RENAL MEDULLARY LYMPHATICS: MICRORADIOGRAPHIC, LIGHT, AND ELECTRON MICROSCOPIC STUDIES IN PIGS

JT Cuttino, Jr., M.D., JC Jennette, M.D., RL Clark, M.D., L Kwock, Ph.D.

Department of Radiology and Pathology, University of North Carolina, School of Medicine, Chapel Hill, North Carolina.

ABSTRACT

Although renal cortical lymphatics have been extensively studied, the existence of renal medullary lymphatics has not been well documented. We studied renal microlymphatics in pig kidneys by retrograde ureteral injection of barium-sulfate-gelatin-saline mixture. Lymphatics filled as a result of forniceal rupture and interstitial extravasation. Microradiographs were correlated with histology and electron microscopy.

We traced renal microlymphatic vessels from the arcuate level not only into the cortex but also into the pyramidal region, and therefore concluded that medullary lymphatics existed.

Intrarenal lymphatic morphology has been the subject of many investigations. The distribution of lymphatics in the renal cortex has been defined by light microscopy (1,2), India ink injections (3,4), electron microscopy (2,3,5,6), and microradiography (7). Although the lymphatics of the renal medulla have also been studied, there continues to be debate about their existence. Several authors (1,8,9) claim that they can be demonstrated, whereas others (3,5,6,10) cannot confirm their presence. Physiologic studies (11-13) of renal lymphatic effluent suggest a contribution from the concentrating mechanism of the medulla.

Because of these conflicting reports, we re-examined renal medullary lymphatics using a combination of microradiograph, light microscopic and transmission electron microscopic techniques.

MATERIALS AND METHODS

The ureters of 30 freshly excised pig kidneys were cannulated and injected at 100-150mmHg pressure with a baruim-sulfate-gelatin-saline mixture (14) for approximately 5 minutes. At the end of the radiocontrast injection, the renal artery was perfused with 100ml of 2% glutaraldehyde-paraformaldehyde for 24 hours. After this fixation, the kidneys were sectioned into 1-4mm slices and radiographed using previously described techniques (7,14). Slices were trimmed so that only a papilla and rim of adjacent cortex were present in the radiographic field. No attempt was made to identify lymphatics in papillae with tubular influx. All the barium was removed from the papillary surface prior to radiography. Portions of medullary tissue containing barium-filled vessels suspected of being lymphatics were cut out, and the vessels identified with a dissecting microscope. Blocks of tissue containing these vessels were then sliced perpendicular to the long axis of the vessels into approximately 1mm thick sections, with alternating ones processed for light microscopy or held in fixative for possible ultrasound examination. Hematoxylin and eosin-stained sections of the former were

examined by brightfield and darkfield microscopy to identify the cross sectioned vessels containing barium. Areas in the tissue sections saved for electron microscopy, that corresponded to areas in adjacent sections found to have barium-filled vessels by light microscopy, were then cut into 1mm cubes, fixed in osmium tetroxide, dehydrated, embedded in Polybed 812, sectioned at 1 micrometer, stained with toluidine blue, and examined by light microscopy. Vessels containing contrast were identified and included in block faces for ultramicrotomy. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined with a JEOL T-7 transmission electron microscope. For ultrastructural examination, 20 blocks from 2 kidneys were sectioned. The examination protocol was not designed to quantify medullary lym-phatics, but rather to demonstrate their possible existence.

RESULTS

Whole specimen radiographs (Fig. 1) revealed pyelotubular reflux (predominantly polar), as well as lymphatic and venous filling. However, lymphatic and venous visualization was more apparent on microradiographs of tissue slices. Of the 30 kidneys, lymphatics in the hilar and arcuate areas were filled with barium in 25. Radiographically, hilar and arcuate lymphatics had numerous valves and a characteristic plexus pattern around arcuate veins. Although renal veins may occasionally contain valves, we never identified any in our specimens. Cortical and medullary lymphatics could be traced from the hilar

Fig. 1: Pig kidney after ureteral barium perfusion (barium pyelogram). Tubular reflux into upper pole. Veins are filled in the lower pole (curved arrow). Hilar lymphatics containing valves (short straight arrows) and arcuate lymphatics (long straight arrow) are seen.

