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Histological and Enzyme Histochemical Changes Found in the Renal Tubules of Eels Transferred from Freshwater to Seawater

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Abstract Renal tubules of the eel, *Anguilla japonica*, transferred from freshwater to seawater were examined histologically and histochemically. The epithelial cells of renal tubules in eels adapted to seawater for 10 days were less interdigitated in comparison with those in freshwater. The poor interdigitation of epithelial cells seems to be a structure relating to high water permeability in the renal tubules of eels in seawater. Newly formed nephrons and degenerated ones were especially recognized in eels adapted to seawater for 2 and 4 days. These findings indicate that some of the nephrons equipped with poorly interdigitated epithelial cells are produced newly within a few days after transfer from freshwater to seawater. No significant difference was encountered in the activities and localizations of all enzymes examined between the renal tubules of freshwater and seawater eels. The enzyme activities and localizations did not reflect the physiological changes in the renal tubules of eels adapted from freshwater to seawater.

During adaptation from freshwater to seawater, euryhaline teleosts decrease the amount of urine to maintain the osmotic pressure and water of their body fluids (Sharratt *et al.*, 1964; Stanley and Fleming, 1964; Chester Jones *et al.*, 1969; Hickman and Trump, 1969). The reduction of urine amount is perfomred by two phases (Oide and Utida, 1968; Hickman and Trump, 1969). The first phase is the reduction of glomerular filtration rate which takes place immediately after the transfer from freshwater to seawater. In the second phase, water permeability and excretion of divalent ions are increased in the renal tubules, which takes a few days before completion.

The 1st phase has been histologically examined by some investigators (Ogawa, 1968; de Ruiter, 1980). According to de Ruiter (1980), glomeruli become smaller, the lumina of glomerular capillaries decrease in diameter, and the number and size of the endothelial fenestration in the capillaries are reduced. These changes decrease the glomerular filtration rate. However, the 2nd phase remains unknown. The present study was undertaken to examine histologically and enzyme histochemically the renal tubules of eels transferred from freshwater to seawater.

Materials and methods

Twenty-five specimens of the Japanese eel (*Anguilla japonica* Temminck et Schlegel, body weight 140–208 g) were used. Five individuals each were sacrificed every 2, 4, 7, and 10 days after transferring from freshwater to seawater. The remainder (5 eels) were used as the freshwater control. Trunk kidneys of both series were removed and cut into appropriate pieces. Light, scanning electron microscopical, and histochemical examinations were performed on the specimens of the freshwater control eels and eels adapted to the seawater for 10 days, whereas eels adapted to the seawater for 2, 4, and 7 days were examined only by light microscopy.

The samples processed for histochemical examinations were divided into two groups. One group was immediately frozen by precooled dry ice-acetone. Sections (10 μm thick) were cut in a cryostat and immersed in the following reaction media: 1) malate, isocitrate, and β-hydroxybutyrate dehydrogenase reactions by the method of Hess *et al.* (1958), 2) Na-K-ATPase reaction modified for demonstration by light microscopy with the method of Mayahara *et al.* (1979), 3) aldolase reaction by the method of Abe and Shimizu (1964), 4) Ca-ATPase reaction by the method of Padykula and Herman (1955). The other group was fixed in 1.0% glutaraldehyde buffered with 0.1 M sodium phosphate (pH 7.4) at 4°C for 1 hr and subsequently embedded in water soluble resin JB-4 (Polysciences, Inc., Warrington, Pennsylvania) as described by Dobyan *et al.* (1982).

Sections (3 μm thick) were cut and then incubated in reaction media for the following enzymes: 1) carbonic anhydrase by the method of Hansson (1968), 2) acid phosphatase by the method of Gomori (1950). Incubation for all reactions was performed at 30°C for 10–30 min. Control sections for all staining except for Na-K-ATPase and carbonic anhydrase were prepared by immersion in incubation media lacking specific substrates. Control sections for Na-K-ATPase were exposed to 10 mM ouabain added to incubating media. Specificity of carbonic anhydrase activity was confirmed by adding 1 mM acetazolamide to the incubating media.

