MICRODISSECTION OF KIDNEY ZONES AND RENAL TUBULE SEGMENTS OF SQUALUS ACANTHIAS, WITH EMPHASIS ON RENAL HISTOLOGY AND TUBULE SEGMENTATION OF ELASMOBRANCHS

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Separation of tissue zones and single tubule portions by free-hand microdissection from kidney slices using a low power stereomicroscope is a well established technique supplying material for biochemical and physiological studies. We obtained tissue samples from defined renal zones in the little skate, Raja erinacea for determination of concentrations of Na, Ka, urea and water [Elger et al., Bull. MDIBL 23:62-63, 1983; Hentschel et al., Comp. Biochem. Physiol. 84A:553-557, 1986]. Isolated renal tubules of dogfish, Squalus acanthias were used in physiological experiments [Beyenbach and Frömter, Am. J. Physiol. 248:F282-F295, 1985; Friedman and Hebert, Am. J. Physiol. 258:R398-R408, 1990).

We have previously studied the kidney of European dogfish, Scyliorhinus caniculus, and we described the segmentation of the extremely long nephron in relation to renal zones, blood vascular system and collecting duct system [Elger and Hentschel, Verh. Dtsch. Zool. Ges. 75:267, 1982; Hentschel and Elger, Verh. Dtsch. Zool. Ges. 75:263, 1982]. These results were based on the combination of injection techniques with histology, reconstruction using light microscopy of serial sections, and analyses with the electron microscope. Our findings were subsequently bolstered by comparing dogfish kidneys to those of other lower vertebrates [Hentschel and Elger, Adv. Anat. Embryol. Cell Biol. 108:1-151, 1987). Accepted criteria of morphological homology were also applied for comparing nephron segmentation of dogfish with that in the mammalian kidney [for a description of the latter, see, e.g. Kaissling and Kriz, Adv. Anat. Embryol. Cell Biol. 56:1-121, 1979]. Conclusive evidence for the correctness of our definition of nephron segments as related to the collecting duct system was also obtained recently by the description of the ontogenetic development of nephron and collecting tubule [Hentschel, Am. J. Anat. 190:309-333, 1991] and computer-assisted 3-D reconstruction of an entire nephron (Hentschel et al., Anat. Rec., in press).

Histological work of others suggests that profound differences might exist between kidneys of spiny dogfish, Squalus acanthias and European dogfish, Scyliorhinus caniculus [Lacy and Reale, Anat. Embryol. 173:23-34, 1985]. In view of the great interest in spiny dogfish as an experimental animal for physiological, biochemical and immunohistochemical studies of renal transport [see, e.g. Swenson et al., Bull. MDIBL 31:105-107, 1992; Biemesdorfer et al., JASN 3:804, 1992], we were prompted to reinvestigate kidney structures of spiny dogfish, Squalus acanthias.

Kidneys were excised in toto from medium-sized male spiny dogfish. Portions of 0.5 to 1 cm thickness were incubated at least for 1 h in chilled dogfish Ringers. The dogfish Ringers consisted of 280 mM NaCl, 6 mM KCl, 5 mM $CaCl_2$, 3 mM $MgCl_2$, 0.5 mM Na_2HPO_4, 1.0 mM NaH_2PO_4, 330 mM urea, 5 mM glucose, 72 mM trimethylamineoxide, 8 mM $NaHCO_3$ dissolved in 1 liter water. The Ringers was kept on ice at 0°C. Cross sections of kidneys (1 to 2 mm thick) were cut from the tissue samples with razor blades. The zones of mesial tissue and lateral bundles could be well seen at low magnification (x20). The localization of the countercurrent bundles was very variable and changed from section to section. In addition to laterally situated cap-like bundle zone, deep furrows, which were bordered on both sides by the bundles extended into the mesial tissue. This arrangement of the countercurrent system was not found in kidneys of young dogfish and skate. In their small kidneys the bundle zone is always located in a superficial layer ventrolaterally (dogfish) or dorsolaterally (skate) on the mesial tissue. The great complexity of tissue architecture in mature elasmobranchs as revealed by cross sectioning, obviously is the result of growth processes involving the kidney lobules. These lobules are descendent from the originally metamerical organisation of the opisthonephric kidney.

Small blocks of tissue (approximately 0.5 mm^3) were dissected from mesial tissue and bundle zone, respectively. Aliquots were used for (a) microdissection, (b) were lyophilized for future biochemical determinations and (c) were fixed with paraformaldehyde for histology.

The mesial tissue contained 3 types of tubules, which could be freed from the convoluted nephrons by gently teasing the tissue with sharpened needles (Fig. 1) (1) thick (60 to 80 μm), yellowish tubules, which showed a bi-refringent border at the luminal side, (2) thin yellowish to clear tubules (approximately 30 to 50 μm in diameter), (3) thin clear tubules. Frequently, tubules were dissected which consisted at one end of type (1) and at the other end of type (2). The transition between both portion occured gradually. Tubules type (1) and (2) characteristically contained flames of cilia, which were rapidly beating in the direction of the thinner end in the mixed portions.

