

STRUCTURE OF THE INITIAL LYMPHATICS OF THE HUMAN URINARY BLADDER WITH INVASIVE UROTHELIAL TUMORS

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ABSTRACT

The ability of urothelial tumors of the urinary bladder to metastasize via the lymphatic circulation and the extent of metastatic involvement of regional lymph nodes is an important parameter in the staging and prognosis of these neoplasms. Accordingly, we examined the site and morphology of initial lymphatic vessels in the mucosa of the human urinary bladder in patients with invasive transitional cell carcinoma. Lymphatics in the papillary tumoral mass was also examined. Endoscopic transurethral biopsies from the urinary bladder of 120 patients with invasive transitional cell papillary carcinoma were utilized for this study. Biopsy from the uninvolved lateral wall of the same patient was utilized as a control. On histopathology of biopsies of neoplastic tissues, initial lymph vessels were seen in the deeper region of the mucosa but not in the subepithelial layer nor in the stroma of the tumoral papillae. The latter were often associated with arteriolar and venular vessels. When edema and inflammation occurred in peritumoral regions, lymphatics showed a dilated lumen, non-indented wall with dissociated perivascular collagen and elastic fibers. Tumoral permeation or embolization of lymphatics was seen in 12% of patients with invasive tumors, and these lymphatic vessels did not display significant morphologic changes. The absence

of initial lymphatics in the stroma of tumoral papillae and in infiltrated subepithelial regions of the urinary bladder may explain the absence of lymph node metastasis in early-stage invasive urothelial tumors.

The ability of tumor cells to permeate and infiltrate the surrounding microvasculature with formation of nearby or distant metastases is an important biologic phenomenon and a major cause of death in cancer. Many human neoplasms metastasize via the lymphatic circulation and the extent of metastatic involvement of regional lymph nodes has become an important parameter in the staging and prognosis of human primary neoplasms. Approximately 95% of neoplasms of the urinary bladder are of urothelial origin and most of them are malignant. Because they are common, often multiple and recurrent, tumor management is difficult and outcome unpredictable (1). Transitional cell carcinomas ordinarily progress by invasion of the bladder muscular wall and then involve the prostatic urethra, distal ureters and in ~40% of male patients spread to prostatic ducts (2). Metastases in the regional lymph nodes have been observed in 14% of patients with superficial neoplasms (3). Whereas some studies have been performed on lymphatic involvement in metastasis formation and on tumor cell adhesion factors with special reference to endothelial cells (4,5), little is

TABLE 1
Quantitative Data on Initial Lymphatic Vessels

Mucosal Region	Normal Mucosa		Tumoral Mucosa	
	Number	Diameter ($\mu\text{m}+\text{SE}$)	Number	Diameter ($\mu\text{m}+\text{SE}$)
Superficial	10	30.2 + 0.82	12	27.8 + 1.23
Deep	17	48.3 + 1.35	26	57.4 + 0.91

known about the structure of lymphatic vessels involved in urothelial tumors of the urinary bladder. In this context, the purpose of the present investigation was to study after endoscopic transurethral biopsies and histopathologic examination the site, morphology, and fine structure of lymphatic vessels of the mucosa of the human urinary bladder with developing invasive urothelial tumors.

MATERIAL AND METHODS

Endoscopic transurethral biopsies from infiltrated mucosal areas of the urinary bladder from 120 patients (aged 42-75 years) with invasive papillary urothelial tumors and from normal lateral walls of the same patients were utilized. The endoscopic biopsies were taken under normal conditions of volume and pressure (~5 cm H₂O). Three to 6 pieces of bladder wall involving the tumoral mass together with the possibly infiltrated mucosa were resected. Two superficial pieces of tissue were also resected from apparently uninvolved regions of the urinary bladder. Biopsy specimens from 80 patients were fixed in 10% neutral formalin and embedded in paraffin. Eight to 10 μm thick sections were stained with hematoxylin and eosin and Gomori silver impregnation for reticulin for urothelial tumor identification. The tumors were classified and graded using the AFIP grading classification (1).

