Japanese Journal of Ichthyology
Vol. 33, No. 3 1986

魚類学雑誌
33巻3号 1986年

Histological and Enzymatic Studies on the Nephron of the Lungfish, *Lepidosiren paradoxa*

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(Received October 30, 1985)

Abstract Nephron of South American lungfish was examined histologically and enzyme-histochemically. Cells of the first and second proximal segments exhibited poor interdigitation forming narrow intercellular spaces, whereas the distal segment consisted of deeply interdigitated cells with wide intercellular spaces. Activities of aerobic enzymes (malate, isocitrate, NADH, and β-hydroxybutyrate dehydrogenases), Na-K-ATPase, and carbonic anhydrase were mostly detected in the distal segment. In contrast, hexokinase activity was mostly seen in the 1st and 2nd proximal segments. In the collecting tubule, two types of cells were distinguished by their histological and enzyme-histochemical features. One type showed deep interdigitation and intense carbonic anhydrase activity. The other did not have heavy interdigitation and carbonic anhydrase activity. However, both cell type exhibited intense activities of aerobic enzymes. These structures and enzyme distributions in the lungfish nephron indicate that the lungfish is more specialized in nephron than teleosteans and elasmobranchs, though, slightly similar to the latter.

Dipnoan is an air-breathing fish and a relative of the fish from which the early amphibian is thought to have evolved. Therefore, it is necessary to study the nephron in considering the transition of renal function from the fish to amphibian. Nephrons of freshwater teleosts, marine elasmobranchs, and frogs have been examined histotopographically and enzyme-histochemically by some investigators (Bargmann and Welsch, 1972; Lönnerholm and Ridderstråle, 1974; Anderson and Loewen, 1975; Ridderstråle, 1976; Endo and Kimura, 1982a, b; Endo, 1984; Endo and Kimura, 1984). These reports have provided significant insights their renal functions.

The lungfish nephron has been histologically examined by some investigators (Bargmann, 1934; Guyton, 1935; Grafflin, 1937; Hickman and Trump, 1969). According to Guyton (1935), it is composed of a glomerulus, neck segment, first and second proximal segments, intermediate segment, distal segment, and a collecting tubule. However, the fine structures and enzyme distributions in each segment have not been discussed in previous reports. The present study will compare the fine structures and enzyme distributions in every segment of lungfish nephron with those of teleosts, elasmobranchs, and frogs.

Materials and methods

Three specimens of the South American lungfish *Lepidosiren paradoxa* (body weight 64–98 g, sexually immature) were purchased and used for histological examinations. The specimens were perfused via the heart with physiological saline solution followed by 1.0% glutaraldehyde buffered in 0.1 M sodium phosphate (pH 7.4). Kidneys were removed and cut into appropriate pieces. Samples processed for light microscopical surveys were postfixed in Bouin's solution, embedded in paraffin, sectioned at 5 μm thick, and stained with Mayer's hematoxylin and eosin.

Samples used for scanning electron microscopical observations were immersed in buffered 2.5% glutaraldehyde and then prepared as follows: 1) HCl-collagenase treatment by Evan *et al.* (1978), 2) cryofracture treatment using dimethylsulfoxide by Tokunaga *et al.* (1974). The specimens were dehydrated by graded alcohol series, immersed in isoamyl acetate, dried in a critical point dryer (Hitachi HCP-2) with liquid CO_2, and then coated with gold in an ion sputter (JEOL JFC-1100). Observations were performed by JEOL JSM T-20 scanning electron microscopy.

Other three specimens of lungfish (body weight 37–72 g) were used for enzyme-histochemical examinations. Kidneys were removed, cut into appropriate pieces, and divided into two groups. The first group was fixed in 1.0% glutaraldehyde buffered in 0.1 M sodium phosphate (pH 7.4) for 1 hour at 4°C and embedded in water soluble

resin JB-4 (Polysciences, Inc., Warrington, Pennsylvania) as described by Dobyan *et al.* (1982). Sections were cut, 3 μm in thick, and were incubated in the following reaction media: 1) acid phosphatase activity by the method of Gomori (1950), 2) carbonic anhydrase activity by the method of Hansson (1968).

