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Structures and Functions of Segments in Some Teleostean Nephrons

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Abstract The nephrons of four species of teleosts were examined histologically and histochemically for enzymes. In the carp the epithelial cells on every segment were deeply interdigitated with adjacent cells. The epithelial cells seem to exhibit high sodium permeability. Na-K-ATPase activity was localized in the distal tubules. Active transport of sodium seems to take place in the distal tubules, whereas the first and second proximal tubules may passively absorb the sodium. The renal tubules of marine teleosts are probably permeable to water, because the epithelial cells showed the poor interdigitations. Macromolecular materials are expected to be absorbed in the 1st proximal tubules of the carp, a marine catfish, and the yellowtail, but not for a frogfish, because the segments of the former three fishes exhibited intense activity of acid phosphatase and had pinocytotic invaginations in their cell apex. Distribution of carbonic anhydrase in the 1st and 2nd proximal tubules of the carp and yellowtail indicates that the secretion of hydrogen ion is performed in the 1st and 2nd proximal tubules. In the marine catfish, on the other hand, the distal tubules are expected to secrete the hydrogen ion from the localization of carbonic anhydrase in these regions.

Freshwater teleosts are hyperosmotic to the external medium and in danger of excess water inflow. Elimination of water and conservation of salt are a primary function of the freshwater teleostean nephrons. The nephron consists of a glomerulus, neck, first proximal tubule, second proximal tubule, distal tubule, and initial collecting tubule (Edwards, 1929, 1935; Marshall, 1934; Ogawa, 1962). The glomerulus filters water, salt, and so on from the blood stream, which is assisted by blood pressure (Hickman and Trump, 1969). The distal tubule is the principal site for active absorption of salt (Endo and Kimura, 1982; Nishimura and Imai, 1982).

When ancestral teleosts were adapting to seawater, they faced salt inflow. Therefore, they must have conserved water and excreted salt. Structural modifications of the nephrons may solve this problem. One modification is the loss or shortening of the distal tubule (Marshall, 1934; Ogawa, 1959). Another modification is poor vascularization of glomerulus in many marine teleosts and aglomerularism in some others (Marshall, 1934; Edwards, 1935; Ogawa, 1958; Elger and Hentschel, 1981).

In mammalian nephrons, the some functions of segments have been suggested by the structures and enzyme distributions (Ericsson, 1965; Ernst, 1975; Bachmann and Kriz, 1982; Dobyan et al., 1982). Although many studies on the teleostean nephrons have revealed the structure and modifications correlating with the habitats of fishes, few functional analyses on every segment have been examined in the previous studies. The present study, thus, examined the teleostean nephrons histologically and enzyme histochemically.

Materials and methods

Three specimens of the carp, *Cyprinus carpio* (body weight 83–130 g), two specimens of the yellowtail, *Seriola quinqueradiata* (body weight 750–1,150 g), four specimens of a marine catfish, *Plotosus lineatus* (body weight 15–84 g), and three specimens of a frogfish, *Phrynelox tridens* (body weight 49–102 g), were used in this study. The animals were perfused via bulbus arteriosus with physiological saline solution and then with 1.0% glutaraldehyde buffered in 0.1 M sodium phosphate (pH 7.4). The trunk kidneys were removed from these specimens and cut into pieces in appropriate size.

The samples processed for light microscopical examinations were fixed by Bouin's solution and embedded in paraffin. Serial sections (10 μm

thick) were cut and stained with Mayer's hematoxylin and eosin. The specimens processed for scanning electron microscopical examinations were immersed in buffered 1.0% glutaraldehyde and prepared as follows: 1) HCl-collagenase treatment method of Evan et al. (1978), 2) cryofracture treatment method of Tokunaga et al. (1974). Afterward, 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 the aid of JEOL JSM T-20 scanning electron microscope.

