Cell Tissue Res (1997) 287:193–201

Cell & Tissue Research

Three-dimensional organization of the developing vasculature of the kidney

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Received: 20 May 1996 / Accepted: 11 July 1996

Abstract. Kidney function depends on a well-developed vascular system. Any impairment of the blood supply disturbs the integrity and function of the organ. The differentiation of renal vessels has been investigation for many years, but little is known about the relationship between nephrogenesis and vessel development. In the present work the spatial organization of the differentiating vessels was analyzed in precisely oriented tissue sections and in optical sections acquired by laser scan microscopy. Developing vessels as well as small capillaries were visualized with two endothelium-detecting antibodies. Small vessels running in parallel towards the organ capsule were detected in numerous cortico-medullary-oriented tissue sections. Cross-sections of the nephrogenic zone showed a regularly arranged network, which was composed of cells detected by both monoclonal antibodies. Parts of this network were localized in regions of the nephrogenic zone which have been assumed to be free of vessels or vessel-like structures for a long time. These results were confirmed by the laser-scan-microscopic analysis of complete cortex explants. The extraordinarily regular arrangement of the endothelial network in the nephrogenic zone allowed us to reconstruct the developing vascular system. The results presented here underline the close relationship between nephrogenesis and vessel development.

Key words: Kidney – Development – Vascular system – Endothelium-detecting antibodies – Rabbit

Introduction

A close interaction between the vessel system and the organ parenchyma is essential for the proper functioning of the kidney. Both harmful metabolic products and useful nutrients use the bloodstream to leave or to reach the organ. After passage through the glomerular filter, the useful and harmful metabolites are separated. The composition of the primary ultrafiltrate of the urine is changed by the controlled reabsorption of reuseable components, as well as by the active secretion of harmful waste products into the lumen of the tubule system.

Kidney function without an intact vessel system is unthinkable. In this context it is of great interest to know the mechanisms behind the development of the renal vascular system. In contrast to the vascular system, the development of the organ parenchyma of the kidney has been investigated intensively (Saxén 1987). Renal organogenesis is inititated by the mutual induction of the nephrogenic mesenchyme and the ureteric bud. The mesenchyme gives rise to the nephrons, while the collecting duct system originates from the ureteric bud. Both components develop in close spatial and temporal proximity. The dichotomous branching of the collecting duct ampullae and the continuous induction of new nephrons result in an intriguingly regular composition of the nephrogenic zone.

It has been assumed for many years that parts of the nephrogenic zone are not supplied by vessels (Osathanondh and Potter 1966; Kazimierczak 1971). Early functional vessels have been detected by different methods within the so-called S-shaped body – a relatively late nephron differentiation stage (Lewis 1958; Jokelainen 1963; Potter 1965). However, the region between the upper pole of the renal vesicle, an earlier nephron differentiation stage, and the fibrous organ capsule seemed to be avascular. Recently, the close coordination of renal vascular development and nephrogenesis was demonstrated (Kloth et al. 1994a). By means of monoclonal antibodies which detect endothelial cells of different developmental stages (EC1, EnPo 1; Kloth et

Dedicated to Professor A. Oksche on the occasion of his 70th birthday.

This investigation was supported by a grant from the Deutsche Forschungsgemeinschaft (KI 741/7-1).

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al. 1992, 1994b), it could be shown for the first time that all parts of the nephrogenic zone were supplied by vessels or at least vessel-like structures.

The present work describes the three-dimensional spreading out of the developing vasculature in neonatal rabbit kidneys. The unipapillary rabbit kidney is not completely developed at the time of birth. Maturation takes place during the first 3 weeks postnatal (Mehrgut et al. 1990). Beyond the fibrous organ capsule embryonic collecting duct ampullae, nephrons of different developmental stages, and mesenchymal cells are found (Saxén 1987). This tissue zone is 100 to 200 μm thick and is called the nephronic zone. Beyond this region we can observe nephrons at more advanced stages of development and then the well-known zonal organization of the mammalian kidney. Thus, the continuous progression of tissue differentiation can be easily analyzed on precisely oriented sections.

Matured and developing vessels were detected with two monoclonal antibodies (EC1, EnPo 1). In combination with confocal laser scan microscopy the detailed analysis of the nascent vascular network in the nephrogenic zone provided a reconstruction of the three-dimensional structure of the developing vascular network possible.

Materials and methods

Production of monoclonal antibodies

Monoclonal antibodies EnPo 1 and EC1 were raised by immunizing Balbc mice with crude rabbit kidney homogenates (Kloth et al. 1992, 1994b). The fusion of spleen cells and the cloning of the hybridoma cells were performed as described by Köhler and Milstein (1975).

Cloned hybridomas were cultured in RPMI 1640 (Gibco-BRL Life Technologies, Eggenstein, Germany) supplemented with 2 mM L-glutamin, 10^{-5} M β-2 mercaptoethanol and 10% fetal calf serum (Boehringer, Mannheim, Germany) in a Heraeus incubator (humid 5% CO_2/95% air atmosphere at 37° C).

Indirect immunoperoxidase method

Kidneys were removed from 1- to 3-day-old rabbits and embedded in a drop of TissueTek O.C.T. 4583 Compound (Miles, Elkhart, USA). The tissue was immediately frozen in liquid nitrogen for later sectioning.

