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THE LYMPHATIC VESSELS AND THEIR RELATIONSHIP TO LYMPH FORMATION IN THE HUMAN URINARY BLADDER

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ABSTRACT

After endoscopic transurethral biopsies of normal human urinary bladder, an extensive network of small initial lymphatic vessels was depicted by means of light and electron microscopy. Using light microscopy, lymphatic vessels were seen in the mucosa and submucosa and formed a complex network in the detrusor muscular coat. These lymphatics were characterized by an irregular and attenuated wall and increased in number and size from the superficial to the deeper region of the bladder. Ultrastructurally, the lymphatic wall was characterized by endothelial cells joined together end-to-end or by complicated interdigitations. Often intercellular channels and gaps between two contiguous endothelial cells were present. A broad network of elastic and collagen fibers joined the lymphatic endothelial wall to the neighboring connective tissue. Nevertheless, as far as the fibrillar component was concerned, the vesical intramuscular lymphatic endothelial wall lacked elastic fibers. These anatomic variations were examined in reference to lymph formation in an organ (the urinary bladder) which undergoes continual changes in volume and pressure.

In 1944, Powell (1) described a well-developed network of lymphatic capillaries in the mucosa and muscular coat of the urinary bladder using dye injection. Subsequently, lymphatics of the vesical wall were depicted by light and electron microscopy (2,3). To clarify vesical lymph drainage, we now examined the fine structure of small lymphatic vessels (SLV) and their distribution in the normal human urinary bladder. Particular attention was directed to the structure and composition of the connective matrix that surrounds the lymphatic vessels in the different regions of the bladder wall.

MATERIALS AND METHODS

Ten male subjects (40 to 60 years of age) who had symptoms of bladder outlet obstruction were utilized for this investigation. Two endoscopic transurethral biopsies from the lateral wall of the normal urinary bladder were performed under a constant bladder distention after introduction of physiological saline at 50 cm H_2O constant pressure. The biopsy trocar was regulated to obtain vesical specimens including both the bladder mucosa and the muscular coat. All samples were fixed in a mixture of glutaraldehyde (2.5%) and paraformaldehyde (2%) in 0.1M sodium cacodylate buffer at pH 7.4 for 4 hrs. at 4°C and postfixed by 1% O_SO_4 in 0.2M collidine buffer at pH 7.4 for 2 hrs at 4°C. The specimens were then dehydrated and embedded in Epoxy resin. Under light microscopy, small lymphatic vessels (SLV) were identified on semithin sections stained with toluidine blue as described previously (4,5). Approximately 50 SLV were examined. At least 20 selected