The tissue pieces processed for histochemical examinations were divided into two groups. One was frozen by precooled dryice acetone and sections (10 μm thick) were cut in a cryostat for examining intensity of enzyme activity. The other was embedded in water-soluble resin JB-4 (Polysciences, Inc., Warrington, Pennsylvania) as described by Dobyan et al. (1982) and sections (3 μm thick) were cut on a microtome for observing enzyme localization precisely. The cryostat and resin sections were immersed in appropriate incubating media at 25°C for 20 min. The histochemical techniques for enzymes included the following demonstrations: 1) Na-K-ATPase reaction modified for examination by a light microscope by the method of Mayahara et al. (1979), 2) acid phosphatase reaction by the method of Gomori (1950), 3) carbonic anhydrase reaction by the method of Hansson (1968). Control sections for acid phosphatase were prepared by immersion in incubating media which lacked specific substrate. Control sectons for Na-K-ATPase were exposed to 10 mM ouabain added to the incubating media. Specificity of carbonic anhydrase activity was confirmed by adding 1 mM acetazolamide to the incubating media.

Results

Carp. The glomeruli were well vascularized and the outer surfaces were covered with primary and secondary processes of podocytes (Fig. 1). In parietal walls of Bowman's capsules, the flat epithelial cells with solitary cilia were delimited by marginal microvilli (Fig. 2). The necks were short and of small diameters (13–20 μm). The epithelium consisted of basophilic and low cuboidal cells with numerous cilia. The diameters of lumens were small (3 μm). The nuclei were round. The first proximal tubules were long. The diameters of these tubules and the lumens became larger toward the second proximal tubules (20–58 μm in tubule diameters, 10–23 μm in lumen diameters). The epithelia were lined with slightly eosinophilic and columnar cells with high microvilli and many cilia (Fig. 3). Pinocytotic invaginations were recognized in the cell apex. The oval nuclei were localized in the basal portions of the cells. The second proximal tubules were very long and had large diameters (33–58 μm). The lumen diameters were large in the initial regions of these tubules (23 μm), whereas the central and terminal regions exhibited small lumen diameters (3 μm). The epithelial cells were columnar and eosinophilic, and contained many small mitochondria. The apical surfaces were provided with short microvilli and a few cilia. The round nuclei were observed in the middle portions of cells. Intermediate tubules were slender (18–23 μm) and short. These tubules were lined with slightly eosinophilic and cuboidal cells with numerous and long cilia. The diameters of lumens were small (3 μm). The nuclei were round. The distal tubules were very long and the diameters became larger toward the initial collecting tubules (25–40 μm). The epithelia were composed of slightly eosinophilic and cuboidal cells with numerous mitochondria. The mitochondria were of large sizes. Sparse and short microvilli were observed in the apical surfaces (Fig. 4). The lumen diameters in the proximal portions of the distal tubules had small and constant (5 μm), whereas in the distal portions became larger toward the initial collecting tubules (5–10 μm). The nuclei were round and located in the central portions of the cells. The initial collecting tubules had slightly thick basement membranes. The other morphological features were similar to those of the distal tubules. The two (sometimes one) distal tubules were joined to connect with the initial collecting tubule. The lateral surfaces of epithelial cells on every segment were heavily folded and thereby the epithelial cells were deeply interdigitated with adjacent cells (Fig. 5).

