Nephrogenesis Is Induced by Partial Nephrectomy in the Elasmobranch Leucoraja erinacea

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Abstract. The mammalian kidney responds to partial nephrectomy with glomerular and tubular hypertrophy, but without renal regeneration. In contrast, renal regeneration in lower vertebrates is known to occur. Understanding the underlying mechanisms of renal regeneration is highly important; however, a serviceable animal model has not been developed. A neonephrogenic zone has been identified in the European lesser spotted dogfish, Scyliorhinus caniculus (Hentschel H. Am J Anat 190: 309–333, 1991), as well as in the spiny dogfish Squalus acanthias and the little skate, Leucoraja erinacea. The zone features the production of new nephrons complete with a countercurrent system. To analyze this nephrogenic region of elasmobranch fish further, a renal reduction model was established. The neonephrogenic zone in the adult kidney of the little skate resembles the embryonic metanephric kidney and contains stem cell-like mesenchymal cells, tips of the branching collecting duct system, and outgrowth of the arterial system. Four stages of nephron development were analyzed by serial sections and defined: stage I, aggregated mesenchymal cells; stage II, S-shaped body-like structure with high-prismatic epithelial cells; stage III, segmental nephron segregation; stage IV, functioning nephron. The stages were analyzed after partial nephrectomy. In addition, cell proliferation was assessed by incorporation of bromo-deoxyuridine (BrdU). New nephrons developed in animals undergoing partial nephrectomy. Growth was greatly stimulated in the nephrogenic zone, both in the remnant tissue and in the contralateral kidney within 10 wk. Mesenchymal cell aggregates increased significantly per renal cross-section compared with controls (stage I, 0.64 ± 0.28 versus 0.27 ± 0.25; P < 0.005; n = 10 animals per group). The same was the case for S-shaped body-like cysts (stage II, 0.24 ± 0.19 versus 0.08 ± 0.09; P < 0.02). Cellular proliferation in the neonephrogenic zone of the contralateral kidney was also greatly enhanced (14.42 ± 3.26 versus 2.64 ± 1.08 BrdU-positive cells per cross-section, P < 0.001). It is concluded that the skate possesses a nephrogenic zone containing stem cell-like mesenchymal cells during its entire life. Partial nephrectomy induces renal growth by accelerating nephrogenesis. This unique model may facilitate understanding renal regeneration.

In mammals, nephron formation is terminated at birth or shortly thereafter (1). Subsequent growth is generally restricted to tubular epithelial replacement by proliferation and/or to angiogenic processes involved in vascular reconstruction (2,3). The limited regenerative potential results in a greatly reduced capacity to cope with renal disease. Injured glomeruli do not regenerate, and glomerular destruction is the major cause of final nephron loss (4,5). The remaining nephrons respond with compensatory glomerular and tubular growth. Large-scale nephron loss is therefore not accompanied by new nephron generation (“neo” nephrogenesis) in mammals. However, lower vertebrates have superior abilities in that regard. Adult sexually mature bony fish (teleosts) can develop new nephrons as shown in light and electron microscopic studies (6,7). Formation of de novo nephrons has been reported in goldfish kidneys after the administration of nephrotoxic substances (8). We previously found nephrogenic tissue in the little skate, Leucoraja erinacea, an elasmobranch cartilaginous fish (9), as well as in the spiny dogfish, Squalus acanthias. Furthermore, a detailed analysis of nephron anlagen and developing nephrons in the lesser spotted dogfish, Scyliorhinus caniculus, has been published (10,11). Because elasmobranch kidneys exhibit great structural complexity (12) and resemble by this and other morphologic features mammalian kidneys, these animals could serve as a model for nephrogenesis. We therefore studied nephrogenesis in adult skate kidney and tested the hypothesis that this process is stimulated by renal size reduction.
Materials and Methods

Animals

Female skates, *Leucoraja erinacea* (Mitchell 1825), were netted in Frenchman Bay, Maine, or purchased from the Marine Station Woods Hole, MA, USA. The body size as measured by the disc diameter (fin span) ranged from 20 to 32 cm. The body weight ranged from 310 to 790 g. The animals were kept in large circular tanks with running aerated seawater and were acclimatized for a few days before the experiments. The animals were fed a diet of frozen shrimp.

