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TOPOGRAPHY AND ULTRASTRUCTURE OF KIDNEY LYMPHATICS IN SOME HIBERNATING BATS

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ABSTRACT

The kidney lymphatic system of some bats consists of intraparenchymal (interlobar, arcuate, and interlobular) and extraparenchymal vessels (capsular and prehilar connective). These vessels drain lymph via precollecting and prenodal collecting lymphatics into a hilar lymph node. There are no lymphatics in the renal medulla. The lymphatic vasculature (precollecting vessels excluded) is characterized by an endothelial wall lacking basal lamina and fenestrations. The endothelial cells, mostly rectangular in shape, are joined together by overlapping, end-to-end, and complex interdigitating junctions. Cytoplasmic expansions profile and thickness, intercellular junctions and particularly the different categories of uncoated vesicles (free or opened on luminal or abluminal surface) show qualitative and quantitative seasonal variations. Luminal and abluminal cytoplasmic processes appear (when analyzed in tridimensional reconstructions) as "intraendothelial channels." The increased number of these structures during summer characterizes them as dynamic elements and supports the concept of an active role played by them in transendothelial transport. Nevertheless, the main functional role is still ascribed (in addition to membrane transport mechanisms) to the vesicular system, also defined as the "vesicular route." We did not find any open intercellular junctions.

Despite many studies of the renal lymphatic system, uncertainty still exists regarding intra- and extraparenchymal peripheral absorbing vessel distribution as well as the legitimate role of tubular reabsorbate in lymph formation. Although previous studies based on dye or polymer injections permitted tridimensional reconstruction (1-5) and were useful to define morphologic interrelationships, they, nonetheless, left several issues unsettled: in particular the existence of lymphatics in the medulla and constituents of the nephron as well as the continuity between cortical and capsular vessels. Electron microscopy, and to a lesser extent, light microscopy (6-9), have to date only partially clarified the problem. In fact, as O'Morchoe exhaustively reports in a monograph (10), the existence of lymphatics in the cortex is unanimously accepted, but many disagree on the distribution in renal corpuscles and along the tubular system. Furthermore, there is no agreement about the existence (11-13) or absence (2,7) of medullary lymphatics. In fact, some physiological studies assert that the renal medulla contributes to renal hilum lymph formation (14,15). A recent study on dogs (16)

has described lymphatics among Henle loops and medullary ray collecting tubules at the cortico-medullary zone, but has failed to visualize them in the medulla. According to O'Morchoe (17), even if medullary lymphatics exist, their structural and functional role seems minimal in view of their rarity.

The aim of the present study was to assess renal lymphatic topography to augment Ottaviani's work (18) and to better define lymphatic ultrastructure in some species of bats in order to collect, through comparative topography and seasonal cytologic changes, useful data about lymphatic endothelial permeability particularly as related to trans-endothelial transport.

MATERIALS AND METHODS

Forty-six (46) adult bats (*Pipistrellus kuhli kuhli*, *Rhinolophus ferrum equinum*, and *Eptesicus serotinum*) were captured over several years (1980-86) in the provinces of Parma and Brescia. The bats were divided into 3 groups. The first group (18 bats) was captured and killed during the hibernation period (January and February); the second group (6 bats) was captured in the same season and kept in the laboratory for 2-3 days before killing. During this time they were not fed. The third group (22 bats) was captured during June, July, and August. Five of these bats had prenodal collecting lymphatics ligated for 5 hrs. A tridimensional mold of the renal lymphatic system was obtained in 6 bats (3 from the winter group and 3 from the summer group) by retrograde injection of Neoprene Latex 842/A (Du Pont) via a hilar node. Red and blue Neoprene were injected at the same time into the renal artery and vein, respectively, following the technique described by Ottaviani and Giacomelli (19). Furthermore, the lymphatic system of 3 bats (of the summer period) was visualized by retrograde injection of Prussian blue 15% in trementine oil.

The bats were anesthetized with sodium pentobarbital (Nembutal-Abbot). The kidneys were perfused through the aorta with tyrode solution and subsequently fixed with glutaraldehyde 3.5% in cacodylate buffer (pH 7.4) for 25 min. During perfusion, the latter solution was also dripped onto the renal surface as suggested by O'Morchoe and Albertine (20). Kidneys were excised and thereafter cut transversely to obtain small blocks of parenchyma (2-3mm). After 5 hrs wash in this buffer, the blocks were post-fixed by immersion with osmic acid in sodium cacodylate buffer (pH 7.4) for 2 hrs. After dehydration in acetone they were embedded in Durcupan. Sections 1μ thick stained with toluidine blue 1% in sodium carbonate 0.5% were analyzed to identify blood vessel bundles containing lymphatics and by a Reichert FM 90 microscope in order to center the lymphatic vessel and cut the specimens. Thin sections were stained with lead citrate according to Reynolds (21) and examined by a Phillips 300 electron microscope. Tridimensional reconstruction of several segments of endothelial wall (about 300μm in length) was carried out in 4 lymphatics (2 arcuate and 2 interlobular) by means of the wax-disk technique (22) recently revised by Werner (23). Furthermore, we analyzed 12 groups (240 serial sections each) of micrographs derived from 10 lymphatic vessels, 5 of them derived from bats killed during hibernation and the remaining from bats killed during the summer. From these micrographs the numerical densities, volume densities, and mean diameter for the different categories of the uncoated vesicles were determined by means of a Zeiss Videoplan image analyzer which was programmed to provide this information.

