Lymphology 13 (1980) 100–106

An Integrated Light and Electron Microscopic Study on the Existence of Intramedullary Lymphatics in the Dog Kidney*

K.H. Albertine, Ph.D. and C.C.C. O'Morchoe, M.D., Ph.D.

Department of Anatomy, Loyola University Stritch School of Medicine, Maywood, Illinois

Summary

The existence of intramedullary lymphatics was investigated in canine kidneys by two detailed morphological approaches. The first used a combined light and electron microscopic analysis of medullary tissue fixed *in vivo* and obtained from animals with dilated as well as freely-flowing intrarenal lymphatics. Examination of 100 vascular bundles and 100 interbundle areas from 90 medullary tissue strips elicited only one strip containing lymphatic vessels. These lymphatics, in a vascular bundle, were confirmed ultrastructurally and were followed in serial one micron sections which revealed their course to be immediately adjacent to or within the arcuate region. The dearth of medullary lymphatics was in marked contrast to the existence of cortical lymphatics found in similarly processed tissue. By the second approach sections of arcuate lymphatics were examined for medullary tributaries. In serial sections from 60 tissue blocks arcuate lymphatics received only cortical tributaries. Reconstruction diagrams made from 8 blocks showed 14 tributaries from the cortex and none from the medulla. It was concluded from this study that an intramedullary system of lymphatics does not exist within the dog kidney. It is proposed that a medullary contribution to renal lymph occurs by movement of fluid through the medullary interstitium to lymphatics associated with arcuate or possibly interlobar blood vessels.

Introduction

The intrarenal distribution of lymphatics has been the subject of considerable debate with respect to both cortex and medulla. Recent evidence has clarified the distribution and density of lymphatic capillaries in the canine renal cortex by both qualitative and quantitative morphological approaches (1, 2). Previous morphological studies on the medulla, however, have failed to agree even on the existence of renal intramedullary lymphatics. As a result, two opposing morphological theories on a medullary contribution to renal lymph receive support. The proponents of one theory (3, 4, 5, 6) maintain that such a contribution occurs directly through intramedullary lymphatics which ascend to the arcuate region and there enter arcuate lymphatics. The alternative concept is that intramedullary lymphatics do not exist (7, 8, 9, 10). Under such circumstances, either lymph formation does not occur in the medulla or any medullary contribution to renal lymph occurs by movement of interstitial fluid from the medulla to lymphatic vessels of the arcuate region. Physiological studies however, provide evidence that a medullary contribution to renal hilar lymph does indeed exist (5, 11, 12, 13, 14, 15). Such investigations have revealed that the concentration of sodium and chloride is higher in renal hilar lymph than in renal venous plasma under control conditions, and that this difference can be abolished by the administration of furosemide. Accordingly, a hypothesis has been proposed that renal hilar lymph stems at least in part from the outer medullary interstitium. This interstitial region is influenced by the electrolyte pump in the ascending thick limb of *Henle* which actively pumps chloride, followed electrochemically by sodium but without an osmotically equivalent volume of water, into the surrounding interstitial space. The ensuing hyperosmolality of the outer medullary interstitium is thought to be the cause of the hilar lymph-to-plasma concentration difference for sodium and chloride. The present study was designed to investigate the morphological counterpart to the

*This work was supported by a grant from the National Institutes of Health, #AM 18473.

0024–7766/80 1400–0100 $ 02.00

physiological data by employing two detailed morphological approaches in dog kidneys.

Methods

The left kidneys of 12 mongrel dogs of either sex were used. For the first approach, three groups of kidneys were investigated. Group 1 comprised three kidneys with unilateral ureteric obstruction of three days duration and with renal lymphatic ligation for five hours. Group 2 consisted of three kidneys with renal lymphatic ligation only. Group 3 contained three kidneys with unimpeded lymph and urine flow. The experimental procedures used in groups 1 and 2 have been shown previously to dilate the intrarenal lymphatics, thus making them more visible in tissue sections (1, 2, 15). Kidneys in the third group were expected to reveal the renal lymphatic system under near-normal physiological conditions. All the kidneys were fixed *in vivo* with acrolein which was dripped onto the renal surface and simultaneously infused retrogradely through the ureter (1, 16). Ten medullary tissue strips measuring 1 x 1 x 4 mm were excised from each kidney, immersed in 2% glutaraldehyde, post-fixed in 1% osmium tetroxide and embedded in EPON 812. All the flat-embedded blocks were examined by light and electron microscopy for intramedullary lymphatics. At least one hundred vascular bundles and one hundred interbundle areas were examined.

