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The Distribution and Ultrastructural Morphology of Lymphatic Vessels in the Canine Renal Cortex*

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Summary

Ultrastructural observations were made of canine renal cortical lymphatic vessels following specific labeling. The major findings were 1) the large number of the discreete openings in the lymphatic among arteries, veins, tubules and Bowman's capsules and 3) the disreet openings in the lymphatic endothelial wall. It is concluded that the renal cortex contains a significant lymphatic plexus and that cortical lymph may be derived from a variety of intrarenal structures.

Early investigations concerning the intrarenal distribution of lymphatic vessels were accomplished by injecting a contrasting medium into the renal parenchyma (stab injection method) and observing its uptake by lymphatic vessels. As pointed out by *Peirce* (1) following a stab injection tissue spaces, blood capillaries, tubules and venules as well as lymphatic vessels may be filled with ink. Although *Peirce's* study greatly clarified the intrarenal distribution of lymphatic vessels, it also utilized the stab injection method. *Peirce's* conclusions were largely substantiated by the work of *Bell et al.* (2) in which distribution was studied after lymphatic vessels were injected directly with Indian ink. The latter study showed lymphatic plexuses in the intralobular spaces of the cortex with extension in the immediate vicinity of *Bowman's* capsule. Although this study involved specific labelling of lymphatic vessels, the nature of the preparations did not allow the identification of the lymphatic endothelium. Thus it was impossible to assure that structures described as lymphatic vessels were not ink filled tissue spaces. In addition, the electron micrographic (EM) studies of *Rhodin* (3) have suggested that the renal cortex is poorly supplied with lymph capillaries. In the present study, EM techniques were combined with specific labelling of lymphatic vessels in an attempt to establish the presence or absence of cortical lymphatic vessels and their possible relations to other renal structures.

Methods

Dogs were anesthetized with sodium pentobarbital (30 mg/kg) and the left kidney exposed through a flank incision. India ink was introduced into a capsular lymphatic vessel via a small polyethylene catheter (*Clay Adams* PE 10). Gentle stroking in a retrograde direction caused the ink to flow past the valves, filling a length of a lymphatic. In successful preparations small lymphatics entering the parenchyma were also filled. The animal was sacrificed and 10 ml of 6.25% glutaraldehyde in phosphate buffered saline, pH 7.2, was immediately injected into the renal artery. The kidney was then removed and macrosectioned to reveal the areas of carbon filled lymphatics. Selected areas of these macrosections were trimmed and fixed in 1% phosphate buffered osmium tetroxide

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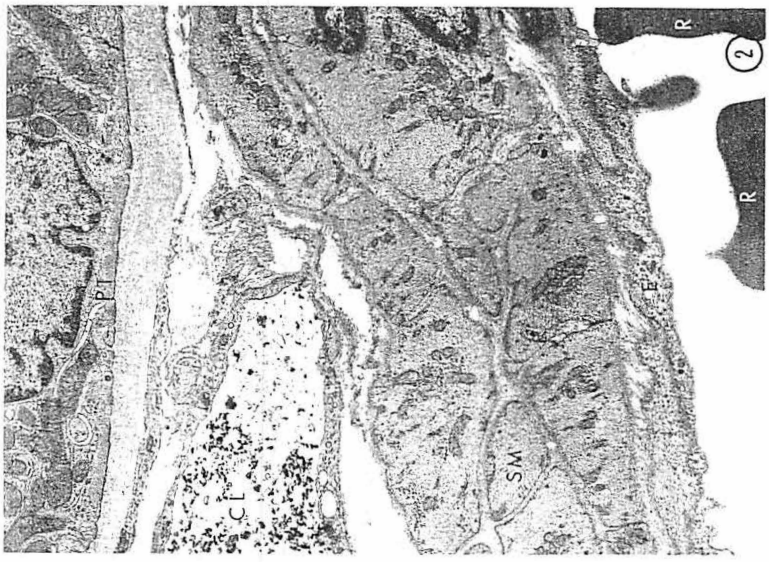