For morphological and ultrastructural studies, biopsies from 40 patients 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, post-fixed in 1% OsO₄ in 0.2 M collidine buffer at pH 7.4 for 2 hours at 4°C, dehydrated and embedded in epoxy resin. Forty-five lymphatic vessels were presumptively identified from their morphological characteristics by light microscopy on semithin sections stained with toluidine blue (6). These lymphatic vessels were identified and their lymphatic nature confirmed on ultrathin sections collected on grids and contrasted with uranyl acetate and lead citrate by a Zeiss EM 10 electron microscope.

The diameter (of area-equivalent circle) of initial lymphatic vessels and their number for a given area unit were measured in the superficial and the deep regions of the mucosa. Quantitative data were obtained measuring the lymphatic vessels on semithin sections by a computerized automatic image analyzer. The data were then compared with those obtained in the same mucosal regions of normal urinary bladders by the same computerized program as previously described (6,7).

RESULTS

The number per mucosal area unit and diameter of initial lymphatics are quantified in *Table 1*.

Morphology of the Lymphatic Vessels in the Normal Regions

In human urinary bladder, it is difficult

to distinguish the mucosal and submucosal layers because the discontinuous muscularis mucosae is formed by only a few groups of smooth muscle cells (8). In a previous study, we distinguished a superficial or subepithelial region and a deep region of the lamina propria separated by a discontinuous muscular layer (7).

In the control samples lymphatic vessels showed distribution and morphologic characteristics already observed and described in normal human urinary bladder wall (6,7). In fact, in semithin sections no lymphatics were observed in the subepithelial areas of the mucosa whereas thin lymphatic vessels were seen near the discontinuous layer of the muscularis mucosae (*Fig. 1*). In the deeper areas of the mucosa, lymphatic vessels were larger in diameter, occurred in greater number and were sometimes pushing against the muscular coat. Nevertheless, all these microvessels were initial lymphatics because they lacked a muscular sheath around the endothelial wall. On electron microscopy, these lymphatics showed an indented and thin wall; endothelial cells were extremely attenuated with scanty cytoplasm. In the luminal and abluminal side of the endothelial wall, several micropinocytotic vesicles were seen. Adjacent endothelial cells showed different types of contacts: end to end

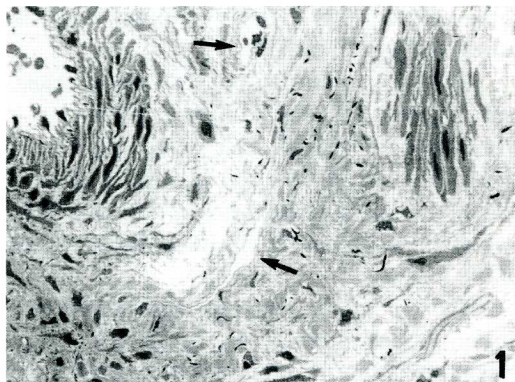
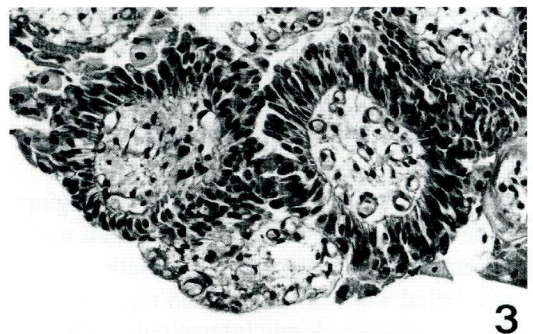
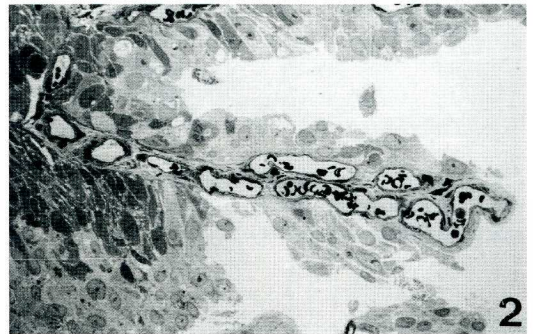