A second investigative approach was also employed, in which the arcuate lymphatics were traced in serial sections and examined for medullary tributaries. In this second approach, six kidneys with unimpeded lymph and urine flow were fixed with acrolein, as described above. Ten blocks (5 mm^3) were excised from the arcuate region of each kidney, embedded in Araldite 502, serially sectioned at 3 μm thickness and stained with Delafield's hematoxylin and eosin-Y for light microscopy. Thin sections from additional tissue blocks (1 x 2 x 2 mm) were prepared and stained for transmission electron microscopy. Arcuate lymphatics seen in serial sections were meticulously followed and reconstructed diagrammatically in a search for medullary tributaries.

Results

Light and electron microscopic examination of 90 tissue strips from the first approach failed to reveal evidence of intramedullary lymphatic vessels. Indeed in only one block were any lymphatics seen. In this instance, three lymphatics, all in the same tissue strip were observed within the upper end of a vascular bundle composed of ascending and descending vasa recta. These vessels were tentatively identified as lymphatics by light microscopy (Fig. 1), largely on the basis of their clear lumen which was in contrast to the neighboring blood vessels that contained plasma and/or cells. Figure 2 is an electron micrograph of one of the lymphatics in an adjacent thin section. Positive identification of these vessels as lymphatics was based on the well established morphological criteria of a non-fenestrated endothelium that lacks a continuous subjacent basal lamina, which is in contradistinction to the well developed basal lamina of the surrounding blood vessels (17, 18, 19). Serial 1 μm thick sections from this tissue block revealed that the lymphatics began as blind capillaries high in the outer medulla. Their diameter increased in the direction of the juxtamedullary cortex, suggesting that these vessels coursed through the arcuate region.

A quantitative analysis of the results from the first approach was performed (Table 1). The number of cortical and medullary lymphatics from the three experimental groups were compared to gain an appreciation of the relative frequency of the two vessel types. Some of the cortical values have already been reported (1). Although many cortical lymphatics were observed, on an average one in every two or three tissue blocks, only one of the ninety medullary blocks contained lymphatics (Table 1).

In the second part of the study, recognition of arcuate lymphatics was based primarily on the presence of valves (Fig. 3), although occassional thin sections from some of the blocks were cut adjacent to thick sections and used for ultrastructural confirmation (Figs. 4 and 5). The distinction between valves and the confluence of two tributaries, often impossible

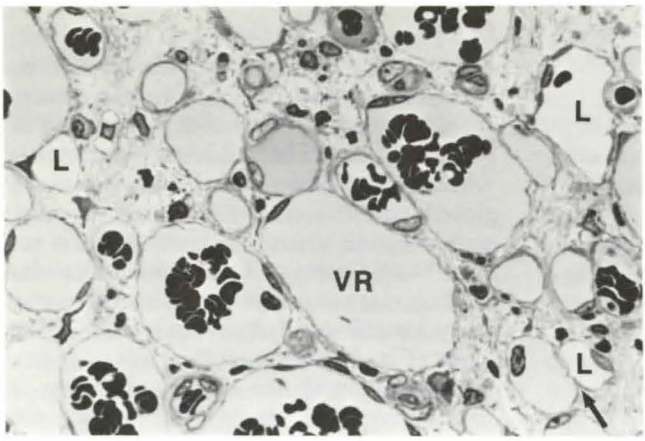

Fig. 1 Light micrograph from the arcuate region of a ureter-obstructed kidney. Three lymphatics (L) are located among vasa recta (VR). [Note the paler staining characteristics of the lymphatic luminal contents as compared with those of the blood vessels.] The arrow denotes the vessels shown in Fig. 2. x 550