For reduction of renal mass, we surgically removed renal tissue. The animals were anesthetized by adding 0.1 g/L tricaine (Sigma) to the seawater. During anesthesia, the seawater was cooled with ice, and deep anesthesia was achieved after 40 min. We developed two operations. Fish were mounted upside-down on a styrofoam board. The gills were superfused with a tricaine drip (0.05 g/L seawater) via the oral cavity. The body was covered with paper towels soaked in ice-cold seawater. The posterior body cavity was opened by a midline incision through the ventral body wall. The pelvic girdle was transected, and the intestine and gonads were carefully laid aside to expose the right kidney. The large efferent intrarenal veins of the renal portal system, which were visible through the peritoneal epithelium, were cauterized. A 2/3 nephrectomy in one kidney was performed with scissors after cauterization of the intrarenal arteries and veins. Great care was taken not to injure the multiple renal arteries entering the kidney from the dorsal aorta. The viscera and the pelvic girdle were repositioned. The body wall was closed with two sutures. One ran in the subdermal musculature and one in the skin. A nonresorbable surgical thread was used in all instances. After the operation, the fish were placed in their holding tanks. They recovered from anesthesia after 5 to 10 min.

For the second technique, the animals were laid on their ventral surface. The gills were dripped with anesthetic via the spiracles. A longitudinal incision was made through the skin and underlying musculature (longissimus dorsalis) in the pelvic region, 2 cm lateral from the midline. The dorsal surface of the right kidney was thereby directly exposed. Two thirds of this kidney was removed without injury to the peritoneal membrane. The incision was closed by a double suture. Sham operations were performed in one group of animals. Unoperated fish served as additional controls. Fish were maintained in their tanks with constant feeding. After 10 wk, 20 animals (10 nephrectomized [NX] and 10 control skates) were sacrificed, and their kidneys were examined.

Preservation of Kidney Tissue

The kidneys were fixed by vascular perfusion via the conus arteriosus as described previously (12). In brief, the vascular system was rinsed with modified elasmobranch Ringer solution (13) at a pressure of 100 cm H₂O for several minutes. For minimizing osmotic effects during the clearing of the vasculature, 2% polyvinyl pyrrolidone was added. After the blood was cleared from the circulatory system, the fixation solution was introduced without a pressure drop and perfused for approximately 5 min at the same pressure as the rinsing solution. The chilled fixation fluid contained paraformaldehyde (3 to 4%) and picric acid (0.5%) in...
Figure 2. (A) Histologic renal cross-section. The nephronogenic zone (arrow) with newly developing glomeruli is located along the ventrolateral border of the bundle zone. Here, new nephrons are added and effect the growth of renal tissue in a lateral direction. Paraffin section, Masson-Goldner, light micrograph (LM). (B) Schematic cross-section. The two major zones termed “lateral bundles” (i.e., the renal countercurrent system, shown in pink) and “mesial tissue” (blue), and the “nephrogenic tissue” (yellow) are shown. Intrarenal arteries deriving from multiple renal arteries (RA) give rise to bundle arteries (BA) and afferent glomerular arterioles (aff A). The renal portal system enters the kidney by afferent renal and intrarenal veins (ARV, AIRV), supplies the mesial tissue by capillary sinusoids (small arrows), and leaves by the efferent intrarenal and renal veins (EIRV and ERV). eff A, efferent arterioles. The nephrons, including the glomeruli, decrease in size and age from the medial to the lateral side. The nephrogenic tissue is the site of new glomerular and tubular formation. The course and segmentation of a mature nephron is shown at the left: EDT LDT, early and late distal tubule segments; IS, intermediate segment; NS, neck segment; PI and PII, proximal tubule segments I and II. The collecting tubule runs through the bundle and joins the collecting duct (CD) tree. The youngest branches of the CD system are located adjacent to the nephrogenic tissue (adapted from reference 12). (C) Nephrogenic zone at higher resolution. The nephrogenic zone (NZ) is located between the coiled countercurrent bundles (LB) of individual nephrons and the tubules of the mesial tissue (MT). A mature glomerulus (GL) is seen in the central part of the figure. Semithin (0.5 μm) plastic section, toluidene blue staining, LM. (D) The nephrogenic zone contains mesenchymal cells in different stages of development. The aggregated mesenchymal cells of the first developmental stage (arrow) and the corresponding end branch of the collecting duct system (CDT) are shown. The developmental stage is embedded in a collagen-rich interstitial tissue (Co). Semithin (0.5 μm) plastic section, toluidene blue staining, LM. (E) Mesenchymal cells of developmental stage I adjacent to the collecting duct tip (CDT). The cells (asterisks) are enveloped by laminated cell processes of fibroblasts (FB). Thin (60 nm) plastic section, transmission electron micrograph.
0.1 M Sörensen phosphate buffer, pH 7.4. The osmolality of the buffer of the fixative was adjusted with sodium chloride and sucrose to that of the pre-rinse (950 mOsmol/L). After fixation, the body was opened, the kidneys were exposed, and the size and shape of the kidneys were photographed in situ from the ventral side. The organs were carefully removed, weighed, and documented from the dorsal side.