The specimens processed for histological observations were fixed in Bouin's solution and 1.0% gultaraldehyde buffered in 0.1 M sodium phosphate (pH 7.4). The specimens fixed in glutaraldehyde were prepared as follows: 1) HCl-collagenase treatment of Evan *et al.* (1978), 2) cryofracture treatment of Tokunaga *et al.* (1974). Then, these specimens were postfixed by phosphate buffered 1.0% osmium tetroxide. They were dried by a critical point dryer (Hitachi HCP-2) with liquid CO_2 and were coated with gold in an ion sputter (JEOL JFC-1100). Observations were made by JEOL JSM T-20 scanning electron microscopy. The specimens immersed in Bouin's solution were embedded in paraffin, cut serially (10 μm thick), and stained with Mayer's hematoxylin and eosin.

Results

Histological observations. In eels in freshwater, the neck was short and composed of slightly basophilic, cuboidal cells with numerous cilia. The first proximal tubules had columnar and slightly eosinophilic epithelial cells. The apical surfaces of cells were covered with high microvilli and many cilia. In the second proximal tubules, the columnar and eosinophilic epithelial cells had small microvilli and a few cilia in the apical surfaces. Intermediate tubules were short. The epithelial cells were eosinophilic and cuboidal. The apical surfaces of cells were provided with cilia. The distal tubules consisted of the slightly eosinophilic, cuboidal cells with very small microvilli. The initial collecting tubules had slightly eosinophilic, columnar epithelial cells with very small microvilli and slightly thick basal membrane. The epithelial cells of every tubule except for neck and intermediate tubules were deeply interdigitated in the lateral surfaces and had wide intercellular spaces (Fig. 1). Therefore, the basolateral portions of neighboring cells could be recognized between the wide intercellular spaces (Fig. 2).

Both new neprhons in the process of formation and degenerated nephrons were observed in eels adapted to seawater for 2, 4, 7, and 10 days. In the new nephrons of formation, the glomeruli, very small in diameter, had highly basophilic cells, and were not well vascularized (Fig. 5). The epithelial cells of the renal tubules were highly basophilic and their diameters were very small (Fig. 6). Mitoses were often recognized in the epithelial cells. The proportion of new neprhons to the old ones containing the degenerated ones was 3–18: 100, 2–9: 100, 0–7: 100, and 0–1: 100 in eels adapted to seawater for 2, 4, 7, and 10 days, respectively. In the degenerated nephrons, the mesangial cells and capillary walls of glomeruli were swollen. Disappearance of microvilli, development of vacuolation and edema, and pycnosis of nuclei occurred in the epithelial cells of degenerated renal tubules. Necrotized nephrons were phagocytized by large numbers of granulocytes (Figs. 7, 8). The proportion of degenerated nephrons to normal ones except for new ones was 3–13: 100, 4–21: 100, 4–5: 100, and 0–3: 100 in the eel adapted to seawater for 2, 4, 7, and 10 days, respectively.

In eels adapted to seawater for 10 days, the morphology of renal tubules is similar to that of freshwater eels except for the diameters of renal tubules, interdigitation and intercellular spaces of their epithelial cells. The renal tubules decreased in diameter to about 90% compared to those in freshwater. The interdigitation and intercellular spaces of cells were less developed in the seawater eel than freshwater examples (Figs. 3, 4). These morphological changes occurred prominently in the terminal portions of 1st proximal tubules and initial portions of 2nd proximal tubules.

Enzyme histochemical observations. In the renal tubules of eels in freshwater acid phosphatase activity appeared in the 1st proximal tubules with intense activity. Weak Ca-ATPase activity was observed in the 2nd proximal tubules. The distal and initial collecting tubules exhibited weak Na-K-ATPase activity. Weak carbonic anhydrase activity occurred in the distal and initial collecting