The acid phosphatase activity was moderate

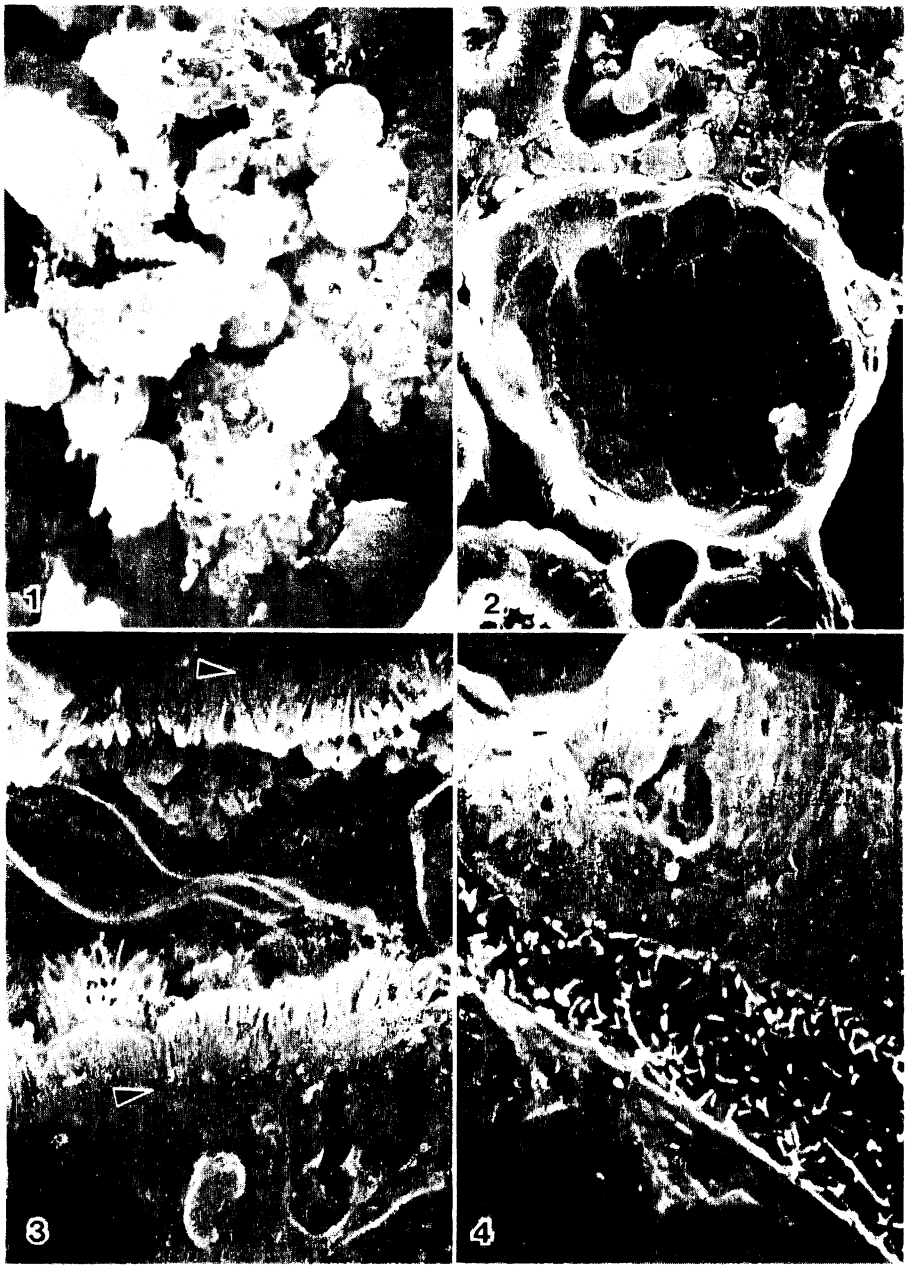

Fig. 1. Glomerulus of the carp (*Cyprinus carpio*). The surfaces of capillaries are covered with podocytes having fine primary and secondary processes. ×2,900.

Fig. 2. Parietal wall of the Bowman's capsule in the carp. Squamous epithelial cells with solitary cilia are delimited by marginal microvilli. ×800.

Fig. 3. The first proximal tubules of the carp. Microvilli and cilia cover the apical surfaces of epithelial cells. Pinocytotic invaginations (arrows) are observed in the cell apex. ×4,000.

Fig. 4. The distal tubule of the carp. The epithelial cells with large and many mitochondria are provided with sparse and short microvilli. ×4,300.

in the apical portions of the epithelial cells of the 1st proximal tubules and very low in the 2nd proximal tubules. The carbonic anhydrase activity was intense in the brush border of epithelial cells in the 1st and 2nd proximal tubules (Fig. 6). The Na-K-ATPase activity was intense in the apical portions of the epithelial cells of the distal and initial collecting tubules (Fig. 7).