Fig. 2: Section through medullary region. Barium impacted in a calyx partially obscures papillary tip. Interlobular lymphatics can easily be identified by the presence of valves (straight arrow). These can be traced to arcuate and medullary lymphatics (curved arrows) (x5 magnification).

and arcuate lymphatics (Fig. 2). Cortical lymphatics were filled in 15 and medullary lymphatics in 10 of the 25 kidneys with hilar and arcuate lymphatic filling. Medullary lymphatics could best be identified in sections of isolated papillae (Fig. 3). Other structures filled with barium (e.g., interlobular veins, vasa recta, and tubules) could be differentiated by their characteristic branching and well-established distribution. There was also focal accumulations of contrast in the interstitium.

As described above in the Materials and Methods section, medullary vessels, suspected on the basis of microradiography of being lymphatics, were examined first by light microscopy and then by electron microscopy. Barium filled vessels having ultrastructural features of lymphatics were found by light microscopy to lie within the medullary vascular bundles (Fig. 4). Light microscopy of 1 micrometer plastic sections (Fig. 5) revealed contrast material within
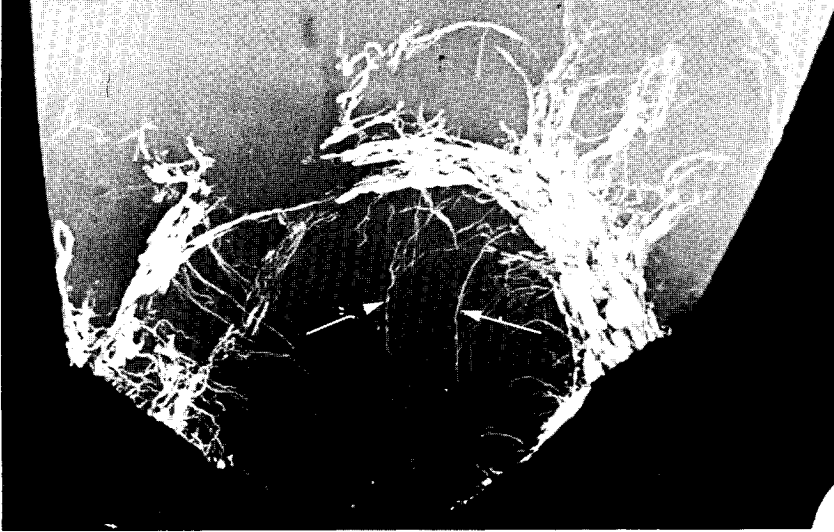

Fig. 3: Papillary section of another kidney. Barium impacted in calyx has carefully been removed. Many arcuate lymphatics are present. Several medullary lymphatics (arrow) extending into mid-medulla are present. No tubules or veins are filled in this specimen. (x5 magnification).
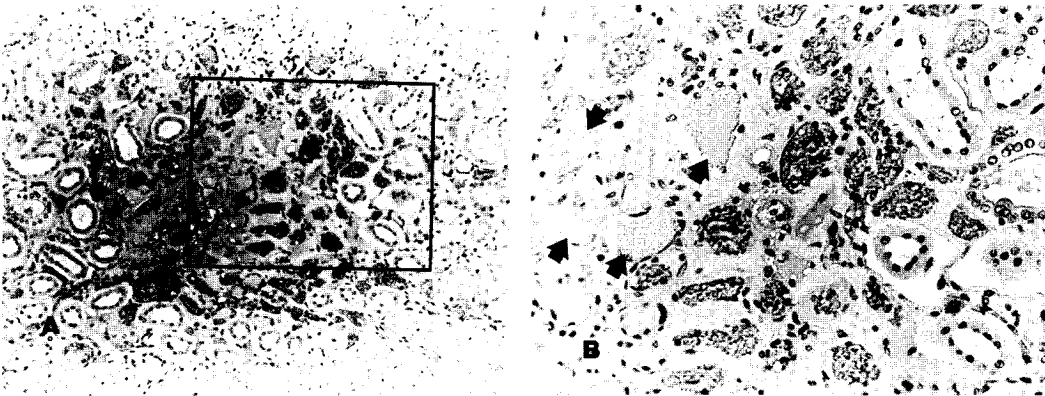

