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Electron Microscopic Observation of the Renal Lymphatic Capillary after Injection of Ink Solution

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Summary

After injecting colloidal ink solution into the renal cortex of the rat, followed by an intraaortal perfusion fixation, the fine structure of the lymphatic capillary was investigated. Whereas the lumen of the blood vessel is free from the ink particles, that of the lymphatic contains them, making it easier to locate the latter. The vessel is characterized by failed endothelial fenestration, absent or scanty basal lamina, fibrils and collagen fibers attached to the endothelium, luminal and abluminal projection and a loose endothelial junction which could be opened by stimuli. Its characteristic structure has something to do with lymphatic transport of the large particulates from the tissue under various pathological conditions.

The fine structure of the renal lymphatic vessel was shown for the first time by *Rhodin* (9) in human nephrotic kidney. *Huth* has investigated the rabbit after ligature of hiler lymphatics; *Kritz* et al. (4) used various animals including the rat, after administration of an osmotic diuretic. The lymphatic capillary is reported to be characterized by absent or scanty basal lamina as well as an absent endothelial fenestration.

This investigation was undertaken to evaluate the above characteristics and also to establish a more practical method to locate the kidney lymphatic.

Materials and Methods

Under ether anesthesia, 0.1 ml of lukewarm colloidal ink solution (0.040 to 0.067 g of carbon in 10 ml of saline solution) was slowly (0.1 ml per minute) injected (2, 5, 6) into the left renal cortex of eight adult Sprague-Dawley rats. After 20 minutes, perfusion fixation was performed by manually injecting 10 to 20 ml of lukewarm 6% glutaraldehyde in phosphate buffer (pH 7.2-7.4) into the lower abdominal aorta. The left common iliac vein was cut down to let it bleed and the perfusion was continued until the content of the left renal vein became transparent. The whole kidney was removed and placed in the above fixative for half an hour at 4°C. Then specimens were excised from the border of the injected area, cut into small pieces and fixed further for 2 hours in the same solution. Postfixation was carried out by 2% OsO_4 in phosphate buffer for 2 hours at 4°C. Staining was performed by 1% phosphotungustic acid and 0.5% uranyl acetate and lead monoxide. The lymphatic capillary was first examined by cutting 1 μ thick sections stained with toludine blue. The same procedure was performed after injecting 0.1 ml warmed physiological saline solution into the same tissue for control (2 rats).

Result

Lymphatic endothelium: (Fig. 1 and 2) The photograph shows cell organelles including coated vesicles. The lymphatic endothelium is characterized by luminal (Fig. 5