RESULTS

Light microscopy

Retrograde injection of Prussian blue and Neoprene Latex via a hilar

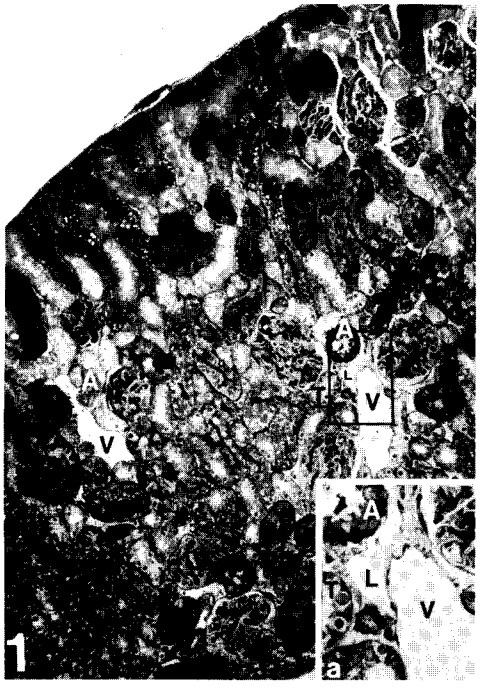

Fig. 1. R.f. equinum-*kidney-cortex. Two interlobular vascular bundles containing a lymphatic vessel (L) in the connective tissue (see also inset a) surrounding the artery (A) and the vein (V). A portion of the lymphatic endothelial wall borders the outer surface of a distal convoluted tubule (T). x150; x350.*

node shows mid-caliber intraparenchymal lymphatics (interlobar lymphatics). These vessels accompany interlobar arteries and veins and drain from the arcuate lymphatics lying at the outer limit of the outer medulla. Small-caliber lymphatic vessels arise from the same zone and then penetrate the renal cortex closely linked to interlobular blood vessels but never reaching the renal capsule. No lymphatics were detected in the renal medulla, but some were found in the renal capsule and the connective tissue interposed between cortical tissue and renal calyx. The lymph of these vessels drains via precollecting and prenodal collecting lymphatic into a single (sometimes double) hilar node.

This topographical distribution is confirmed by histological sections: lymphatics are uniformly present in the connective tissue encircling interlobar, arcuate and interlobular blood vessesl *(Figs. 1,2,4)*. It is important to emphasize that the lymphatic vessel encounters different relations along its course through the abundant connective

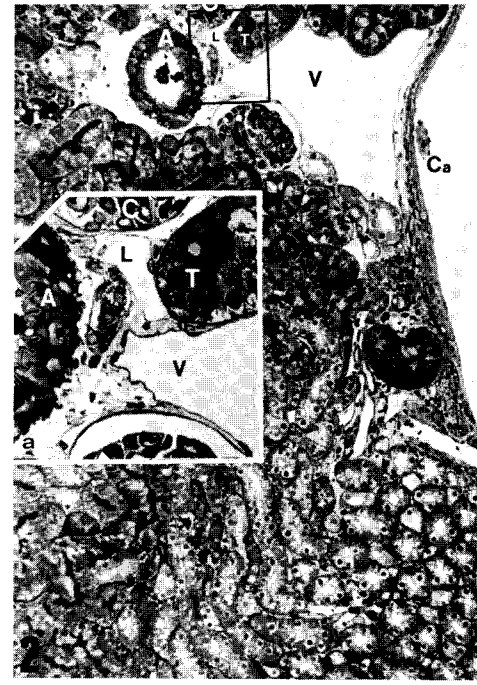

Fig. 2. P.k. kuhli-*kidney-cortex. At the top, vascular bundle at the limit between cortex and outer medulla: lymphatic (L) lies in the connective tissue between arcuate artery (A) and vein (V). Inset a (black rectangle--upper portion) distinctly shows the relationship of lymphatic (L) with blood vessels (A and V), a renal corpuscle (C) and a proximal convoluted tubule (T). Ca=renal calyx. x150; x350.*

tissue enwrapping the blood vessel bundle: sometimes it runs simply between the artery and the vein, whereas at other times a segment of the lymphatic touches the outer surface of a renal corpuscle, or the basement membrane of a proximal or distal convoluted tubule *(Figs. 1,2--insets a and 4)*. We did not observe lymphatics along the branch running from the interlobular artery to the renal corpuscle,