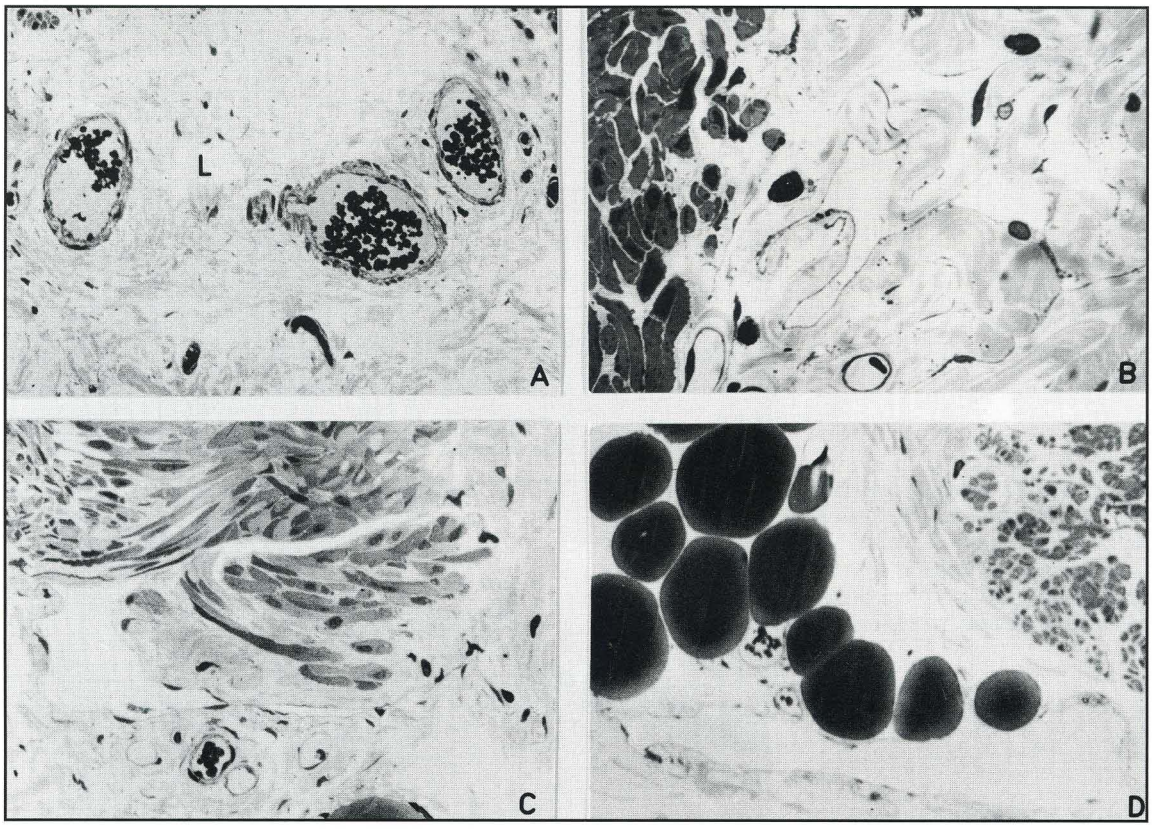

Fig. 1. Semithin sections from the human urinary bladder: A) A small lymphatic in the deeper region of the lamina propria between two large blood vessels (L=lymphatic) (500x); B) a lymphatic vessel in close proximity to the muscular layer (500x); C) a large lymphatic is seen in the muscular layer between smooth muscle cells (350x); D) a lymphatic of the subserosa is comparatively large with characteristics of a collecting vessel (200x).

TABLE 1
Quantitative Data on Small Lymphatic Vessels (SLV) of the Human Urinary Bladder

Vesical Region	SLV (n)	Diameter (± SEM)
Subepithelial	10	30.2±0.82
Submucosal	17	48.3±1.35
Muscular	18	70.3±1.20
Subserosal	5	185±4.21

lymphatics were observed on ultrathin sections, contrasted with uranyl acetate and lead citrate, by electron microscope Zeiss EM109.

The quantitative evaluation of SLV was performed with a computerized automatic image analyzer (IBAS I and II Kontron-Zeiss) as described previously (6). The morphometric analysis was performed at 3 levels: subepithelial region of the lamina propria; submucosa (deep region of the lamina propria); and detrusor muscle.

RESULTS

Light Microscopy

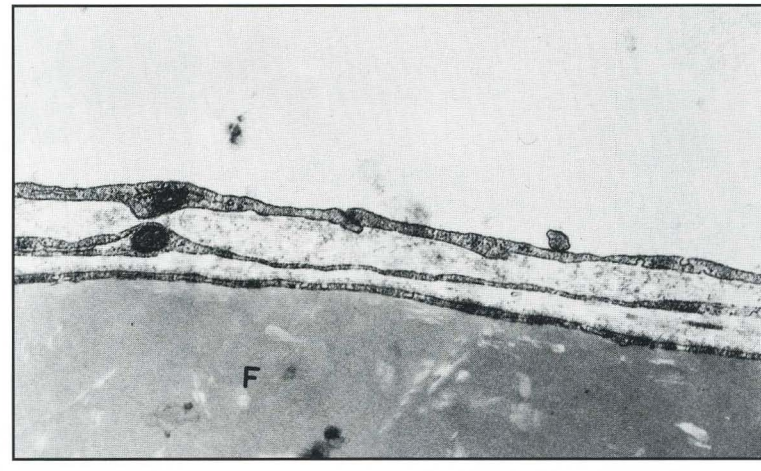

Fig. 2. A very thin endothelium characterizes this lymphatic vessel in which two contiguous endothelial cells are joined end-to-end (F=fat cell). (17000x)

