STRUCTURE OF THE LYMPHATIC MICROCIRCULATION IN THE HUMAN URINARY BLADDER WITH DIFFERENT INTRALUMINAL PRESSURE AND DISTENSION

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ABSTRACT

The localization, morphology and fine structure of initial lymphatic vessels in the mucosa of the empty and distended urinary bladder were studied. Endoscopic trans-urethral biopsies of the empty (collapsed) bladder showed under light and electron microscopy numerous intramural lymphatics with a dilated lumen and thin profile. Contacts between endothelial cells were single, overlapping, interlocking, and open while the perivascular connective tissue was filled by fascicles of collagen fibers. In the most superficial layer (subepithelial mucosa), lymphatics were not seen. Biopsies obtained under elevated intraluminal pressure and distension showed on light and electron microscopy lymphatic vessels with small lumens characteristically reduced to irregular slits. Endothelial cell contacts were simple or overlapping; open junctions were rare. The perivascular connective tissue was dense and collagen and elastic fibers often abutted one another. These findings support that with a distended or expanded urinary bladder, the effect of increased intraluminal pressure on the superficial (mucosal) layer radially pulls on the connective tissue that in turn compresses the initial lymphatics thereby restricting lymph transport.

The human urinary bladder wall consists of transitional epithelium, a lamina propria with a discontinuous muscularis mucosae, a smooth muscular wall and subserosa. No sharp distinction exists between the mucosa and submucosa (1-3). Whereas there are numerous blood capillaries at the interface between the epithelium and lamina propria and in the subepithelium mucosa, lymphatics are not present at these superficial sites in the human and rat urinary bladder (4). Lymphatic vessels are seen, however, in the deeper regions of the mucosa and in the muscular and subserosal sheaths. Moreover, lymphatics progressively increase in number and diameter from the deep mucosal layer to the subserosa (1-4). Distention of the wall of hollow viscera undergoes complex changes in their internal anatomic structure. Consideration along these lines is particularly relevant as it applies to a highly distensible organ such as the urinary bladder which contains a rich supply of both blood and lymphatic vessels. Because lymphatics in the urinary bladder mucosa lack a smooth muscle layer (4), they can properly be considered as initial lymphatics and probably expand and collapse in response to mechanical modifications of the vesical wall.

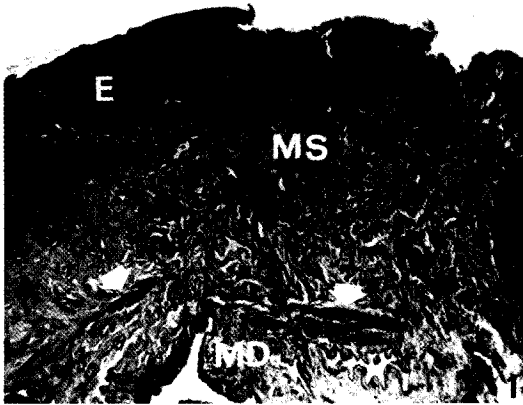In this context, we studied the localization, morphology and fine structure of initial

Fig. 1. The border between the superficial and deep regions of the bladder mucosa is depicted by a discontinuous muscularis mucosae. Semithin section from a full expanded bladder wall. (x60). E: Transitional epithelium. MS: Superficial region of the mucosa. MD: Deep region of the mucosa.

lymphatic vessels in the mucosa of the human urinary bladder under varying conditions of distension and intraluminal pressure elevation. The lymphatics examined were limited to the paramucosa because of intrinsic sampling limitations via endoscopy.

MATERIAL AND METHODS

Endoscopic transurethral biopsies of the urinary bladder were taken in the course of clinical studies from 15 male patients (age range from 40 to 67 years) with possible prostatic obstruction. The endoscopic and light microscopic appearances of the urinary bladder and vesical biopsy specimens were normal. The investigational protocol was approved by the institutional review board and the patients were informed about the utilization of biopsies for investigational purposes and specifically for examination of lymphatic morphology. Each patient provided informed written consent.