**Histology and Quantification of Developmental Nephron Stages**

The excised kidneys were postfixed in fresh fixative for an additional hour. Each kidney was cross-sectioned with razor blades in eight to ten blocks and processed for paraffin embedding. Serial cross-sections (3 μm) were prepared from each block and stained with Masson-Goldner trichrome stain. In these sections, developmental stages I to IV were identified in accordance to developmental stages in dogfish, *Scyliorhinus caniculus* (10). The amount of developmental stages was estimated in ten nephrectomized and ten control animals. The group of nephrectomized animals consisted of five animals operated from the dorsal side and five animals operated from the ventral side. The controls included five sham-operated and five non-operated animals. The comparison was made between the intact contralateral kidneys of the nephrectomized animals and one kidney of the sham-operated and non-operated controls. Serial cross-sections (8 μm) were

Figure 2. (Cont'd)
prepared from five blocks per kidney. Three sections per tissue block at a distance greater than 300 μm were used to determine the number of developmental stages I and II per renal cross-section.

**Electron Microscopy**

For transmission electron microscopy, tissue pieces of perfusion-fixed kidneys were postfixed in 1.5% glutaraldehyde and 1.5% paraformaldehyde solution, followed by fixation in 1% OsO₄ solution, and subsequent embedding in Epon resin. Thin sections were obtained with an ultramicrotome OMQ3 (Reichert, Vienna) and viewed with Zeiss electron microscope EM10 (Zeiss, Oberkochen).

**Cell Proliferation**

Proliferative activity of the renal tissue was estimated by indirect immunohistochemical detection of incorporated 5-bromo-2′-deoxyuridine (BrdU) in a separate set of ten adult female little skate (body size, 24 to 27 cm; body weight, 500 to 690 g). Ten days after partial nephrectomy of the left kidney in five animals (NX) and sham operation in five control skates, the animals received an intraperitoneal injection of 1.5 mg/100 g BW BrdU dissolved in PBS. After 12 h, the kidneys were fixed by perfusion as described above. Four tissue blocks from the middle portion toward the caudal end of one kidney of control skates and of the contralateral kidney of NX skates were embedded in paraffin, and six to eight independent cross-sections per animal were used for numerical determination of BrdU-labeled cells. The deparaffinized sections were treated with 0.01% trypsin in PBS for 1 min at RT and Universal Blocking Reagent (BioGenex, San Ramon) for 10 min and were incubated with mouse monoclonal anti-BrdU antibody (clone BMG 6H8) containing nucleases for DNA denaturation (Roche) diluted 1:20 in PBS for 30 min at 37°C, followed by anti-mouse Ig-alkaline phosphatase (Roche) for 30 min at RT. The reaction was visualized by color substrate solution containing nitroblue tetrazolium (NBT) salt and 5-bromo-4-chloro-3-indolyl phosphate (BCIP). Cell nuclei labeled with BrdU were counted per kidney cross-section. Three tissue blocks from the ipsilateral kidney of the NX animals were used to study the distribution of proliferative cells in the operated kidney. Parallel sections were stained with trichrome stain after Masson-Goldner for identification of developmental stages.

