THE PROXIMAL TUBULE OF THE WINTER FLOUNDER, PSEUDOPLEURONECTES AMERICANUS, AS REVEALED BY MICRODISSECTION AND QUANTITATIVE HISTOLOGY

Marlies Elger[1], Hartmut Hentschel[2], Rolf K.H. Kinne[2]
[1]Institut für Anatomie und Zellbiologie I, Universität D-69120 Heidelberg, FRG
[2]Max-Planck-Institut für molekulare Physiologie, D-44026 Dortmund, FRG

The winter flounder Pseudopleuronectes americanus is a euryhaline marine teleost fish, which is frequently used for the investigation of renal mechanisms by biochemical and physiological studies. The structure and organization of the nephron was described by light- and electronmicroscopy in several related species (e.g. Parophrys vetulus: Bulger and Trump, Am. J. Anat. 123:195-226, 1968; Paralichthys lethostigma: Hickman and Trump, The Kidney In: Fish Physiology Vol. 1:91-239, 1969; Pleuronectes platessa: Ottosen, Cell Tiss Res. 190:27-45, 1978

For enzymatic and molecular biological studies at the cellular level, we looked for criteria for the discrimination of the nephron segments in isolated tubules by microdissection, in conjunction with histological information of the kidney of this species. Major results will be briefly described below. Microdissection of renal tubules: Small pieces of fresh tissue were dissected in flounder Ringer's (NaCl 140 mmol/l, KCl 2.5 mmol/l, $CaCl_2$ 1 mmol/l, $MgCl_2$ 1 mmol/l, $NaHCO_3$, gassed with 98% O_2 , 2% CO_2).

Two types of nephrons were isolated (Figure 1): (a) Short and coiled nephrons were located in the vicinity of side-branches of the collecting tubule-collecting duct (CT/CD) system. These nephrons presumably were young, newly developed nephrons. This type of nephron prevailed in the anterior portion of the kidney; (b) long nephrons extended from a region near the dorsal surface of the kidney, where glomeruli were located, in the direction of the CT/CD system at the ventral side of the kidney. A distinct zonation of the renal tissue, which could have helped in the separation of PI and PII was not apparent. Instead the major landmark for the detection of PI was the renal corspuscle. However, during quick microdissection, glomeruli were difficult to find. This was not improved by the injection of alcian blue as a vital dye into the circulation, which should have stained anionic sites in the filtration barrier of the glomeruli. The majority of the the glomeruli were tightly embedded in the interstitial tissue (Figure 2). A very short and inconspicous neck segment connected the small glomeruli with the first proximal tubule segment PI. PI was always short and coiled (2 to 4 bends) in the immediate vicinity of the glomerulus. The transition of PI to PII is easily seen by an abrupt increase in tubular diameter. PII of young nephrons is also extensively coiled, PII of older nephrons frequently performed wider bends, which, however by their curvature were generally differing from the straight rami of the furcating collecting duct system.

For histology, kidneys of two sexually mature females, approx. 25 cm total body length, were fixed in situ by dripping an ice-cold mixture of 2% paraformaldehyd and 0.5 % picric acid in 80% ethanol on the surface of the kidney. Serial sections were cut from paraffin-embedded tissue blocks from three regions. The sections were stained with alcian blue-PAS for the histochemical detection of neutral and acid glycoconjugates. On cross sections no dorso-ventral zonation was apparent. The tubules and their segments were irregularly distributed in

the large mass of interstitial tissue. The latter consisted of mononuclear-lymphoid cells, hematoblasts and peculiar melanomacrophage centers. The venous sinusoids and the intrarenal arteries and arterioles contained blood cells. All nephronic segments belonged to the proximal tubule, as was clearly seen by the staining in bluish magenta of the brush border. PI was markedly different from the segment PII by its narrow tubular profile, and, more specifically by the staining of intracellular granules, which represented secondary lysosomes.

Stereological examination uncovered that glomeruli plus renal tubules (i.e. proximal tubule segments PI and PII and CT/CD) may occupy less than one third of the total volume of the kidney. Within this fraction of volume, glomeruli were present with a fraction of 0.1, PI with 0.1, collecting tubules with less than 0.1. Thus more than three quarters of the volume of renal tubules in the kidney of winter flounder may be PII cells.