Two superficial pieces of tissue were taken from the trigone and lateral wall of the urinary bladder incorporating transitional epithelium with mucosa. Ten male subjects underwent endoscopic biopsy under resting conditions of volume and pressure (5 cm H_2O intraluminal vesical pressure) whereas in five subjects biopsy was taken under high volume and intraluminal pressure conditions (15 cm H_2O for 20 minutes). The biopsy samples were fixed in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodylate buffer at pH 7.4 for 4 hours at 4°C and postfixed by 1% OsO_4 in 0.2 M collidine buffer at pH 7.4 for 2 hours at 4°C. They were dehydrated and embedded in Epoxy resin.

Lymphatic vessels were identified from their morphologic characteristics (4) by light microscopy using semithin sections stained with toluidine blue. Selected lymphatic vessels were subsequently observed on ultrathin sections collected on grids and contrasted with uranyl acetate and lead citrate, and an aqueous solution of 2% orcein by a transmission Zeiss EM 10 electron microscope. For quantitative evaluations, initial lymphatics from each biopsy specimen were examined with stereological techniques using a computerized automated image analyzer (IBAS I and II Kontron, Zeiss). Morphometric analysis was performed in the superficial and deep regions of the lamina propria. The border between these regions was depicted by the discontinuous muscularis mucosae *(Fig. 1)*. The diameter (of area-equivalent circle) of vessels and their numbers for a given area unit were measured in the mucosa as previously described (4,5).

RESULTS

Table 1 quantifies the diameter and number of vesical initial lymphatics per mucosal area unit under resting conditions and with increased intraluminal pressure and wall distension. Morphometric results of the empty bladder have been taken from a previous investigation (4).

Empty Urinary Bladder (Basal Pressure and Volume)

	EMPTY BLADDER (Collapsed wall)		EXPANDED BLADDER (Distended wall)	
TABLE 1 **Quantitative Data on Small Lymphatic Vessels of the Human** **Urinary Bladder Under Basal and High Pressure-Volume Conditions**				
Vesical region	Number	Diameter (μm$\pm$SE)	Number	Diameter (μm$\pm$SE)
Superficial mucosa	10	30.2$\pm$0.82	8	21.8$\pm$0.61
Deep Mucosa	17	48.3$\pm$1.35	16	42.2$\pm$0.40

In transverse semithin sections through the collapsed mucosa, the transitional epithelium was composed of 4-7 layers of cells joined to each other by junctions of the adherence type. The interface between urothelium and lamina propria was scalloped by blood capillaries lying between indentations of the basal urothelial cells. In these subepithelial (superficial) areas, no lymphatics were observed. On close examination of toluidine blue stained sections, numerous initial lymphatics were detectable primarily in the deeper regions of the mucosa of the vesical wall *(Fig. 2)*. They were often associated with arteriolar and venular vessels but were distinguished from blood vessels by their irregular contours, thin wall, absence of a muscular sheath, and a lumen without red blood cells but often filled with proteinaceous debris.

The bulk of terminal lymphatics in the empty urinary bladder showed a dilated, irregular lumen and their number increased from the superficial to the deeper layers of the vesical mucosa. Electron microscopy also showed these lymphatics to have a dilated lumen, a moderately irregular and thin profile and the endothelial cells were attenuated with scanty cytoplasm *(Fig. 3)*. On the luminal and abluminal side, endothelial cells displayed rare micropinocytotic vesicles.

Adjacent endothelial cells showed varying types of contact with each other: end-to-end, overlapping cytoplasmic processes, and fork-like interlockings with intercellular channels. Typical wide open junctions were also seen. The loose connective tissue surrounding the lymphatic wall was filled by bundles of collagen and elastic fibers that reach the lymphatic capillary wall. Elastic fibers were more abundant around lymphatics within the deep mucosa and were tightly joined to the abluminal side of the endothelium by thin anchoring filaments.