Table 1
Number of cortical and medullary lymphatics

Group	Region	#Blocks	#LY
Ureter-obstructed	Cortex	90	43
	Medulla[a]	30	3
Lymphatic ligated	Cortex	90	34
	Medulla	30	0
Free lymph flow	Cortex	90	38
	Medulla	30	0

[a]100 vascular bundles and 100 interbundle areas examined in each medullary region.
LY = lymphatic.

in single sections by light microscopy, was achieved by the use of serial sections. As serial sections were followed, cortical tributaries could readily be seen to join the arcuate lymphatics as they descended with the interlobular blood vessels (Fig. 6). In this position, the lymphatics frequently branched and rejoined, and thereby made a complex network around the blood vessels. The initial arcuate lymphatics usually had secondary relationships with neighboring renal corpuscles and tubular elements (Figs. 3 and 4) but this was not true for the later segments of these vessels. As the arcuate lymphatics approached the interlobar vessels, connective tissue septa became organized into sleeves that tended to isolate the vascular elements from much of the renal parenchyma. A detailed examination of 60 tissue blocks which contained arcuate lymphatics failed to reveal any tributaries that ascended from the medulla. Reconstructions (e.g. Fig. 7) made from eight of the serially sectioned blocks showed 14 cortical tributaries

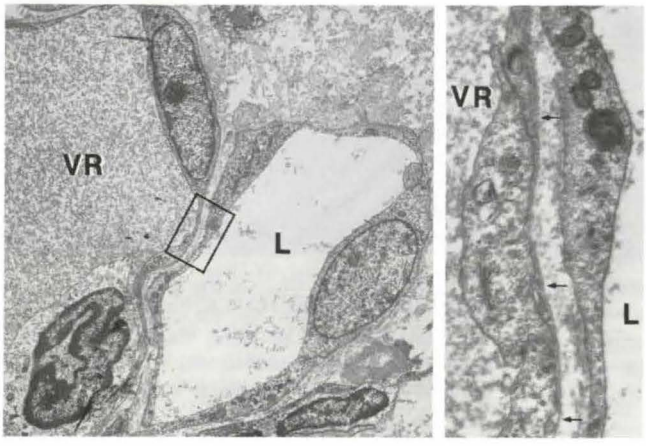

Fig. 2 Electron micrographs of a lymphatic and vas rectum from Fig. 1. (A) Low power view of the lymphatic (L) and vas rectum (VR). Note that the lumen of the lymphatic is clear whereas that of the vas rectum contains plasma proteins and a cell. 3,460. (B) High power micrograph of the area bounded by the box in panel A. A continuous basal lamina (arrows) is subjacent to the endothelium of the vas rectum and is not evident beneath the lymphatic. x 12,500.

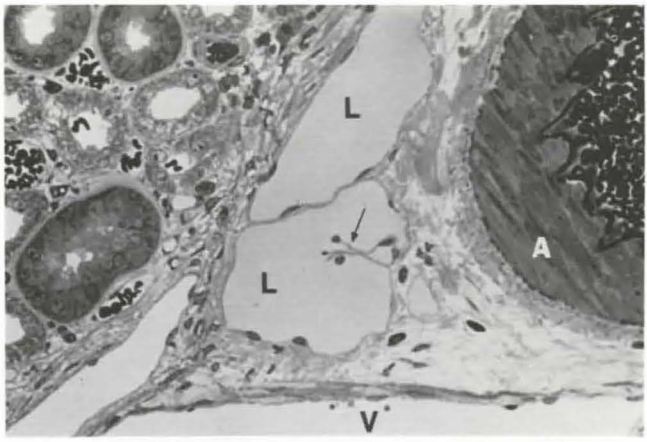

Fig. 3 Light micrograph of two adjoining lymphatics from a kidney with unimpeded lymph flow. The lymphatics (L), identified by the presence of valves, are beside an arcuate artery (A), vein (V) and renal parenchyma. x 260.