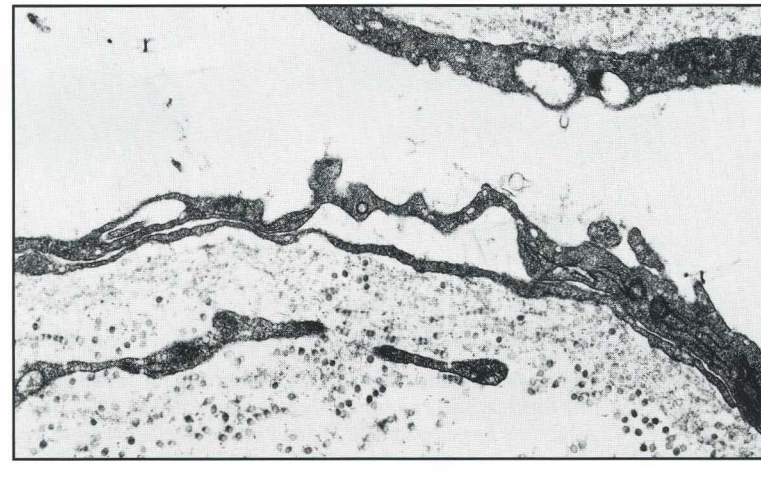

Fig. 3. In this mucosal lymphatic, two contiguous endothelial cells delimit a large channel. The lymphatic is surrounded by a thin fibrillar network of collagen fibers and anchoring filaments (17000x).

A well-developed lymphatic vessel network was found in the vesical wall. The distribution between mucosal and submucosal SLV was difficult to distinguish because the separation of these layers in human urinary bladder was unclear because of an irregularly arranged muscularis mucosae (7). Accordingly, we designated "subepithelial SLV" as the lymphatics lying immediately beneath the epithelium and "submucosal SLV" as the lymphatics lying on the remaining lamina propria up to the detrusor muscle. Lymphatic vessels were seen in all regions. They increased in number and size from the mucosal layer to the detrusor muscular layer *(Table 1)*. In the subepithelium, lymphatics were rare. Several SLV were consistently present in the bladder submucosa. They were often adjacent to and intermingled with arterioles and venules but were distinguishable from blood vessels by their very thin, indented walls *(Fig. 1A)*. In the deeper regions of the submucosa, lymphatics abutted the detrusor muscular layer *(Fig. 1B)*. *Table 1* summarizes the quantitative data on the number and size of SLV in the different regions of the vesical wall. In the detrusor layer, wide lymphatics were seen in the loose interstitial connective tissue which penetrated into the muscular bundles *(Fig. 1C)*. These lymphatics were often difficult to recognize because the lumen was occluded over a long stretch. In some specimens in which the

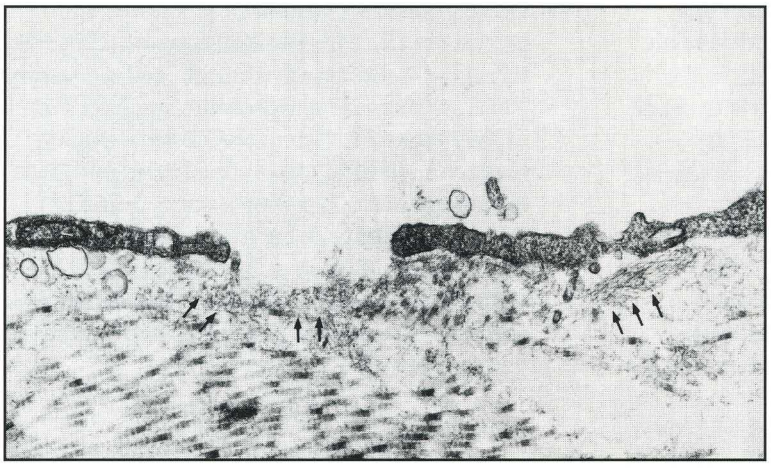

Fig. 4. Closeup of lymphatic showing a gap between two contiguous endothelial cells. Anchoring filaments are tightly joined to the abluminal side of the endothelial cells (arrows) 17000x).