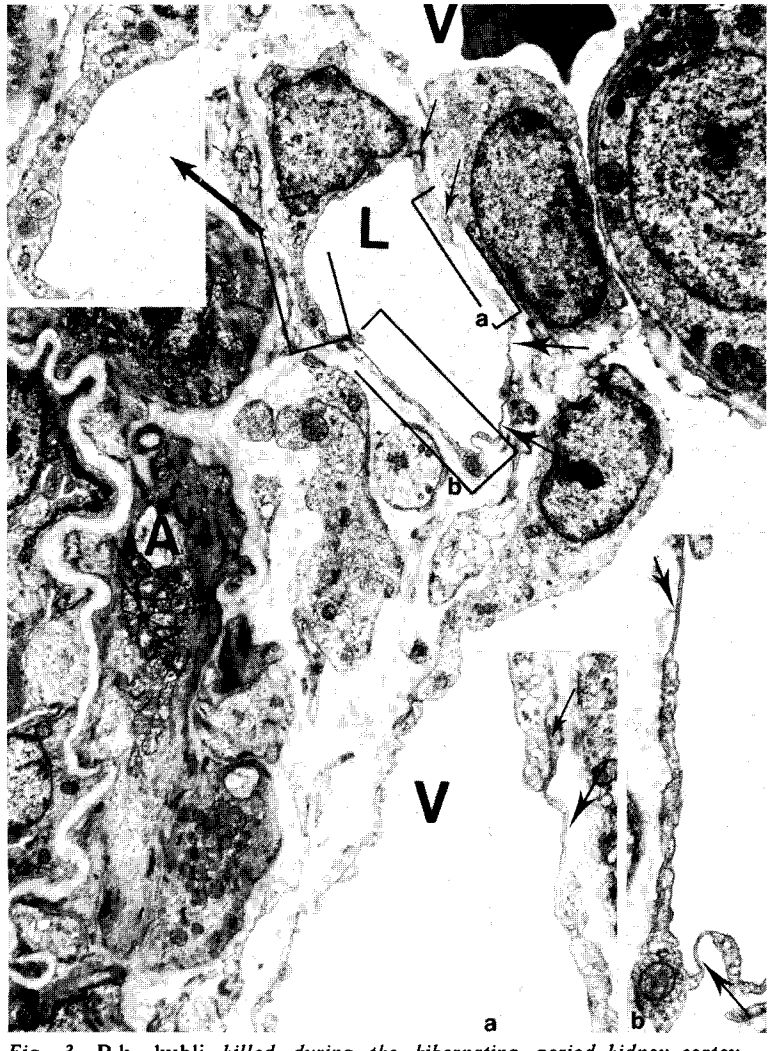

Fig. 3. **P.k. kuhli** *killed during the hibernating period-kidney-cortex. Lymphatic vessel (L) between interlobular artery (A) and vein (V). The endothelial wall is characterized by a linear profile and extremely thin segments of the cytoplasmic expansions (large arrows). Luminal and abluminal cytoplasmic membranes (of the above mentioned segments) are separated by a thin cytoplasmic layer without vesicles, RER and ribosomes (see also inset a and b). Intercellular junctions are of the overlapping and interdigitating type (small arrows). Luminal and abluminal vesicles are rare. x10,000; x27,500; x27,500.*

a finding also noted in other mammals (13,24).

Ultrastructure

Intra- and extraparenchymal lymphatic vessels, apart from diameter and seasonal cytological aspects, share common ultrastructural characteristics. Thus, they consist of a monolayer of flat endothelial cells lacking continuous basal lamina and fenestrations *(Figs. 3,6,8)*. The endothelial wall is formed by more cells, polyhedric or rectangular in shape, as demonstrated by tridimensional models built on serial thin sec-

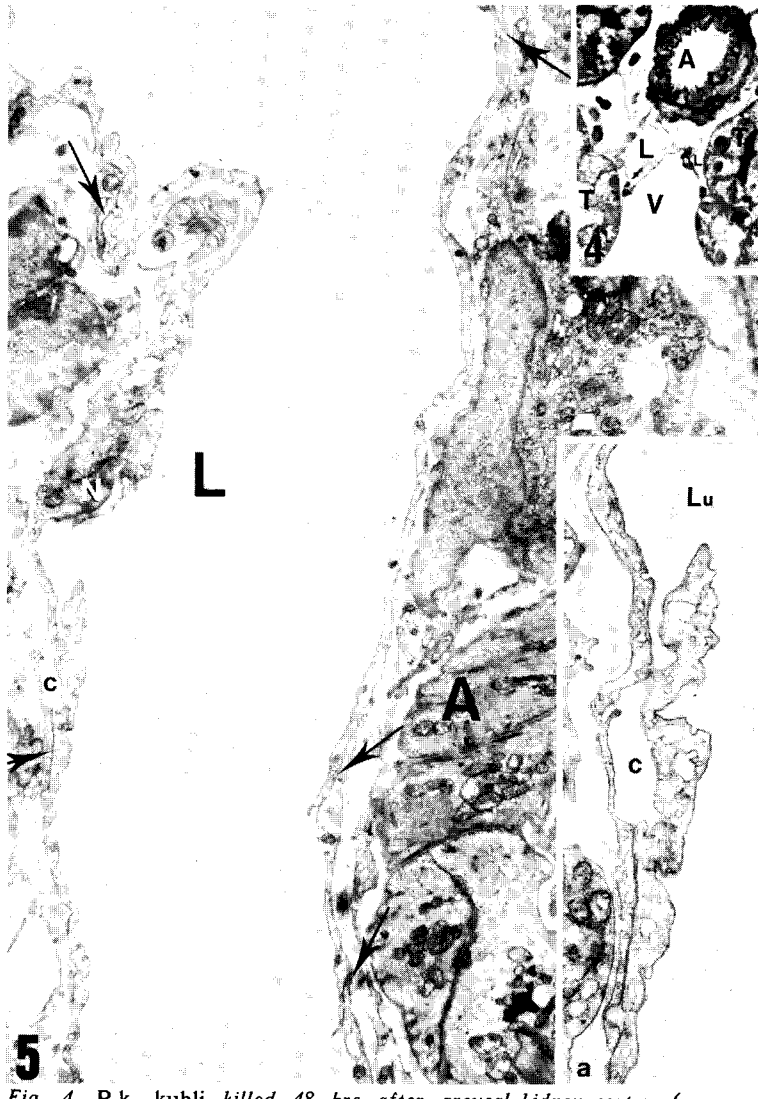

Fig. 4. P.k. kuhli *killed 48 hrs after arousal-kidney-cortex (upper right). Intraparenchymal lymphatic (L) between arcuate artery (A) and vein (V). A part of their endothelial wall touches a distal convoluted tubule (T).*