Fig. 2 Renal Cortex, Carbon Injection. A cortical lymphatic (CL) situated between a proximal tubule (PT) and an artery. Smooth muscle SM, arterial endothelium (E) and red blood cells (R) are shown. X 16,250

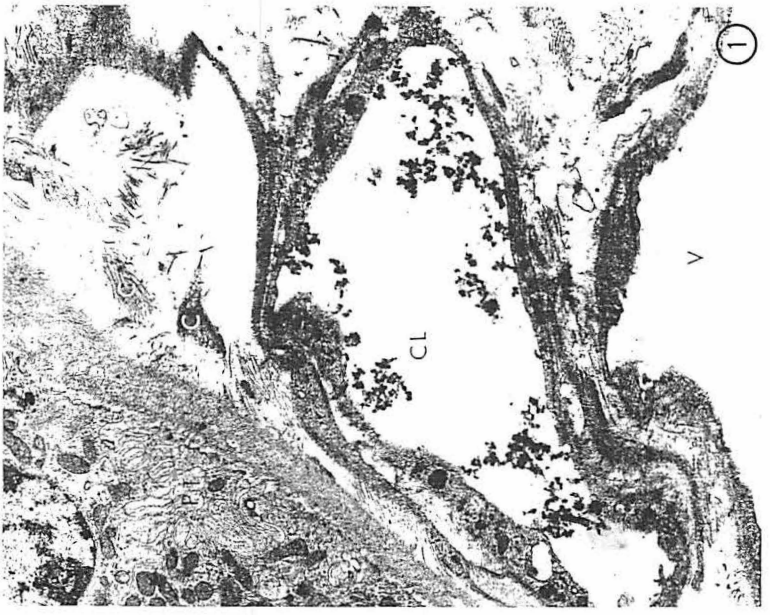

Fig. 1 Renal Cortex, Carbon Injection. A cortical lymphatic (CL) containing carbon particles, positioned against a proximal tubule (PT). Note the overlapping of the endothelial cells and the numerous cytoplasmic organelles. Blood vein (V) and collagen fibers (C) are also shown. X 14,000

for 2 hours. The blocks were then embedded flat in araldite, sectioned and stained with uranyl acetate and lead citrate (4). Micrographs were taken at various magnifications on a Hitachi HU-11B electron microscope.

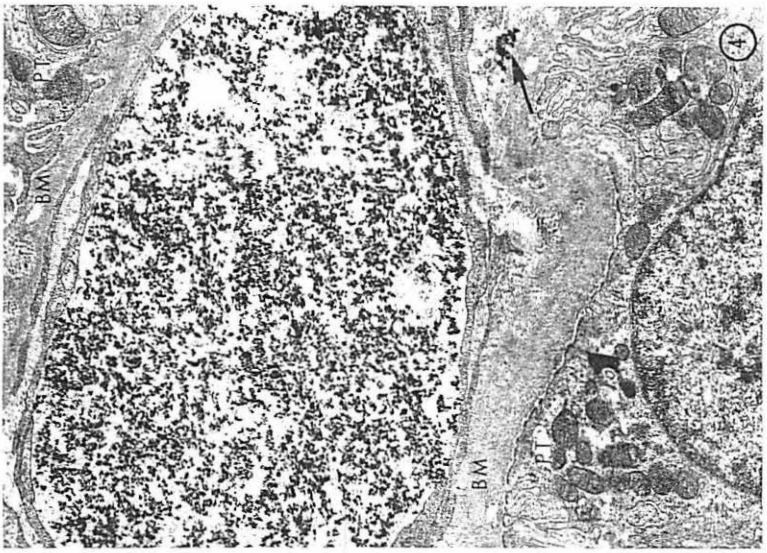

Fig. 4 Renal Cortex, Carbon Injection. A lymphatic capillary tightly positioned between two proximal tubules (PT). Note the lymphatic endothelium is separated from tubular epithelium by only the width of the basement membrane (BM). Extravasated carbon (→) may be seen. X 21,000