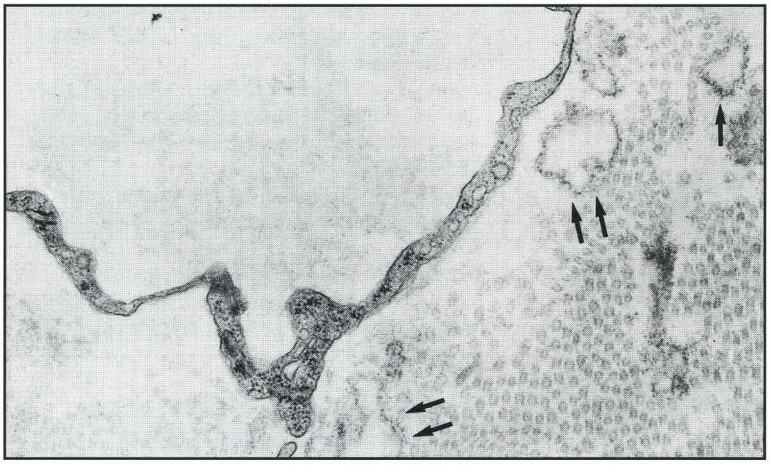

Fig. 5. Bundles of elastic fibers (arrows) and some collagen fibers abut the lymphatic endothelium. (17000x).

subserosa was seen, the lymphatics were quite large and showed the characteristics of collectors *(Fig. 1D)*. Their wall was supported by neighboring tissue structures; consequently, they had a winding appearance.

Electron Microscopy

In all lymphatic vessels examined, the endothelial cells showed a very thin and typically irregular profile. They were characterized by scanty cytoplasm; near the nuclei, organelles such as mitochondria, ribosomes and Weibel-Palade bodies were seen. The micropinocytotic vesicles were usually scarce both in the luminal and in the abluminal side of the endothelium. In the subepithelial and submucosal lymphatics, the contiguous endothelial cells were joined together primarily by simple contact (i.e., "end-to-end") *(Fig. 2)* or by overlapping. Some endothelial gaps also occurred where the endothelial wall was prominently distended *(Fig. 4)*. In the muscular and subserosal lymphatics the neighboring endothelial cells were joined together by more complex interdigitations between two or more cytoplasmic edges. Here, the endothelium was markedly indented and intercellular channels were common *(Fig. 3)*.

The loose connective tissue surrounding the lymphatic was filled with abundant

fascicles of collagen and elastic fibers that joined the lymphatic endothelial wall. Elastic fibers surrounded lymphatics mainly in the subepithelial and submucosal areas *(Fig. 5)*. In fact, bundles of elastic fibers were tightly joined to the abluminal side of the endothelial cells by thin anchoring filaments. The endothelium of the lymphatics located in the muscular sheath generally lacked elastic fibers. The lymphatic endothelium was surrounded by a thin network of collagen fibers with anchoring filaments attached to the abluminal surface *(Figs. 3,4)*. All the lymphatics examined lacked valves. Moreover, the larger lymphatics located in the muscular layer and in the submucosa did not have smooth muscle in the lymphatic wall.

DISCUSSION

Our findings on small lymphatic vessels (SLV) of the human urinary bladder can be summarized as follows: 1) SLV are less numerous and smaller in the mucosa than in the detrusor muscular layer and in the subserosa; 2) SLV ultrastructural characteristics are similar in each area examined; 3) SLV in the detrusor muscle and in the subserosa lack elastic fibers.

As to finding 1: The results agree with those previously described (8,9) and suggest that SLV drains interstitial fluid from the more superficial to the deeper regions of the bladder.

As to point 2: vesical SLV show the ultrastructural features of initial lymphatics, namely, a single endothelial lining but without either smooth muscle or intraluminal valves. The presence of junctions along the endothelial wall of bladder SLV suggest that they open during influx of interstitial fluid into the lymphatic lumen, and close when lymph inflow closes (11). The opening and closing of these junctions are linked to elastic filaments that anchor the endothelial cells at the bladder mucosa to adjacent connective tissue (12-14).

Besides this fibrillar network, SLV are also regulated by vasomotion of adjacent arterioles (15). This fibrillar network, however, is lacking in the bladder muscle and subserosa. This ultrastructural dichotomy suggests that in the mucosal region of the human urinary bladder anchoring elastic fiber filaments force expansion of the lymphatic vessel influx of interstitial fluid as lymph. On the other hand, in the deeper regions (muscle and subserosa) of the bladder, contractions of the bladder wall and adjacent arterial pulsations help propel lymph onward.

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