Fig. 5. P.k. kuhli *killed 48 hrs after arousal-kidney-cortex. Electron micrograph showing lymphatic endothelial wall with wavy profile, intercellular junctions of overlapping and interdigitating type (arrows) and an intraendothelial channel (c) with its luminal opening (see also inset a). Uncoated free and abluminal vesicles are numerous in the cytoplasmic expansions. N=endothelial cell nucleus. Lu=lymphatic vessel lumen. x350; x12,000; x18,000.*

tions. Each endothelial cell is characterized by: a) a quite bulky central part containing the nucleus and an abundant perinuclear cytoplasm containing Golgi apparatus, most rough endoplasmic reticulum (RER), mitochondria and some ribosomes; b) laminar and extensive cytoplasmic expansion where the peri-

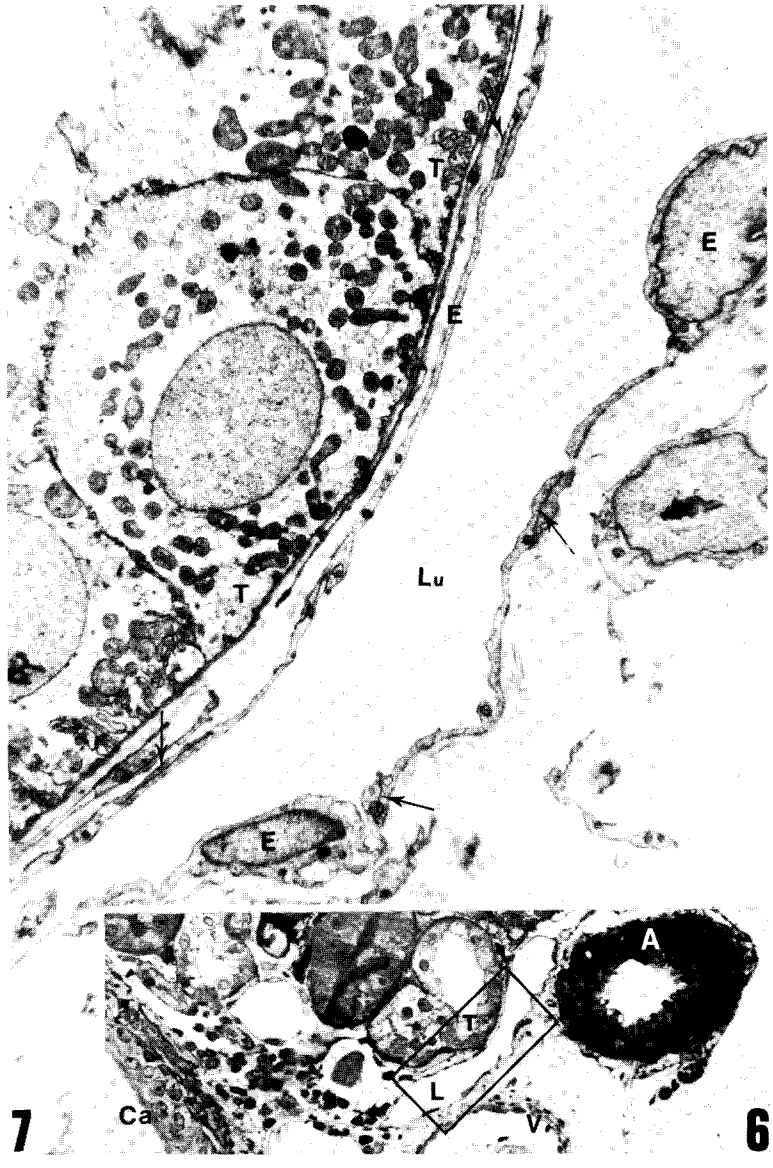

Fig. 6. P.k. kuhli *killed during the hibernating period-kidney-cortex (lower inset). Extrahepatic lymphatic vessel (L) lying in connective tissue between the outer surface of distal convoluted tubules (T) and two blood vessels (A and V). Ca=renal calyx. x350.*

Fig. 7. P.k. kuhli *killed during the hibernating period-kidney-cortex. Magnification of the lymphatic represented in* Fig. 6 *(black rectangle) showing the endothelial wall with a linear profile formed by more cells (E) joined by overlapping junctions (arrows). Lu=lymphatic vessel lumen. T=distal convoluted tubule cells. x350; x10,000.*

pheral edges are joined to the neighboring cell by end-to-end, overlapping, and interdigitating junctions. One or more specialized junctional complexes (tight or gap junctions) are consistently present at the site of contact between two cells. These specialized junctional complexes occur with

different frequency in the three types of intercellular contacts. Tight and gap junctions occur together in the interdigitating intercellular contacts, whereas tight junctions are peculiar to the end-to-end and overlapping intercellular contacts. We observed slight accumulations of connective fibers in limited areas of abluminal plasma membrane outer surface.

