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Ultrastructural and Histochemical Changes of Mucous Cells in the Gill Epithelium of the Seawater-Adapted Guppy (*Poecilia reticulatus*)

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Ultrastructural and histochemical changes of mucous cells in the freshwater and seawater-adapted guppy (*Poecilia reticulatus*) gills were observed by the light, scanning- and transmission-electron microscopes.

The mucous cells were usually located in the epithelium of primary lamellae projected from the gill arch. The rough endoplasmic reticulum and Golgi complex were highly developed in immature mucous cells. The mature mucous cells were nearly filled with the mucous granules. In the freshwater guppy, the histochemical properties of the mucous cells were a mixture of the neutral mucin, sialomucin and sulfomucin. When guppy was adapted to the seawater, the content of acid glycoproteins (sialomucin and sulfomucin) was decreased. In addition, the number of mucous cells in the seawater-adapted group was less than a third of those in the freshwater one. These results suggest that the seawater-adapted guppy would react to the changed osmotic stress of the seawater. And also, the environmental change by the increased salt concentraion might lead to reduce the chance of infections.

KEY WORDS: Mucous Cell, Neutral Mucin, Sialomucin, Sulfomucin

The gill of fish is a complex organ which has functions of respiration, osmoregulation, excretion of metabolic nitrogen and acid base balance (Laurent and Dunel, 1980; Heisler, 1984; Randall and Daxboeck, 1984; Maina, 1990; Kim *et al.*, 1993). For these functions, the gill epithelium is consisted of at least four different cell types which are pavement cells, chloride cells, neuroepithelial cells and mucous cells (Laurent, 1984). Especially, mucous cells are found in the epidermis, gill and digestive canal in the teleost fish, and many studies of their morphology (Mittal and Munshi, 1971; Zaccone, 1972; Yamada and Yokote, 1975; Fletcher *et al.*, 1976), functions (Rosen and Cornford, 1971), and the chemical contents of the mucin (Harris and Hunt, 1973; Gona, 1979) have been reported.

Structures of the mucous cells in the fish are mostly studied about cells located in the epidermis rather than the gill epithelium (Henrikson and Matoltsy, 1968; Downing and Novales, 1971; Schwerdtfeger, 1979; Blackstock and Pickering, 1980; Zaccone and Licata, 1983; Whitear and Mittal, 1984).

Functional studies of the mucous cells in the gill epithelium show that these cells secrete the mucous material to the surface of the gill epithelium forming a mucous layers which has a protective function as a boundary between the gill epithelim and its surrounding water (Jakowska, 1963), and through this layer, excretion of CO_2 and ammonia, control of ionic strength and gas exchange are achieved (Wright *et al.*, 1989; Handy and Eddy, 1991; Randall *et al.*, 1991;

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Lumsden *et al.*, 1994).

According to the histochemical studies, mucous granules in mucous cells of many teleosts contain glycoproteins (Asakawa, 1970; Bremer, 1972; Zaccone, 1972; Harris *et al.*, 1973; Carmignami and Zaccone, 1974; Ojha and Munshi, 1974), and there are some differences in the contents of the mucus not only in different species but also in the same species(Mattheij and Stroband, 1971; Mittal and Munshi, 1971; Zaccone, 1972; Yamada and Yokote, 1975; Fletcher *et al.*, 1976). It was found that when the fish was adapted to the acidic water (about pH 3.0) the formation of mucus was activated to form a mucous layer on the surface of the gill which could help diffusion of oxygen during respiration (Ultsch and Gros, 1979). It was also reported that in fishes which shift their inhabitations between fresh water and sea water through their life cycle, there are changes in the number of mucous cells as well as the chemical contents of the mucus according to the salt concentration of surrounding water (Mattheij and Sprangers, 1969; Wendelaar Bonga, 1978; Solanki and Benjamin, 1982).

In this study, the characteristics of the distribution and fine structures of mucous cells in the gill epithelium of guppy (*Poecilia reticulatus*) are observed, and the changes in the number and the chemical components of the mucus of the mucous cells when guppy, a fresh water tropical fish, was adapted to sea water by gradually increased salt concentration are analysed.