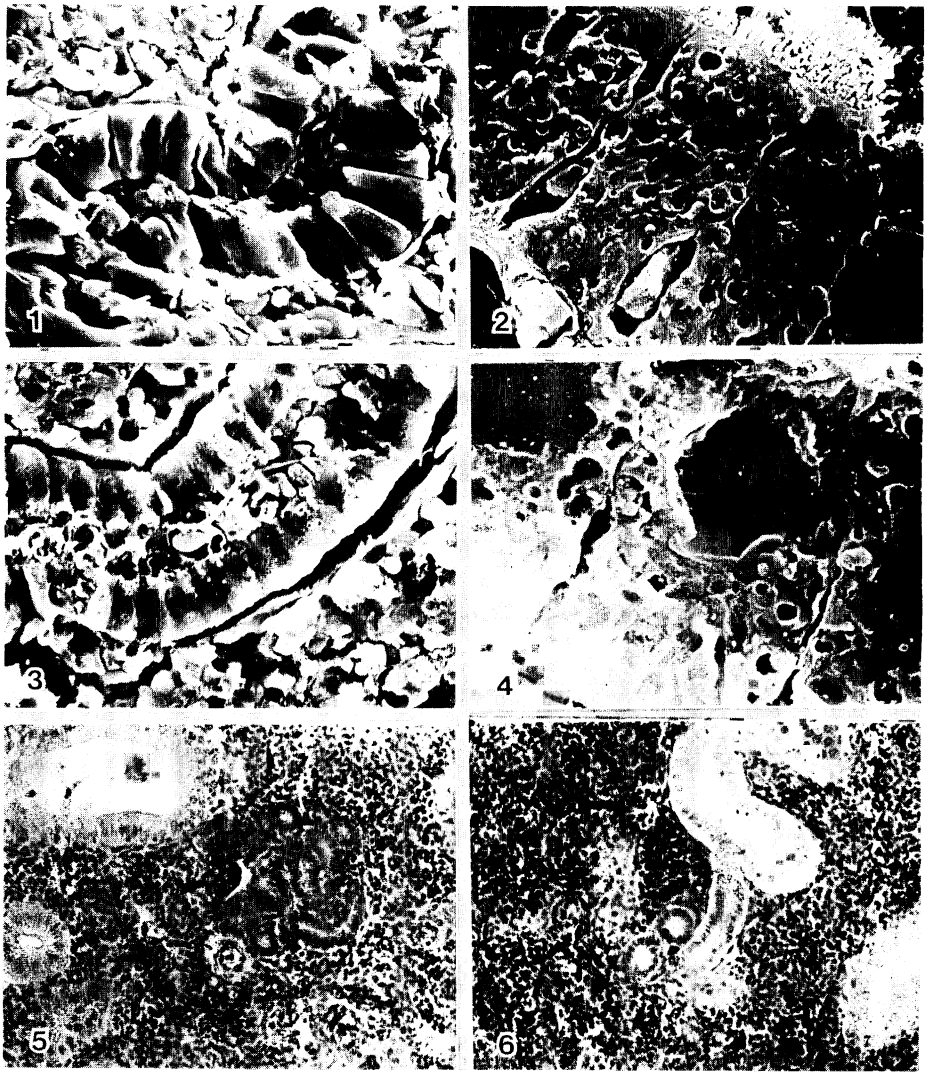

Fig. 1. Second proximal tubule in eel (*Anguilla japonica*) of freshwater control. The epithelial cells are deeply folded in the lateral surfaces. ×670.

Fig. 2. Second proximal tubule in the eel of freshwater control. The baso-lateral portions of neighboring cells are observed between the wide intercellular spaces. ×3480.

Fig. 3. Second proximal tubule in eel adapted to seawater for 10 days. The epithelial cells are poorly folded in the lateral surfaces. ×680.

Fig. 4. Second proximal tubule in the eel adapted to seawater for 10 days. The intercellular spaces of the epithelial cells are narrow. ×5040.

Fig. 5. Newly produced glomerulus (arrow) in the eel adapted to seawater for 2 days. The glomerulus is small and not so vascularized. ×160.

Fig. 6. Newly produced renal tubule in the eel adapted to seawater for 2 days. The renal tubule is very slender and the epithelia are basophilic. ×160.

tubules, but the other tubules had very weak to none activity (Fig. 9).