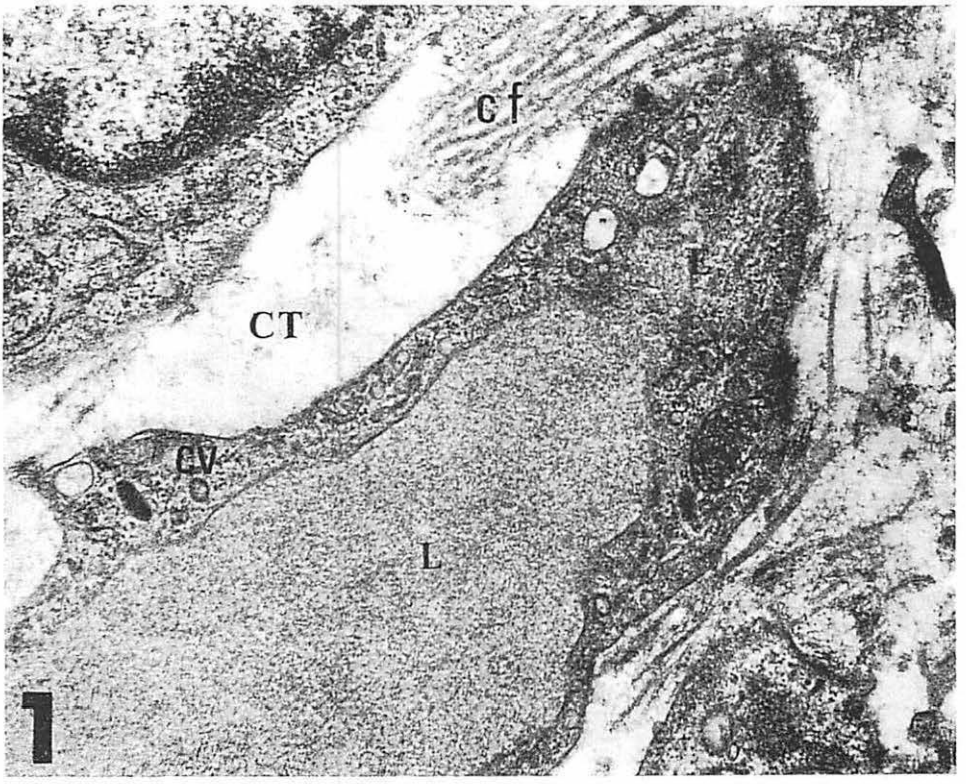

Fig. 1. A lymphatic capillary with fine electron dense particles in its lumen and coated vesicle in the endothelium. x 27,500

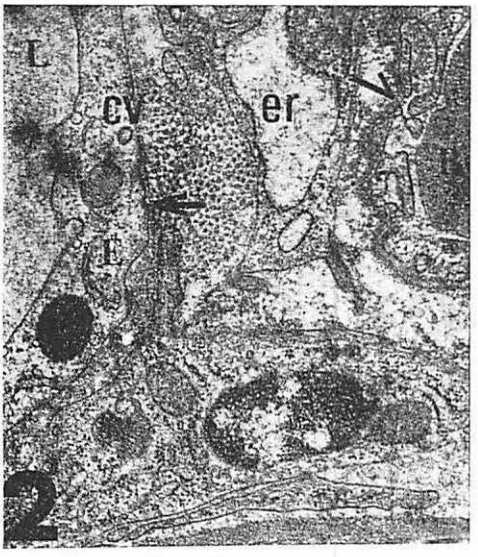

Fig. 2. A coated vesicle and the basal lamina associated with the so-called half-desmosome (↗). Notice the blood capillary with a continuous basal lamina and endothelial pores (↓). The rough ER of a connective tissue cell is remarkably dilated(er). x 20,000.

and 6) and abluminal projections of endothelium. Neither open junctions nor fenestrations of the endothelium could be found as *Huth* has mentioned (3). Four different types of junctions have been observed (7); overlapping (Fig. 3 and 4), end-to-end (Fig. 5), side-to-end (Fig. 6) and interdigitated.

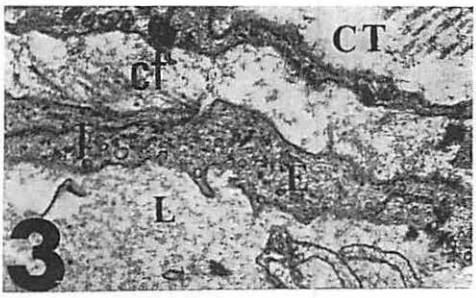

Fig. 3. Collagen fibers attached to the endothelium at the junction and numerous vesicles of the endothelium. x 23,000.

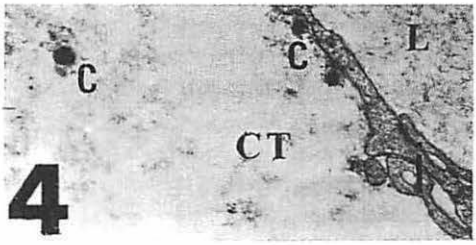

Fig. 4. Ink particles are seen in the connective tissue area and some of them are near to the lymphatic endothelium. x 18,000.

Lymphatic lumen: fine electron dense particles, which appear to originate from blood, make the lumen darker than the adjacent area. Fibrin, blood cells, destroyed cells and cell organelles as well as ink particles (Fig. 6) were seen in the lumina.

Basal lamina are absent and if present, they are poorly developed (Fig. 2 and 6) and sometimes associated with the half-desmosome (Fig. 2).

Fiber and fibril: Fibrils and collagen fibers are attached to the endothelium of the lymphatic capillary with the other ends merging into the ground substance of the connective tissue area. Some are located at the endothelial junction (Fig. 3).

Connective tissue area: there is marked extra- and intracellular edema. The connective tissue cells show enlarged endoplasmic reticulum (Fig. 2) and vacuoles. Plasma cells are occasionally encountered. All particles and cells found in the lymphatic lumina are also observed in the connective tissue area.