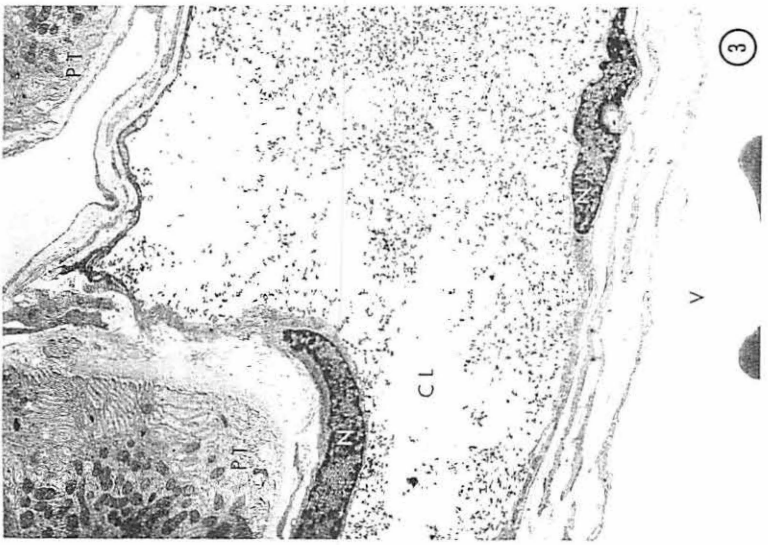

Fig. 3 Renal Cortex, Carbon Injection. This micrograph shows a large cortical lymphatic vessel (CL) adjacent to two proximal tubules (PT) and a large blood vein (V). Nuclei (N) are seen in the endothelium of the lymphatic wall. X 15,000

Results

Renal cortical lymphatics were characterized by a lack of a continuously definable basement membrane and an attenuated endothelial cell wall in which the nuclei protruded into the lumen. The endothelial cytoplasm contained moderate numbers of mitochondria, endoplasmic reticulum and lysosome-like structures. The free space in the cytoplasm was

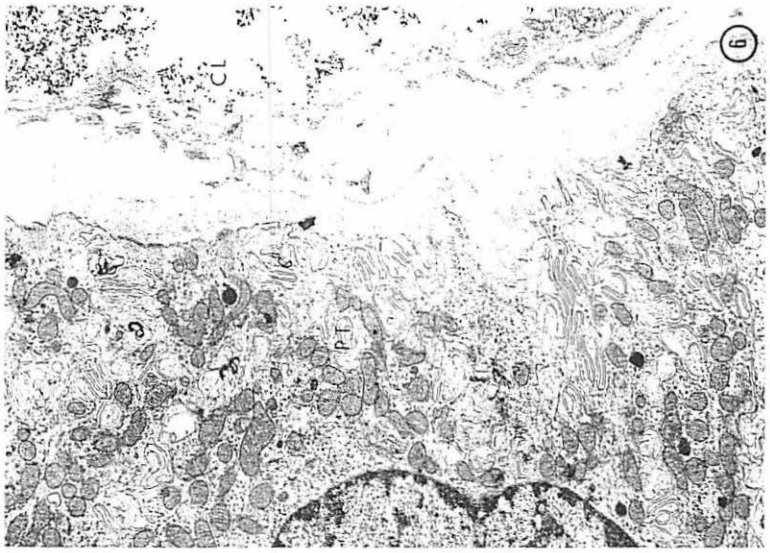

Fig. 6 Renal Cortex, Carbon Injection. This figure is a high magnification of a proximal tubule (PT) and an associated lymphatic (CL) Note the overlapping of lymphatic endothelial cells and their closeness to the tubular basement membrane. X 13,750