Marine catfish. Although the glomeruli and capillary lumens were small, the other structures of renal corpuscles resembled those of the carp. The necks were short and slender (25 μm). The lumen diameters were small (5 μm). The cells of epithelium with oval nuclei were cuboidal, basophilic, and had long cilia. The first proximal tubules were very long. The tubule and lumen diameters became smaller toward the second proximal tubules (35–50 μm in tubule diameters, 5–25 μm in lumen diameters). The epithelium consisted of slightly eosinophilic and cuboidal cells with long microvilli and cilia. The density of cilia were higher than those of the carp. Pinocytotic invaginations were observed in the cell apex. The oval nuclei were sited in the middle of cells. The second proximal tubules were very long and of large diameters (45 μm). The epithelial cells were eosinophilic and columnar, and covered with short microvilli and a few cilia. The lumens were uniformly moderate diamters (15 μm). The nuclei were round and situated in the middle portions. The distal tubules were divided into two portions on the basis of diameters, slender and large portions. The slender portions had moderate length and surrounded the large portions. The diameters of slender portions were 30 μm and the lumen diameters were small (10 μm). The large portions were short and of large diameters (80 μm). The lumen diameters were large (25–30 μm). The epithelial cells were slightly eosinophilic and cuboidal, and contained small mitochondria. Finger print-like microridges and a single cilium covered the apical surfaces of cells (Fig. 8). The nuclei were round and seen in the middle portions of cells. The initial collecting tubules had thick basement membranes and were composed of eosinophilic and cuboidal epithelial cells with round nuclei. The cilia lacked in the apical surfaces of cells. The other morphological features were common to those of the distal tubules. Single distal tubule attached directly to the collecting tubules. The epithelial cells on every segment were not interdigitated between adjacent cells.

The acid phosphatase activity was moderate in the apical portions of the epithelial cells of the 1st proximal, and low in the 2nd proximal tubules. The carbonic anhydrase activity was moderate in the apical and lateral portions of the epithelial cells of the distal and initial collecting tubules (Fig. 9). The Na-K-ATPase activity was very low in the apical portions of the epithelial cells of the slender portions of distal tubules.

Yellowtail. The glomeruli and the lumen diameters of capillaries were small. The primary and secondary processes of the podocytes were larger than those of the carp (Fig. 10). The necks were short and of small diameters (23 μm). The diameters of lumens were small (8 μm). The cuboidal cells of epithelium were low and basophilic, and had many, long cilia. The nuclei were oval. The first proximal tubules were moderate length and had large diameters (40–50 μm). The slightly eosinophilic and columnar epithelial cells were covered with long microvilli and numerous cilia, and had the oval nuclei in the basal portions of cells. Many pinocytotic invaginations were seen in the cell apex. The lumen diameters became larger toward the second proximal tubules (10–20 μm). The second proximal tubules were very long. In the initial and terminal portions of these tubules, the tubules and lumen diameters were larger than those in the middle portions (tubule diameters: 50 μm in initial, 30 μm in middle, 40 μm in terminal; lumen diameters: 20 μm in initial, 10 μm in middle, 20 μm in terminal). The epithelia were composed of eosinophilic and columnar cells with short microvilli and a few cilia. Many and small mitochondria were observed in the epithelial cells. The nuclei were oval and located in the middle of cells. Initial collecting tubules had thick basement membranes. The epithelial cells were slightly eosinophilic and columnar, and contained many mitochondria. The apical surfaces were provided with short microvilli. The nuclei were round and located in the middle portions of cells. The lateral surfaces of the epithelial cells on