From these results we can conclude: Winter flounder are teleosts with a low number of small glomeruli, a short proximal tubule segment PI and a large amount of proximal tubule PII cells. Thereby more than 80% of cells harvested from the tubules by separation procedures can belong to this secretory segment, which also is rather easy to obtain by microdissection.

Table1. Volume densities of renal structures as estimated by point counting on six histological cross-sections from kidneys of two specimens of Pseudopleuronectes americanus. (Mean value + SE.)

Nephron and CT/CD	Glomeruli	PI	CT/CD
0.285 +0.014	0.028 + 0.005	0.03 + 0.008	0.018 + 0.007

Figure 1. Microdissected tubules from winter flounder, Pseudopleuronectes americanus. A small glomerulus (*) leads via a narrow neck portion to the first brush border segment, the proximal tubule segment PI. PI is short and located in the vicinity of the glomerulus. The second proximal segment PII is distinctly thicker than PI. PII comprises the major length of the nephron and joins at its end the collecting tubule-collecting duct system (arrow). x 100

Figure 2. Histological section through the middle portion of the kidney of winter flounder, Pseudopleuronectes americanus. Alcian blue-PAS staining.
Two glomeruli (*) are sectioned. The proximal tubule segment PI cells display intracellular granules and a well stained brush border. PII cells are largely devoid of stainable granules, they are high-prismatic and have a pronounced brush border, which stains bluish. In the collecting tubule (arrow), the entire apical cytoplasmic zone is stained redish-blue, indicating the production of mucosubstances by the epithelial cells. MC - melanomacrophage centers, VS - venous sinusoids. x 250

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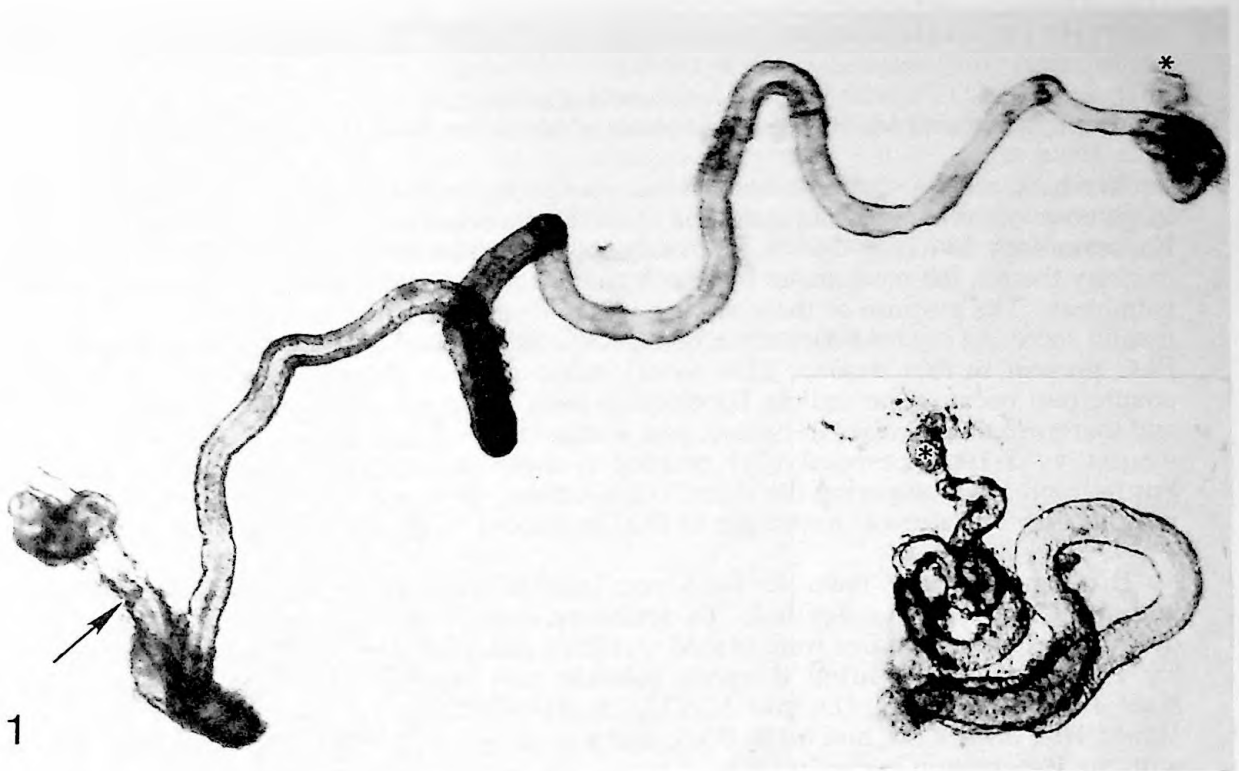