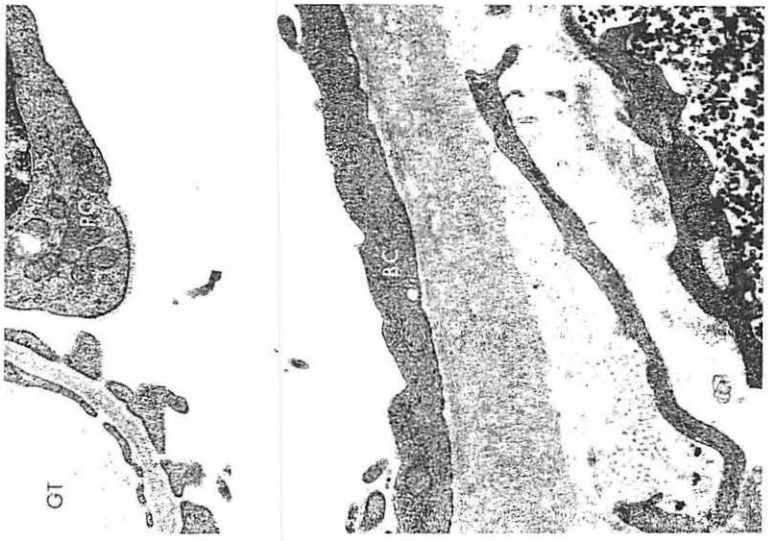

Fig. 5 Renal Cortex, Carbon Injection. This figure shows the close association of cortical lymphatics (CL) with Bowman's capsule (BC). A glomerular tuft (GT) with associated podocyte (PC) is seen. X 30,400

filled with ribosomes and small vesicles. There were numerous small filaments lying parallel to the long axis of the endothelium (Fig. 1) in some parts of the cytoplasm adjacent to the plasma membrane.

The endothelial cells formed a wall in which there was much overlapping and interdigitation. These cells were attached to adjacent cells in the wall by a variety of closed junc-

Fig. 8 Renal Cortex. Carbon Injection. This figure depicts an opening (→) in the lymphatic wall which has allowed the extravasation of a large amount of carbon. The lymphatic shown (CL) is positioned between two proximal tubules (PT). Inset is a high magnification of this same endothelium demonstrating a Zona occludens (ZO) and a Zona adhaerans (ZA). Picture X 13, 100, Inset 23,600

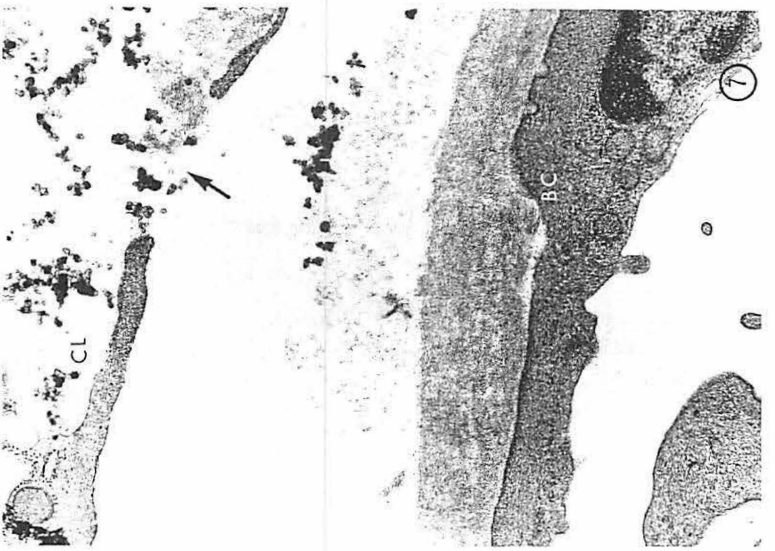

Fig. 7 Renal Cortex, Carbon Injection. A high magnification of an opening (→) in the lymphatic endothelial wall (CL) adjacent to Bowman's capsule (BC) is shown. Note that the cells are apparently undamaged at their points of separation. X 39,000

tions (Fig. 8), the most common being a zona adhaerens. There were also frequent zona occludens and an occasional macula adhaerens (desmosome). In rare cases there was evidence of a concentration of small filaments on the ablumenal side of the lymphatic endothelium and though no hemidesmosomes were observed, the filaments were similar in morphology and location to "lymphatic anchoring filaments" (Fig. 1).

Large lymph vessels followed the pattern of the blood vessels in the interlobular space (Fig. 2 and 3). Small lymphatics were observed to follow branches of interlobular veins and arteries to form a relatively dense network within the lobule. While most cortical lymphatics tend to follow blood vascular components, some sections showed small lymph capillaries without an accompanying blood vessel (Fig. 4).