**Statistical Analyses**

Statistical difference was tested between nephrectomized and control animals by t test.

**Results**

In Figure 1, the ventral aspect of the kidneys is shown in situ after removal of the intestine (panel A). Two separate flat roughly bean-shaped organs are located in the caudal retroperitoneum. We determined the renal mass in fifteen female skates (300 to 800 g body weight). Total kidney weight ranged from 1 to 3 g corresponding to 0.3 to 0.4% of body weight. Kidney weight and body weight were closely correlated, and a linear correlation over a large range of body weight was observed ($r = 0.91, P < 0.001$) (panel B).

The renal architecture, nephron segmentation, the relationship of the nephrons to the renal zones, the renal arterial and venous portal systems, and the structure of the nephrogenic zone are shown in Figure 2. Cross-sections revealed a flat elliptical shape and demonstrated a cap of tubular bundles (lateral bundles) located on the mesial tissue mass (panel A). These bundles represent the renal countercurrent system. They are situated along the dorsolateral surface of the kidney in a continuous zone beginning at a distance of approximately 1 mm from the medial edge of the kidney and extending around the lateral margin to the ventral surface for up to 1 mm. The afferent intrarenal veins and the glomeruli separate the bundle zone and the mesial tissue zone. The nephrons were heterogeneous. The most elaborate nephrons with the largest glomeruli were observed at the medial location. The small nephrons were located at the lateral edge of the kidneys (see also reference 9). Developing tubules and nephron anlagen were frequently present in the vicinity of the outermost small glomeruli. By careful stereomicroscopic examination of the decapsulated renal surface, a narrow band of whitish tissue was identified along the convex lateral surface of the kidney. This tissue demarcated the border between the pale bundle zone and the reddish-brown mesial zone. Because the band contained nephron anlagen, we termed this zone “nephrogenic tissue.” In young animals, the nephrogenic tissue formed a ribbon-like structure along the entire kidney. In older animals, the band was discontinuous.

Figure 2B shows a schematic drawing of a renal cross-section. Blood supply and the nephron course are demonstrated in the two major kidney zones: the lateral bundle zone and the mesial tissue. Several renal arteries arise from the dorsal aorta. Intrarenal arteries give rise to afferent glomerular arterioles and to bundle arteries. The mesial tissue is supplied by capillary sinusoids of the renal portal system, which enters the kidney by afferent renal and intrarenal veins. The bundle vessels, including the central vessels of the individual bundles and the efferent arterioles, join the mesial sinusoid system. The efferent intrarenal veins drain the sinusoid system. The zone of neonephrogenesis with newly developing glomeruli is located near the most peripheral branches of the arterial system (compare also reference 9).

Figures 2C and 2D show a nephrogenic zone at the lateral margin of the kidney at higher resolution. The nephrogenic zone was wedged between the ventrolateral margin of the bundle zone and the mesial tissue. Blind ends of the collecting duct system invaded the nephrogenic tissue. They frequently showed mitosis of epithelial cells. Occasionally, two tips originating from the collecting duct ends were observed. Growth of the collecting duct system by formation of new tips in conjunction with formation of nephron anlagen appeared to be exclusively located in this nephrogenic zone. We did not observe neonephrogenesis in other regions of the kidney.