For the immunohistological detection of endothelial antigens, 8 μm cryosections were cut using a cryomicrotome (Microm, Heidelberg, Germany). The sections were placed on coverslips and were fixed (Kujat et al. 1993) and incubated as has been described in detail (Kloth et al. 1994b). Primary antibodies were applied overnight in the form of undiluted culture supernatants. Bound primary antibody was detected using biotin-conjugated donkey anti-mouse Ig antiserum (Dianova, Hamburg, Germany).

Co-incubation experiments were performed with the cytokeratin-19-specific antibody Ks 19.2.105 and EC1 to detect collecting duct epithelium. The anti-cytokeratin antibody was a generous gift from Prof. R. Moll, University of Halle. Within the nephrogenic zone, only collecting duct ampullae were labeled by Ks 19.2.105, while mesenchyme and developing nephrons showed no immunostaining (Moll et al. 1991).

Controls. In order to ensure the specificity of the antibody labeling, different controls were included in the experiments. Pre-immune serum as well as irrelevant primary antibodies were applied. Furthermore, control sections were incubated with the detecting antibody and the enzyme complex alone. None of the control sections showed any positive antibody labeling of vessels. More than 1000 sections obtained from 100 different kidneys were analyzed. Oriented serial cross-sectioning was performed with 10 kidneys. No differences of the spatial structure of the developing vessels were observed in different cortex areas. The described vascular network was abundant throughout the nephrogenic zone of the whole organ.

Confocal laser scan microscopy

Renal tissue explants for confocal laser scan analysis were obtained by stripping off the fibrous organ capsule (Fig. 1b). This technique for preparing nephrogenic tissue omits proteases (Minuth 1987). Thus the tissue composition is not changed: both the extracellular matrix and cell membrane proteins remain intact. The explants were approximately 150 μm thick. Six explants obtained from different kidneys were analyzed.

The tissue was incubated in blocking buffer (PBS, pH 7.2, 1% bovine serum albumin, Sigma; 10% horse serum, Boehringer) for 1 h. Then the explants were incubated overnight in undiluted culture supernatants of either antibody EC1 or EnPo 1. Prior to the application of the detecting antibody (donkey anti-mouse Ig fluorescein isothiocyanate; Dianova; 1:200 in blocking buffer, 2 h) the tissue pieces were washed intensively for 30 min. The explants were spread out on coverslips, embedded in FITC-guard (Testoc, Chicago, Ill., USA) and analyzed with a confocal laser scan microscope (MRC 500, BioRad, Munich, Germany; in combination with a Zeiss IM 35 microscope, Oberkochen, Germany).

Optical sections of 1 μm thickness were obtained by scanning the explant along the z-axis from the fibrous organ capsule down to a depth of 100 μm. The optical sections were processed using software from BioRad. Volume rendering of the laser scan images was done with Voxel View/E 2.2 (Vital Images, Fairfield, Iowa, USA) on a SGI Iris Indigo computer (Silicon Graphics, Mountain View, Calif., USA).

The three-dimensional models were drawn with I-DEAS Solid Modeling software (Structural Dynamics Research Corporation, Milford, Ohio, USA) on a Sparc station IPX (Sun Microsystems, Mountain View, Calif., USA). Results were documented using Kodak Ektachrome 64T film.

Tissue preparation for semithin sections

Freshly prepared renal tissue was fixed in a solution of 3% glutaraldehyde (Sigma) in 0.1 M cacodylate buffer. For post-fixation 1% osmium tetroxide (Merck) was used. Following fixation of the tissue the samples were immersed in 1% uranyl acetate solution (Merck), dehydrated in a graded ethanol series and embedded in Epon (Polyscience, Warrington, USA). Semithin sections were cut with a Reichert ultramicrotome OMU III (Basel, Switzerland). The sections were stained with Richardson's solution [solution A: 1% methylene blue in 1% Na-tetraborate (Merck); solution B: 1% Mallory's Azur II in destilled water; equal amounts of solution A and B were mixed]. For documentation Agfa Pan 25 film was used.

Results

The three-dimensional structure of the developing vascular network in the kidney was investigated using organs from neonatal rabbits and two monoclonal antibodies (EnPo 1, EC1) which detect endothelial cells (Kloth et