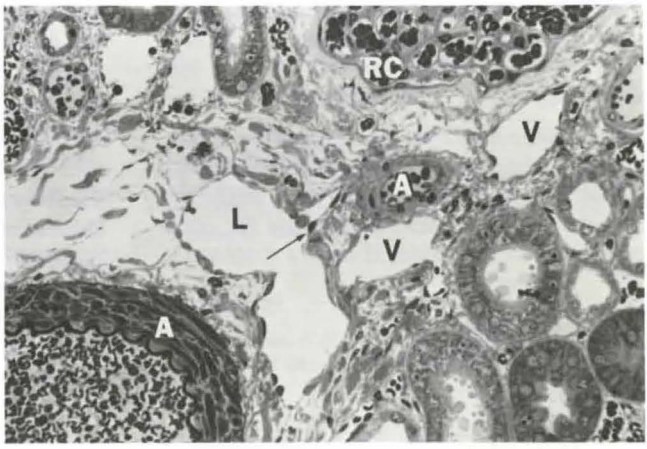

Fig. 4 Light micrograph of initial arcuate lymphatics from a kidney with unimpeded lymph flow. A small tributary (arrow) has joined a larger lymphatic (L). The tributary is located by a small artery (A), veins (V) and renal corpuscle (RC). x 225.

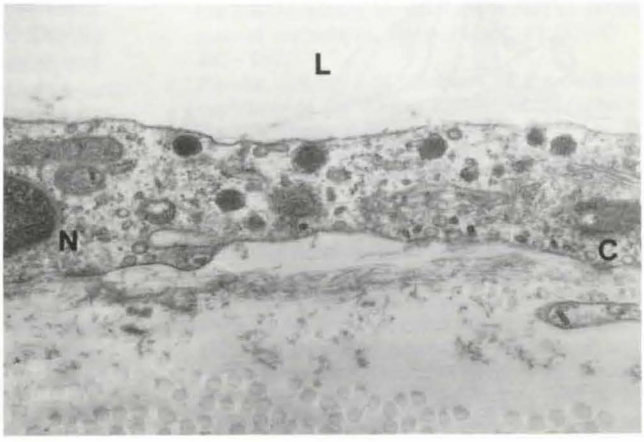

Fig. 5 Electron micrograph from an adjacent thin section of the small tributary in the region indicated by the arrow in Fig. 4. A portion of the lymphatic (L) endothelial cell nucleus (N) and centriole (C) are seen. x 20,500.

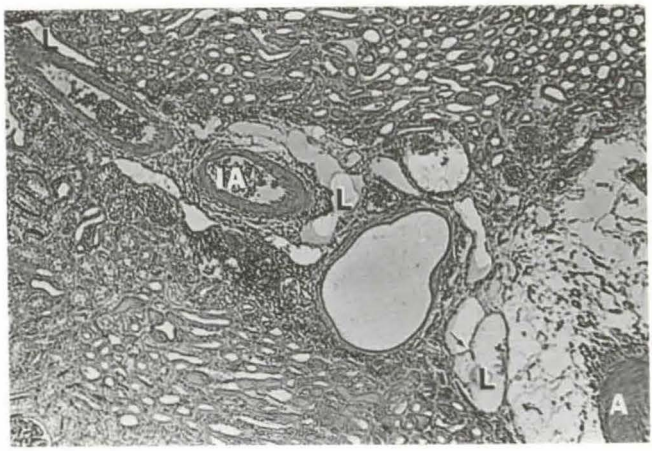

Fig. 6 Light micrograph of interlobular tributaries to the arcuate lymphatics from a kidney with unimpeded lymph flow. The interlobular lymphatics (L) formed a complicated network around the arteries (A). Valves (arrow) were visible in the arcuate lymphatics. x 48

to arcuate lymphatics, but in no instance were medullary tributaries seen.

Discussion

The possible existence of a lymphatic drainage from the renal medulla carries many functional implications, especially as it might affect the countercurrent mechanism for concentration of the urine. If such a drainage exists, its role in the concentrating mechanism has yet to be revealed. Alternatively, the absence of such a drainage raises questions on the mechanism for removal of interstitial protein which is known to exist in significant quantities in the renal medulla.