The other group was immediately frozen by precooled dryice acetone. Sections were cut (10 μm in thick) in a cryostat and immersed in the following reaction media: 1) malate, isocitrate, β-hydroxybutyrate, and NADH dehydrogenase reactions by the method of Nachlas *et al.* (1958), 2) hexokinase reaction by the method of Meijer (1967), 3) Na-K-ATPase reaction modified for examination by light microscopy with the method of Mayahara *et al.* (1979). The incubations of all reactions except for carbonic anhydrase were performed for 20–30 min at 30°C. The carbonic anhydrase reaction was treated at room temperature for 10 min. Control sections of all dehydrogenase and acid phosphatase reactions were prepared by immersion in incubating media which lacked specific substrates. Control sections for Na-K-ATPase and carbonic anhydrase activities were exposed to reaction media in which respective inhibitors (10 mM ouabain and 1 mM acetazolamide) were added.

Results

Histological observation. The glomerulus was well vascularized and possessed a few mesangial cells (Fig. 1). The surface was compactly covered with podocytes having primary and secondary processes. In the Bowman's capsule, cells of the parietal wall had isolated cilia. The neck segment was slender and short. The epithelium was composed of eosinophilic and cuboidal cells with many cilia.

The first proximal segment was long and wide, and consisted of cuboidal and eosinophilic cells (Fig. 2). The apical surfaces of cells, covered with long microvilli and a few cilia, protruded into the lumen. The slightly eosinophilic and columnar cells of the secondary segment were provided with short microvilli and a few cilia on the apical surfaces. Lateral surfaces of cells of the 1st and 2nd proximal segments were poorly interdigitated and possessed sparse microvilli (Fig. 3). There were a few foot processes in the baso-lateral portions. In addition, these cells had developed junctional complexes and narrow intercellular spaces between adjacent cells (Fig. 4). The morphology of the intermediate and neck segments were similar.

The distal segment was composed of cuboidal and eosinophilic cells (Fig. 5). On the apical surfaces of cells, isolated cilia and short microvilli were found in the central and peripheral portions, respectively. The lateral surfaces of cells were deeply interdigitated and had dense microvilli (Fig. 6). These epithelial cells possessed poor junctional complexes and wide intercellular spaces between adjacent cells (Fig. 7). The collecting tubule consisted of deeply or poorly interdigitated cells (Fig. 8). Both cell structures, except for interdigitation, resembled those of the distal segment cell.

Enzyme-histochemical observation. Moderate Na-K-ATPase activity was observed in the cytoplasm of epithelial cells of the distal segment (Fig. 9). Other segments did not show such activity. Moderate to weak activity of carbonic anhydrase appeared in the lateral and basal membranes of cells of the 1st proximal segment and the apical and basal portions of cells of the 2nd proximal segment. The epithelial cells of the distal segment exhibited intense activity in their cytoplasm. In the collecting tubule, the deeply interdigitated cells possessed the intense activity in their cytoplasm as the distal segment cells. On the other hand, poorly interdigitated cells displayed activity in the basal membranes, but not in the cytoplasm (Fig. 10). Intense to weak activity of acid phosphatase was observed in the apical portions of cells of the 1st proximal segment. The 2nd proximal segment showed very weak activity, and no activity appeared at all in the other segments.

Intense activities of malate, isocitrate, β-hydroxybutyrate, and NADH dehydrogenases were seen in the epithelia of the distal segment and collecting tubule (Figs. 11, 12). Weak activity appeared in the 1st and 2nd proximal segments, and the neck intermediate segments exhibited very weak activity. Moderate hexokinase activity occurred in the epithelial cells of 2nd proximal segment. The 1st proximal segment had weak activity, and the distal segment and collecting tubule showed very weak activity. Activity was absent in the neck and intermediate segments. The glomerulus was negative to all the reactions.