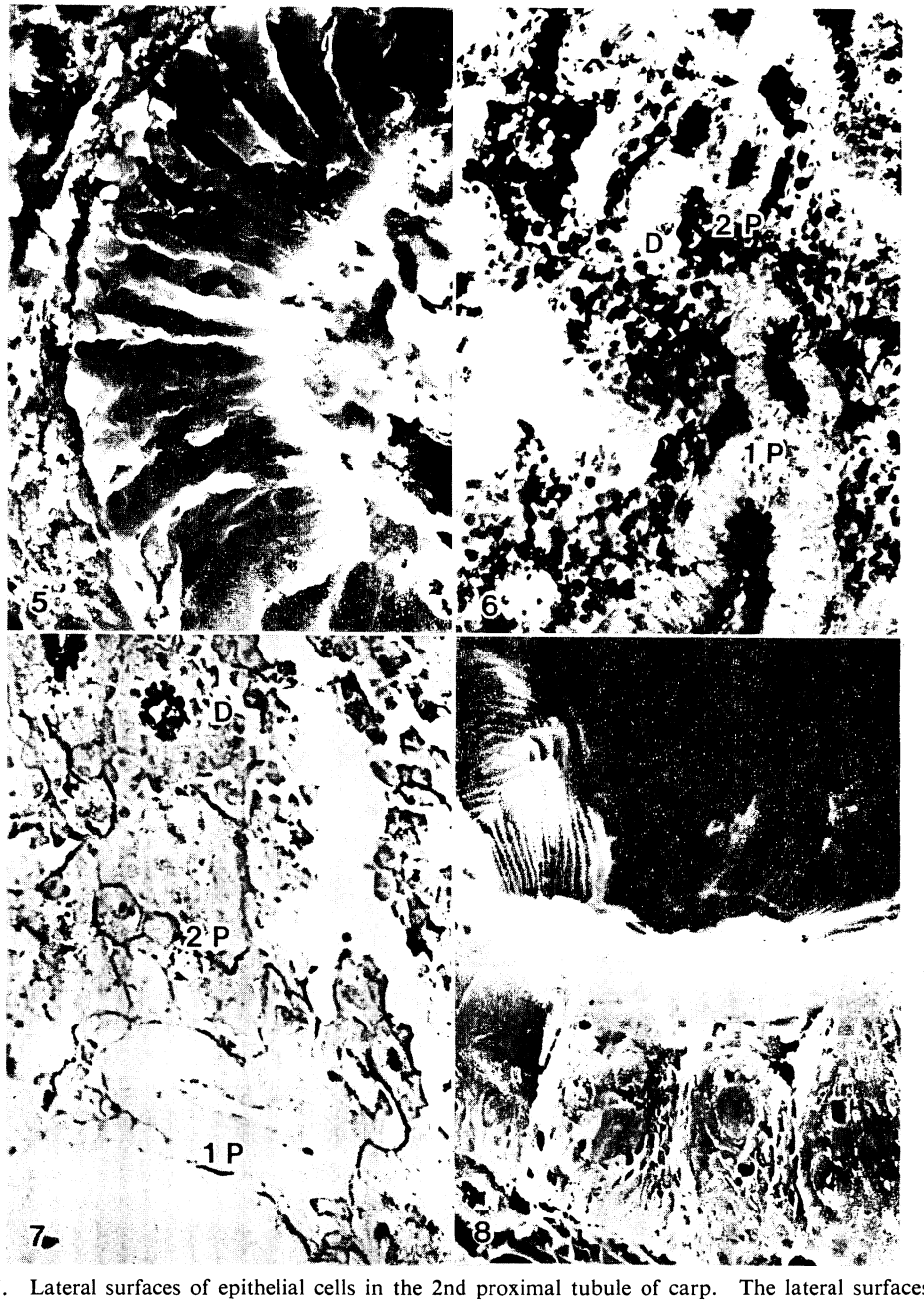

Fig. 5. Lateral surfaces of epithelial cells in the 2nd proximal tubule of carp. The lateral surfaces are heavily folded and interdigitated. ×1,700.

Fig. 6. Carbonic anhydrase reaction in the carp. The intense activity is localized in the brush border of the 1st (1P) and 2nd (2P) proximal tubules. Distal tubules (D) show no activity.

Fig. 7. Na-K-ATPase reaction of the carp. Intense activity is distributed in the distal tubule, whereas the 1st and 2nd proximal tubules do not exhibit the activity. Abbreviations as in Fig. 6. ×200.

Fig. 8. Distal tubule of the marine catfish (*Plotosus lineatus*). The epithelial cells are lined with finger-print-like microridges. ×3,500.

every segment were not folded like those in the carp (Fig. 11).

The acid phosphatase activity was intense in the apical portions of the epithelial cells of the 1st proximal tubules (Fig. 12) and low in the 2nd proximal tubules. The carbonic anhydrase activity was intense in the brush border of the 1st and 2nd proximal tubules. The Na-K-ATPase activity was very low in the apical portions of the epithelial cells of the initial collecting tubules.

Frogfish. The proximal tubules were long and slender (28 μm). The epithelial cells were cuboidal and eosinophilic, and covered with single cilium and very sparse and short microvilli. Round nuclei were seen in the middle of cells. The diameters of lumens were small (8 μm). The initial collecting tubules were lined with eosinophilic and cuboidal epithelial cells. Very sparse and short microvilli and a few cilia covered the apical surfaces. The round nuclei were sited in the middle portions of cells. The basement membranes were thin. Two or three proximal tubules converged to one initial collecting tubule. The epithelial cells of the proximal and initial collecting tubules were not interdigitated.

None of the reactions examined in the present study were demonstrated in neither the proximal nor initial collecting tubules.