Blood capillary: It is sourrounded by the non-interrupted basal lamina and possesses endothelial fenestrae (Fig. 2). Its lumen is less dense than that of the lymphatic. No ink particles were detected in the lumen of the blood capillary.

Control: The injected area is macroscopically recognized only by means of hemorrhage with an indefinite border. No side-effect of the injected ink on the structure of the lymphatic could be detected. A direct comparison of the specimen was made after injection of saline solution alone (Fig. 7).

Discussion

No ink particles were observed in the lumen of the blood vessel, partly because the blood vessel take ink less frequently than the lymphatics; and if it occurs, ink is carried away by the circulating blood stream and phagocytized elsewhere; part of them may have been washed away by the perfused fixative. The vacant lumen of the blood vessel is clearly distinctive from that of the lymphatic containing fine ink particles,

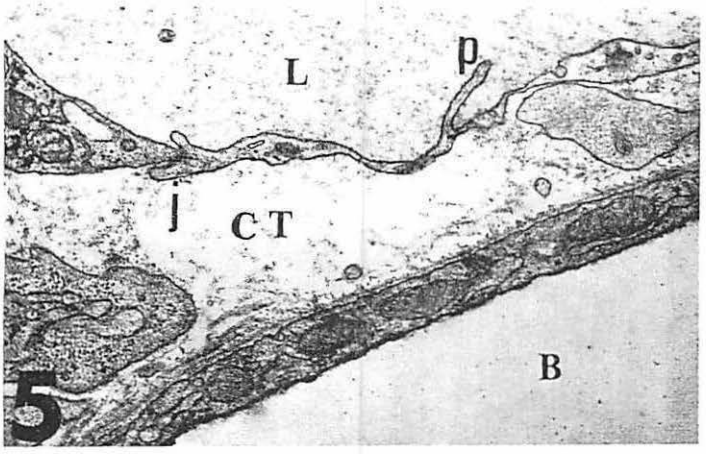

Fig. 5. An endothelial junction from end-to-end type and a luminal projection. x 20,000