Fig. 4: A: A photomicrograph of renal medulla showing cross-sectioned vascular bundle in the center of the field (H & E, X150). B: Higher magnification of the area within the rectangle on A, showing blood-filled vasa recta and lymphatics (arrows) filled with lymph and granular contrast material (H & E, X500).
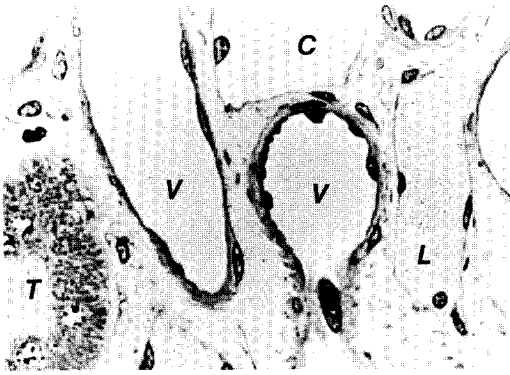
Fig. 5: Photomicrograph of 1 micrometer plastic section of a medullary vascular bundle showing a lymphatic containing contrast material (L), vasa recta (V), probable capillary (C), and a tubule (T) (Toluidine blue, X1200).
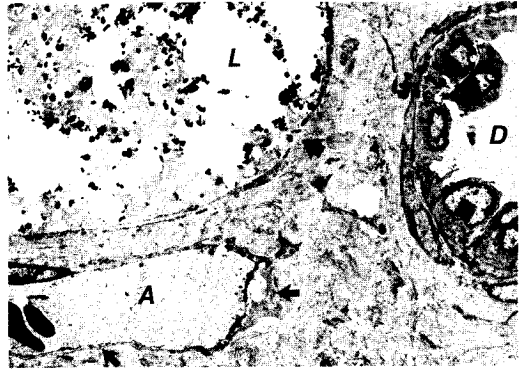

Fig. 6: Electron micrograph of a medullary vascular bundle showing a lymphatic containing contrast material (L), descending vasa recta (D), and ascending vasa recta (A). Note the better defined basement membrane around the A (small arrows), and the gap between the lymphatic endothelial cells (large arrow) (X3000).
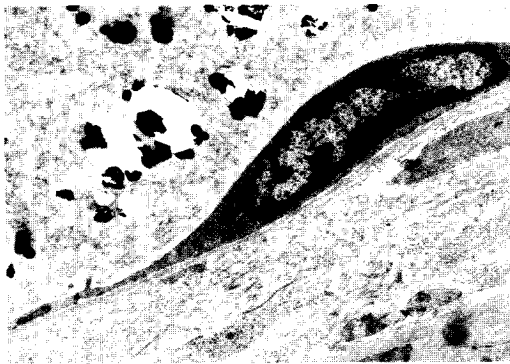