Serial section analysis confirms the data of injection experiments of traditional substances. We noted a complete lack of continuity between interlobular and capsular lymphatics; moreover, in contrast to what was observed in the rat (25,26), we failed to visualize lymphatics related to the juxtaglomerular complex or to the afferent arteriole. Nonetheless, interlobular and arcuate lymphatics may abut (with a part of the endothelial wall) the outer surface of renal corpuscles or convoluted tubules (distal or proximal) and some collecting tubules.

Winter hibernation period

Intraparenchymal lymphatics are characterized by endothelial cells whose cytoplasmic expansions are laminar with a linear profile. Some areas of these expansions are slender (40nm) to such a point that luminal and abluminal plasma membranes are separated only by an extremely thin cytoplasmic layer in which free ribosomes are rare while vesicles, mitochondria, and RER tubules are absent *(Fig. 3 and insets a,b)*. The cytoplasmic expansion (except for the areas described above) is 110nm thick on the average and shows a clear cytoplasm containing free ribosomes, uncoated vesicles (60-70nm in diameter), and some mitochondria. Dense bodies and abluminal or luminal vesicles are rarely represented. The numerical densities, volume densities, and mean diameter for the different categories of uncoated vesicles (analysis performed on 5 lymphatic vessels) are shown in *Table 1*.

Table 1.
Hibernating

Vesicles	V_v $\mu m^3/\mu m^3$	N_v $\#/\mu m^3$	D nm
Abluminal	0.0006	3.6	60
Cytoplasmic	0.021	128	62
Luminal	0.002	12	60

V_v = volume density; N_v = numerical density; D = mean diameter

We classified as "luminal" or "abluminal" the vesicles which either touched or were continuous with the plasma membrane. "Cytoplasmic" vesicles appeared to be totally surrounded by endothelial cytoplasm in multiple serial sections (27). The cytoplasm surrounding the nucleus (with chromatin stacked in the peripheral karyoplasm) showed tubular RER, a poorly developed Golgi apparatus, some mitochondria presenting a clear matrix and some lysosomal dense bodies. The endothelial junctions were mainly of the overlapping and interdigitating type while end-to-end junctions were rare. The portions of the abluminal endothelial wall showing accumulations of collagenous fibers and dense connective ground substance were rare and limited in extent. Similarly, extraparenchymal lymphatics lying in the capsule and in the connective stroma between renal calyx and cortical tissue *(Figs. 6 and 7)*, except for precollecting lymphatics, showed an endothelial wall lacking continuous basal lamina. The endothelial cytoplasmic expansions had a linear profile, and luminal and abluminal vesicles were scarcely represented. Luminal and abluminal cytoplasmic processes which usually form intraendothelial channels were also rare.

Hibernating bats kept awake and not fed before killing

In this group, intraparenchymal and extraparenchymal lymphatics show a wavy endothelial wall almost without the slender areas described above. The

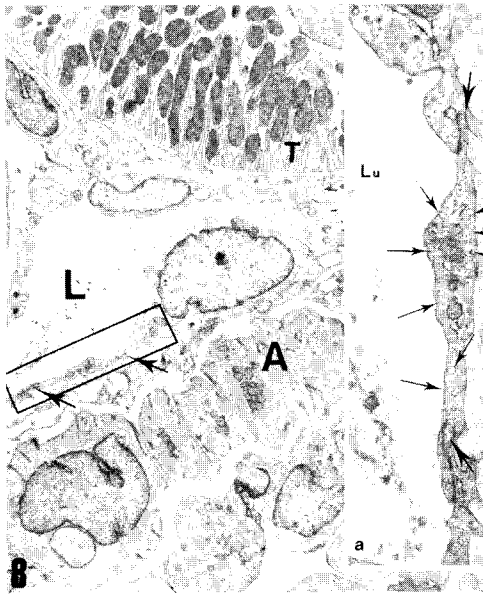

Fig. 8. P.K. kuhli *killed during the summer period-kidney-cortex. Lymphatic (L) between interlobular artery (A) and a distal convoluted tubule (T). The thick endothelial wall (see also inset a) is formed by more cells joined to each other by mainly interdigitating junctions (large arrows). The cytoplasmic matrix shows a rather well-developed RER, ribosomes and many luminal and abluminal vesicles (small arrows). Lu-lymphatic vessel lumen. x10,000; x27,000.*

intercellular junctions are mainly of the overlapping and interdigitating type *(Figs. 4 and 5)*. We did not observe open intercellular junctions. Frequently we observed luminal and abluminal cytoplasmic processes *(Fig. 5, inset a)* similar to those described by Niiro et al (9) in the hamster kidney endothelial wall. These morphological features correspond in thin section tridimensional reconstruction to the intra-endothelial channels previously described in the small intestine and urinary bladder (28-32). Perinuclear and cytoplasmic expansions present a clear matrix with RER tubules and micropinocytotic vesicles lying free or in relation to luminal and abluminal membranes. Quantitative analysis shows that the numerical densities, volume densities, and mean diameter of the uncoated luminal, abluminal, or cytoplasmic vesicles not to vary significantly when compared to that found during the hibernating period.