Distended Urinary Bladder (Elevated Pressure and Volume)

In transverse semithin sections through the expanded mucosa, the urothelium was thin and distended and composed of two or three layers of transitional cells. On light microscopy, the initial lymphatics of the mucosa were few and isolated and located with difficulty because the lumina were generally reduced to irregular slits with thin wall and in a dense connective matrix *(Fig. 4)*. In the deep mucosa, a network of irregular lymphatic vessels interposed among numerous blood vessels was more readily observed. Lymphatics penetrated into the connective tissue around blood vessels as

flattened channels expanding where the interstitium was more abundant *(Fig. 5)*. These lymphatic vessels uniformly had an attenuated wall consisting solely of an endothelial sheath. Ultrastructure *(Fig, 6,7)* showed lymphatic capillaries with a thin profile, delimited by very attenuated endothelial cells with rare micropinocytotic vesicles. Contacts between adjacent endothelial cells were simple. The varieties were end-to-end or overlapping, but complex intercellular channels connecting the vessel lumen and the surrounding interstitium were absent. Open junctions were rare. The connective tissue surrounding the lymphatic wall was more dense in the distended than in the empty bladder whereas the fascicles of collagen and elastic fibers intertwined in close proximity. This fibrillar complex was connected to the endothelial wall by rare anchoring filaments.

DISCUSSION

The human urinary bladder possesses a well developed lymphatic system with initial lymphatics primarily in the deep mucosa and in the submucosal layers (3,4,7). Because the urinary bladder is an organ which undergoes fairly extensive volume and pressure changes, we examined the effects of the variation of intraluminal pressure and volume on vesical
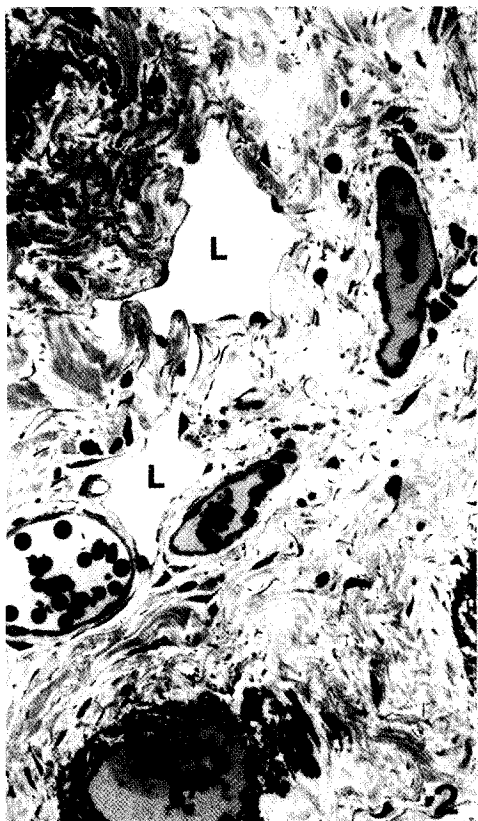

Fig. 2. Semithin section from the collapsed empty urinary bladder wall. Dilated initial lymphatic vessels (L) are located in the deep mucosa near a group of blood capillaries. (x300)