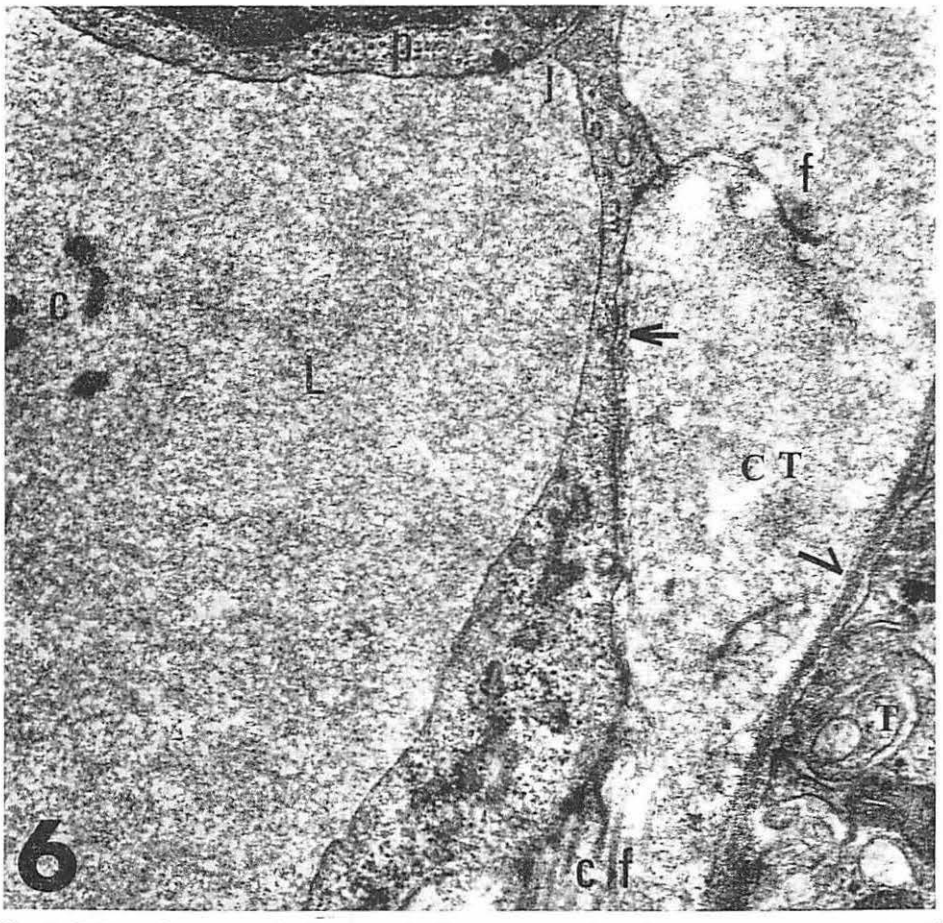

Fig. 6. Collagen fibers and a fibril are attached to the endothelium. There is a scanty basal lamina (↗) around the endothelium. Some ink particles in the lumen, a luminal projection of the endothelium and the continuous basal lamina of the renal tubule (/). x 24,000.
(Fig. 1 to Fig. 6: the renal cortex of the rat after injection of ink solution)