Fig. 7: Electron micrograph of a medullary lymphatic showing electron-dense contrast material in the lumen, nonfenestrated endothelium, and scanty basement membrane (X18000).
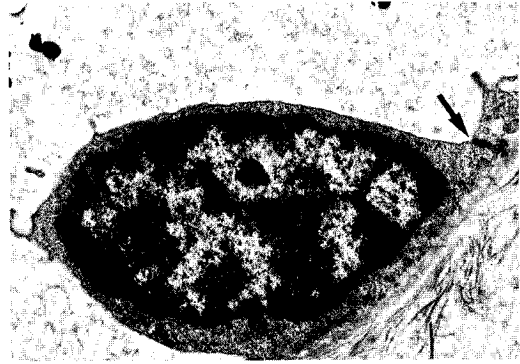

Fig. 8: Electron micrograph of a medullary lymphatic showing an endothelial cell with a bulbous projection into the lumen, and a zonula adherens junctional complex (arrow) (X23000).
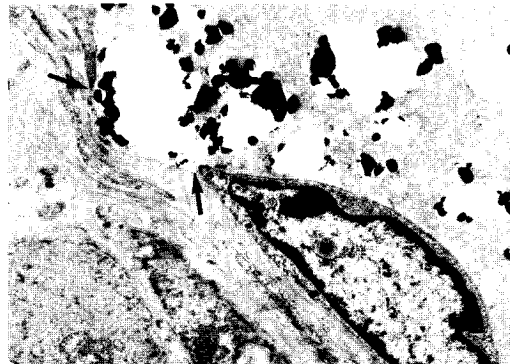

Fig. 9: Electron micrograph of a medullary lymphatic showing a gap between two endothelial cells (between arrows). Also note the absence of a basement membrane (X20000).
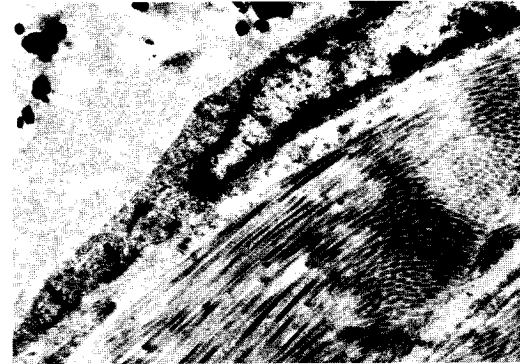

Fig. 10: Electron micrograph of a medullary lymphatic; showing an endothelial cell with areas of increased cytoplasmic density (arrows) and adjacent extracellular filaments (anchoring filaments) extending towards subadjacent interstitial collagen (X24000).

very thin-walled vessels that could be distinguished from tubules and thick-walled vasa recta, but not from capillaries or thin-walled (i.e., ascending) vasa recta.

Electron microscopy was used to confirm the presence of medullary lymphatics by demonstrating the characteristic lymphatic ultrastructure of the barium filled vessels. Lymphatic vessels were identified in both kidneys examined ultrastructurally, and in 12 of 20 blocks sectioned for electron microscopy. Barium was identified in lymphatic vessel lumens as irregular grains and spherules of extremely electron-dense material, often with adjacent defects in the plastic mountant medium (Fig. 6-10). Most endothelial cells lining lymphatic vessels had flattened, elongated nuclei (Fig. 7,10), usually with the long axis of the nuclei parallel to the long axis of the lymphatic. Occasional endothelial cell nuclei and adjacent cytoplasm were more bulbous and projected into the lymphatic lumens (Fig. 8). Lymphatic endothelial cytoplasm not adjacent to the nucleus was markedly attenuated, but not fenestrated (Fig. 7). At junctions between lymphatic endothelial cells, there were either junctional complexes, typically zonular adherens (Fig. 8), or gaps between cells. These gaps sometimes had overlapping of adjacent cells, but at other times resulted in an area of apparent continuity between lymphatic lumen and interstitium (Fig. 6,9). The basement membranes of lymphatic vessels were discontinuous and poorly defined (Fig. 6-10), with endothelial cells sometimes appearing to be in contact with interstitial collagen (Fig. 9,10). Occasionally, there were areas of increased electron density along the abluminal aspect of the lymphatic endothelial cells associated with poorly defined fine filaments extending into the adjacent interstitium (Fig. 10). These structures may be anchoring filaments. No pericytes were present along lymphatic vessels. No attempts were made to precisely quantify the number of medullary lymphatics.