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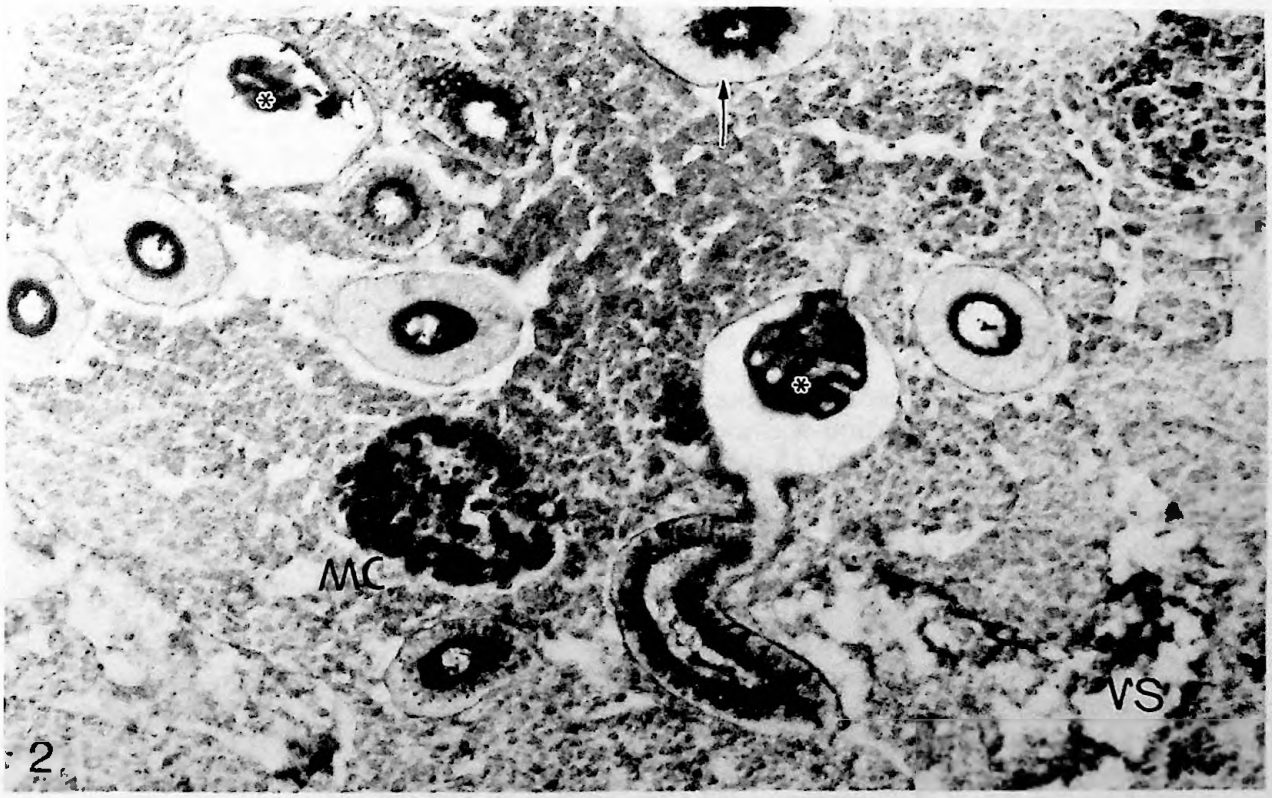

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