Discussion

In the present study new morphological features of teleostean nephrons were recognized, although most of the findings agreed with the previous observations by many investigators (Edwards, 1929; Ogawa, 1959, 1962; Hickman and Trump, 1969; Elger and Hentschel, 1981; Endo and Kimura, 1982). The distal portions of the 1st proximal tubules and initial portions of the 2nd proximal tubules in the carp exhibited larger lumen diameters than the remaining portions of the 1st and 2nd proximal tubules. The structure of 1st and 2nd proximal tubules in the yellowtail resembled that of the carp, whereas in the marine catfish the initial portions of the 1st proximal tubules had larger lumen diameters than in the others. The large lumen diameters would produce slow urine flow and thereby facilitate the absorption of various materials to the surrounding epithelium.

Epithelial cells of every segment in the carp were heavily interdigitated between adjacent cells. The renal tubules of seawater teleosts were remarkably poor for the interdigitating epithelial cells. In mammalian Henle's loop, the deeply interdigitating epithelial cells are permeable to sodium, whereas the water passes through noninterdigitating epithelial cells (Nagle et al., 1981; Bachmann and Kriz, 1982). The carp renal tubules may be permeable to sodium whereas the renal tubules of seawater teleosts may be highly permeable to water. In freshwater teleostean nephrons, the distal tubules are the primary regions absorbing sodium actively (Endo and Kimura, 1982; Nishimura and Imai, 1982). The many and large mitochondria, and Na-K-ATPase activity of distal tubules of carp suggest the active transport of sodium in these regions. In the 1st and 2nd proximal tubules, on the other hand, the sodium absorption seems to be passive, because the Na-K-ATPase activity was not localized in these regions. Although the apical surfaces of carp distal tubules are lined with microvilli, in the marine catfish fingerprint-like microridges covered the cellular apices of distal tubules. The occurrence of microridges may be an adaptation owing to reduction of surface area.

Acid phosphatase activity and pinocytotic invaginations were primarily observed in the 1st proximal tubules of the carp, marine catfish, and the yellowtail, but not for the frogfish. In the former fishes, macromolecular materials seem to be absorbed in the 1st proximal tubules. The absorption of macromolecular materials in their regions is in accordance with the results of Hickman and Trump (1969). The brush border of 1st and 2nd proximal tubules of the carp and yellowtail showed the intense activity of carbonic anhydrase. The secretion of hydrogen ion may take place in both the proximal and distal tubules of mammals (Arruda and Kurtzman, 1981). However, the distribution of carbonic anhydrase in the carp and yellowtail suggests that the hydrogen ion is principally secreted in the 1st and 2nd proximal tubules. In the marine catfish the distal tubules seem to secrete the hydrogen ion, because the presence of carbonic anhydrase was demonstrated in these tubules.

