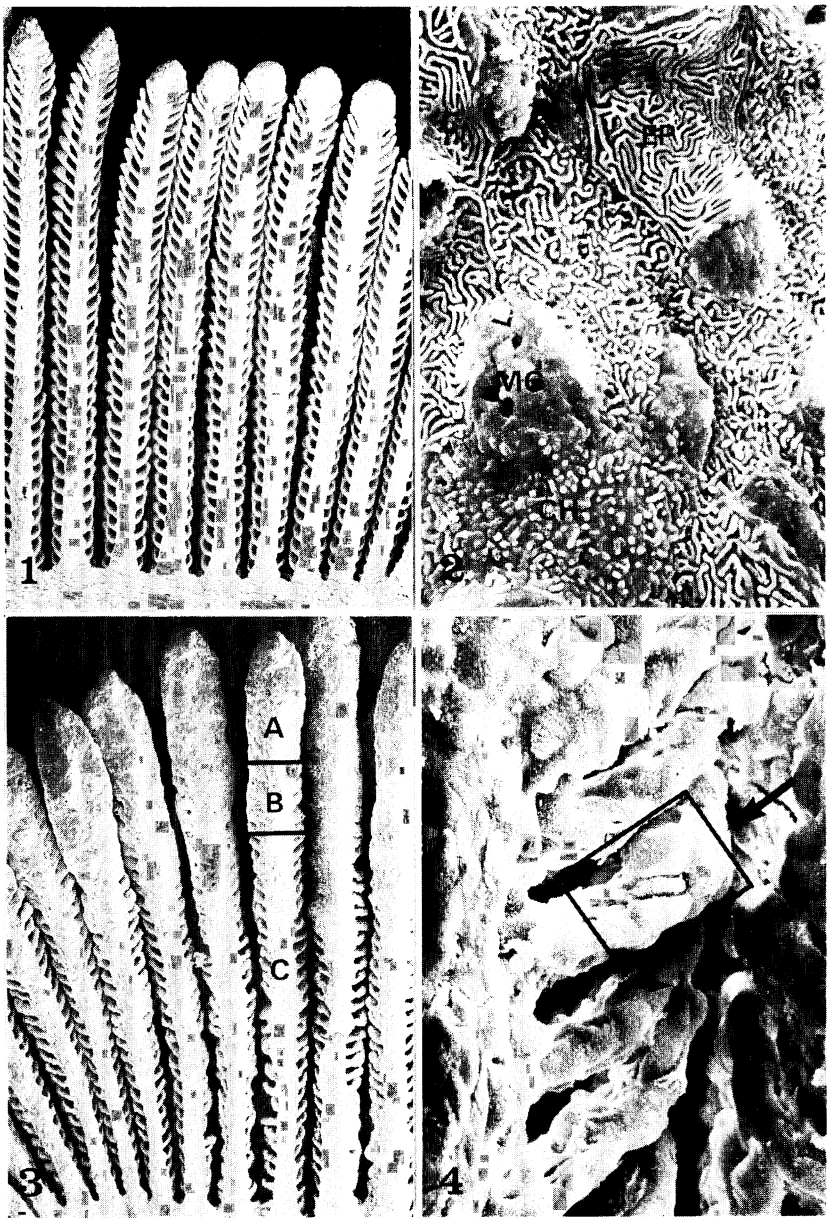

Fig. 1. Normal gill filaments with leaf-like gill lamellae on the upper and lower sides. ×96.

Fig. 2. Surface ultrastructure of gill filament epithelium. EP: epithelial cell, CH: chloride cell, MC: mucous cell. ×4,400.

Fig. 3. Gill filaments in bacterial gill disease. Each filament exhibits a club-like contour. The filaments are divided into three regions, A, B and C for the sake of description, in an advanced disease condition. ×80.