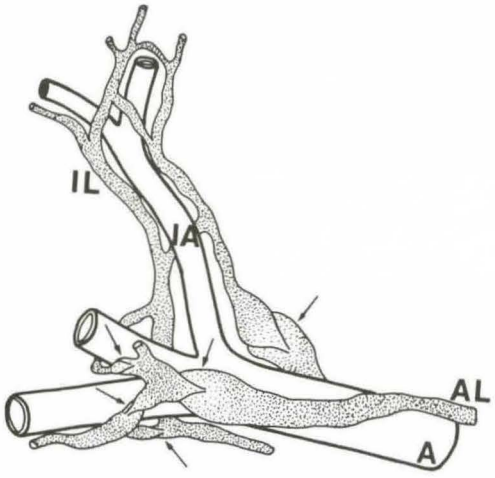

Fig. 7 Reconstruction diagram from one tissue block, that illustrates interlobular tributaries to arcuate lymphatic vessels. The lymphatics (stippled) were followed in serial 3 μm thick sections. The interlobular lymphatic vessels (IL) formed complicated networks around the interlobular arteries (IA) and then anastomosed to form arcuate lymphatics (AL) which possessed valves (arrows)

The literature contains reports of morphological studies which describe in varying degrees of detail a medullary lymphatic system (3, 4, 5, 6), as well as reports of contrasting studies which have failed to find any such system (7, 8, 9, 10). Unfortunately, lymphatic vessels are characteristically elusive in tissue sections, at least at the light microscopic level. Thus studies which fail to find lymphatic components tend to be less convincing than those which do. In order to equate a failure to find lymphatics with their non-existence, the search must be rigorous and the methodology employed must be one which is known to reveal lymphatic vessels when they are present. This was the purpose of the present study – that is, to conduct a detailed search for lymphatic vessels in the renal medulla using a technique which had already been shown to demonstrate consistently lymphatic vessels in the renal cortex (1). Despite these two conditions as well as the use of an additional reconstruction method, no lymphatic vessels were found. One apparent exception was presented by the three lymphatics found at the corticomedullary boundary of one vascular bundle in one of the kidneys. An explanation for this exception is not

obvious, however, the lymphatics were confined to the outermost end of the bundle, and the lack of lymphatics in at least one hundred other bundles studied showed that it was the exception rather than the rule.

Failure to find lymphatics in the present study, despite the rigorousness of the study, cannot be taken as positive proof that no such vessels exist within the renal medulla. However, in our opinion, it can be taken – especially when compared to findings in the renal cortex (Table 1) – to indicate that if any lymphatics do exist in the canine medulla they are so infrequent as to be functionally insignificant.

The question remains as to how the results of the present study may be correlated with those of previous studies which appear to confirm the existence of medullary lymphatics. One possibility which always has to be considered is that of species differences. Additional factors may, however, be more important. For example, the most detailed morphological description of medullary lymphatics is contained within the report by *Rawson* in 1949 (3). Yet this report was based on evidence obtained from one human kidney in which the lymphatic system was apparently permeated by carcinomatous spread from the stomach. Recognition of lymphatics was necessarily based on light microscopic evidence only, yet in our experience intrarenal veins are so thin walled that their differentiation from lymphatics is well-nigh impossible without additional evidence. Thus the medullary lymphatics described by *Rawson* (3) may have developed in response to extensive malignant invasion of the kidney, or alternatively may have been mistakenly identified. *Rhodin* (4) in a review type article in 1965 referred to his electron microscopic observation that the medulla contained a rich lymphatic plexus in comparison to that in the renal cortex. The original description of his observations has unfortunately not been published so that it is difficult to analyze the difference between his results and ours. *Cockett* and his colleagues (5) have also reported the existence of a medullary as well as a cortical network of lymphatics. They used a casting injection method with subsequent dissolution of the parenchyma. It is possible that in such preparations difficulties may arise in distinguishing lymphatics belonging to plexuses associated with the arcuate and interlobar blood vessels, which course through the medullary region, from true medullary lymphatics.