Material and Methods

Aquarium fish guppy (*Poecilia reticulatus*) were obtained from commercial aquarium and maintained in the lab., supplying oxygen and feeding theramin once a day.

For the experiment of environmental adaptation, fishes which were once kept in the fresh water tank for one week were adapted in the sea water of final salt concentration of 32‰ for about a week by gradually increasing salt concentraion. The gills were dissected from at least 3 fishes each kept in either fresh water or sea water without anesthesia. The sea water (salt concentration: 32‰) collected from adjacent ocean near Wallmeedo at Inchon, was kept at 4°C and mixed with the fresh water for gradual increase of salt concentration. The salt concentration was measured by salinity refractometer (S-Mill).

For scanning electronmicroscopic examination of the gill, the tissue was fixed in 2.5% glutaraldehyde-paraformaldehyde in 0.1M phosphate buffer (pH 7.2) for 3 hrs, postfixed 2% osmium tetroxide for 1 hr, and then dehydrated in ascending ethanol. Dehydrated tissues were dried in critical point drier (Polaron 300) with liquid CO_2, and coated with gold by Ion Sputter JFC-1100. JSM-35C scanning electronmicroscope was used for the observation. For transmission electronmicroscopic observation, the tissue was fixed and dehydrated as the same as for SEM. The dehydrated tissue was embedded in Epon 812 mixture and polymerized in the polymerizer (Reichert-Jung) for 72 hrs at 60°C. The polymerized tissue block was sectioned with 1 μm thickness and stained with 1% toluidine blue to determine the area for electronmicroscopic observation. The ultrathin specimens were prepared using LKB ultratome and stained with uranyl acetate and lead citrate. JEM-1200 EX (JEOL) transmission electronmicroscope was used for examination.

For histochemical analysis of the mucous material in the mucous cells from the gill epithelium, the gill was fixed in 10% neutral formalin, and dehydrated in a series of ethanol, and then embedded in paraffine. The tissue was sliced as a serial section with 5 μm thickness. To detect the histochemical components of the mucous granules in the mucous cells, sections were stained with periodic acid-Schiff reaction (PAS: McManus, 1946), alcian blue pH 2.5 (AB 2.5), alcian blue pH 2.5 - PAS reaction (AB 2.5 - PAS: Mowry, 1963) and aldehyde fuchsin pH 1.7 - AB 2.5 (AF 1.7 - AB 2.5: Spicer and Meyer, 1960). The color tones for the criteria of the histochemical decision were classified as red (R), red with trace of purple (RP), purple (P), blue with trace of purple (BP) and blue(B)(Spicer and Sun, 1967). The intensities of each color were categorized as 1 (weak), 2 (medium) and 3

(strong). Unstained cells were classified as 0 (Sato and Spicer, 1980; Suganuma *et al.*, 1981).