Histological investigation of sections (0.5 μm) from epon-embedded tissue blocks revealed that tubules type (1) and (2) had an epithelium of brush border cells with interspersed multiciliary cells. Type (3) tubules lacked both brush border cells and multiciliary cells. The histology of the mesial tissue of Squalus was remarkably similar to that of Scyliorhinus, with the exception of a greater number of cross sections of tubule (2) in Squalus than in Scyliorhinus.

It is concluded that the yellowish, nearly opaque tubules in the microdissection experiments (tubules (1) and (2)) belong to the proximal tubule segment PII. They represent two subsegments, namely PIIa and PIIb. Type (3) tubules in contrast are portions of the late distal tubule segment, which arrives from the early distal tubule. The latter segment is the diluting segment [Thurau and Acquisto, Bull. MDIBL 9:60-63, 1969; Hebert and

Friedman, Bull. MDIBL 25:128-130, 1985]. The early distal tubule is exclusively present in the lateral bundles. The latter are very similar by their architecture and histology to those of Scyliorhinus and Raja, as could be seen on histological sections [see also Hentschel, this bulletin].

The nephron segmentation and its course in relation to mesial tissue and lateral bundle zone as well as the collecting tubule collecting duct system is summarized in the schematic drawing (Fig. 2).

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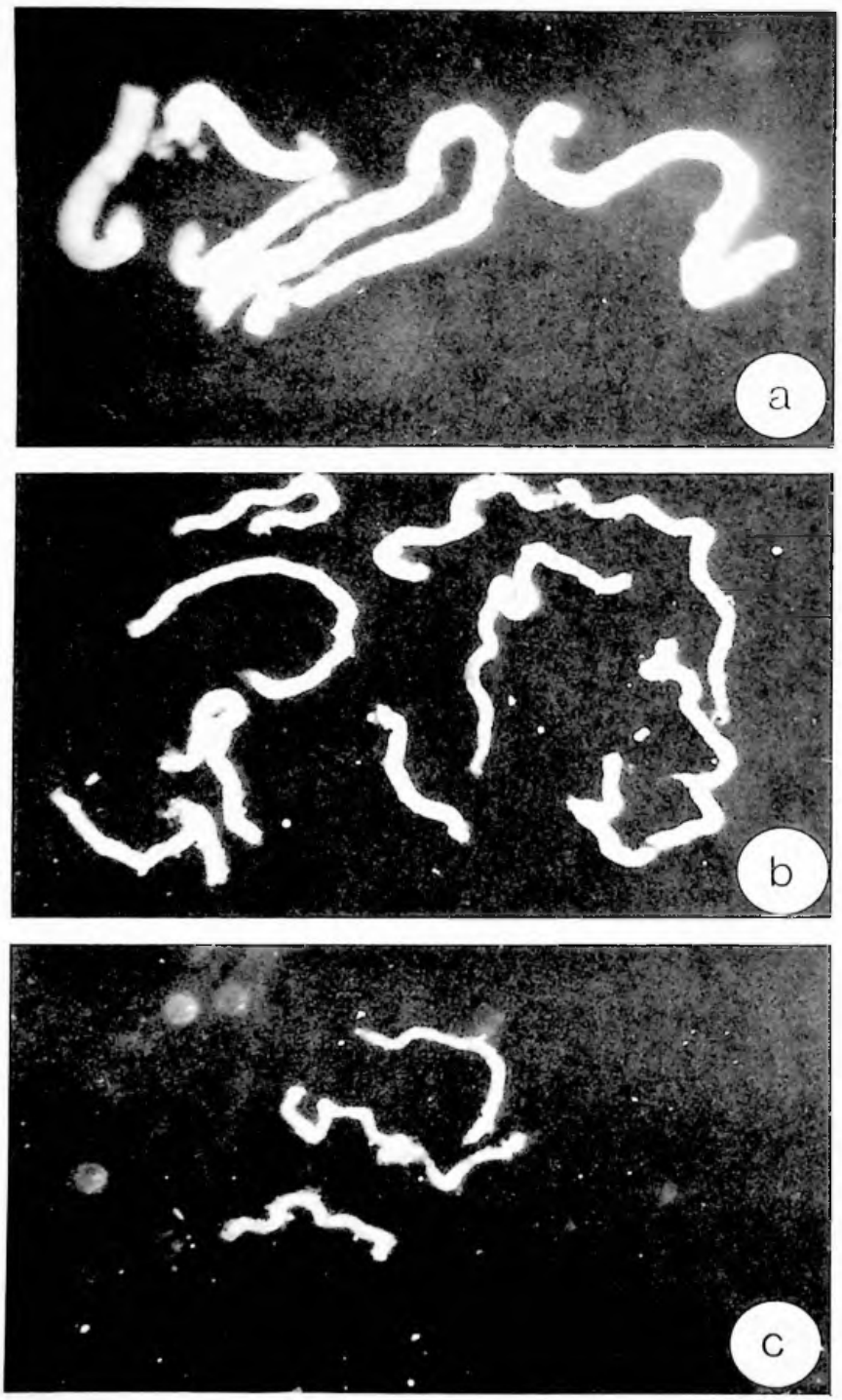

Figure 1. Microdissected portions of tubules of mesial tissue of *Squalus acanthias*. x120. a) Proximal tubule segment PIIa. b) Proximal tubule segment PIIb. Two tubules show transition to the following intermediate segment (arrow heads). c) Portions of late distal tubules. Characteristically these clear thin tubules always lacked flames of cilia.