Fig. 3. Ultrathin section from the collapsed empty urinary bladder wall. A dilated lymph capillary with a fork-like intercellular connection (arrow). Collagen and elastic orcein stained fibers surround the vessel wall. (x12,000)
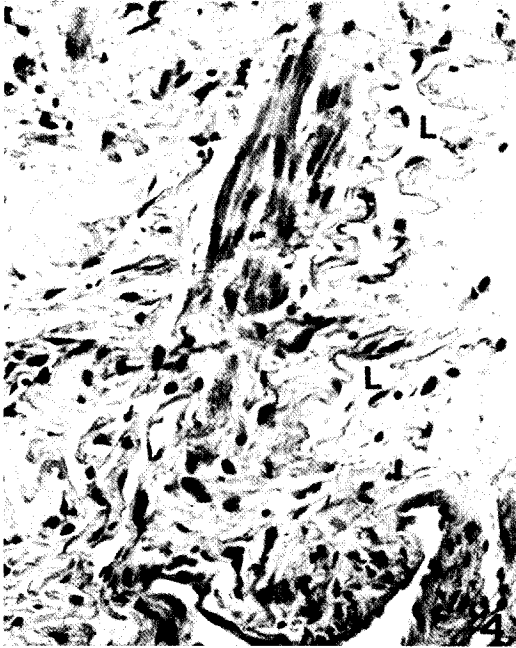
Fig. 4. Semithin section from the distended full expanded urinary bladder wall. Several lymph capillaries (L) immersed in compact connective tissue show a collapsed lumen and irregular contour. (x300)

Fig. 5. Semithin section from an expanded urinary bladder wall. A lymph capillary (L) with an irregular profile is seen in the interstitium surrounded by blood vessels. (x300).

lymphatic vessel morphology. The lymphatic network is a unidirectional drainage system that returns interstitial fluid back to the venous system. Accordingly, we assumed that deformations in modifications of terminal lymphatics may be important factors in regulating lymph formation and flow (8-10). We also assumed that the translocation of interstitial fluid across the initial lymphatic wall occurred when a positive gradient of hydrostatic pressure was created between tissue fluid and lymph. A positive hydrostatic pressure gradient may be generated by several mechanisms including arteriolar pulsations, intrinsic lymphatic pumping or "external" muscular pumping (10) and by modifications of the wall of a hollow organ under different pressure and volume conditions (11). Lymph vessels in the mucosa of the urinary bladder are initial lymphatics

because they lack a muscular sheath. This characteristic previously demonstrated in other distensible organs such as the lung (12), makes it likely that active compression by tissue components surrounding the lymphatics is necessary for promotion of lymph flow. Two major mechanisms likely regulate lymph formation and transport in the urinary bladder, namely distension and relaxation of the vesical wall and modification(s) of the muscular layers of the urinary bladder wall under varying physiologic conditions during filling and emptying.

The morphologic modifications and different configurations of terminal lymphatics observed in this study under different urinary bladder pressure and volume conditions may be interpreted in light of findings observed with skeletal muscle contraction which alters lymph flow by
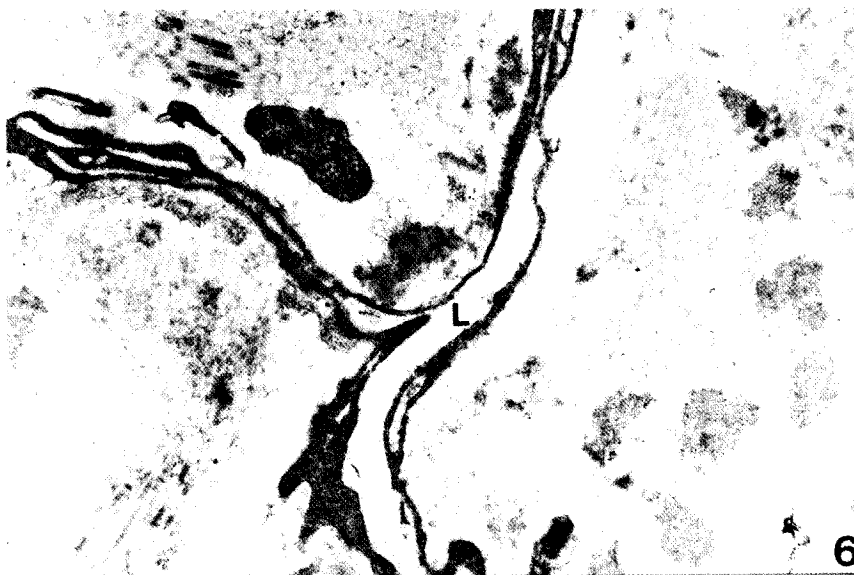
Fig. 6. Ultrathin section from an expanded urinary bladder wall showing a collapsed and irregular lymph capillary (L). (x15,000)