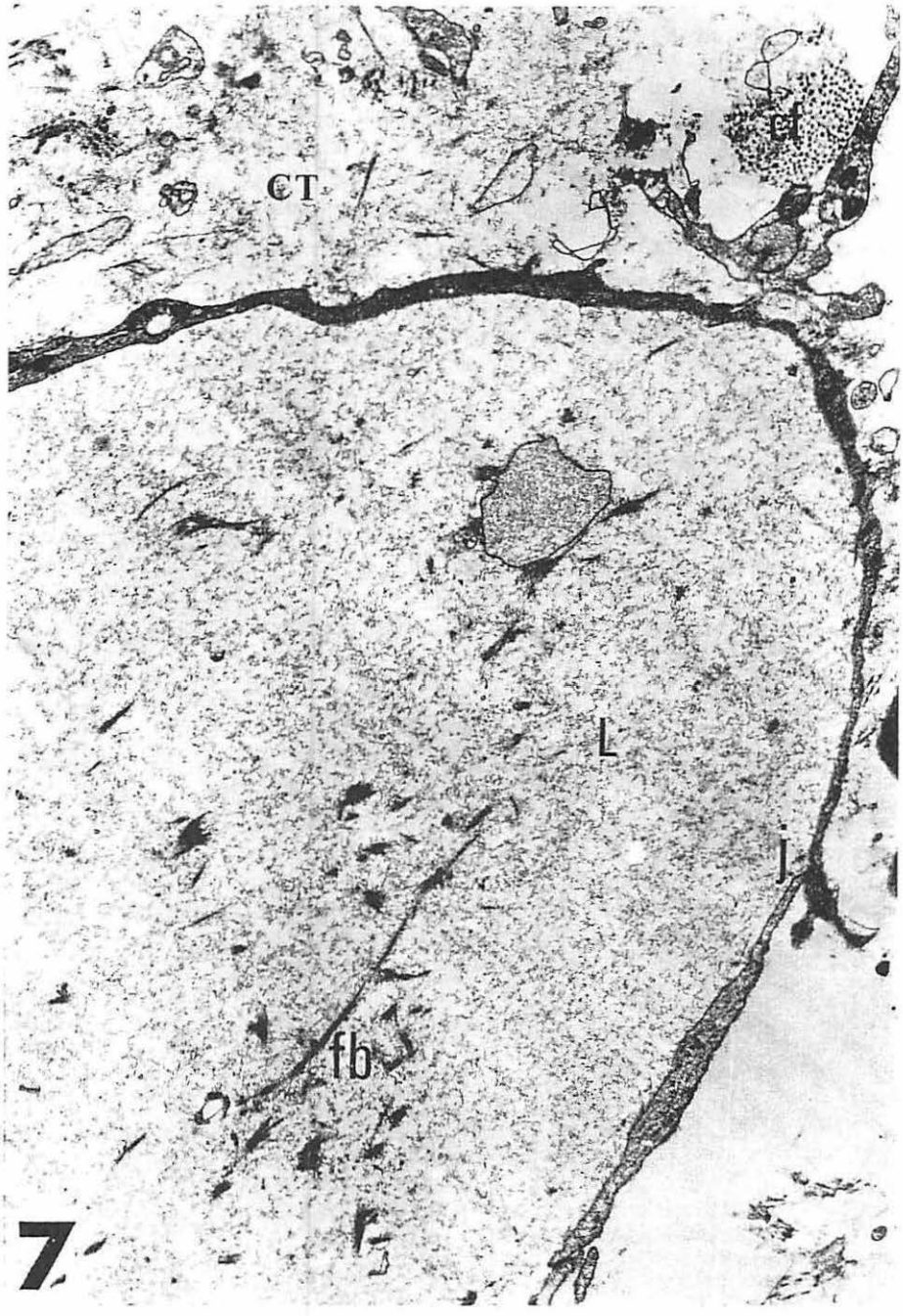

Fig. 7. Fibrin in the lymphatic lumen. Some destroyed cells and cell-organelles in the connective tissue area. Renal cortex of the rat after injection of saline solution. x 10,000.

Abbreviations used in Figures: B: blood vessel, c: ink particle, cf: collagen fiber, CT: connective tissue area, cv: coated vesicle, E: lymphatic endothelium, f: fibril, fb: fibrin, j: endothelial junction, L: lymphatic lumen, p: endothelial projection, T: renal tubulus.

making it easier to locate the latter vessel. Further study is indicated to determine whether the lymphatics with their peripheral ends lying in the injected and injured area, are really intact or not. The specimens taken from the early recognized expanding border of the ink-injected region revealed no morphological evidence for injury in the fine structure. This microinjection method for the lymphatic followed by perfusion fixation is very practical for studying the normal lymphatic, since the injected area is clearly seen. Ink is found in the lumen of the lymphatic but not in the blood vessel.

Collagen fibers are attached to the endothelium of the lymphatic capillary at the endothelial junction. The other ends merge into connective tissue. This part of the endothelium probably functions as a valve, opening and closing the junction, when the adhased collagen fibers are pulled, as *Casley-Smith* has suggested (1). The basal lamina of the lymphatic capillary is absent or, if present, scanty. The endothelial junction is occasionally loosely connected at one point, whereas the blood capillary is surrounded by continuous basal lamina; its junction is tightly closed. The lymphatic with its characteristic structure can take particulates and cells into the lumen without difficulty. This ability is secondary to its basic function. Lymphogenic metastasis of malignant neoplasma and transport of various tissue products to the lymph node is a common occurrence. Control specimens were taken from the injured area, because the injected border was not sharply recognized. This specimen did not present a true control in its strict sense.

The characteristics of the lymphatic capillary are summarized as follows:

1. The endothelial pore is absent.
2. Frequent luminal and abluminal projections of the endothelium with an irregular shape of the lumen are noted.
3. Four types of endothelial junction including a loose type which could be opened by stimuli are seen.
4. Absent or scantly basal lamina occasionally associated with the so-called half-desmosome are observed.
5. Fibrils and collagen fibers are attached to the endothelium.
6. The lumen appears darker than that of the blood capillary, so far as this microinjection method with perfusion fixation is concerned.

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Influence of Lymph Flow Rate on Concentrations of Proteins and Dextran in Dog Leg Lymph

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Summary

Control measurements of lymph:plasma concentration ratios (R) for total protein, albumin, globulin and Dextran-110 over a more than 60-fold range of spontaneous lymph flows (L) in legs of 72 dogs show an inverse relation of R and L which is in qualitative agreement with the prediction of *Drinker's* theory of lymph formation and some of its more recent elaborations. At low to moderate lymph flow rates, the relation conforms to the simplified relation R = PS/(PS + L) where PS is a permeability-surface area product. PS for plasma globulins (mainly gamma, effective radius 55 Å) is about half that for serum albumin (35.5 Å) and PS for Dextran-110 (71 Å) is about one-sixth. The decrease of PS with increasing molecular size characterizes the sieving properties of the blood-lymph barrier. There is considerable variation of PS values among preparations, even under supposedly normal conditions. Legs with high lymph flows tend to have high permeabilities and show diminished sieving.

According to *Drinker's* theory of lymph formation, the concentration of proteins and other large molecules in lymph, relative to plasma, ought to be inversely related to lymph flow rate, provided capillary permeability to these substances remains constant (1). Experimental support for this contention is limited, however. *White* et al. (2) reported that total protein concentration of dog leg lymph decreased as lymph flow increased when unanesthetized dogs with cannulated leg lymphatics were allowed to walk