Intense malate dehydrogenase activity appeared in the distal and initial collecting tubules. The other tubules showed moderate to weak activity of this reaction (Fig. 10). The distributions of isocitrate and β-hydroxybutyrate dehydrogenases resembled that of the malate dehydrogenase, al-

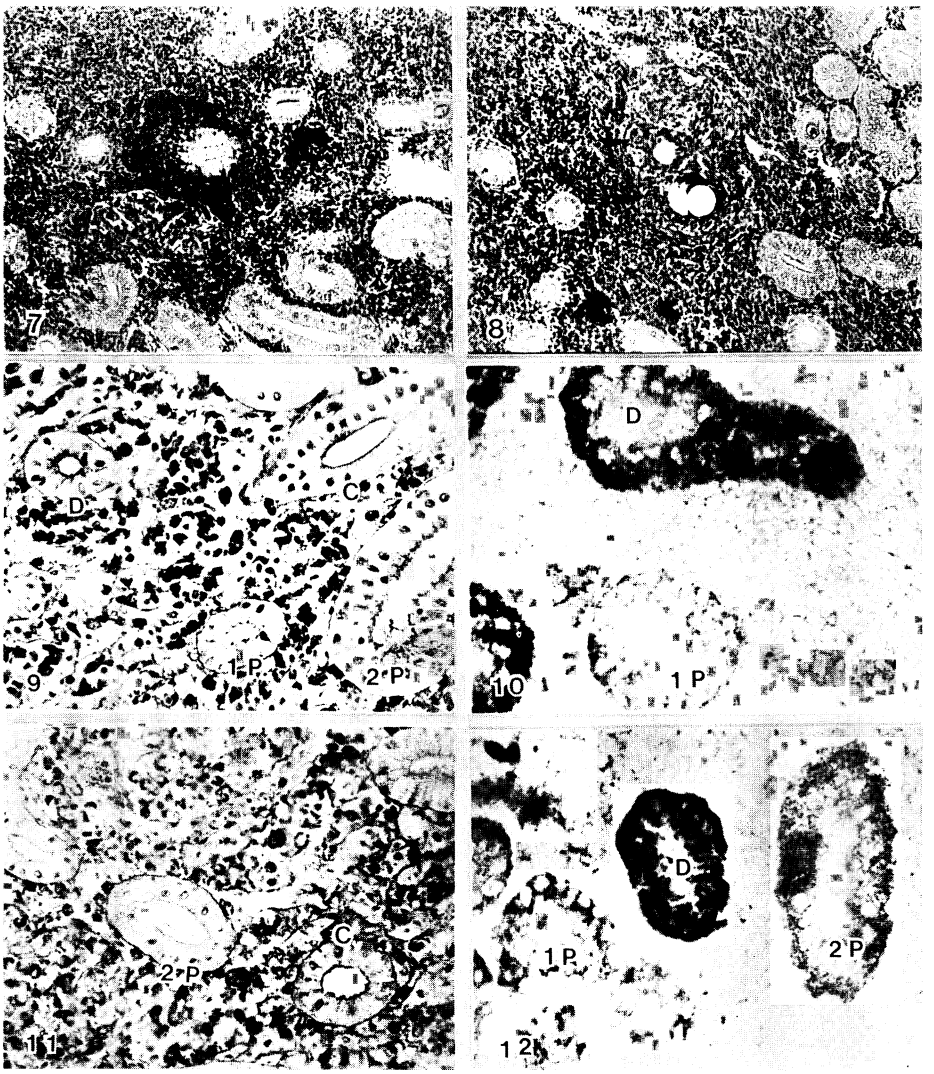

Fig. 7. Necrotized renal tubule in the eel adapted to seawater for 4 days. The renal tubule is phagocytized by large numbers of granulocytes. ×80.

Fig. 8. Necrotized glomeruli (arrows) in the eel adapted to seawater for 4 days. The glomeruli are phagocytized by large numbers of granulocytes. ×80.

Fig. 9. Carbonic anhydrase reaction in the freshwater eel. The weak activity is seen in the distal and initial collecting tubules. 1P, first proximal tubule; 2P, second proximal tubule; C, initial collecting tubule; D, distal tubule. ×160.