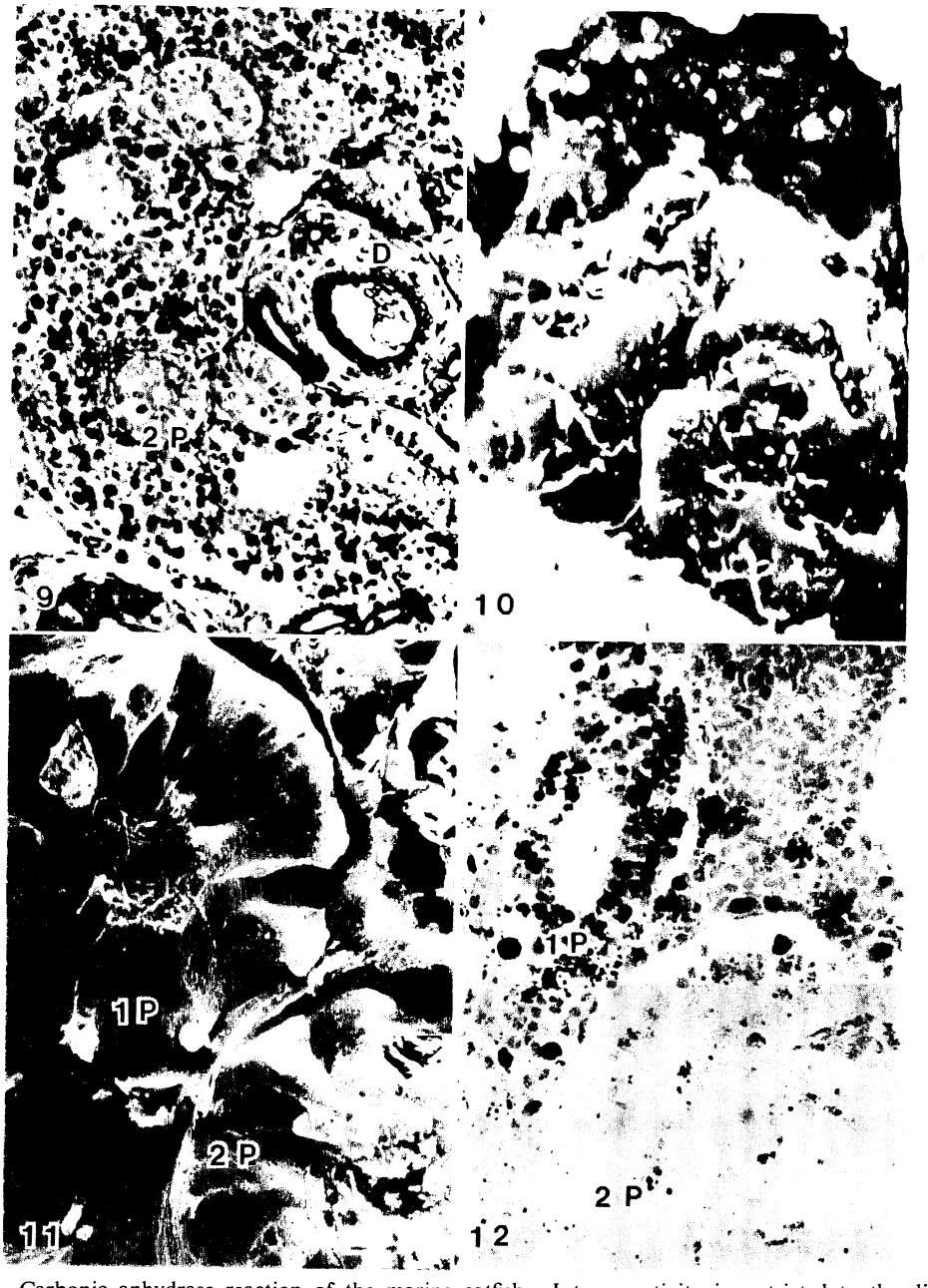

Fig. 9. Carbonic anhydrase reaction of the marine catfish. Intense activity is restricted to the distal tubules. Abbreviations as in Fig. 6. ×200.

Fig. 10. Glomerulus of the yellowtail (*Seriola quinqueradiata*). The podocytes have the larger primary and secondary processes than those of the carp. ×3,800.

Fig. 11. Lateral surfaces of epithelial cells in the 1st and 2nd proximal tubules of the yellowtail. The lateral surfaces are poorer than those of the carp. Abbreviations as in Fig. 6. ×1,800

Fig. 12. Acid phosphatase reaction of the yellowtail. The reaction is displayed in the 1st proximal tubule with intense activity but the 2nd proximal tubules show low activity. Abbreviations as in Fig. 6. ×200.

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硬骨魚ネフロンの各部位の構造と機能

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コイ，ゴンズイ，ブリおよびイザリウオのネフロンを組織学的，酵素組織化学的に調べた．

これら魚種の尿細管各部位とその内腔は一定の場所で太くあるいは細くなっていた．また，コイの尿細管上皮細胞は嵌合がよく発達していたが，ブリ，ゴンズイおよびイザリウオの尿細管上皮細胞の嵌合は発達が悪かった．Na-K-ATPase 活性はコイの遠位部で強く，ブリの集合管とゴンズイの遠位部には非常に低い活性が見られた．コイとブリの近位部第1節と第2節に強いカルボニックアンヒドラーゼ活性が分布していたが，ゴンズイでは遠位部と集合管にその中程度の活性が認められた．強い酸性フォスファターゼ活性がブリの近位第1節にあり，コイとゴンズイの近位部第1節は中程度の活性を持っていた．イザリウオのネフロンはいずれの酵素反応にも反応しなかった．これらの結果から尿細管の各部位の機能を推測した．

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