The nephrogenic zone contained mesenchymal cells and prospective tubular cells in different stages of development (Figure 2D). Around the collecting duct tips, undifferentiated mesenchymal cells aggregated to form the first developmental stage (see below). The developmental stages were adjacent to a prominent mass of collagen-rich interstitial tissue, which was also reference 9). The region around the early stages was poorly vascularized. The early...
Figure 3. Stages of nephron development in the nephrogenic zone. (A) Stages I and II. A newly formed collecting duct (CD) tip surrounded by aggregated mesenchymal cells (I) can be seen on the left. Stage II was defined as the subsequent development of tubular structures from early cyst formation until the appearance of the glomerular anlage. A late stage II (II) is delineated by square brackets. The prospective podocyte layer (arrow) is evident in the glomerular anlage. Notice the extremely high columnar cells of the prospective proximal tubular portion. (B) Stages III and IV. Differentiation of the tubule into segments and appearance of vascular structures in the developing glomerulus (III) can be observed. Stage IV is present with a young well-perfused glomerulus (GL), a lateral bundle (LB) with two tubular hairpin loops, and two short tubular coils added to the mesial tissue (MT). Paraffin sections, Masson-Goldner staining, LM.
aggregating mesenchymal cells were nonpolarized cells with a comparatively large nucleus and a narrow cytoplasmic zone (Figure 2E). They were tightly surrounded by flat, laminate cell processes of adjacent fibroblasts or by neighboring mesenchymal cells.

For further definition of the developmental stages in the skate kidney, and as a basis for quantification of nephrogenic responses to different stimuli, we arranged the developmental stages according to their size and complexity. We developed a system of four major stages with further subdivisions into early and late phases to grade our findings, as shown in Figures 3 and 4. Stage I was defined as a distinct small structure of less than 100 μm in diameter, consisting of a condensed mass of mesenchymal cells in the vicinity of a new bud of the collecting duct system (Figures 3A and 4A). In the first phase (stage I-1), the mesenchymal cells were irregularly arranged. In stage I-2, cells at the very tip of the collecting duct bud began to align and to epithelialize with formation of basement membrane and development of apical tight junctions.

Stage II was defined as the subsequent development of tubular structures from early cyst formation and the appearance of the glomerular anlage. We defined three subsequent phases of these structures according to their length. In the first phase (stage II-1), the cyst was an ovoid structure with a central lumen. At the presumptive proximal end, a mass of unpolarized mesenchymal cells formed a plug. In the second phase (stage II-2), the elongated cysts usually performed two bends like the letter S and an additional bend at each end (S-shaped body-like cyst). The most advanced cysts (stage II-3) displayed four alternating tubular loops closely adhering to each other (Figures 3A and 4B). The gap in the proximal end of the cyst was closed by the formation of the glomerular epithelial primordia. An accumulation of undifferentiated mesenchymal cells was still attached to the prospective Bowman’s capsule. The exceptionally high epithelial cells (cell height, >50 μm) and the presence of a distinct lumen in the proximal portion were helpful for identification of all phases of stage II on single sections.

Stage III was characterized by the progressive differentiation of nephron segments, including the occurrence of vascular structures in the developing glomerulus (Figure 3B). Pronounced elongation of the tubule was accompanied by growth of two loops in direction of the renal capsule to become the hairpin loops of one individual bundle. The growth of the two opposite loops was directed to the mesial tissue. Stage IV consisted of young nephrons with well-perfused glomeruli and well-differentiated tubular segments (Figure 3B). Stages III and IV proved difficult to quantify. Although the tubular profiles and glomeruli were easily discernible by their lesser diameter and smaller size compared with adult nephrons, the length of the mesial convolutions and the bundle were already too large to allow quantification by numerical counts. For example, the growing countercurrent bundle tended to coil. Thus, in any given section, one to five cross-sections appeared that were not always identifiable as belonging to one nephron. In addition, the developing mesial coils could not be assigned to the same nephron’s loop bundles.

After defining the different stages of nephron development, we then tested the hypothesis that the nephrogenesis can be induced by renal mass reduction. Figure 5 shows a schematic diagram, the operation itself, and the kidney 10 wk after partial nephrectomy. The loss of renal mass was still manifest, and the mean renal weight was approximately half that of control kidneys (Table 1). As a result, a considerable gap partly filled with pale connective tissue was visible. The contralateral kid-
ney did not differ macroscopically from the roughly bean-shaped control kidneys.