Results

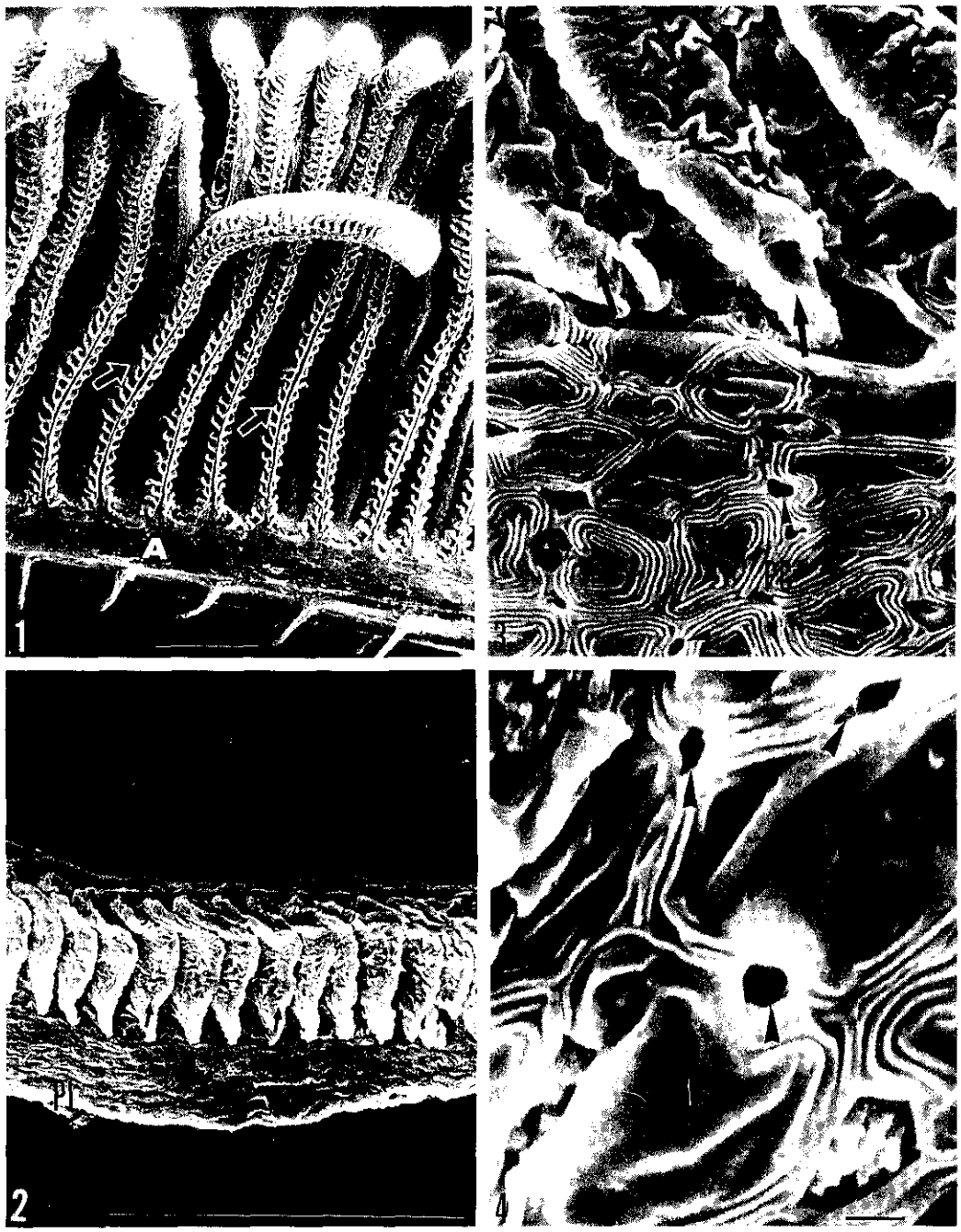
The gill of guppy was consisted of 4 pairs of gill arches. From each gill arch, several primary lammellae were protruded, and the secondary lammellae were protruded from each primary lamellae bilaterally (Figs. 1, 2). The primary lamellae were covered with the stratified epithelium which are consisted of 4-5 layers of epithelial cells. The secondary lamellae were formed with the pillar capillary which is extruded from the basal lamina of the primary lamellae and one layer of respiratory cells which covers the pillar capillary (Fig. 3). There were pavement cells which had multiple layers of microridges regularly arranged as concentric circles, chloride cells with apical microvilli among the pavement cells and the opening for the secretion of mucus from the mucous cells located between chloride cells in the surface of the primary lamellar epithelium (Figs. 3, 4).

Mucous cells in the gill epithelium of the guppy were assorted according to the number of the mucous granules in the cytoplasm. The immature mucous cell of early stage was oval in shape and occupied with a nucleus which shows slightly irregular shape observed at the middle and periphery of the nucleus (Fig. 5). In the cytoplasm, especially rough-endoplasmic reticulum was well developed, and large numbers of ribosomes, mitochondria and a few mucous granules were distributed around the nucleus (Fig. 5). With the maturation of mucous cells, the middle part of the cell was getting filled with mucous granules consisted of homogenous fuzzy like material, and the nucleus and other cellular organells were pushed to the corner (Figs. 6, 7). In maturing cells where cytoplasm was mostly filled with mucous granules, well developed Golgi complex was observed between the nucleus and mucous granules (Fig. 6). Mucous granules in matured mucous cells were globular in shape and had almost the same size with various electron density (Fig. 7). There were some cells which have an empty space in the cytoplasm due to the partial secretion of the mucous granules from the cells to the epithelial surface (Fig. 8).