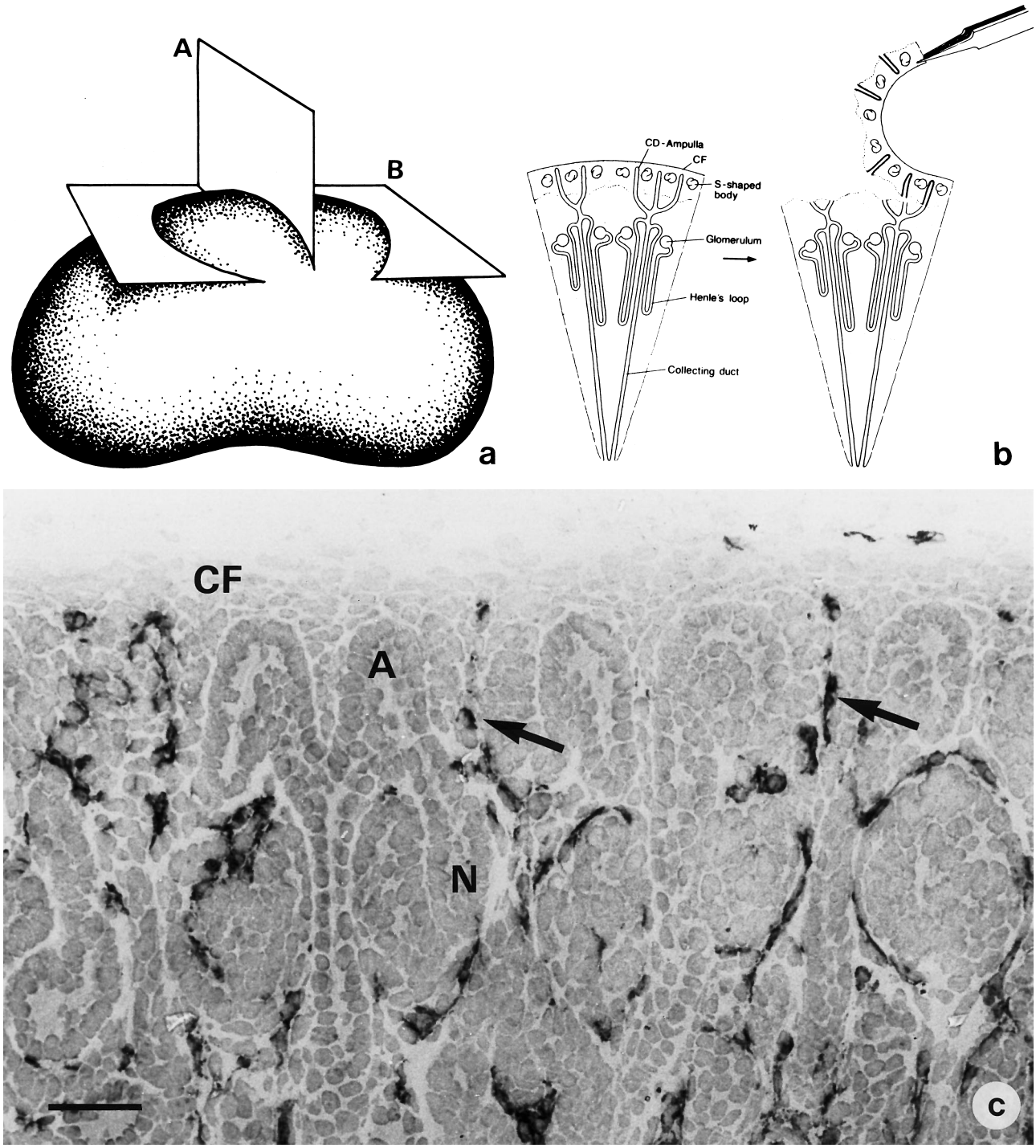
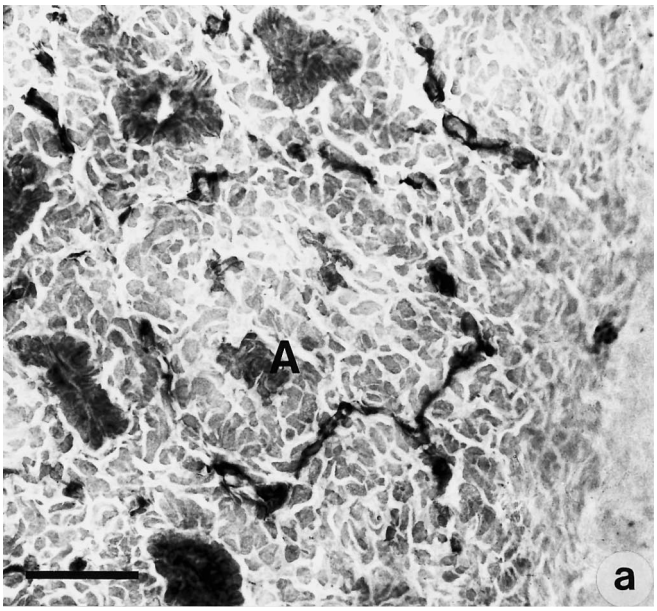
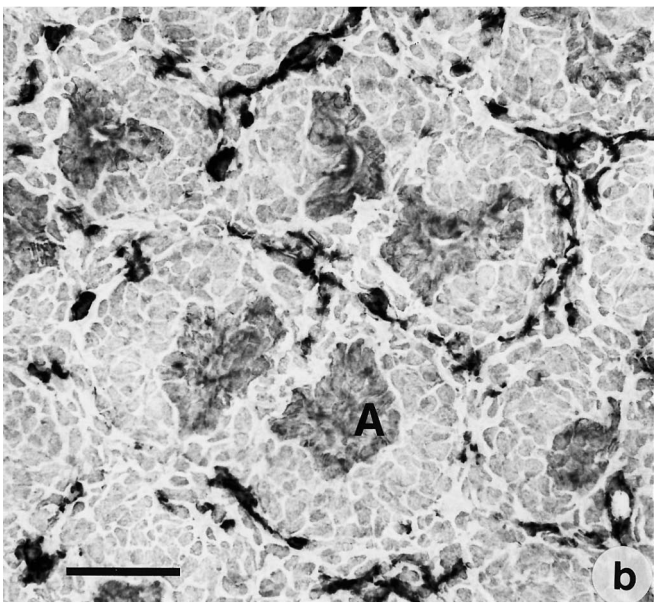