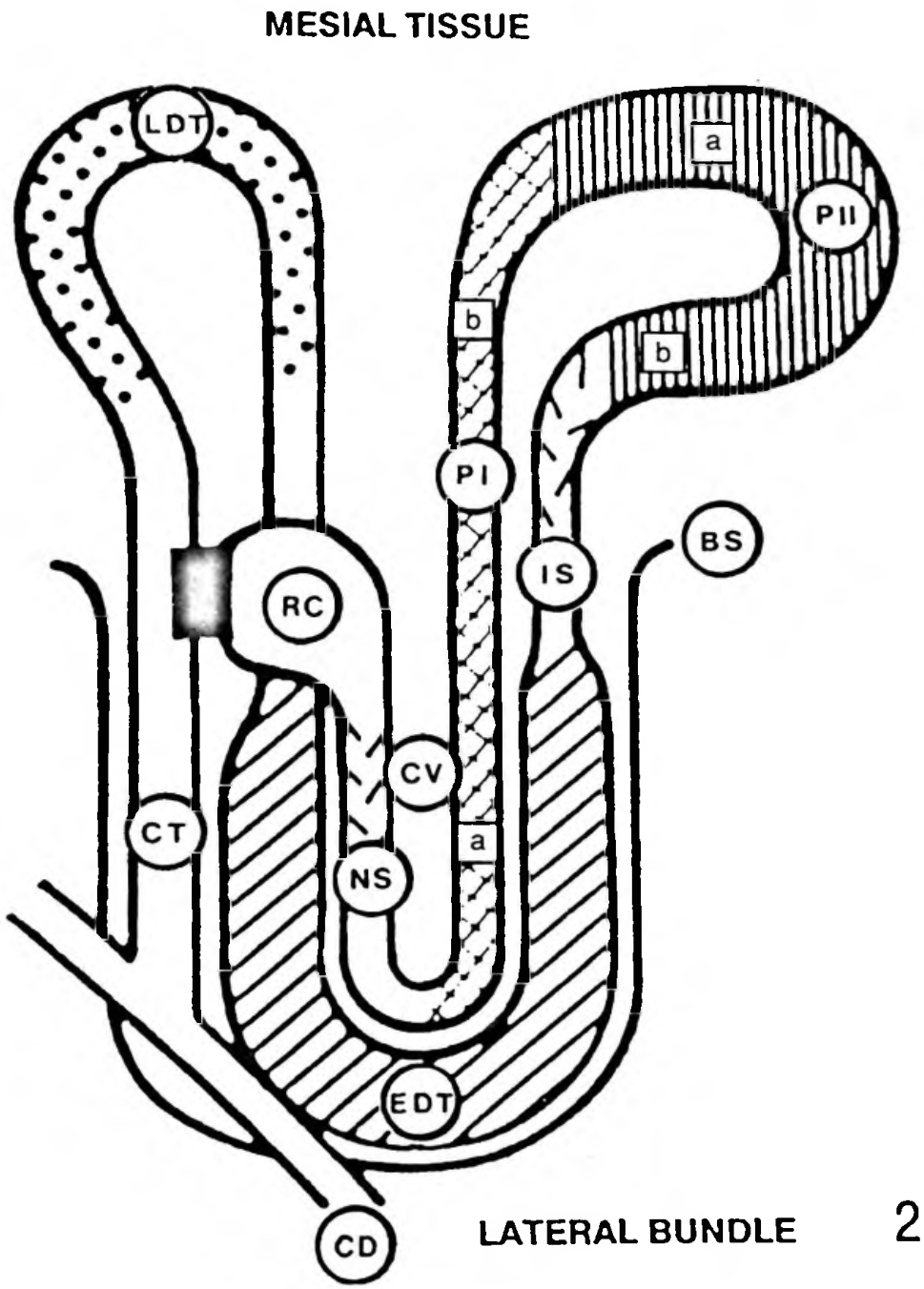

Figure 2. Schematic drawing of a nephron of dogfish. BS = bundle sheath; CD = collecting duct; CT = collecting tubule; CV = central vessel; EDT = early distal tubule; LDT = late distal tubule; NS = neck segment; PI = proximal tubule segment I with subsegments PIa and PIb; PII = proximal tubule segment II with subsegments PIIa and PIIb; RC = renal corpuscle [see also Hentschel et al., Anat. Rec., in press].