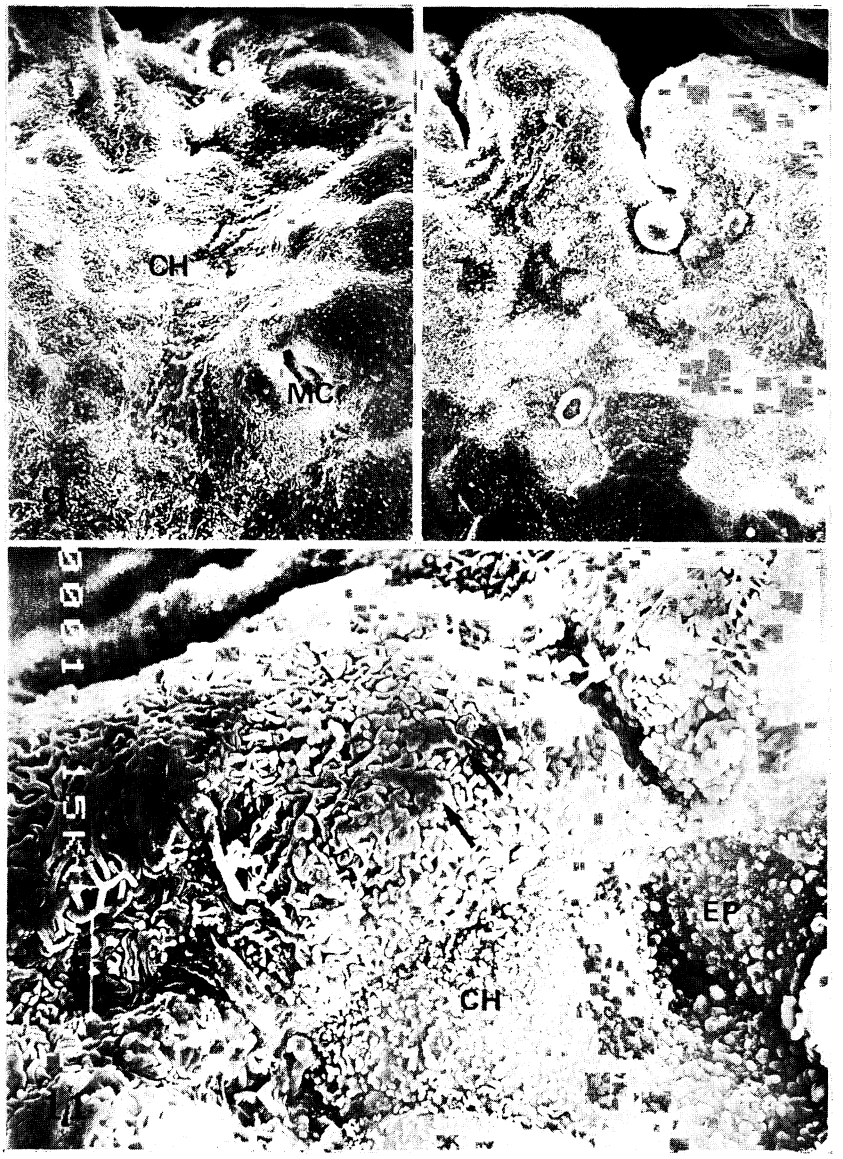

Fig. 9. Region B neighboring on region A. This shows surface ultrastructure of the boundary area of fused lamellae. The majority of epithelial cells show no typical microridge structure. CH: chloride cell, MC: mucous cell discharged secretory granules. ×1,200.

Fig. 10. Region B neighboring on region C. The tips of gill lamellae are seen, suggesting fusion due to proliferation of interlamellar epithelial cells. ×1,200.