Fig. 1. Normal mucosa of a control vesical wall. Presumptive lymphatic vessels (arrow) between one artery and some smooth muscle cells of the discontinuous muscularis mucosae. 120x

overlapping or fork-like interlacing were the most prevalent. Large gaps between contiguous endothelial cells (open junctions) were occasionally present in dilated lymph vessels. Anchoring filaments and fascicles of collagen and elastic fibers reached the capillary wall.

Morphology of the Lymphatic Vessels in the Urothelial Tumors

The extended papillomatous protrusions of the urothelial tumors were formed by multiple layers of polygonal cells connected by irregular and thin extensions and by an axial connective stromal framework. The specimen was sometimes edematous and showed numerous blood capillary vessels with a dilated lumen filled by erythrocytes and coagulated plasma proteins (*Figs. 2,3*). No lymphatic vessels were present in these proliferative areas nor in the subepithelial layer of the mucosa rich in dilated blood



Figs. 2-3. Longitudinal (2) and transverse (3) sections of papillary protrusions of urothelial tumors with numerous and dilated blood vessels. 120x; 100x

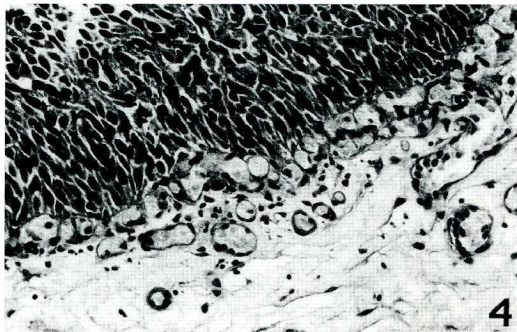


Fig. 4. Numerous enlarged blood capillaries in the subepithelial area of the mucosa in a specimen of intraepithelial vesical carcinoma. No lymphatic vessels are detectable in this region. 100x.

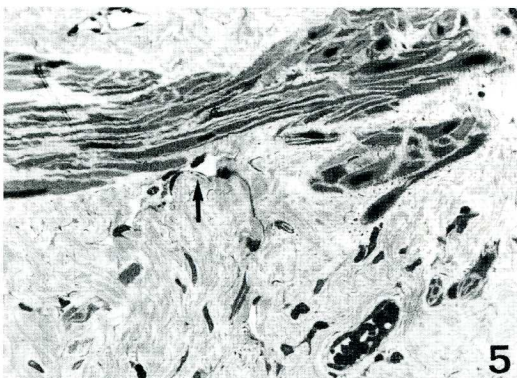


Fig. 5. A thin and irregular presumptive lymphatic vessel (arrow) near the discontinuous muscularis mucosae (Tumoral bladder). 120x

capillaries (Fig. 4). In the mucosal region containing a thin and discontinuous layer of smooth muscle cells corresponding to the muscularis mucosae, few initial lymphatics with a thin and indented endothelial wall were present. They had features similar to those observed in the same region in the control samples (Fig. 5). In the deeper regions of the mucosa, lymphatic vessels with a wide and empty lumen displayed a smooth endothelial wall with a thin and regular profile and were closely intermingled with numerous arteriolar and venous vessels with dilated and congested lumens (Fig. 6). These larger lymphatic vessels sometimes had

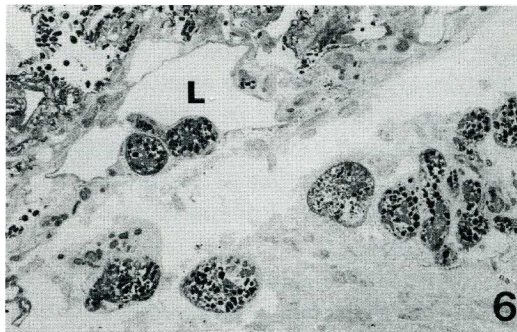


Fig. 6. A large and dilated but empty presumptive lymphatic vessel (L) near congested blood vessels in the deeper region of the mucosa (Tumoral bladder). 80x