Fig. 4. Region B in bacterial gill disease. Fusion (small arrow) and hypertrophy (large arrow) of gill lamellae are seen. The rectangular area is enlarged in Fig. 7. ×880.

on the free cell surface and often piled one upon another or in bead-like chains which frequently continued with short irregular or distorted microridges (Fig. 12). The relatively thick and short cytoplasmic projections were irregular in shape and scattered on the cell surface. They

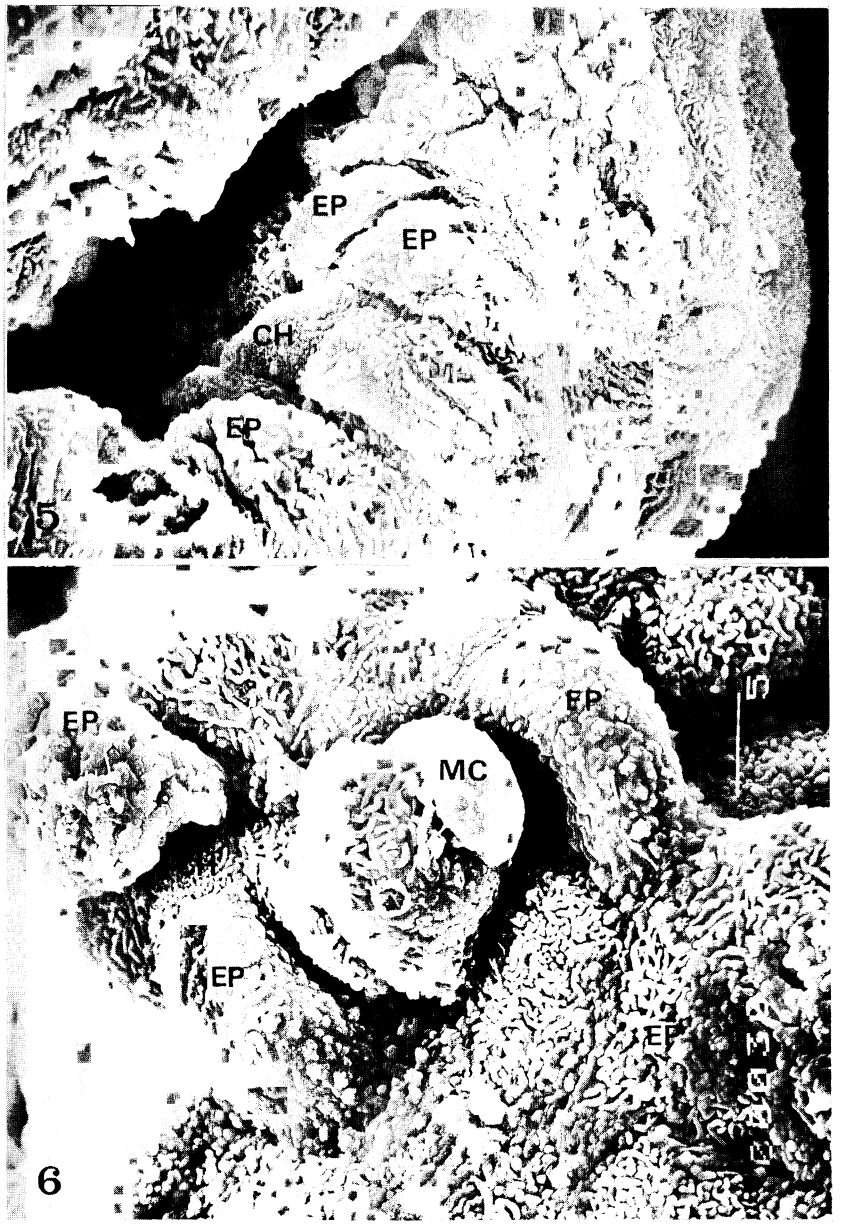

Fig. 5. Hypertrophy in gill lamella. Between domed cells furrows exist of various depths and widths. Note indistinctness of microridge structure in the domed epithelial cells (EP). CH: chloride cell. ×3,680.