Fig. 11. Fusion and degradation of microridges. Arrows show fusion of microridges. CH: chloride cell, EP: epithelial cell facing the surface after exfoliation of the outermost layer of epithelial cells. ×5,280.

microridges were few and varied from cell to cell. No distinguishable differences were found in structural features of the chloride cell surface between the gill filament and lamellar epithelia. Mucous cells exhibited a smooth and small surface, gentle undulations or a sparsely scattered granule or particle texture; this texture was possibly caused by plasma membrane

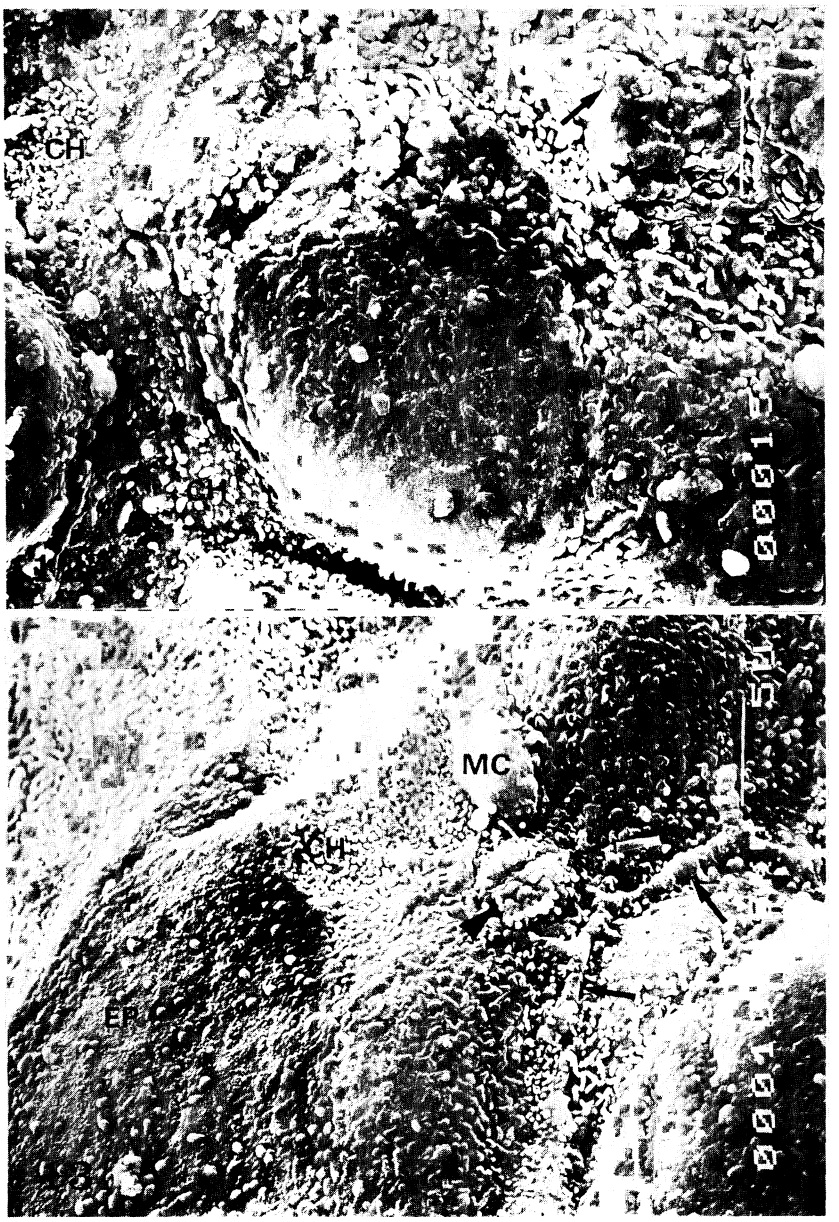

Fig. 12. Transformation of microridges into fine granular elements (arrow-heads). The elements are piled high (arrow). CH: chloride cell, EP: epithelial cell with traces of microridge structure. ×5,760.

Fig. 13. Rampart-like cytoplasmic eminences among cell boundaries (arrows). Arrowhead shows aggregation of fine granular elements. CH: chloride cell, EP: epithelial cell with vesicular projections on the flat surface, MC: mucous cell. ×5,760.

vesiculation. Another characteristic was the frequent appearance of rampart-like cytoplasmic eminences among the cell boundaries (Fig. 13). Intercellular spaces were not observed, and the eminences were at places transformed into bead-like chains or small undulations.