Fig. 7. Ultrathin section from an expanded urinary bladder wall showing an overlapping contact between attenuated adjacent endothelial cells in a collapsed capillary (L). (x10,000)

muscle fiber elongation and shortening (11,13). Compressibility during urinary muscle contraction is a functional characteristic of lymph capillaries; otherwise they would be ineffective as drainage pathways for enhanced lymph production from the muscle interstitium. Thus, lymph flow is enhanced during skeletal muscle contraction whereas lymphatics dilate and take up fluid when the muscle relaxes to a resting state (10,14).

In the empty urinary bladder the wall is collapsed and the smooth muscle layer is foreshortened (4). In this condition the terminal lymphatics appear dilated with numerous open junctions and cytoplasmic micropinocytotic vesicles suggesting active absorption and storage of lymph. In contrast, during urinary bladder distension, the elevated intraluminal pressure and volume radially pulls on the connective tissue and

elastic fibers in the mucosa that in turn squeezes the lymphatics and thereby limits the entry of lymph into more proximal collectors. The morphologic counterpart of this phenomenon is the collapse of initial lymphatics which are often reduced to barely visible thin slits in the connective tissue and loss of endothelial open junctions.

REFERENCES

1. Powell, TO: Studies on the lymphatics of the female urinary bladder. Surg. Gynec. Obstet. 78 (1944), 605-609.
2. Azzali, G, G Romita, R Gatti: Ultrastruttura dei vasi linfatici nella vescica orinaria. Acta Biomedica de l'Ateneo Parmense 54 (1983), 105-115.
3. Fawcett, DW: The urinary system. In: *Textbook of Histology*, 11th Ed. W.B. Saunders, Philadelphia (1986), pp. 787-790.

4. Poggi, P, C Marchetti, A Tazzi, et al: The lymphatic vessels and their relationship to the lymph formation in the human urinary bladder. Lymphology 28 (1995), 35-40.

5. Poggi, P, C Marchetti, A Calligaro, et al: Morphometric data on the lymph capillaries of the ventricles of the rabbit heart. Acta. Anat. 136 (1989), 185-189.

6. Inoue, T, G Gabella: The interface between epithelium and lamina propria in the rat urinary bladder. Arch. Histol. Cytol. 55 (1992), 157-163.

7. Reuter, VE: Urinary bladder and ureter. In: *Histology for Pathologists*, Sternberg, SS (Ed.), Raven Press, New York (1992), pp. 709-720.

8. Garlik, DG, EM Renkin: Transport of large molecules from plasma to interstitial fluid and lymph in dogs. Am. J. Physiol. 219 (1970), 1595-1605.

9. Ladd, MP, FJ Kottke, RS Blanchard: Study of the effect of massage on the flow of lymph from the foreleg of dog. Arch. Phys. Med. Rehabil. 33 (1952), 604-612.

10. Mazzoni, MC, TC Skalak, GW Schmid-Schönbein: Effects of skeletal muscle fiber deformation on lymphatic volumes. Am. J. Physiol. 259 (1990), 1860-1868.

11. Schmid-Schönbein, GW, BW Zweifach: Fluid pump mechanisms in initial lymphatics. News in Physiol. Sci. 9 (1994), 67-71.

12. Marchetti, C, P Poggi, MG Clement, et al: Lymphatic capillaries of the pig lung. TEM and SEM observations. Anat. Rec. 238 (1994), 368-373.

13. Jacobsson, S, I Kjellmer: Flow and protein content of lymph in resting and exercising skeletal muscle. Acta. Physiol. Scand. 60 (1964), 278-285.

14. Skalak, TC, GW Schmid-Schönbein, BW Zweifach: New morphological evidence for a mechanism of lymph formation in skeletal muscle. Microvasc. Res. 28 (1984), 95-112.

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