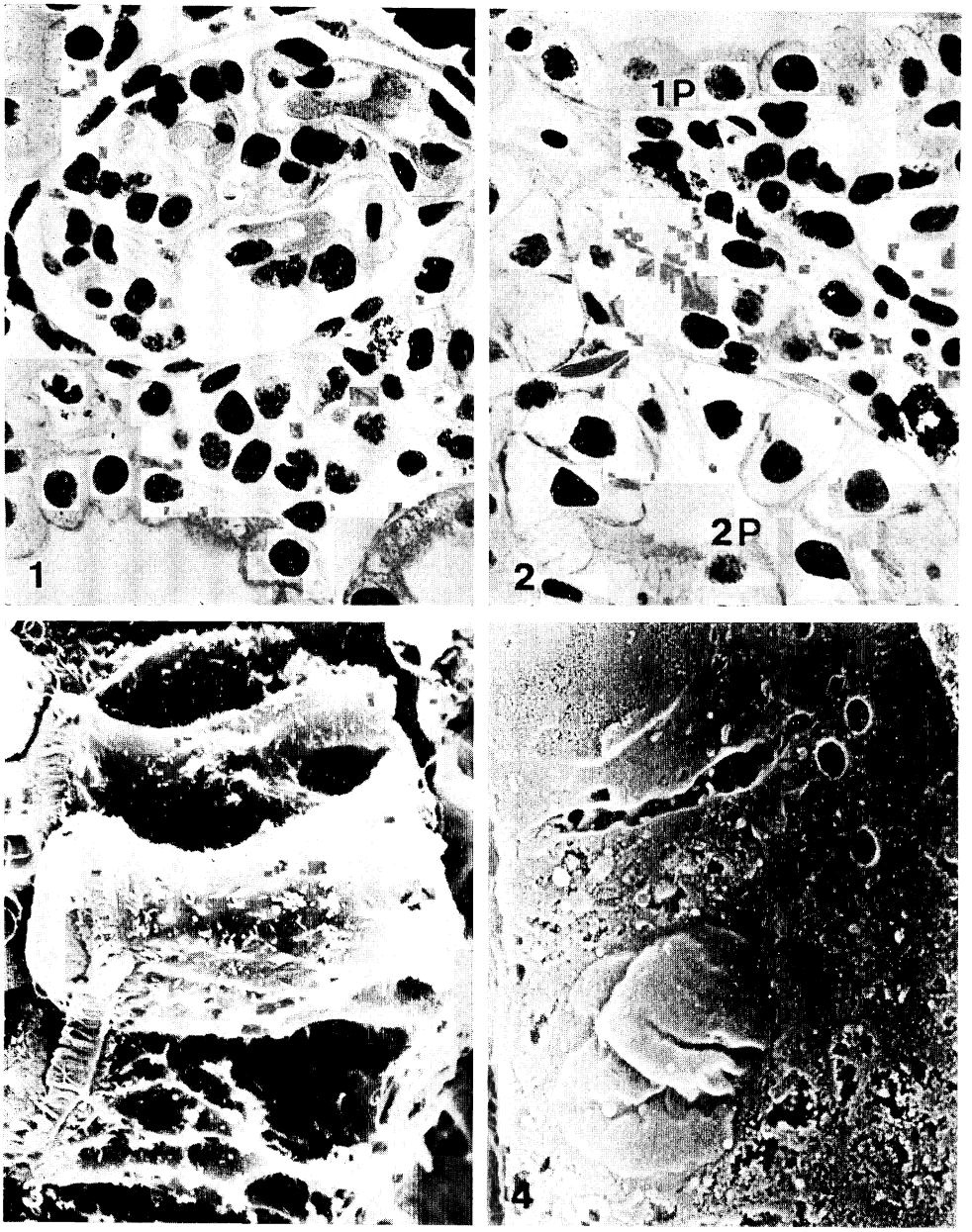

Fig. 1. Glomerulus and neck segment of the South American lungfish *Lepidosiren paradoxa*. The glomerulus is well vascularized and has a few mesangial cells. The neck segment consists of cuboidal cells with round nuclei. G, glomerulus; N, neck. ×350.