Summertime

Interlobular, arcuate, and interlobar vessels show a more winding endothelial wall in comparison to the previous seasonal aspects. Furthermore, the endothelium is thicker (230nm) and the intercellular junctions *(Fig. 8, large arrows)*, mainly overlapping, are fixed by gap and tight junctional complexes. We observed many luminal and abluminal cytoplasmic processes. The endothelial cell cytoplasmic expansions *(Fig. 8, inset a)* show a clear matrix particularly rich in ribosomes and uncoated vesicles (60-70nm). Rough vesicles are very rare. The free uncoated vesicles fill most of the cytoplasmic matrix and are often arranged in a chain-like manner across the endothelial surfaces. A large number of luminal and abluminal vesicles directly open on the lumen or the interstitium, respectively. These seasonal morphological differences are confirmed by an analysis of different groups of thin sections derived from 5 lymphatics. *Table 2* shows the numerical densities, volume densities, and mean diameter for the different categories of uncoated vesicles. The RER is well developed, and its tubules often look dilated. Numerous actin-like filaments are detectable in the abluminal peripheral cytoplasm. Dense lysosomal bodies are present in the perinuclear cytoplasm. Lipofuscin-like dense bodies

Table 2.
Summer

Vesicles	V_v $\mu m^3/\mu m^3$	N_v $\#/\mu m^3$	D nm
Abluminal	0.003	13.6	68
Cytoplasmic	0.012	46.4	70
Luminal	0.007	28.5	68

V_v = volume density; N_v = numerical density; D = mean diameter

are rare. Ultrastructural features similar to those mentioned above are found in the endothelial wall of the extraparenchymal lymphatic vessels. Lymphatics lying in the renal capsule and connective tissue located between cortex and renal calyx continue as precollecting lymphatics. The latter show an endothelial wall containing a continuous basal lamina externally coated with a monolayer of smooth muscle cells, and continue as far as prenodal collecting lymphatics from which they differ in displaying more layers of smooth muscle. An almost identical ultrastructural pattern was observed during hibernation. Lymphatic stasis as a consequence of ligating a prenodal collecting lymphatic did not alter the ultrastructure in the lymphatic endothelium except for slight vessel dilatation.

DISCUSSION

These preparations show that the renal lymphatic vascular system of bats consists of intraparenchymal (interlobar, arcuate, and interlobular) and extraparenchymal (capsular and prehilar connective) vessels. Cortical lymphatics run adjacent to the blood vessels, as also described in dog and man (33-35), rat, hamster, rabbit (4,9,36,37), and pig (5). There are no lymphatic vessels in the renal medulla in agreement with previous observations in man and other mammals (36-39). Nevertheless, we find interlobular and arcuate lymphatics adjacent to renal corpuscles, convoluted tubules, and some collecting tubules as reported in rat and dog kidney (4,7,8). As far as the source of renal lymph production is concerned, our results suggest a perivascular (24,40,41) rather than a peritubular (9,40) origin. The lack of continuity between outer cortical and capsular lymphatics is in contrast to previous studies performed on other mammals (26,42) and particularly on other hibernating species such as the marmot and dormouse (Azzali, unpublished). This discrepancy may relate both to species-related differences and to the lack of connective tissue in the renal capsule.

As far as ultrastructure is concerned, intra- and extraparenchymal lymphatics, except for precollecting and prenodal collecting lymphatics, show an endothelial wall formed by monolayer flat cells lacking continuous basal lamina and fenestrations. The endothelial cells, rectangular in shape, are characterized by: a) a central body containing the nucleus which is surrounded by abundant cytoplasm containing ordinary cytoplasmic organelles; b) extended laminar cytoplasmic expansion whose peripheral edges are fixed to adjacent cell edges by end-to-end, overlapping, or interdigitating junctions. Thus these lymphatic vessels, as in other mammals (7,9,20), share common ultrastructural features (and as a consequence common functional features) which have been considered a peculiarity of the initial tract of the vessel (i.e., the capillary or initial lymphatic). Therefore, we suggest that the renal lymphatic vasculature exclusive of precollecting lymphatics and following vessels should be considered as part of the "absorbing lymphatic peripheral apparatus" (ALPA) (43,44).

The lymphatic endothelium also shows significant seasonal change. In fact, we found different qualitative and quantitative morphological ultrastructural features concerning the profile and thickness of both the cytoplasmic expansion and the intercellular junctions. Furthermore, cytoplasmic organelles, particularly the different categories of uncoated vesicles (60-70nm) show seasonal modifications. Thus, these vesicles (luminal and abluminal) are rare during hibernation whereas they are abundant in summer; they are often arranged in a chain-like fashion across the luminal and abluminal wall but without forming true intracellular channels. The higher numerical and volume densities concerning cytoplasmic vesicles observed in hibernation as

compared to summer probably relates to peculiar morphological aspects (e.g., expansion thickness) of hibernation itself. Our data are in agreement with previous observations performed on renal hilar lymphatics under experimental conditions of low temperature (45). Taken together the findings suggest that, in our biological model, the different transendothelial transport mechanisms are not consistent throughout the year. During hibernation in particular, active and/or passive membrane transport mechanisms may prevail over the "vesicular route" (44,46,47) and the intraendothelial channels which appear prominent in summer. We did not find any open intercellular junctions as described in dogs and pigs (3-5). The seasonal variations of the cytoplasmic expansion thickness and intercellular junctions represent a morphological expression of a functional picture (48,20) involving variable shifting of interstitial contents to lymphatic lumen. The endothelial wall shows luminal and abluminal cytoplasmic processes as described earlier in rats (4) and hamsters (9). These processes, analyzed by means of tridimensional reconstructions of serial thin sections, proved to be "intraendothelial channels" similar to those described in other organs (49-51). The dramatic increase of these structures during summer, as compared to their scarcity during hibernation suggests a dynamism whereby lymphatic endothelium can alter its internal structure (in response to still unknown physical or chemical factors) by virtue of endothelial cell high plasticity (52). In other words, the transendothelial transport mechanism possibly acts under particular conditions or need as a preferential pathway from interstitium to lymphatic lumen for fluids, solutes, and macromolecular aggregates. The foregoing ultrastructural changes of the lymphatic endothelium further suggest that the different metabolic and habitat conditions of hibernating mammals parallel the changes in renal lymph formation. A possible decrease in lymph formation during hibernation could, in accordance with Kayser (53), be ascribed to a sharp decrease in blood flow and, as a consequence, diminution in capillary filtrate.