In region A, hyperplastic lesions rendered it impossible to discriminate the gill lamella from the filament. The surface of the lesional tissues was comparatively smooth and epithelial cells

were frequently exfoliating. Aggregation of domed mucous cells presented a "cobblestone" appearance in some places (Fig. 14). The fusion of two or more filaments was occasionally observed, generally at the tips (Fig. 15). The degree in fusion varied from distinguishable to indistinguishable filaments. The majority of epithelial cells were comparatively flat and polygonal, and scattered vesicular projections were observed on their surface, as seen in the epithelial cells in more advanced lesions of region B. Surface compartments among the projections had a texture as though they were closely packed with an infinite number of particles. Of course, there were several exceptions which had a distorted microridge structure, the traces in microridge structure, or the seat of irregular pebbles. Chloride cells were generally covered with short, thin frequently irregular and closely packed projections. Their number decreased much more than in region B. Mucous cells projected at places comparatively small, smooth faces, but these often had gentle undulations or a granular texture.

Discussion

SEM observations on the surface ultrastructure of gill epithelia in healthy rainbow trout previously revealed a pattern of microridges on the surface of epithelial cells, microvillus-like cytoplasmic projections on the chloride cell surface and occasionally granular openings on mucous cells (Olson and Fromm, 1973; Kimura and Kudo, 1979). These findings could also be applied to the case of the present healthy fingerlings, except for variations in surface ultrastructure between trout adults and fingerlings. The preparation process of the present specimens could not always distinguish the sharp boundaries among the cells. We have interpreted this to indicate less shrinkage of the gill tissue. Therefore, the thickness of microvillus-like projections in chloride cells and the width of microridges in epithelial cells in the present healthy fingerlings seemed worthy of measurement and proved to be about 460 nm thick and 275 nm wide with space between of about 390 nm, respectivery. These measurements might be useful as one criterion in understanding the surface alteration of diseased gill epithelium. TEM observations on the outermost epithelial cells have exhibited irregularly arranged microvillus-like projections on their free surface, tangential sections of which reveal branching and anastomosing structure (Kudo and Kimura, 1983a). From this viewpoint, microvillus-like projections on the free surface of the outermost epithelial cells are identical with a cross section of microridges seen in the present SEM observations. Therefore, the microvillus-like projections must be terminologically kept separated from those on the free surface of chloride cells.

SEM observations of abnormal epithelia in bacterial gill disease revealed that surface ultrastructure varies most remarkably in epithelial cells. The microridge structure may be a typical surface characteristic of the outermost layer of epithelial cells in healthy trout fingerlings and adults, but its pattern is not uniform in individual cells. Nevertheless, the outermost layer of epithelial cells in lesions showed structural changes of microridges in a uniform direction, varying from the typical, labyrinth-like pattern through distortion or transformation into bead-like chains to decay and disappearance. Of course, the process of progression from hypertrophy to hyperplasia did not cause the simultaneous alteration of microridges in all epithelial cells and, as a result, the tissue surface of hypertrophic lesions exhibited a mosaic pattern in these cells among which other types of cells were interposed. Variations of the surface ultrastructure might be related to the total stimulation period of the epithelial cells due to adhesion of the bacterial cells. This is presumed from the fact that the beginning of natural infection of the bacterial cells occurs in the gill lamellae near or at the tip of the gill filaments and progresses towards the base. In lesions of club-like filaments the hyperplasia in region A was most serious and became mild in regions B and C. These findings have been confirmed in an experimental infection of the disease (Kudo and Kimura, 1983b).