DISCUSSION

We have established a convenient technique for visualizing intrarenal lymphatics. It is well known that overdistension of the ureter will result in pyelolymphatic backflow. Lymphatics fill as a result of rupture of the calyceal fornix, interstitial extravasation of contrast, and subsequent lymphatic filling. The contrast also gains access to veins, probably by rupture of small capillaries. Tubular filling can also be seen. The identification of lymphatics radiographically is based on the ability to recognize the characteristic valves. The individual vessel is then traceable with the aid of a dissecting microscope into the various regions of the kidney.

In order to obtain specimens with a greater proportion of lymphatic filling, ureteral injections were done at high pressures. A previous study (14) showed that at lower injection pressures tubular backflow predominates. Arterial perfusion with glutaraldehyde-paraformaldehyde was done to achieve rapid fixation and wash most of the barium mixture out of the arteries and veins. Although fixation was not of maximal quality for electron microscopy, adequate preservation was obtained to assess the ultrastructural features of barium-filled vessels. The pig model was chosen because of easy availability of pig kidneys (commericial slaughter house) and close resemblance to human kidneys with respect to size and multipapillary structure.

Careful sectioning of the kidneys resulted in pure papillary specimens. This eliminated possible confusion from superimposed cortical structures in the microradiographs. Sections were thick enough (1-5mm) to enable tracing of the vessels into the arcuate area (Fig. 2). Once a suspected medullary lymphatic was observed microradiographically, the area in question was isolated as described above for more definitive light microscopy and ultrastructural analysis.

We were able to trace lymphatics from the valve-containing hilar lymphatics into the mid-medullary area. Light microscopy (Fig. 4) showed these vessels to be within vascular bundles closely related to vasa recta. Thin plastic section and electron microscopic studies confirmed these vessels

to be lymphatics.

These medullary lymphatic vessels have ultrastructural characteristics previously observed in renal cortical (2,4-6) and extrarenal (16) lymphatics. These features include thin nonfenestrated endothelium, both junctional complexes and gaps between endothelial cells, discontinuous or poorly-formed basement membranes, and anchoring filaments (Fig. 6-10). These ultrastructural findings distinguish medullary lymphatics from descending vasa recta, which have thick continuous endothelium joined by zonulae occludens (17), the ascending vasa recta, which have thin fenestrated endothelium joined by zonulae occludens (17), and the thin limbs of Henle, which have interdigitating epithelium joined by zonulae occludens (18).

We thus conclude that in the pig, medullary lymphatics do exist. The inability to fill lymphatics beyond the mid-medulla may be due to failure to perfuse completely these minute structures. Similarly, failure to recognize medullary lymphatics in some kidneys reflects, in our opinion, inadequate perfusion rather than their absence. Whereas the data do not show the number or total distribution of medullary lymphatics, they do suggest their presence. The origin of medullary lymphatics remains speculative although Schmidt-Nielson (16) suggests that they form from interstitial cells of the medulla.

Our technique of renal microlymphatic visualization has several advantages over other methods of studying renal lymphatic distribution and morphology. Direct lymphatic injection techniques are limited to antegrade injection because numerous valves in the arcuate region inhibit filling of the medullary area. Our technique of forniceal rupture followed by interstitial extravasation and lymphatic filling permits access to lymphatics in the arcuate area. From there we are able to fill (in retrograde fashion) cortical and medullary lymphatics which are proximal to the valve-containing larger lymphatic vessels.