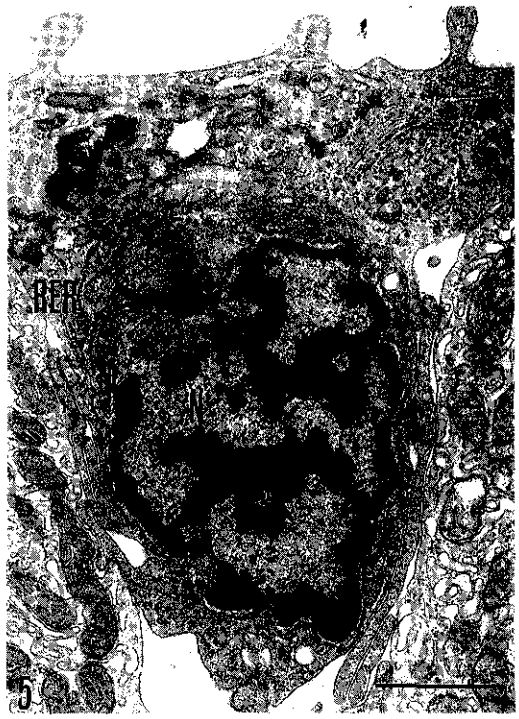
In the experiments to study the changes in the number of mucous cells according to the adaptation of the guppy gills either to the fresh water (salt concentration; 0‰) or to the sea water (salt concentration; 32‰), the mucous cells which were located at the epithelium of the primary lamellae were counted per 100 secondary lamellae for the reasonable comparison. These results showed that the number of mucous cells in the gill epithelium of the fish adapted to the sea water was decreased to 3 times less than that of the fish of fresh water (Fig. 9, $p<0.01$).

Table 1 shows the results of various histochemical analysis to study the composition of the mucous material. When the gills were double stained with AF 1.7 and AB 2.5, most of mucous cells were stained strongly with AB 2.5 appearing as dark blue, while it was rare to be stained with AF 1.7 (Table 1, Fig. 10). The mucus in the mucous cells from the sea water adapted guppy showed medium reaction with PAS reaction, medium stain in both case with AB 2.5, and AF 1.7 - AB 2.5 double stain; however, in the case of AB 2.5 - PAS reaction, it showed more intensely stained mixture of blue purple and red purple staining patterns (Table 1, Fig. 10).

In summary, mucous cells in the epithelium of guppy gill were observed to contain 3 types of glycoprotein of neutral mucin, sialomucin and sulfomucin, and each mucous cell seems to have more than one type of the glycoprotein (Fig. 10). Most of mucous cells of the fresh water guppy gill mainly contained neutral mucin while some of the cells were observed to have a large amount of sialomucin and a small amount of sulfomucin (Fig. 10, a-d). However, in the sea water adapted guppy, even though the histochemical properties showed similar to that of the fresh water guppy, the intensities of the staining of the sialomucin and sulfomucin were reduced as compare with that of the fresh water (Fig. 10, e-h).