Fig. 2. First and second proximal segments of the lungfish. In the 1st proximal segment, the epithelial cells protrude slightly into the lumen. The epithelial cells of 2nd proximal segments show highly cuboidal to columnar shapes. 1P, first proximal segment; 2P, second proximal segment. ×350.

Fig. 3. Lateral surfaces of the cells in 2nd proximal segment. They have sparse microvilli and are slightly interdigitated between adjacent cells. There are a few processes in the lateral-basal portions of cells. ×1900.

Fig. 4. The epithelial cell of 2nd proximal segment. The narrow intercellular space and developed junctional complex between adjacent cells are recognizable. ×4600.

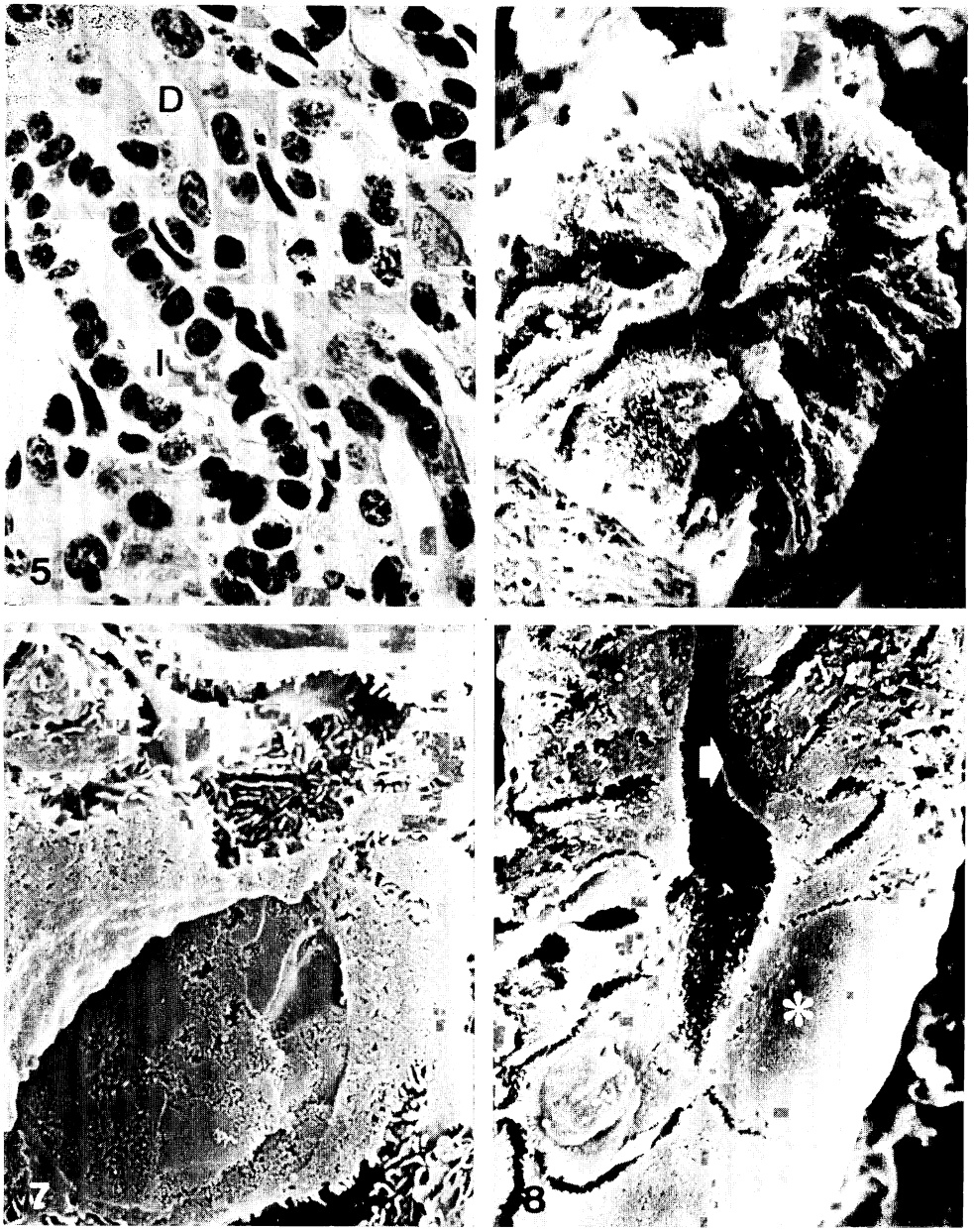

Fig. 5. Intermediate and distal segments in the lungfish. The distal segments are composed of cuboidal cells without brush border and cilia. In the intermediate segment, the cells have many cilia on the apical surfaces. D, distal segment; I, intermediate segment. ×350.

Fig. 6. Lateral surfaces of cells in the distal segment. They have dense microvilli and are heavily interdigitated between adjacent cells. ×2200.

Fig. 7. The epithelial cell of distal segment. The wide intercellular spaces and poor junctional complex are seen between adjacent cells. ×4800.

Fig. 8. Two types of cells in the collecting tubule. One cell type (arrow) is deeply interdigitated between neighboring cells, whereas another (*) does not show interdigitation. ×1900.