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REFERENCES

1. Peirce, EC: Renal lymphatics. Anat. Rec. 90 (1944), 314-335.
2. Bell, RD, MJ Keyl, FR Shrader, et al: Renal lymphatics: The internal distribution. Nephron 5 (1968), 454-463.
3. Nordquist, RE, RD Bell, RJ Sinclair, et al: The distribution and ultrastructural morphology of lymphatic vessels in the canine renal cortex. Lymphology 6 (1973), 13-19.
4. Ohkuma, M: Electron microscopic observation of the renal lymphatic capillary after injection of ink solution. Lymphology 6 (1973), 175-181.
5. Cuttino, JT, JC Jennette, RL Clark, et al: Renal medullary lymphatics: Microradiographic, light, and electron microscopic studies in pigs. Lymphology 18 (1985), 24-30.
6. Dieterich, HJ, W Kriz: Zum Problem der Fixierung des Nierenmarks. Licht- und elektronenmikroskopische Untersuchungen an der Außenzone der Rattenniere. Acta Anat. 74 (1969), 267-289.
7. Albertine, KH, CCC O'Morchoe: An integrated light and electron microscopic study on the existence of intramedullary lymphatics in the dog kidney. Lymphology 13 (1980), 100-106.
8. O'Morchoe, CCC, KH Albertine: The renal cortical lymphatic systtem in dogs with unimpeded lymph and urine flow. Anat. Rec. 198 (1980), 427-438.
9. Niiro, GK, HM Jarosz, PJ O'Morchoe, et al: The renal cortical lymphatic system in the rat, hamster, and rabbit. Am. J. Anat. 177 (1986), 21-34.
10. O'Morchoe, CCC: Lymphatic drainage of the kidney. In *Experimental Biology of the Lymphatic Circulation*, Ch. 10, Johnston, MG, (Ed.), Elsevier Science Publishers (1985), 261-301.
11. Kutsuna, M, M Kiyozumi, S Yamasita: Feinere Verteilung der Lymphagefässe im Innern der Niere. Taiwan Igakkai Zassi 38 (1939), 390-396.
12. Rhodin, JAG: Fine structure of the