Fig. 1a–c. The organization of the developing vasculature in oriented tissue sections **a** Schematic illustration of section orientations in the neonatal kidney. Plane *A*: cortico-medullary section; plane *B*: tangentially oriented cross-section. **b** The nephrogenic zone of the neonatal rabbit kidney is located beyond the fibrous organ capsule (*CF*). It consists of collecting duct ampullae, developing nephrons, mesenchymal and endothelia cells. This zone can be easily prepared by stripping off the fibrous organ capsule. The organization and the protein composition of the tissue remain intact when this preparation technique is used. **c** Cortico-medullary-oriented section of the nephrogenic zone. The specimen was incubated with the endothelium-specific antibody EC1 and a horseradish peroxidase conjugate for visualization of the vessels. Beyond the fibrous organ capsule (*CF*) numerous small vessels were detectable. Parallel vessels running toward the capsule are a characteristic feature of these sections (*arrows*). Between the parallel oriented vessels developing nephrons (*N*), mesenchyme and collecting duct ampullae (*A*) were found. ×300. *Scale bar:* 50 μm





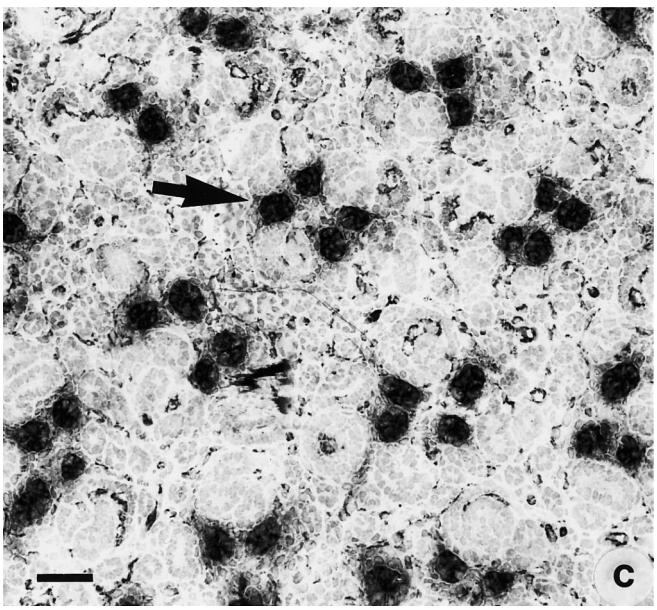
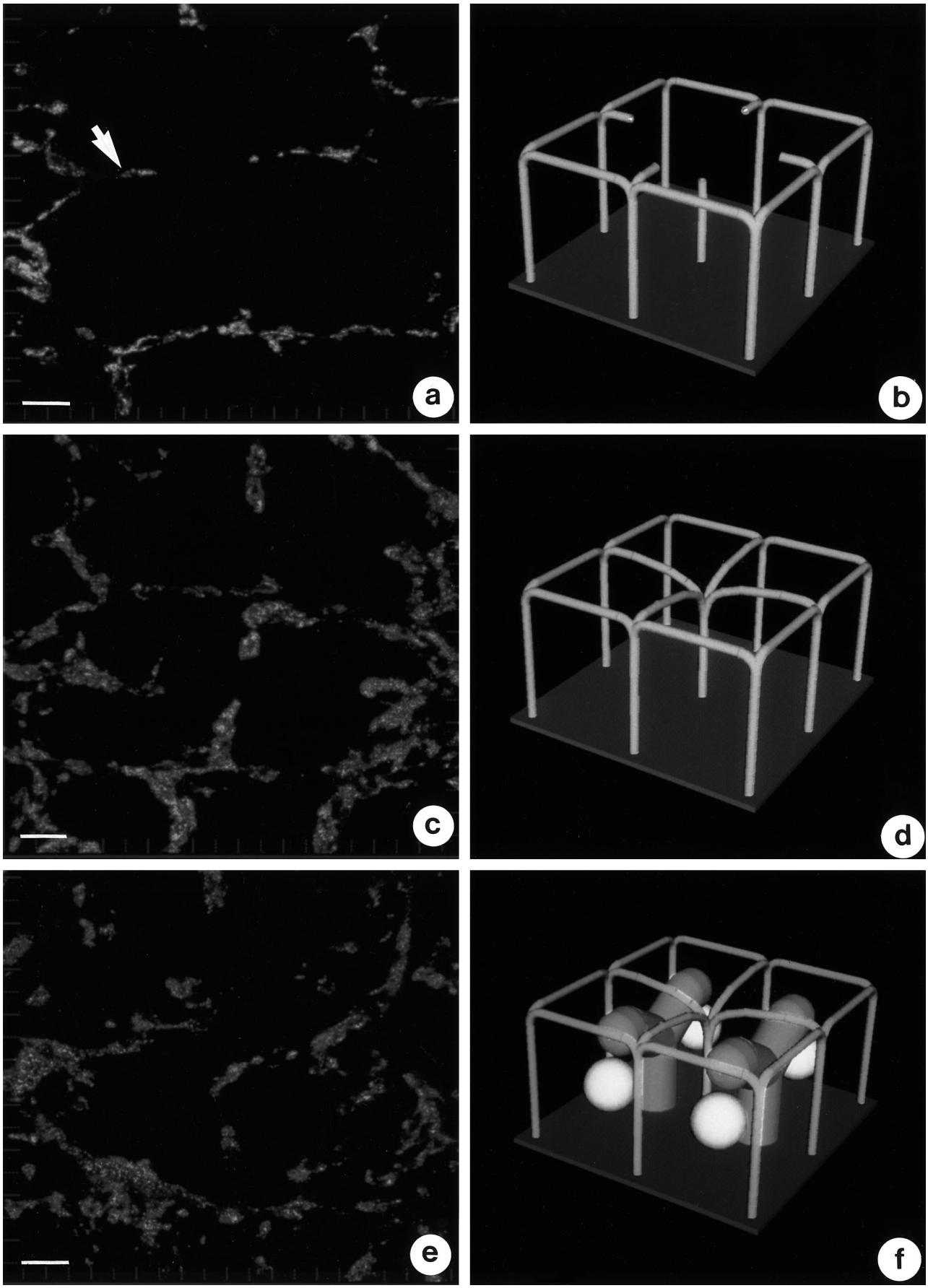