Cross-sections of the contralateral kidneys revealed no changes in areas of mature tissue. However, in the nephrogenic tissue, a conspicuously large number of developmental stages were evident in partially nephrectomized animals. Two to five profiles of nephrogenic stages were seen on a renal cross-section as shown in Figure 6. All phases of stages I through IV

Figure 5. Nephrectomy in the skate. (A) Schematic view of ventral aspect of the kidneys. The nephrogenic zone runs in a small band along the ventrolateral edge (arrows). Multiple renal arteries (RA) arise from the aorta (A), and several renal veins join the cardinal vein (V). Two thirds of one kidney were surgically removed, leaving the medial margin containing the large renal vessels in place. (B) Operated skate. The right kidney is exposed by an incision through the dorsal body wall. A major portion of the kidney with the lateral nephrogenic zone was surgically removed. (C and D) Kidneys of control animal and NX animal with partial nephrectomy 10 wk after surgery, dorsal aspects. The surface of the kidneys is smooth with scarce lobulation. The intrarenal (endocrine) organs are located at the medial margin (asterisks). Ten weeks after surgery, the operated kidney has begun to rebuild from the caudal portion, where remnants of developmental tissue were intact (arrow). Perfusion-fixed kidneys.
were enhanced. The ramifying blind collecting duct ends were thicker than in control animals. The condensed mesenchymal stage I masses formed extensive caps with generally more than 200 cells around the collecting duct tips.

Growth quantification in the nephrogenic zone was performed (a) on the frequency of developmental nephron stages I and II and (b) on cell proliferation. The renal cross-sections of controls and intact contralateral kidneys from partially nephrectomized animals revealed values (n = 10 animals per group; mean ± SD) for stage I of 0.27 ± 0.25 and 0.64 ± 0.28, respectively (P < 0.005). Mean values for stage II were 0.08 ± 0.09 for controls and 0.24 ± 0.19 for partially nephrectomized animals (P < 0.02) (Figure 7). Small but distinct islands of lymphomyeloid cells were observed in the vicinity of the nephrogenic tissue in the contralateral kidney of partially nephrectomized animals in addition to islands with blood forming tissue adjacent to large intrarenal arteries.

Cellular proliferation in the contralateral kidney and in the operated kidney was studied by immunohistochemical demonstration of incorporation of BrdU 10 d after nephrectomy. Control skates showed very rarely BrdU-labeled cells in the mature portion of the tissue. A low number of reactive cells were present in the nephrogenic zone. Many histologic sections were lacking dividing cells entirely. In the contralateral kidney of nephrectomized animals, the mean number of labeled nuclei per kidney cross-section was significantly increased (Figure 8). The majority of the reactive cells were located in the center of the nephrogenic zone and in adjacent young nephrons.

We also analyzed the growth and proliferative response in the ipsilateral, operated kidney. As expected, regions of massive degeneration, areas of dilated tubules, and foci with apparently healthy nephrons were present. However, in the small caudal portion where both the mature and the original nephrogenic zone were basically intact and not disturbed by the operative procedure, an increase in the number of developmental stages was found. Renal cross-sections of this portion revealed for stage I a mean value of 0.46 ± 0.40 and for stage II a mean value of 0.42 ± 0.39 (mean ± SD, n = 10 animals). Proliferation of the developmental stages was vigorous, as many cells in developing tubules and glomeruli displayed BrdU incorporation. Serial sectioning of these ipsilateral, operated kidneys did not show signs of proliferation in areas of mature tissue. Specifically, the dilated tubules were essentially devoid of BrdU labeling.

Discussion

We have characterized a nephrogenic zone in the adult kidneys from the skate. The nephrogenic zone represents a niche within the kidney where stem cell-like cells reside. The tissue responds to partial reduction of renal mass with the formation of new nephrons. The morphogenetic process that we term neonephrogenesis appears to be an important mechanism for renal growth, as well as for repair of injured kidney. Renal hypertrophy, which is a common response to renal mass reduction in higher vertebrates and man (14–16), contributed only slightly to the reconstitution of renal mass in the skate. Our morphologic analyses demonstrated that a zone of embryonic renal tissue persists in adult skates. Evidently, the advanced complexity of elasmobranch kidneys does not preclude nephrogenesis in the adult kidney.