Fig. 10. Malate dehydrogenase reaction in the freshwater eel. The distal tubule exhibits the intense activity, but the activity is weak in the first proximal tubule. ×320.

Fig. 11. Carbonic anhydrase reaction in the eel adapted to seawater for 10 days. The intensity of activity is similar to that of the freshwater eel. ×160.

Fig. 12. Malate dehydrogenase reaction in the eel adapted to seawater for 10 days. The intensity of activity is similar to that of the freshwater eel. ×320.

though the activities of both dehydrogenases were weaker than the malate dehydrogenase. Weak aldolase activity was displayed in the neck, 1st and 2nd proximal, and intermediate tubules. The other tubules showed very weak to none activity.

The patterns of enzyme distributions and

activities in eels adapted to seawater for 10 days were common to those in freshwater (Figs. 11, 12).

Discussion

The present examination reveals that interdigitation and intercellular spaces of epithelial cells were poorer in the renal tubules of eels adapted to seawater for 10 days than in those in freshwater. The decrease in diameter of the renal tubules in eels in seawater is probably due to their poor interdigitation and intercellular spaces. The structural differences of the epithelial cells are perhaps correlated to an increase in water permeability, as in mammalian Henle's loops sodium is transported in the heavily interdigitated cells forming wide intercellular spaces, whereas the simple shaped cells with few interdigitation are consistent with the free water permeability (Dieterich *et al.*, 1975; Barrett *et al.*, 1978; Nagle *et al.*, 1981; Bachmann and Kriz, 1982).

In eels adapting to seawater, new nephrons in the process of formation were recognizable. This finding agrees with that of eels in freshwater injected with prolactine reported by Olivereau and Lemoine (1968). Degenerated neprhons were also observed in eels adapted to seawater. These findings indicate that some of the renal tubules having poorly interdigitated cells in eels in seawater may be newly produced during the adaptation to seawater. The new formation of nephrons almost takes place within a few days after transfer to seawater, because the nephrons in the process of formation and degenerated ones are primarily observed in eels adapted to seawater for 2 and 4 days.

Enzyme distributions and activities of eel nephrons except for Na-K-ATPase and carbonic anhydrase are closely similar to those of freshwater teleostean nephrons (Endo and Kimura, 1982, 1984). The activity of Na-K-ATPase is more weaker than the freshwater teleosts, although both resemble in the distributions (Endo and Kimura, 1982). The activity and distribution of carbonic anhydrase reaction are different from the freshwater teleosts. The carbonic anhydrase reaction occurred in the distal and initial collecting tubules showed very weak activity, although freshwater teleostean nephrons usually have the very intense activity in the proximal tubules (Endo and Kimura, 1984).

These patterns of enzyme distributions and activities were constant even when the eel were adapted to seawater for 10 days. The renal tubule of freshwater fish reabsorbs sodium actively and eliminates water, whereas the renal tubule of seawater fish reabsorbs water and secretes divalent ions (Hickman and Trump, 1969; Nishimura and Imai, 1982; Nishimura *et al.*, 1983). Although these physiological changes must occur in the renal tubules of eels transferred from freshwater to seawater, they were invalidated by the enzyme distributions and activities examined in the present study.

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淡水から海水へ適応させたウナギに見られた尿細管の組織学的・酵素組織化学的変化

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淡水ウナギと海水へ 2, 4, 7 および 10 日間適応させたウナギ尿細管を組織学的ならびに酵素組織化学的に検討した。

淡水ウナギの尿細管上皮細胞には，嵌合がよく発達し広い細胞間隙が見られる．一方，海水に 10 日間適応させたウナギの尿細管は，嵌合の発達の貧弱な上皮細胞から構成されている．このような形態上の差は，水の透過性の増加と関連があるものと考えられる．

海水適応の過程で，新生されたと思われるネフロンと変性したネフロンとが多数見られた．海水に 10 日間適応させたウナギのネフロンの中には，適応中に新生されたものもあると見なされる．

淡水ウナギと海水に 10 日間適応させたウナギの尿細管における各種の酵素活性を調べたところ，その分布や強さには差が認められなかった．

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