Fig. 2a–c. Oriented cross-sections of the nephrogenic zone. All specimens were incubated with the monoclonal antibodies EC1 and Ks19.2.105. Endothelial cells were labeled by EC1, while the collecting duct ampullae reacted with Ks19.2.105. Bound primary antibodies were detected by horseradish peroxidase conjugates. **a** A section adjacent to the fibrous organ capsule. A network of endothelial cells was detected in a tissue zone that until now has always been assumed to be avascular. The network consisted of regularly arranged large meshes. In this section, a large mesh with a diameter of about 160 µm is visible. The endothelial cells encircle first cuts of ampullary tips of the collecting ducts (*A*) and mesenchymal cells. The ampullary tips were labeled by Ks19.2.105. Within the mesh very few endothelial cells were found. ×250. *Scale bar:* 60 µm. **b** A cross-section of a tissue layer which lies 30 µm deeper. Smal meshes of endothelial cells were detected by the antibody EC1. Within each small mesh only one cross-section of a collecting duct ampulla (*A*) was found. The oval form of the collecting duct revealed that the ampulla had been sectioned through its broadened tip. In close proximity to the ampulla, mesenchymal cells or developing nephrons were located. Very few endothelial cells were detected within the small mesh. ×250. *Scale bar:* 60 µm. **c** One hundred micrometers beneath the fibrous organ capsule no regularly arranged endothelial networks were detectable. Many vessels were observed all over the section. Characteristically grouped collecting ducts (*arrow*) were labeled by Ks19.2.105. These groups of collecting ducts were enclosed by vessels and developing nephrons. ×120. *Scale bar:* 60 µm