The nephrogenic zone of the skate contains the essential structures characteristic in found in the embryonic mammalian metanephros. We defined four stages of nephron development. The evaluation of the four stages in the adult skate corroborated previous light and electron microscopic results in the European dogfish, Scyliorhinus caniculaus (10). Because certain stages of nephrogenesis have been observed in adult specimens of several other fish species, neonephrogenesis is probably a common feature in elasmobranchs and teleosts (6–10).

The nephrogenic mesenchyme is present in stages I-1 and I-2. These stages correspond to the two transient mesenchymal condensations that precede the comma-shaped and S-shaped body in the embryonic mammalian metanephros (1,17,18). Stage I-1 resembles the cap condensate and stage I-2 is very similar to the pretubular condensate (17,19,20). The collecting duct tips that are in contact with the stage I structures are the structural homologues to the tips of the ureteric bud branches. The collecting duct tip of the skate differs in several morphologic features from the mammalian ureteric bud branches. The collecting duct tip is usually a solid structure, compared with the ampulla-like tip in rodents and humans (20,21). The tip is encompassed by a sheath of fibroblasts that becomes the bundle sheath of the elasmobranch countercurrent system (10).

Stage II was defined as S-shaped body-like cysts in agreement with our observations of Scyliorhinus. Our results showed considerable variability in this developmental stage, apparently representing different phases. We suggest that at the end of stage I, the pretubular condensate grows into a roughly spherical cyst that is in contact with the collecting duct tip at the distal end. The proximal end features a plug of unpolarized cells. This renal vesicle-like structure grows rapidly to become an S-shaped body-like cyst with two additional bends. The

**Table 1. Kidney weights**

<table>
<thead>
<tr>
<th>Animals</th>
<th>Weight (g/kg body weight)</th>
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<tbody>
<tr>
<td>Control animals (Co)</td>
<td></td>
</tr>
<tr>
<td>left kidney weight</td>
<td>1.727 ± 0.116</td>
</tr>
<tr>
<td>right kidney weight</td>
<td>1.764 ± 0.175</td>
</tr>
<tr>
<td>total kidney weight</td>
<td>3.491 ± 0.261</td>
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<tr>
<td>Nephrectomized animals (NX)</td>
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<tr>
<td>left operated remnant kidney weight</td>
<td>0.872 ± 0.124(^b)</td>
</tr>
<tr>
<td>right non-operated kidney weight</td>
<td>1.998 ± 0.261(^c)</td>
</tr>
<tr>
<td>total kidney weight</td>
<td>2.869 ± 0.279(^d)</td>
</tr>
</tbody>
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\(^a\)Results are mean ± SD. n = 5 animals per group; t test.  
\(^b\)P < 0.001 versus means of left and right kidney of Co. Ten weeks after surgery, the weight of operated kidneys of NX animals was still lower than the kidney weight of control animals.  
\(^c\)P < 0.1 (n.s.) versus means of left and right kidney of Co. The contralateral non-operated kidney showed a tendency to increase in weight. 
\(^d\)P < 0.01 versus total kidney weight of Co.
Figure 6. Nephrogenic zone from control animal (panel A) and of contralateral non-operated kidney from NX animal (panel B). (A) In control animals, few developmental nephron stages are present. A stage I is located adjacent to the lateral bundle (LB) of a stage III to IV. Three cross-sections of the same bundle (consisting of five tubular profiles each) are seen. A young glomerulus (GL) of stage IV is located nearby in the mesial tissue (MT). (B) Growth rate and formation of new nephrons is enhanced in the nephrectomized animal. The mass of aggregated mesenchymal cells (MES) is enhanced, and several stages II (II) and at least one stage III (III) are present. The mesial tissue (MT, delineated by a bracket) is irrigated by venous sinusoids (VS), whereas the zone containing the mesenchymal aggregates is virtually devoid of vasculature. Paraffin sections, Masson-Goldner staining, LM.
epithelia of the collecting duct tip and the adjacent distal end of the cyst merge. Very marked growth soon leads to a structure with four narrow bends. Thus, in this phase of stage II, the cyst far exceeds the typical S-shape. The presumptive proximal end still displays a plug of unpolarized cells. In the terminal phase of stage II, a portion of the unpolarized cells epithelializes and becomes the glomerular epithelia of stage III. A feature common to all phases of stage II is that the high prismatic epithelial cells appeared uniform. The numerous mitotic figures and the incorporation of BrdU give evidence for rapid growth in all regions of the cyst.