Fig. 6. Hypertrophy in the boundary area between gill filament and lamella. Surface ultrastructure varies in the individual epithelial cells (EP). CH: chloride cell, MC: mucous cell. ×4,880.

could have been formed by the piling-up of fine granular elements as described above (Fig. 12). The bizarre ultrastructure originating from disorganization or degradation of the microridge structure extended at places on the lesion to a few grouped epithelial cells, so that the whole surface of hyperplastic lesions consisted of various mosaic patterns. The surface ultrastructure was so complex that it was impossible to characterize definitely. Other features in

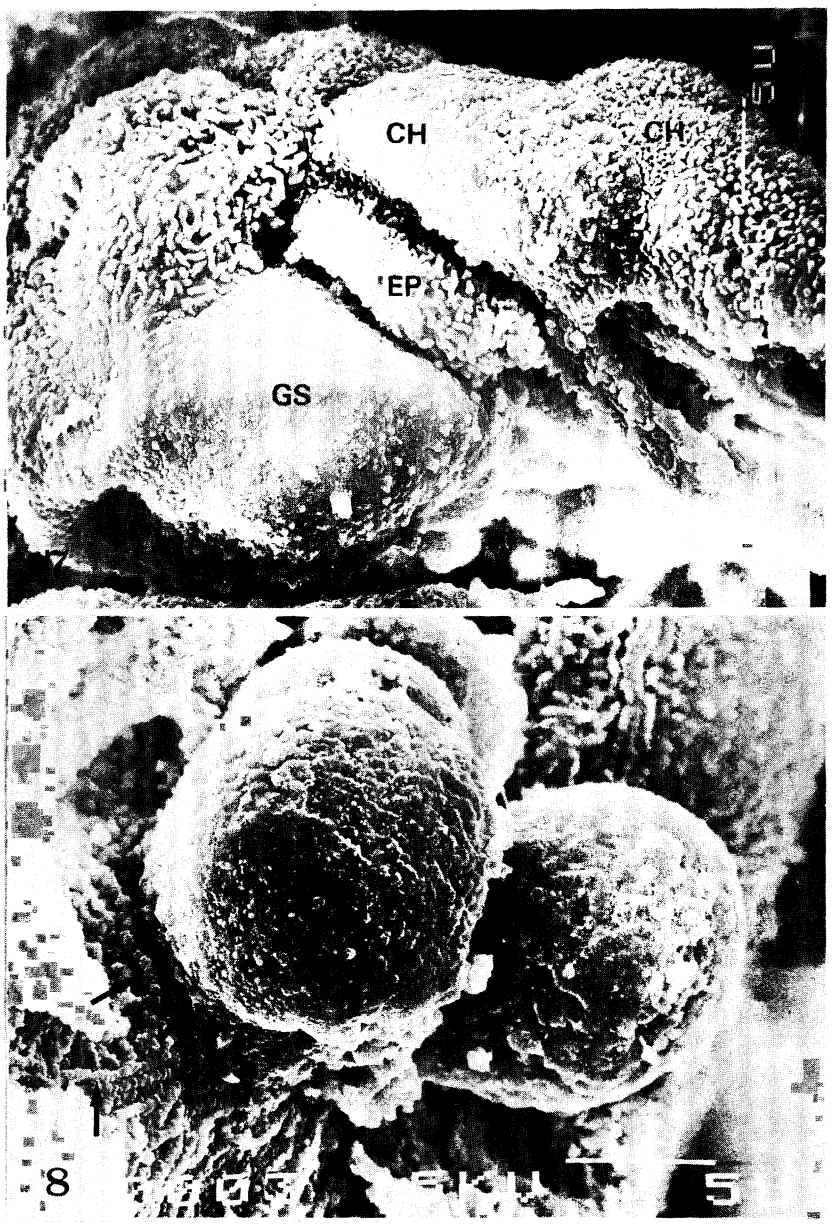

Fig. 7. Hypertrophy in gill lamella. The surface of chloride cells (CH) varies in ultrastructure. EP: epithelial cell, GS: early stage of a globoid structure. ×4,880.
Fig. 8. Globoid structure. Arrows show microridges transformed into bead-like chains. ×6,800.

the surface ultrastructure were as follows: faint traces of microridge structure, various degrees of surface prominence and often a few thick irregular projections (Fig. 12) or a flat surface where vesicular projections were sparsely scattered (Fig. 13). The free surface of chloride cells was generally granular in texture. This was caused by the presence of numerous thin, microvillus-like, various size projections (about 310 nm thick) and the presence of extremely short microridges with several narrow gourd-like parts (Fig. 11). The gourd-like parts may be due to the fusion of some short blunt-ended projections with one or two narrow parts. Such