Figs. 1-4. Scanning electronmicrographs of the guppy (*Poecilia reticulatus*) gill.
Fig. 1. The primary lamellae (arrows) projected from the gill arch (A). Bar = 100 μm.
Fig. 2. The secondary lamellae (arrows) extruded from the primary lamella (PL). Bar = 100 μm.
Fig. 3. The secondary lamellae (arrows) and the surface of the primary lamella. The ridges of pavement cells (PC) are well observed and the opening of the mucous cells(arrowheads) are shown between pavement cells. Bar = 1 μm.
Fig. 4. High magnification of the surface of primary lamella. Notice the opening of the mucous cells(arrowheads). Bar = 1 μm.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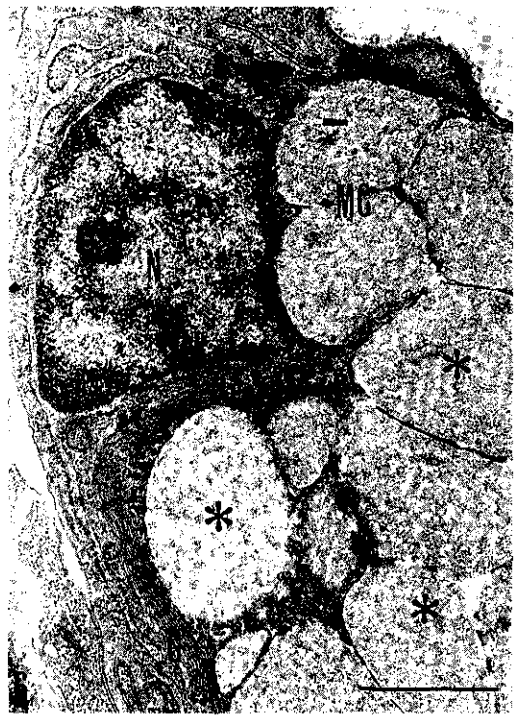
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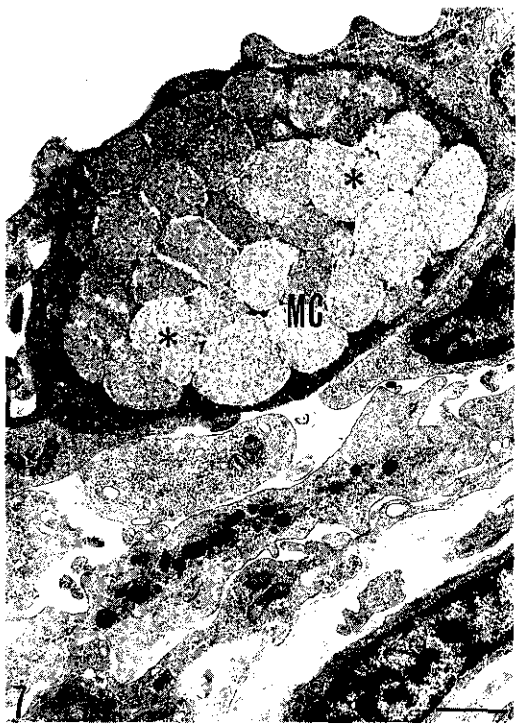
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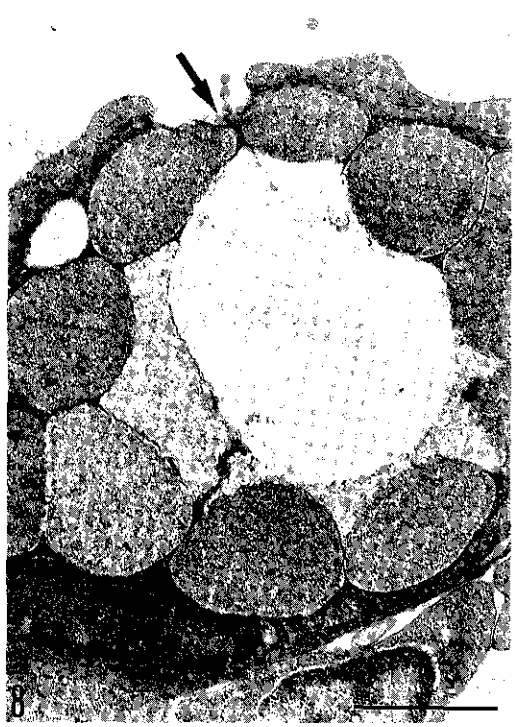
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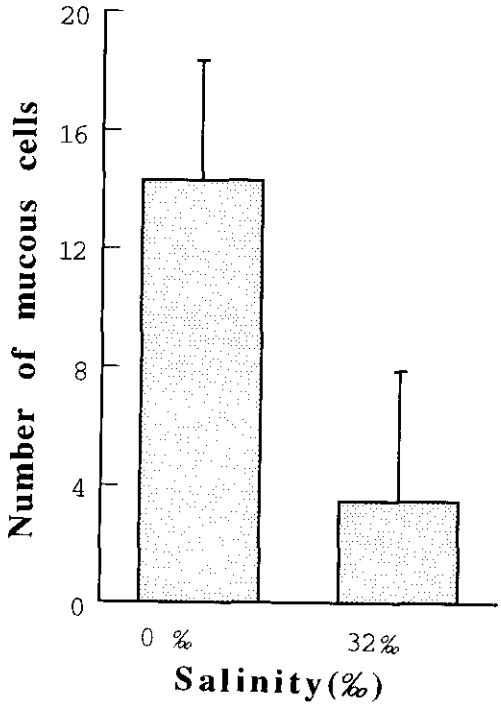


Fig. 9. The number of mucous cells per 100 secondary gill fillaments in the freshwater-and seawater-adapted guppy (*Poecilia reticulatus*).