Stage III is characterized by the segregation into tubular segments and the appearance of vessels. The specific architecture of the elasmobranch nephron (22,23) is established at this stage. Further growth leads to stage IV, the young nephron. This stage is relatively immature with respect to cell size and tubular length; however, the segments can now be considered as a functioning excretory unit. The addition of newly formed nephrons in the periphery of the elasmobranch kidney, as originally uncovered in the young skate (9), was described in detail in adolescent young dogfish (10). The present study demonstrates the potential of nephron growth and differentiation in the adult skate kidney.

Mesenchymal cells capable of nephrogenesis must establish contact with collecting duct tips for growth and branching to occur (24,25). It can be anticipated that mesenchymal cells competent for nephron development presumably provide the progenitor cells also in the skate. These cells are presumably derived from a continuous pool of embryonic stem cell-like cells within the nephrogenic zone of the elasmobranch kidney. We suggest that these cells are precursors of competent stage I mesenchyme. We further suggest that the mesenchyme provides self-renewing cells for ongoing nephrogenesis. Such cells would fulfill requirements of stem cells (26). Future studies involving lineage studies will address these hypotheses.

The contralateral kidney of the partially nephrectomized skate increased in size, a common finding in mammalian kidneys (16,27–29). Information from lower vertebrates is limited. A nephrectomy study in the common carp, Cyprinus carpio, a teleost, was focused on blood cell formation. The contralateral kidney of the carp showed distinct hypertrophic growth after several months, and the excised portion in the kidney undergoing partial nephrectomy did not regenerate (30). Similar results were reported for the newt, Notophthalmus viridescens, a urodelic amphibian. In this animal, nephrogenesis was not observed (31).

In this study, we were able to confirm our earlier findings that neonephrogenesis indeed occurs in the adult elasmobranch (9,10). Our new observations of BrdU incorporation in skate provide further evidence for formation of nephrons de novo in the nephrogenic zone. Mitotic activity as visualized by this technique was high in various stages of nephron development and young nephrons of nephrectomized animals. Older portions of the kidney were virtually devoid of BrdU labeling. Similar results were obtained in the remnant portion of the operated kidney. This clearly shows that it is only the lateral
zone that can be stimulated to substantial renal regeneration with production of new nephrons. In this region, nephron development and growth of the collecting duct system together with growth of the renal vasculature can be stimulated. We suggest that the undifferentiated cells residing in the mesenchyme of the lateral kidney zone can provide targets for signals that initiate cell division after stimulation by nephrectomy. This will be followed by the formation of initial stages of nephron development. The formation of new nephrons was also observed in adult teleosts (6,7,32). In contrast, nephrogenesis in mammals has been described to terminate shortly after birth. The reasons for loss of this ability in higher vertebrates are unclear. During prenatal growth of the metanephric mammalian kidney, nephrogenesis can be greatly augmented according to recent uninephrectomy experiments with unborn sheep (33). In these animals, compensatory renal growth of the contralateral kidney is associated with an increase in final nephron number due to prolonged nephrogenic growth in the embryonic metanephiros. Our experiments suggest that the elasmobranch kidney is equipped with a nephrogenic zone, in which similar “embryonic growth” persists life-long. The nephrogenic potential can be activated by removal of renal tissue.

We believe that our findings provide exciting perspectives. We provide a model in which new nephron production can be observed. We believe that identifying the stem cells capable of nephrogenesis in this model is possible. Their identification will facilitate studying the signaling molecules responsible. Gene expression studies should be helpful in this regard. Because teleost fish also possess nephrogenic potential, our studies may be transferable to zebrafish, the genome of which is currently available. However, elucidation of the elasmobranch genome also stands on the horizon. We suggest that models such as these will improve our understanding of basic functions and eventually will contribute to novel therapies.

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