Microridges had a general tendency to disappear in the course of the disease; early changes were disorganization and thickening, followed by fusion which rendered the cell surface flat so that the microridges appeared to be lost. On the other hand, exfoliation of epithelial cells also caused disappearance of the microridge structure in hyperplastic lesions; the exfoliation

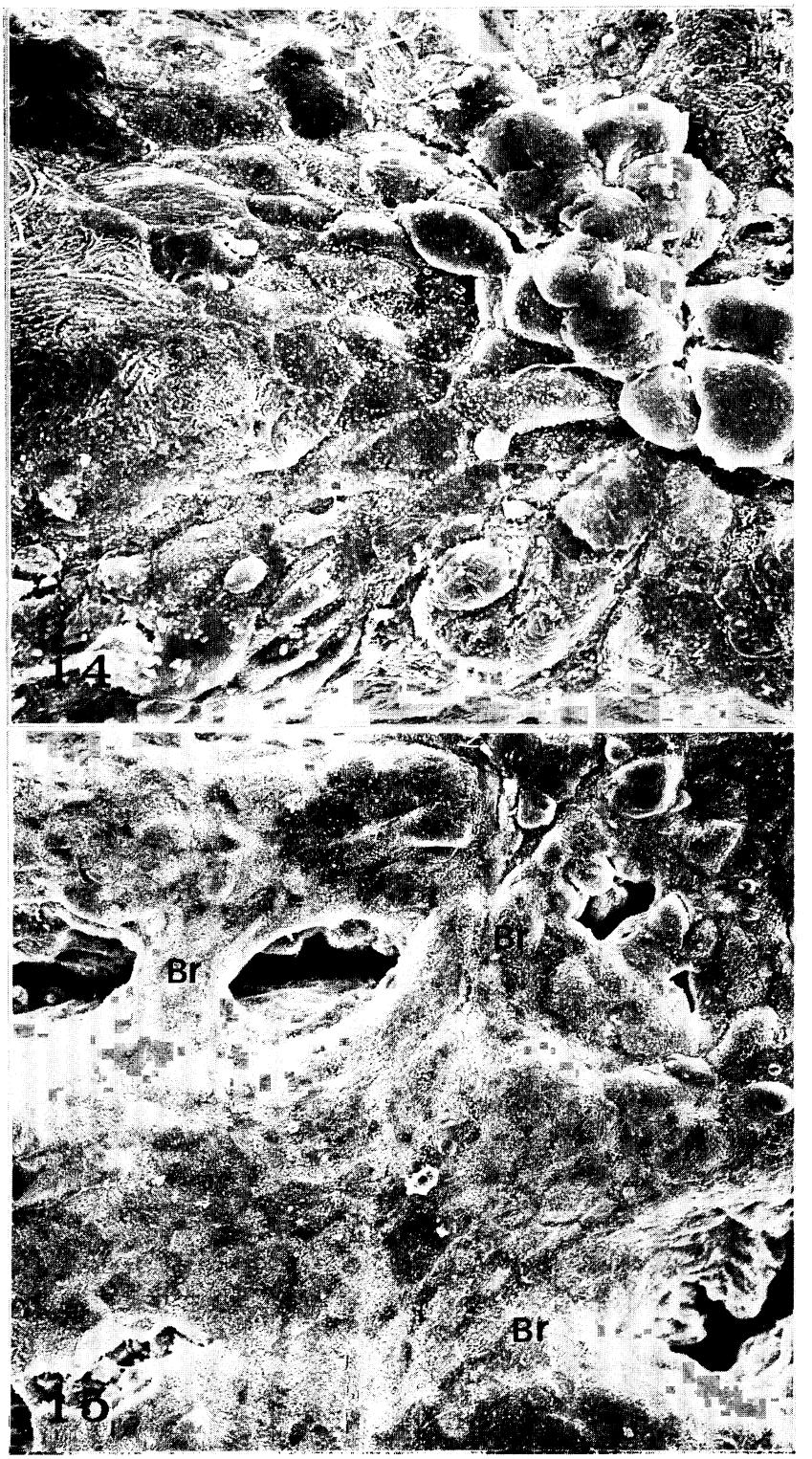

Fig. 14. Cobblestone appearance due to aggregation of domed mucous cells in region A. ×1,700.
Fig. 15. Fusion of three gill filaments. The bridges (Br) consisting of epithelial cells span the gill filaments. ×800.