A lack of medullary lymphatics may at first appear difficult to reconcile with physiological data (2, 5, 11, 12, 13, 14) which indicate an outer medullary component of renal hilar lymph. Yet interstitial fluid movement is an inevitable part of lymph formation, and could explain the data if fluid movement occurs from outer medulla to arcuate lymphatics in the boundary zone or to interlobar lymphatics on their way to the sinus of the kidney. The results of the study reported here suggest that this may be the only mechanism to account for the physiological data, at least in the dog.

Acknowledgements

Thanks are expressed to Ms. *H.M. Jarosz* for her technical assistance and to Ms. *J. Bancroft* for her illustrative work.

References

1 *Albertine, K.H., C.C.C. O'Morchoe:* The distribution and density of the canine renal cortical lymphatic system. Kidney Int. 16 (1979) 470–480
2 *Holmes, M.J., P.J. O'Morchoe, C.C.C. O'Morchoe:* Morphology of the intrarenal lymphatic system. Capsular and hilar communications. Am. J. Anat. 149 (1977a) 333–352
3 *Rawson, A.J.:* Distribution of the lymphatics of the human kidney as shown in a case of carcinomatous permeation. Arch. Pathol. 47 (1949) 283–292
4 *Rhodin, J.A.G.:* Fine structure of the peritubular capillaries of the human kidney. In: Progress in Pyelonephritis. E.H. Kass, ed. F.A.Davis Co., Phila., 1965, 391–458
5 *Cockett, A.T.K., A.P. Roberts, R.S. Moore:* Evidence for two intrarenal lymphatic networks. Invest. Urol. 7 (1970) 266–270
6 *Huth, F.:* Beiträge zur Orthologie und Pathologie der Lymphgefäße der Nieren. Beitr. Path. Anat. 136 (1968) 341–412
7 *Peirce, E.C.:* Renal lymphatics. Anat. Rec. 90 (1944) 315–335
8 *Bell, R.D., M.J. Keyl, F.R. Shrader, E.W. Jones, L.P. Henry:* Renal lymphatics: The intrarenal distribution. Nephron 5 (1968) 454–463

9 *Kriz, W., H.J. Dieterich:* Das Lymphgefäßsystem der Niere bei einigen Säugetieren. Licht- und elektronenmikroskopische Untersuchungen. Z. Anat. Entwickl.-Gesch. 131 (1970) 111–147
10 *McIntosh, G.H., B. Morris:* The lymphatics of the kidney and the formation of renal lymph. J. Physiol. (London) 214 (1971) 365–376
11 *Santos-Martinez, J., E.E. Selkurt:* Renal lymph and its relationship to the countercurrent multiplier system of the kidney. Am. J. Physiol. 216 (1969), 1548–1555
12 *O'Morchoe, C.C.C., P.J. O'Morchoe, N.M. Heney:* Renal hilar lymph. Circ. Res. 26 (1970) 469–479
13 *Bell, R.D.:* Cortical and medullary canine renal lymph formation during acetylcholine induced renal vasodilation. Lymphology 4 (1971) 74–78
14 *O'Morchoe, C.C.C., P.J. O'Morchoe, E.J. Donati:* Comparison of hilar and capsular renal lymph. Am. J. Physiol. 229 (1975) 416–421
15 *Holmes, M.J., P.J. O'Morchoe, C.C.C. O'Morchoe:* The role of renal lymph in hydronephrosis. Invest. Urol. 15 (1977b) 215–219
16 *Albertine, K.H., C.C.C. O'Morchoe:* Renal lymphatic ultrastructure and translymphatic transport. Microvasc. Res. 19 (1980) 338–351
17 *Casley-Smith, J.R., H.W. Florey:* The structure of normal small lymphatics. Quart. J. Exp. Physiol. 46 (1961) 101–106
18 *Fraley, E.E., L. Weiss:* An electron microscopic study of the lymphatic vessels in the penile skin of the rat. Am. J. Anat. 109 (1961) 85–101
19 *Leak, L.V., J.F. Burke:* Fine structure of the lymphatic capillary and the adjoining connective tissue area. Am. J. Anat. 118 (1966) 785–810

Dr. Kurt H. Albertine, Cardiovascular Research Institute, University of California, San Francisco, California 94143