Discussion

The gill of fresh water teleost is an important organ which has functions of gas exchange and control of ionic strength. Recently, the epithelial surface of gill has been proved to have an important role in the respiration, especially in the excretion of CO_2 and ammonia (Piiper *et al.*, 1986; Wright *et al.*, 1989). The surface of fish gill is covered with mucus, and this substance acts as an ion exchange material absorbing H^+ (Handy *et al.*, 1989) contributing to the buffering function of the gill in its microenvironment (Playle and Wood, 1989).

Results of the present study showed that mucous cells in the gill of guppy were found mainly in the primary lamellae and were not found in the secondary lamellae. The distribution of the mucous cells in the gills of rainbow trout and eels was coincided with the present study (Yamada and Yokote, 1975; Fletcher *et al.*, 1976), while a few numbers of mucous cells were also observed in the secondary lamellae in flounder and plaice (Fletcher *et al.*, 1976). This suggests that the pattern of the distribution of mucous cells in the gill epithelium of fishes is different in various species.

Table 1. Histochmical reaction of the gill epithelial mucous cells in freshwater- and seawater-adapted guppy (*Poecilia reticulatus*)

	PAS	AB2.5	AB2.5-PAS	AF1.7-AB2.5
Freshwater-adapted guppy	2-3R	2-3B	2-3B/2-3BP	2-3B/2-3BP
Seawater-adapted guppy	2R	2B	2-3RP/2-3BP	2B/2BP

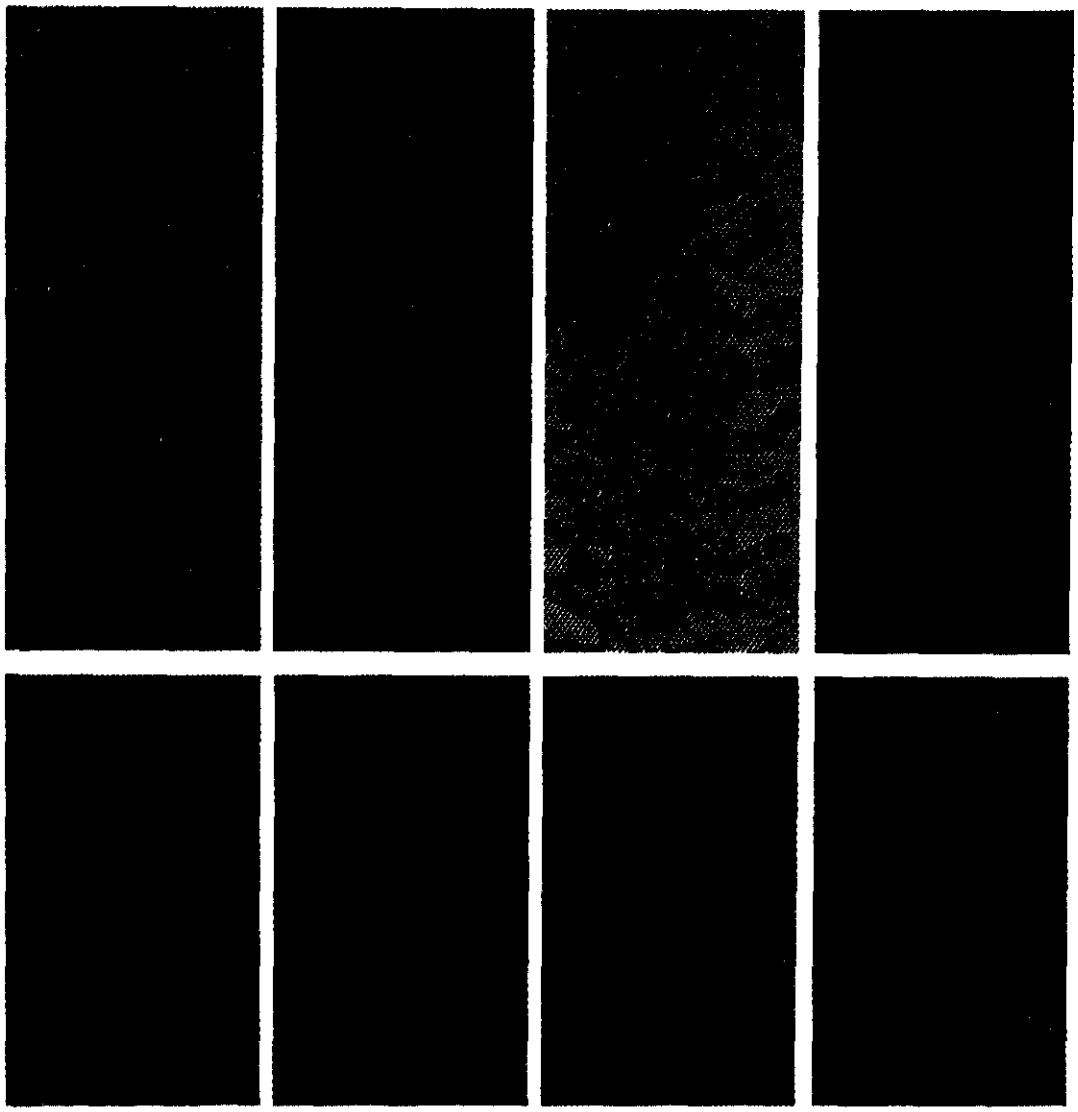
PAS (periodic acid-Schiff reaction), AB2.5 (alcian blue pH2.5), AB2.5-PAS (alcian blue pH2.5-periodic acid-Schiff reaction), AF1.7-AB2.5 (aldehyde fuchsin pH1.7-alcian blue pH2.5); Degree of staining: 3=intense, 2=moderate; Color abbreviation: R=red, RP=redpurple, BP=bluepurple, B=blue