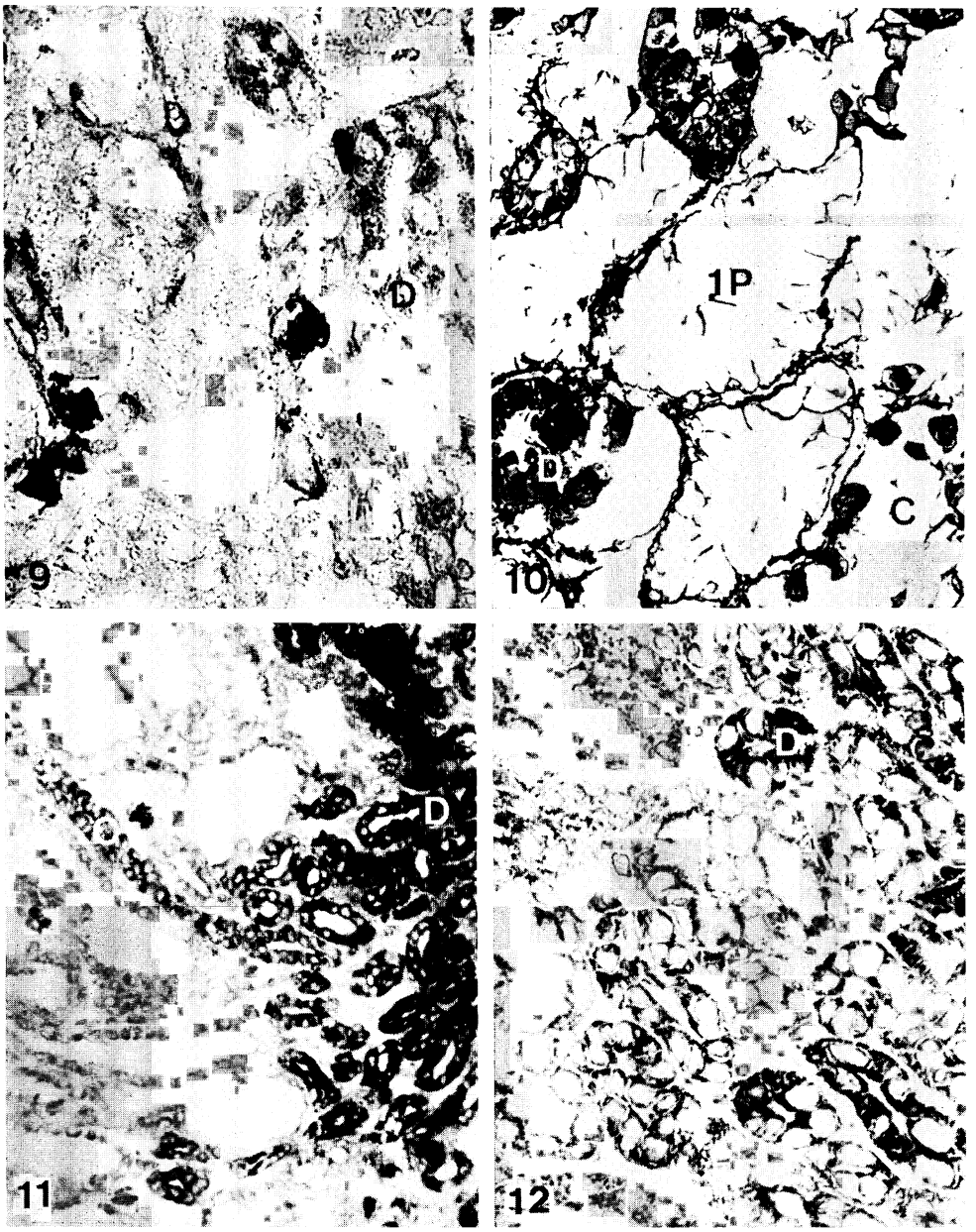

Fig. 9. Na-K-ATPase reaction in lungfish nephron. Moderate activity is detected in the distal segments. D, distal segment. ×175.

Fig. 10. Carbonic anhydrase activity in lungfish nephron. In the distal segments, intense activity is seen in the cytoplasm of cells. The collecting tubules consist of cytoplasmic staining and unstaining cells. C, collecting tubule; 1P, first proximal segment. ×175.

Fig. 11. Malate dehydrogenase reaction in lungfish nephron. Intense activity is recognized in the distal segments and collecting tubule. ×70.

Fig. 12. NADH dehydrogenase reaction in lungfish nephron. The distal segments exhibit intense activity, whereas scarcely any activity can be demonstrated in the 1st and 2nd proximal segments. ×175.

Discussion

The morphology of nephron in the South American lungfish was first studied by Bargmann (1934) and Guyton (1935). Their observations are closely similar except for the presence of intermediate segment. Guyton (1935) identified the segment, whereas Bargmann (1934) denied it. In the present study, its segment was confirmed distinctively. The other structural features found in the present study were consistent with the reports of Bargmann (1934) and Guyton (1935).

The distal segment of lungfish nephron consisted of heavily interdigitated cells having wide intercellular spaces, as seen in thick ascending limb of mammalian Henle's loop (Barrett *et al.*, 1978; Backmann and Kriz, 1982). Therefore, this segment seems to be highly permeable to sodium. The distributions of Na-K-ATPase and carbonic anhydrase activities in the distal segment suggest that sodium absorption is performed by active transport and H^+/Na^+ exchange processes. However, Na-K-ATPase and carbonic anhydrase activities were observed in the cytoplasm of the distal segment cells, instead of being localized in cell membranes as generally known (Pitts, 1974). These enzyme distributions in the cytoplasm of distal segment cells were probably due to the deep interdigitation of cells.