Fig. 3a–f. Confocal laser scan analysis of renal cortex explants and reconstruction of the developing vascular network. The nephrogenic zone of the neonatal rabbit kidney was prepared by stripping off the fibrous organ capsule (see Fig. 1b). Endothelial cells were visualized by incubating the tissue explant with the monoclonal antibody EnPo 1 and a fluorescein isothiocyanate-conjugated secondary antibody. The flat explant was placed on a slide in a drop of mounting medium and was completely scanned in steps of 1 µm. **a, b** The reconstruction (**a**) consists of the five optical sections beyond the fibrous organ capsule. One large mesh of endothelial cells (diameter: 160 µm) is shown. Within this tissue zone we found large, interconnected meshes over the whole area of the explant. Typical short endothelial bands (*arrow*) reached from the margins to the center of the mesh. The relationship between the large mesh and the parallel vessels observed in cortico-medullary tissue sections (see Fig. 1c) is shown in the model (**b**). **c, d** A reconstruction of optical sections of the zone 50–60 µm beyond the organ capsule is shown. Within this tissue zone small endothelial meshes of about 80 µm of diameter were detected. Analysis of subsequent optical sections revealed the continuous elongation of the short endothelial bands which were observed in the large mesh (**a**). **d** The large mesh is subdivided by four of these bands which met in its center. **e** The reconstruction of five optical sections obtained in a tissue zone about 80 µm below the organ capsule demonstrated a multitude of vessels. The regular arrangement of the developing vascular network in the outer tissue zone was no longer detectable. ×415. *Scale bar:* 20 µm. **f** The relationship between the regularly arranged vessels and the developing nephrons and collecting ducts is shown in this model. Each small mesh encloses a collecting duct ampulla, a nephron and mesenchymal cells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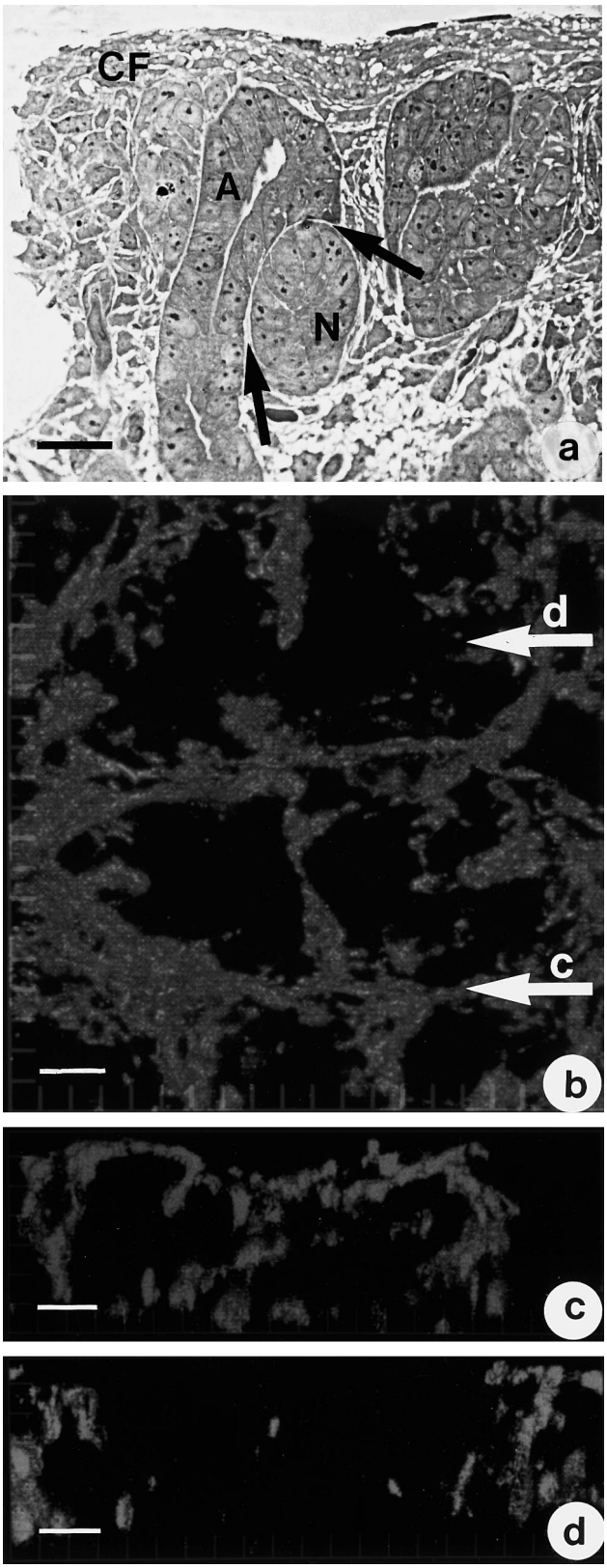
al. 1992, 1994b). They showed a remarkable specificity for endothelial cells of different developmental stages. This made it possible to analyze vessels within the nephrogenic zone of the developing kidney (Kloth et al. 1994a).



Organization of the developing vasculature in tissue sections

Precisely oriented tissue sections were used for this investigation. The kidneys were sectioned in both cortico-medullary and tangential directions (Fig. 1a). The cortico-medullary orientation (Fig. 1a, section plane A) yielded sections consisting of the cortex with the nephrogenic zone and the medulla including the papilla. The tangentially oriented sections (Fig. 1a, section plane B) represented cross-sections through the nephrogenic zone of the developing kidney. All of the cross-sections, even the first cuts through the fibrous organ capsule, were sampled and analyzed.

In cortico-medullary kidney sections (Fig. 1, section plane A) numerous small vessels could be detected by the monoclonal antibody EC1 within the nephrogenic zone (Fig. 1c). We always observed parallel vessels running toward the fibrous organ capsule. Branches of the arteria interlobularis which supply the developing nephrons were connected with these small capillaries. The parallel vessels were found in close proximity to the collecting ducts as well as between the developing nephrons. The distance between vessels was 80–160 μm.

Cross-sections of the nephrogenic zone showed that the parallel oriented vessel branches were connected with a network of endothelial cells localized beneath the fibrous organ capsule (Fig. 2). This network spread out parallel to the organ capsule. Furthermore, the cross-sections (Fig. 1a, section plane B) revealed a high degree of spatial organization of the developing vasculature which has not been described before (Fig. 2). Both antibodies labeled a cellular network located within a tissue zone which has long been assumed to be avascular. The meshes of this network were formed by endothelial cells and could be demonstrated for the first time by the antibodies EC1 and EnPo 1.

Two different networks were detectable: (1) Large meshes with an approximate diameter of 160 μm were found in the first cross-sections beneath the fibrous organ capsule (Fig. 2a). (2) Consecutive sections showed small meshes with diameters of 80–100 μm (Fig. 2b). Adjacent to this zone no meshes could be detected but

Fig. 4a–d. Within the nephrogenic zone, the developing vascular network is found in distinct areas, while other parts of the tissue are nearly free of endothelial cells. **a** As shown in the semithin section, the contact area (*arrows*) between collecting duct ampulla (*A*) and the developing nephron (*N*) is devoid of any cells. Neither mesenchyme nor endothelial cells were observed. *CF*, Fibrous organ capsule. ×500. *Scale bar:* 20 μm. A complete reconstruction of a large mesh subdivided by endothelia bands into four small meshes was analyzed (**b–d**). **b** Reconstruction of 80 optical sections (section thickness: 1 μm). The *arrows* indicate the positions of the cortico-medullary sections shown in **c** and **d**. ×415. *Scale bar:* 20 μm. **c** Cortico-medullary section (Fig. 1a, section plane A) through the periphery of the large mesh. A large number of endothelial cells can be observed. ×394. *Scale bar:* 20 μm. **d** Two small meshes were transversely sectioned. The centers of the meshes are nearly free of endothelial cells. ×394. *Scale bar:* 20 μm

we did see a large number of cross-sectioned vessels (Fig. 2c).