Figs. 5-8. Transmission electronmicrographs of the mucous cells in the gill epithelium of the primary lamellae. Bars = 1 μm

Fig. 5. Immature mucous cell is seen in the primary epithelium and contains mucous granules (asterisk), rough endopoasmic reticulum (RER) and mitochondria (M) in the cytoplasm. The nucleus (N) is located in the center of the mucous cell and shows an irregular shape.

Figs. 6, 7. Fully matured mucous cell (MC) is observed near the surface. The cytoplasm is nearly filled with typical mucous granules (asterisk) which represent the various electron density. The nucleus(N) is located in the corner of the cytoplasm and Golgi complex (G) is remarkable.

Fig. 8. The opening of mucous secretion(arrow) is exposed to the surface.



Figs. 10 a-d. Parts of the gill of the freshwater guppy. The mucous cells are mainly distributed in the primary epithelium of the gill. The mucous cells show strong alcianophility (arrows) and weak fuchsinophility (arrowhead). × 500

Figs. 10 e-h. Parts of the gill of the seawater (32‰ salinity) adapted guppy. When the guppy is fully adapted to the seawater, alcianophility (arrow) and fuchsinophility (arrowhead) of the mucous cells are weakly appeared compared with those of the freshwater guppy. × 500 a, e: Periodic acid-Schiff (PAS) reaction b, f: Alcian blue pH 2.5 (AB 2.5) stain c, g: AB 2.5 - PAS reaction d, h: Aldehyde fuchsin pH 1.7 - AB 2.5 stain

The ultrastructure of the mucous cells in the gill epithelium has never been reported, and the structural studies of mucous cells in fish were achieved mostly in the epithelium of epidermis or digestive tracts (Mittal and Munshi, 1971; Zaccone, 1972; Harris and Hunt, 1973; Yamada and Yokote, 1975; Fletcher *et al.*, 1976; Schwerdtfeger, 1979). Harris and Hunt (1973) reported in their ultrastructural study of the mucous cells in the epidermis of atlantic salmon that the cells began to differentiate initially near the basal epidermis. Extensive development of

rough-endoplasmic reticulum and Golgi complex suggests that both these organelles are involved in the formation of mucus, which is deposited as clear vesicles increasing in number as the cells reach the periphery. Mature mucous cells appear distended with mucous vesicles as they reach the epidermal surface, whereupon their plasma membrane ruptures and the contents are released over the surface of the epidermis. Their reports are mostly coincided with the results of present study except that immature mucous cells were observed more frequently at the middle part of the gill epithelium instead of the basal portion.