The distal segment of lungfish nephron exhibited intense activities of aerobic enzymes (malate, isocitrate, NADH, and β-hydroxybutyrate dehydrogenases). In contrast, hexokinase activity was mostly seen in the 1st and 2nd proximal segments only. These enzyme distributions indicate that the distal segment utilizes ketone bodies and fatty acids as principal energy sources. Such distinctive energy metabolism is probably related to sodium absorption in the distal segment. Furthermore, it is commonly observed in the distal segments of the teleosts (Endo and Kimura, 1982a, b), elasmobranchs (Endo, 1984), and frogs (Bargmann and Welsch, 1972).

Two types of cells were recognized in the collecting tubule of lungfish. The first cell type exhibited the deep interdigitation and intense activity of carbonic anhydrase similar to the distal segment cell. In contrast, the second cell type did not show heavy interdigitation and carbonic anhydrase activity. Since this second cell type was not observed in the distal segment, it indicates that the collecting tubule may be functionally different form the distal segment.

It is known that the collecting tubule of frog (*Rana*) nephron is composed of two types of cells (canaliculi and light cells). The canaliculi cell exhibits deep interdigitation and carbonic anhydrase activity, whereas the light cell does not show this enzyme activity and interdigitation (Bargmann and Welsch, 1972; Lönnerholm and Ridderstråle, 1974; Ridderstråle, 1976). The two types of cells in the collecting tubules of lungfish may correspond to the canaliculi and light cells of frogs, respectively. Both cell types of the collecting tubule of lungfish had intense activities of aerobic enzymes. In frogs, however, the canaliculi cell exhibits intense activities of aerobic enzymes, which were not seen in the light cell (Bargmann and Welsch, 1972). From this view point, lungfish nephron differs slightly from that of the frog. On the other hand, in the collecting tubules of teleosts and elasmobranchs, cells are not distinguished by their histological and enzyme-histochemical features (Endo and Kimura, 1982a, b, 1984; Endo, 1984).

In the descending limb of mammalian Henle's loop, poorly interdigitated cells having narrow intercellular spaces show high water permeability (Barrett *et al.*, 1978; Backmann and Kriz, 1982). The 1st and 2nd proximal segments of lungfish nephron were lined with poorly interdigitated cells having narrow intercellular spaces. Thus, these segments are expected to be highly permeable to water. In addition, the poor interdigitation of cells of the 1st and 2nd proximal segments is common among the lungfish examined in the present study, and in elasmobranchs (Endo, 1984), and frogs (Bargmann and Welsch, 1972), but is not detected in the freshwater teleosts, which have heavily interdigitated cells in their segments (Anderson and Loewen, 1975; Endo and Kimura, 1984).

Comparing the above structures and enzyme distributions of lungfish nephron with those of teleosts and elasmobranchs, the lungfish nephron seems to be more highly specialized than both, although, slightly similar to the latter.

Acknowledgments

I wish to thank the late Dr. Y. Enomoto, University of Miyazaki, and Prof. M. Oguri, University of Nagoya, for their advice and improvement of the manuscript.

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肺魚のネフロンの組織学的・酵素組織化学的研究

延東　真

南米産肺魚ネフロンの近位節第1部と第2部においては，上皮細胞の interdigitation が未発達で，細胞間腔も狭い．一方，遠位節の上皮細胞では，interdigitation が発達し，細胞間腔も広い．また，この遠位節細胞には，Na-K-ATPase，炭酸脱水酵素，および各種脱水素酵素の中程度から強程度の活性が認められた．集合管の上皮組織は，2種類の細胞から構成されていた．第1の細胞には発達した interdigitation があり，また強い炭酸脱水酵素活性が認められた．第2の細胞では interdigitation の発達が悪く，炭酸脱水酵素活性も見られなかった．しかし，両細胞とも，各種脱水素酵素の強い活性をもっていた．以上の構造と酵素分布を真骨魚類や板鰓類のものと比較すると，肺魚のネフロンは真骨魚類や板鰓類のネフロンよりもかなり進化しており，むしろ板鰓類のネフロンに似ていた．また，無尾両生類のネフロンと類似する点も多かった．

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