In order to analyze the spatial arrangement of the developing vessels, the collecting duct ampullae and the maturing nephrons, co-incubation experiments were carried out. An anti-cytokeratin 19 antibody was used to label the collecting duct epithelium (Moll et al. 1991). These experiments underlined the high degree of spatial organization of the different tissue components within this region of the organ. Within the large endothelial meshes, which were observed in the first cross-sections under the fibrous organ capsule, we found only small first slices of collecting duct ampullae (Fig. 2a). These bits of ampullae were surrounded by endothelial and mesenchymal cells which showed no signs of epithelial organization. One large mesh enclosed up to four sections of collecting duct ampullae.

In the following sections, approximately 60 μm beyond the organ capsule, meshes of smaller diameter were found. Each small mesh contained only one complete cross-section of a collecting duct ampulla (Fig. 2b). The oval form of the cross-sections revealed that the ampullae had been cut through their broadened tips. The collecting ducts were surrounded by unlabeled cells which showed in part an epithelial organization. Part of the mesenchymal cells have already converted into a developing nephron.

In tissue layers more than 100 μm below the organ capsule no regular endothelial networks were detectable (Fig. 2c). A multitude of small capillaries and S-shaped bodies were observed instead. However, within this tissue region the collecting ducts were characteristically grouped. Four sections of collecting ducts were always found surrounded by vessels and S-shaped bodies.

Laser scan analysis of the spatial structure of the developing vessels in tissue explants

In order to reconstruct the three-dimensional organization of the developing endothelial network tissue, we prepared explants of the nephrogenic zone. The thin tissue explants consisted of collecting duct ampullae, endothelial cells, developing nephrons, and mesenchyme attached to the fibrous organ capsule (Fig. 1b). The microsurgical preparation technique (Minuth 1987) did not affect the tissue organization or the protein composition of the extracellular matrix and the cell membrane. The explants were flat and 100–150 μm thick. Tissue penetration by the antibodies was verified in optical sections of the whole explant.

Immediately beyond the fibrous organ capsule vessel-like structures were found (Fig. 3a). The antibody-labeled cell bands were described vessel-like because of their similarity to developed vessels (Kloth et al. 1992). However, these vessel-like structures were detected within a tissue region where vessels with a lumen have not been found before.

The vessel-like structures were arranged in a regular network as found in the cross-sections. In optical sections adjacent to the fibrous organ capsule the network was found to consist of large meshes (Fig. 3a), while in deeper tissue layers only small meshes were found (Fig. 3c). The diameter of one large mesh was about 160 μm. This network spread out parallel to the organ capsule. Bands of endothelial cells extended from margins of the large mesh toward its center. These bands divided the large mesh in four small subunits (Fig. 3d). The meeting point was localized in the center between four collecting duct ampullae. It was situated a few micrometers below the level of the large mesh. Stepwise scanning of subsequent tissue layers thus gave the impression of a large mesh continuously being subdivided by endothelial sprouts.

At a depth of 50 μm endothelial bands had reached the center of the large mesh (Fig. 3b). Its division into four equal parts was complete. The regular arrangement of the smal vessels was observed throughout the whole explant. In the small meshes themselves short endothelial bands were again found reaching toward the center of the mesh. Furthermore, few cross-sectioned vessels were observed.

Reconstructions of all sections within a distance of 80 μm from the organ capsule (Fig. 4b) were produced as well as reconstructions of selected regions (Fig. 3a, c, e). The results of the laser scan analysis were confirmed by analyzing the oriented cross-sectioning of the nephrogenic zone. The large meshes were found at a distance of 30 μm from the organ capsule (Figs. 2a, 3a). Small meshes were observed at 60 μm (Figs. 2b, 3c). In adjacent tissue layers beyond a distance of 80 μm from the organ capsule no regular arrangement of the vessels was observed (Fig. 2c, 3e). These tissue regions were supplied by a large number of small vessels surrounding the nephrons and collecting ducts.

The tissue areas enclosed by regular endothelial meshes were nearly free of endothelial cells (Fig. 4b–d). A complete reconstruction of a large mesh which was subdivided into four small meshes is shown in Fig. 4b. It was cut in cortico-medullary sections. The section through the margin of the large mesh showed numerous labeled endothelial cells (Fig. 4c). A transverse section of two small meshes demonstrated that the center of the meshes were free of endothelial cells (Fig. 4d).