Although the statistical distribution of lymph channels in the renal cortex was not evident with these methods, there was distinct association of lymphatics with glomeruli. At no time were these lymphatics observed to enter the glomerulus or Bowman's capsule (Fig. 5). In many fields, carbon filled lymphatics were also found in close proximity to both proximal and distal tubules. In some cases these vessels were separated from tubular epithelium by a distance of one micron or less (Fig. 6). The ultrastructural characteristics of the lymphatic endothelial walls near glomeruli, tubules and blood vessels were similar in all respects.

In some fields, carbon particles were present outside of the endothelial wall (Fig.7 and 8). Micrographs from these areas indicated that the major part of carbon loss was through discreete openings in the endothelial wall. These openings were apparently formed by the separation of endothelial cells at their junctions without evidence of cellular damage. Carbon was not observed to be actively transported by phagocytosis across the endothelial wall, nor was carbon observed to diffuse through endothelial junctions in the areas of intact zonae.

Discussion

The major findings of this study are 1) the large numbers of cortical lymphatic vessels, 2) their distribution among arteries, veins, tubules and *Bowman's* capsules and 3) the discreete openings in the lymphatic endothelial wall. The ultrastructural characteristics of renal cortical lymphatic vessels observed in this study are similar to those described by *Rhodin* (3) and *Kriz* and *Dietrich* (5) in the renal medulla and by *Casley-Smith* (6) and *Leak* and *Burke* (7) in other organs. Our findings differ from the previous studies of *Rhodin* (3) in that he indicated a paucity of cortical lymphatic vessels and thus was unable to appreciate an association with cortical structures. This difference may have resulted from our use of an electron opaque marker which clearly delineated the lymph vasculature from other vessels and grossly demonstrated lymphatic distribution. Since the material studied was selected on the basis of carbon distribution, the data from this study offers no information concerning the uniformity of lymphatic distribution throughout the cortex. The discreete openings in the lymphatic walls might represent a route for rapid uptake of macromolecules and fluid from the interstitial space. Such openings in lymphatic vessels have been described by other workers using different experimental procedures, thus suggesting these findings were not induced by methodology. The association of lymphatic vessels with arteries, veins, tubules and Bowman's capsules suggests that lymphatic fluid may be derived from any or all of these structures.

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Direct Transtissue Intra-Organ Lymphography;Technique and Results

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Summary

Rodriquez Sica and *Sica's* technique of intra-organ lymphangiography is described and has been demonstrated to be effective in dogs. Ultrafluid liposoluble iodine dye is injected directly into the tissues at a slow rate, into areas where the capillary network is abundant, without any incision of the skin. The technique has been carried out in the head and neck, mammary glands, limbs, testis and rectum. Collateral channels, after obstruction of the thoracic duct, have been visualized. Lymphatics of intra-abdominal and retroperitoneal organs were seen. The possibility of using this technique in man is suggested, based upon the absence of lasting inflammatory reaction in the injected organ, and upon excellent tolerance of the procedure by experimental animals.

Lymphangiography has gained wide clinical use and in many instances can be considered a routine procedure. It is used as a diagnostic tool when the lymphatic system is primarily involved, and as a means of evaluating the extent of disease both in primary lymphatic conditions (1) and in those where the lymphatic system is secondarily involved by another pathologic process. The basic technique has been that developed by *Kinmonth* and described by him in 1952, 1954, and 1955 (2). In 1960 *Hreshchyshyn* and in 1961, *Wallace* made modifications in details. The modified *Kinmonth* technique is currently used in clinical practice. Its indications and the details of the procedure will not be discussed.

Kinmonth's method can be difficult to perform. A surgical procedure is necessary so as to insert a catheter into one of the minute lymphatic channels. This surgical procedure usually is not done without difficulty and even though there is little risk, it can cause anxiety in the patient. The dissection of the lymphatic vessels must be done with care. The catheterization of the lymph vessel can be difficult. The procedure has been largely limited to the lymphatic system in the extremities. There are many other areas, such as the mammary glands and the pelvis, where study of the lymphatic anatomy would be of fundamental importance. The Kinmonth technique is founded on the dissection and catheterization of the lymphatics