The ability of our method to trace lymphatics accurately microradiographically adds another dimension to conventional histologic and electron microscopic techniques. Although tissue clearing techniques have not been utilized, they could also be done to "three-dimensionalize" vascular and lymphatic channels. Due to particle size (2 micrometers) and presence of gelatin, barium perfusate is not only an excellent contrast agent, but also an acceptable histological and ultrastructural marker that does not wash out or cause substantial artifacts. By retrograde filling at high pressure lymphatics are distended thereby making them easier to find histologically. Inability of Albertine (5) to locate medullary lymphatics may relate to lack of adequate distention. Our previous studies (15) have shown that extremely high ureteral pressures are required to fill intrarenal lymphatics via ureteral perfusion and interstitial extravasation. In the collapsed state, lymphatics are difficult to distinguish from groups of interstitial cells and capillaries.

REFERENCES

1. Rawson AJ: Distribution of the lymphatics of the human kidney as shown in a case of carcinomatous permeation. Arch. Pathol. 47 (1949), 283-292.
2. Albertine KH, O'Morchoe CCC: Distribution and density of the canine renal cortical lymphatic system. Kidney Int'l. 16 (1979), 470-480.
3. Bell RD, Keyl MJ, Sharder FR, Jones EW, Henry LP: Renal lymphatics: The intrarenal distributon. Nephron 5 (1969), 454-463.
4. Nordquist RE, Bell RD, Sinclair RJ, Keyl MJ: The distribution and ultrastructural morphology of lymphatic vessels in the canine renal cortex. Lymphology 9 (1973), 13-19.
5. Albertine KK, O'Morchoe CCC: An integraded light and electron microscopic study on the existence of intramedullary lymphatics in the dog kidney. Lymphology 12 (1980), 100-106.
6. Kriz W, Dieterich HJ: Das lymphagefaB system der niere bei einigen saugetieren. Lichtund electronenmikroskopische untersuchungen A. Anat. Entwickl.-Gesch. 131 (1970), 111-147.
7. Clark RL, Cuttino JT Jr: Microradiographic studies of renal lymphatics. Radiology 124 (1977), 307-311.
8. Cockett ATK, Roberts AP, Moore RS: Evidence for two intrarenal lymphatic networks. Invest. Urol. 7 (1970) 266-270.
9. Reny-Vamos F, Balagh F: Pyelonephritis. Akademiai Kiado, Budapest, 1979, 16.

10. Peirce CC: Renal lymphatics. Anat. Rec. 90 (1944), 315-335.
11. Santos-Mortinez J, Selburt EC: Renal lymph and its relationship to the countercurrent multiplier system of the kidney. Am. J. Physiol. 216 (1969), 1548-1555.
12. Holmes JJ, O'Morchoe PJ, O'Morchoe CCC: The role of renal lymph in hydronephrosis. Invest. Urol. 15 (1977b), 215-219.
13. O'Morchoe CCC, O'Morchoe PJ, Danati EJ: Comparison of hilar and capsular renal lymph. Am. J. Physiol. 229 (1975), 416-424.
14. Bookstein JJ, Clark RL: Renal Microvascular Disease: Angiographic-Microangiographic Correlates. Little Brown & Co., Boston 1980.
15. Cuttino JT Jr, Clark RL, Fried FA, Stevens PS: Microradiographic demonstration of pyelolymphatic backflow in the porcine kidney. Am. J. Roentgenol. 131 (1978), 501-505.
16. Leak LV: Electron microscopic observations of lymphatic capillaries and the structural components of the connective tissue-lymph interface. Microvasc. Res. 2 (1970), 361-391.
17. Schwartz MD, Karnovsky MJ, Venkatachalam MA: Ultrastructural differences between rat inner medullary descending and ascending vasa recta. Lab. Invest. 35 (1976), 1961-1970.
18. Schwartz MM, Venkatachalm MA: Structural differences in thin limbs of Henle: Physiologic implications. Kidney Int'l. 6 (1974), 103-208.

J.T. Cuttino, Jr., M.D.
Department of Radiology
University of North Carolina
Chapel Hill, NC 27514