Discussion

The development of the renal vascular system has been investigated for many years (Lewis 1958; Osathanondh and Potter 1966; Ekblom et al. 1982; Saxén 1987; Abrahamson et al. 1991), but markers for endothelial cells of different developmental stages are scarce. Thus, the first steps of vessel development as well as the spatial organization of the developing vasculature have remained obscure. In order to be able to detect endothelial cells of different developmental stages, monoclonal antibodies were produced and characterized. The antibody EC1 exclusively labels endothelial cells (Kloth et al. 1994b). Arteries and veins are labeled with the same intensity, but capillaries showed weaker antibody binding than large vessels. The EnPo 1 antigen is expressed intensively by podocytes, capillaries and small vessels, while large arteries and veins are hardly detected (Kloth et al. 1992). However, both endothelium-detecting anti-

bodies labeled a network of small vessel-like structures between the fibrous organ capsule and the upper poles of the renal vesicles (Kloth et al. 1994a). Until now, this zone has always been assumed to be avascular. Thus, the term ''vessel-like structure'' was introduced for bands of cells labeled by the antibodies EC1 and ENPo 1 which were detected within this zone (Kloth et al. 1992). The labeled cell bands were continuous with vessels of more mature tissue layers. They were found between the organ capsule and the renal vesicle – in a tissue region where no vessels with a lumen had been detected until now.

It was demonstrated for the first time that the nephrogenic zone is supplied by a multitude of small vessels and vessel-like structures. Precisely oriented tissue sections and laser scan optical sectioning of tissue explants allowed the reconstruction of the three-dimensional structure of the developing vascular network (Fig. 3f). The schematic drawing represents a simplified model of the developing renal vasculature. However, nothing can be said about the direction of vessel growth at the moment. It is not known whether the endothelial bands, which possess no lumen at this point of differentiation, later give to one or more vessels. Some images prompt us to assume that in some cases two vessels may originate from these bands (Fig. 2b).

The analysis of sections oriented in a cortico-medullary direction (Fig. 1a, section plane A) revealed a multitude of vessels and vessel-like structures within the nephrogenic zone. Cross-sections (Fig. 1, section plane B), on the other hand, revealed that the parts of the tissue located within the endothelial meshes included only very few vessels. By incubation with different antibodies it could be demonstrated that these tissue regions were occupied by collecting duct ampullae, mesenchymal cells or developing nephrons. However, one would except all of these tissue components to be surrounded by a tight endothelial network. Thus, endothelial cells should be present between the collecting duct ampullae and the developing nephrons. What is the explanation for vessel-free areas in the tissue?

Nephrogenesis takes place in close proximity to, but separate from the collecting duct ampulla. The connection between both is established in the renal vesicle stage (Saxén 1987). At this point of development nephron and collecting duct are separated only by a thin and incomplete basement membrane (Lehtonen 1975). Neither mesenchymal cells nor vessels are found in the contact area, which is 30–50 μm thick and is located below the ampullary tip (Fig. 4a). This is about the distance between the tissue zones where large and small meshes are found. Within both types of meshes only few endothelial cells were observed. Just below this zone, the collecting ducts and nephrons are completely enclosed by small vessels. This is the explanation for the sudden change from the regularly arranged vessel network to the multitude of cross-sectioned, difficult-to-survey vessels.

The schematic model (Fig. 3f) represents only the basic parts of the developing vascular network. Between the parallel vessels, which protrude toward the organ capsule, many vessel-like structures were detected. These vessels are not represented in the schematic drawing, but were frequently observed in tissue sections (Fig. 1c). They can be demonstrated in the laser scan images as well (Fig. 4c). Thus, developing nephrons and collecting ducts are tightly surrounded by capillaries and vessel precursors. Only the contact site between the two structures remains free of vessels.

The morphogenic factors involved in the generation of these patterns are the components of the extracellular matrix (Sorokin and Ekblom 1992; Haralabopoulos et al. 1994), cell membrane proteins (Korhonen et al. 1990; Lallier et al. 1994) and soluble factors (Folkman and Klagsbrun 1987; Houck et al. 1992). At present we can only speculate about how the regular arrangement of the developing renal vasculature is achieved.

Two different mechanisms for renal vessel development have been discussed. An ingrowth of vessel sprouts into the nephrogenic zone has been proposed (Ekblom et al. 1982; Sariola et al. 1983). It was assumed that the vessel sprouts find their way to the developing nephrons using distinct extracellular components as guiding structures (Sorokin and Ekblom 1992). However, tissue culture experiments recently revealed that the extracellular matrix itself is not sufficient to support the regular structure of the renal microvascular system (Kloth et al. 1995). The second proposal was that the renal vasculature may originate from the nephrogenic mesenchyme (Emura and Tanaka 1972). A subpopulation of mesenchymal cells in the nephrogenic zone diffusely expressed the EnPo 1 antigen (Kloth et al. 1992, 1994a). Only the mesenchyme which surrounded the ampullary tip – the cell population which will give rise to further nephron generations – was labeled by the antibody. The expression of the EnPo 1 antigen by some of these cells suggests that nephrons and endothelial cells may both originate from the mesenchyme.

The regular, dichotomous branching of the collecting duct gives rise to collecting duct ampullae which are in a comparable differentiation stage (Osathanondh and Potter 1963a, b). They are located at an equal distance to each other. Each ampullary tip is surrounded by a cap of mesenchymal cells. This arrangement provides the framework for the regular organization of the developing vessels.

Acknowledgements. We gratefully acknowledge the skilful technical assistance of Mrs. Christiane Wanke. We wish to thank Dr. Hinrich Sass, Institute of Zoology, University of Regensburg, for his kind support in three-dimensional modelling.

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