In this study, it showed that the single mucous cell contained either neutral or acid glycoproteins alone or in combination. These results are coincided with those from the works of the mucous cells in other tissues (epidermis, gill, digestive duct, and oral epithelium, etc.) of the fish (Zaccone, 1972; Harris and Hunt, 1973; Fletcher *et al.*, 1976; Sikder and Das, 1981; Solanki and Benjamin, 1982; Zuchelkowski *et al.*, 1985; Mittal *et al.*, 1994).

Fletcher *et al.* (1976) reported that there were differences in the mucous components in mucous cells of gill epithelia from 3 species with different living environments. Their study showed that in the gill of plaice living in the sea water the number of the mucous cells containing acidic mucin was similar with that of containing neutral mucin, while in the rainbow trout which inhabits in the fresh water and flounder which lives in the estuary, more cells containing acidic mucin were observed. Sulfomucin which is an acid mucin were increased when the cells were infected with bacteria or fungi (Ojha and Munshi, 1974; Gona, 1979; Solanki and Benjamin, 1982; Ferguson *et al.*, 1992; Mittal *et al.*, 1994). Sulfomucin were found more frequently in the fresh fish than in the sea water fish because its strong electrical charge had an important role in the osmotic regulation in the fresh water by reacting easily with cations (Clamp *et al.*, 1978; Solanki and Benjamin, 1982). When the freshwater fish were adapted to the sea water, the shift of the mucous components were observed, from sulfomucin to sialomucin (Fletcher *et al.*, 1976; Hentschel and Muller, 1979; Solanki and Benjamin, 1982; Zuchelkowski *et al.*, 1985), from neutral mucin or sulfomucin to sialomucin (Gona, 1979; Zuchelkowski *et al.*, 1985). Generally, the number of mucous cells in the epidermis of sea water adapted fish were reduced (Solanki and Benjamin, 1982). In the present study, the contents of acid sialomucin and sulfomucin in the mucous cells were reduced during adaptation to the sea water, and the number of mucous cells was also decreased by 3 times. These results may be due to not only the reaction of guppy to the changed osmotic stress in the sea water but also the reduced chance of infection according to the high salt concentration in the sea water. This suggests that it needs more studies that the changes in the environmental condition of the fish affect the mucous substances both quantitatively and qualitatively.

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해수에 적응된 Guppy (*Poecilia reticulatus*) 아가미 점액세포의 미세구조 및 조직화학적 변화

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담수산과 해수에 적응된 guppy (*Poecilia reticulatus*) 아가미 상피내 점액세포의 미세구조를 주사 및 투과 전자현미경으로 관찰하고, 점액질 조성의 변화를 조직화학적으로 분석하여 다음의 결과를 얻었다.

점액세포는 아가미궁에서 돌출되어 형성된 일차층판상피에 주로 위치하였다. 미성숙점액세포에는 과립형질내세망과 Golgi 복합체가 잘 발달되어 있었으며, 성숙 점액세포는 거의 점액과립들로 가득 채워져 있었다. 담수산 guppy 아가미 상피내 점액세포의 점액질은 중성점액질과, 산성점액질인 sialomucin 및 sulfomucin을 다량 포함하고 있었으며, 바닷물에 적응시킨 경우에서는 sialomucin과 sulfomucin이 약간 감소하였다. 바닷물에 적응된 guppy의 아가미 상피내 점액세포의 수는 담수산과 비교하여 3배이상 감소하였다. 이는 서식환경을 해수로 옮긴 결과, 변화된 삼투적 스트레스에 대응한 결과일 뿐 아니라 담수보다는 높은 염분농도인 해수환경의 감소된 감염기회와도 연관된 결과라고 생각된다.