peritubular capillaries of the human kidney. In *Progress in Pyelonephritis*, Kass, EH, (Ed.), F.A. Davis Co., Philadelphia (1965), 391-458.
13. Rawson, AJ: Distribution of the lymphatics of the human kidney as shown in a case of carcinomatous permeation. Arch. Path. 47 (1949), 283-292.
14. O'Morchoe, CCC, PJ O'Morchoe, NM Heney: Renal hilar lymph. Circ. Res. 26 (1970), 469-479.
15. O'Morchoe, CCC, PJ O'Morchoe, EJ Donati: Comparison of hilar and capsular renal lymph. Am. J. Physiol. 229 (1975), 416-421.
16. Eliska, O: Topography of intrarenal lymphatics. Lymphology 17 (1984), 135-141.
17. O'Morchoe, CCC: On the existence of renal medullary lymphatics. Lymphology 18 (1985), 2-4.
18. Ottaviani, G: Richerche sul sistema linfatico dei Chirroteri. Atti. Soc. It. Progr. Scienze 3 (1932), 104-122.
19. Ottaviani, G, V Giacomelli: Notizie tecniche ed osservazioni anatomo-microscopiche sui rapporti tra vasi sanguiferi e linfatici nei preparati al neoprene. Ateneo Parmense 26, 3 Suppl. (1955), 3-17.
20. Albertine, KH, CCC O'Morchoe: Renal lymphatic ultrastructure and translymphatic transport. Microvas. Res. 19 (1980), 338-351.
21. Reynolds, ES: The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17 (1963), 208-212.
22. Born, G: Die Plattenmodellirmethode. Arch. Mikrosk. Anat. Etnw. Mech. 22 (1883), 584-599.
23. Werner, G: Raümliche Rekonstruktion. Meth. -Elmi 7 (1975), 4-15.
24. Bell, RD, RD Ornitz, R Trautman, et al: The significance of the renal pelvis and intrarenal veins in renal lymph formation. Invest. Urol. 9 (1971), 149-153.
25. Rojo-Ortega, JM, E Yeghiayan, J Genest: Lymphatic capillaries in the renal cortex of the rat. Lab. Invest. 29 (1973), 336-341.
26. Holmes, MJ, PJ O'Morchoe, CCC O'Morchoe: Morphology of the intrarenal lymphatic system: Capsular and hilar communications. Am. J. Anat. 149 (1977), 333-352.
27. Jones, WR, PJ O'Morchoe, CCC O'Morchoe: The organization of endocytotic vesicles in lymphatic endothelium. Microvasc. Res. 25 (1983), 286-299.
28. Azzali, G, G Werner: Ultrastructura del capillare linfatico e sua ricostruzione tridimensionale. Atti Soc. Ital. Anatomia, 1976; Ed. Grafica Toscana, Firenze (1978), 42-43.
29. Azzali, G: The ultrastructural basis of lipid transport in the absorbing lymphatic vessel. J. Submicrosc. Cytol. 14 (1982a), 45-54.
30. Azzali, G: The transendothelial transport of lipids in the absorbing lymphatic vessel. Experientia 38 (1982b), 275-277.
31. Azzali, G: Ultrastructure of small intestine submucosal and serosal-muscular lymphatic vessels. Lymphology 15 (1982c), 106-111.
32. Azzali, G, G Romita, R Gatti: Ultrastruttura dei vasi linfatici della vescica orinaria. Acta Biomed. Ateneo Parmense 54 (1983), 105-115.
33. Kriz, W, HJ Dieterich: Das Lymphgefäßsystem der Niere bei einigen Säugetieren. Licht-und Elektronenmikroskopische Untersuchungen. Z. Anat. Entwickl.-Gesch. 131 (1970), 111-147.
34. Comparini, L, A Bastianini: I vasi linfatici parenchimali del rene umano. (Morfologia microscopica e istotopografia nell'organo normale). Arch. Ital. Anat. Embriol. 72 (1967), 59-91.
35. Comparini, L, C Fruschelli, A Bastianini, et al: Morfologia microscopica e struttura del sistema vascolare linfatico. Arch. Ital. Anat. Embriol. 78 Suppl. (1973), 7-61.
36. Brzezinski, DK: Neue Befunde mit einer verbesserten Darstellung experimentell aufgefullter Lymphkapillaren an Niere, Hoden-Nebenhoden, Dunn-und Dickdarm. Anat. Anz. 113 (1963), 289-306.
37. Babics, A, F Renyi-Vamos: Clinical and theoretical pictures of some renal diseases. Akademiai Kiado Budapest (1964), 1-313.
38. Huth, F: Beitrage zur Orthologie und Pathologie der Lymphgefäße der Nieren. Beitr. Pathol. 136 (1968), 341-412.
39. Bell, RD, MJ Keyl: The lymph in physiology and pathology of the kidney. In *Lymphology, Proc. VI*, Int'l Congress, Thieme G, Stuttgart, Avicenum, Prague (1978), 124-128.
40. Albertine, KH, CCC O'Morchoe: The distribution and density of the canine renal cortical lymphatic system. Kidney Int. 16 (1979), 470-480.
41. Vogel, G, K Gartner, M Ulbrich: The flow rate and macromolecule content of hilar lymph from the rabbit kidney under conditions of renal venous pressure elevation and restriction of renal function studies on the origin of renal lymph. Lymphology 7 (1974), 136-143.
42. O'Morchoe, CCC (1961) cited by O'Morchoe, CCC: Lymphatic drainage of the kidney. In *Experimental Biology of the Lymphatic Circulation*, Johnston, MG (Ed.), Elsevier Science Publishers (1985), 261-301.
43. Ottaviani, G: Ultrastruttura dei vasi linfatici dell'ALPA (Apparato Linfatico Periferico Assorbente). Mon. Zool. Ital. 70 Suppl. (1962), 343-353.
44. Ottaviani, G, G Azzali: Ultrastructure des capillaires lymphatiques. Symp. int. morphologie histochimie paroi vasculaire.

Fribourg 1965, Acta Anat. T. II (1965), 325-360.
45. O'Morchoe, CCC, WR Jones, HM Jarosz, et al: Temperature dependence of protein transport across lymphatic endothelium *in vitro*. J. Cell Biol. 98 (1984), 629-640.
46. Dobbins, WO, EL Rollins: Intestinal mucosal lymphatic permeability: An electron microscopic study of endothelial vesicles and cell junctions. J. Ultrastruct. Res. 33 (1970), 29-59.
47. Yang, VV, PJ O'Morchoe, CCC O'Morchoe: Transport of protein across lymphatic endothelium in the rat kidney. Microvasc. Res. 21 (1981), 75-91.
48. Kalima, TV, Y Collan, SH Kalima: Variations of lymphatic endothelial cell junctions in experimental conditions. In *Progress in Lymphology*, Mayall, RC, MH Witte (Eds.), Plenum Press (1977), 7-12.
49. Azzali, G: Ultrasturuttura del capillare e del vaso linfatico e loro gradiente di permeabilita. Acta Biomed. Ateneo Parmense 47 (1976), 14-15.
50. Romita, G, R Gatti: Sulla fine struttura dei vasi linfatici della vescica orinaria di piccoli cheloni (Testudo graeca ed Emys europaea). Acta Biomed. Ateneo Parmense 54 (1983), 137-146.
51. Azzali, G: Sur le passage transendothelial de lipides et de cellules dans les vaisseaux lymphatiques absorbants. Acta Anat. 120 (1984), 9.
52. Ottaviani, G: Istobiologia dei vasi linfatici. Folia Angiol. 5 (1958), 1-7.
53. Kayser, C: L'hibernation des mammiferes. Ann. Biol. 29 (1953), 109-150.

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