resulted in revealing the underlying epithelial cells whose free surface does not have such structure, as suggested from a previous paper (Kudo and Kimura, 1983b). This exfoliation was most remarkable in region A which represented a morphological maximum in hyperplastic lesions. Further, simultaneous combination of the three, i.e., partial fusion, plasma membrane vesiculation and transfiguration into bead-like chains of microridges caused an increase in ruggedness or a bizarre structure of the cell surface. In more advanced lesions, epithelial cells were covered with closely packed particles or vesicles; to this covering, plasma membrane vesiculation might be related. Thus, the monitoring of this cell type by SEM seems useful to indicate not only the progression of hyperplastic lesions due to bacterial infection but also the progressive degeneration of the cell itself. Changes in microridge structure were, in general, parallel to the progression of hyperplastic lesions which are typical of bacterial gill disease. They imply a functional decline or degradation as respiratory epithelial cells. This would be presumed from dysplasia and carcinoma in situ in the human uterine cervical epithelium, as shown by the lack of true microridges and short anastomosing of microvilli (Ferenczy and Richart, 1973; Williams et al., 1973). As pointed out earlier (see Rucker et al., 1952), the present examination has also suggested that the high mortality of diseased trout fingerlings may be caused by impaired gas exchanges, including actual suffocation at the level of the gill lamellae epithelium, because of the histological disruption caused by the fusion of gill lamellae or filaments and by the proliferation of epithelial cells.

Chloride and mucous cells in bacterial gill disease have a surface ultrastructure distinct from that of normal cells. The chloride cells in region B showed particularly striking variations (Kudo and Kimura, 1983a). In region A, however, they sharply decreased in number and were often difficult to find. This was possibly caused by their exfoliation through degeneration and degradation as well as by their enclosure within the lesion (Kudo and Kimura, 1983a). Therefore, their surface changes in hyperplastic lesions may be not too useful as a criterion in bacterial gill disease. Comparison between TEM and SEM findings, however, may be useful to learn the condition of individual chloride cells. The increase of thin microvilli and very short, thin micro ridges at early lesion stages was followed by their thickening and simultaneous decrease, plasma membrame vesiculation at their tips, and disappearance of both microvilli and microridges in extremely domed chloride cells. The last case might be difficult to discriminate from domed epithelial cells which mostly lack microridge structure. The difficulty in discrimination may be caused by there being no parallel between the progression in bacterial gill disease and surface changes of chloride cells. This may also account for the unsuitability of chloride cells as a criterion in hyperplastic lesions.

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走査型電子顕微鏡によるニジマス稚魚の細菌性鰓病に関する研究*

工藤重治・木村紀彦

正常な稚魚の鰓弁や二次鰓弁の上皮細胞の表面構造は，迷路を思わしめる迂曲および分岐・吻合した細胞質の微小堤に富み，その長さは種々であるが，その幅は平均約 275 nm で，平均約 390 nm の間隔で配列する．しかし，過形成の病巣表面では，その特徴的な微小堤はほとんどなく，その代りに幅が 520 nm の微小堤が多くの部位でくびれ，ビーズ状になっているか，または 550 nm の微小顆粒状になっており，さらに病巣が進んだところではほぼ平滑になっている．

正常な塩類細胞の表面には太さが平均約 460 nm の短かい微絨毛様突起か等大の顆粒状突起があり，これらの間に極端に短かい微小堤が少数みられる．しかし，病巣での塩類細胞の初期の変化は微絨毛様突起が増加し，細くなり（平均約 310 nm），しかもひょうたんのごとくくびれている．これは，微絨毛様突起の先端での小胞化によると思われる．さらに塩類細胞の自由表面が半球状に突出すると，そこには多くの大小不同ないぼ状突起がみられる．突出がさらに進むと，太く短かい突起が少数あるだけで比較的滑らかになる．

正常な粘液細胞の表面は他の型の細胞より幾分高く，むしろ平滑である．しかし，病巣の表面では粘液細胞の表面は突出し，平滑かまたは分泌顆粒によると思われる小さな半球状の隆起が少数みられる．

肥大した二次鰓弁の表面は凹凸が顕著で，そこにみられる半球状態の表面は微細顆粒状または平滑である．進んだ過形成の表面はむしろ平滑である．

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* ニジマス稚魚の細菌性鰓病に関する超微形態学的研究—II