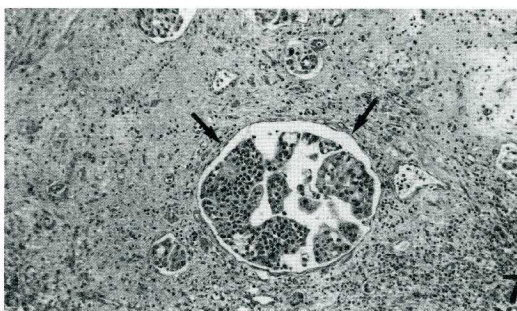


Fig. 7. A presumptive lymphatic vessel (arrows) dilated with tumoral emboli (Tumoral bladder). 70x

valves but they were initial lymphatics because they lacked a muscular layer. In some patients (12%), larger lymphatic vessels contained tumor emboli (Fig. 7).

At the ultrastructural level, blood vessels were characterized by reduplications and increased thickness of the basement membrane (Fig. 8). Electron microscopy confirmed absence of initial lymphatics in the exophytic polypoid lesions and in the subepithelial areas of the mucosa.

The scanty and thin lymphatics near the discontinuous muscular layations of the plasma membrane and luminal protrusions of the nuclei of endothelial cells (Fig. 9). A discontinuous and thin basement membrane, scanty anchoring filaments and packed collagen and elastic fiber bundles surrounded

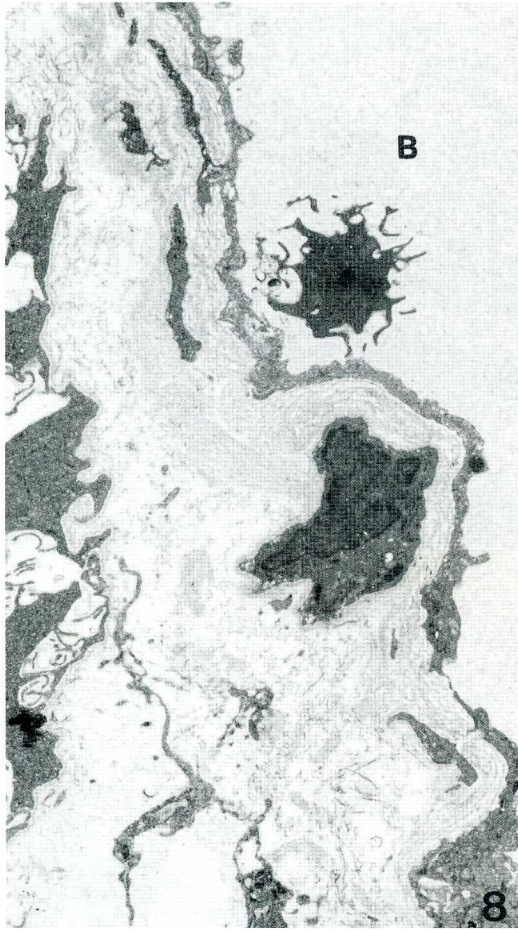


Fig. 8. In the tumoral papillary protrusions a blood capillary (B) with an evident reduplication of the basement membrane. In the vessel lumen a lymphocyte is present. 5000x

the endothelial wall. Endothelial cells with clear cytoplasm with scanty organelles were frequently interposed with endothelia containing denser cytoplasm. Micropinocytotic vesicles were generally numerous in the luminal and abluminal side of the endothelial wall. Simple or complex intercellular junctions (Fig. 10) and sometimes intercellular channels connected contiguous endothelial cells (Fig. 11). Typical open junctions were not seen.

Lymphatic vessels with a dilated lumen observed in the deeper regions of the mucosa

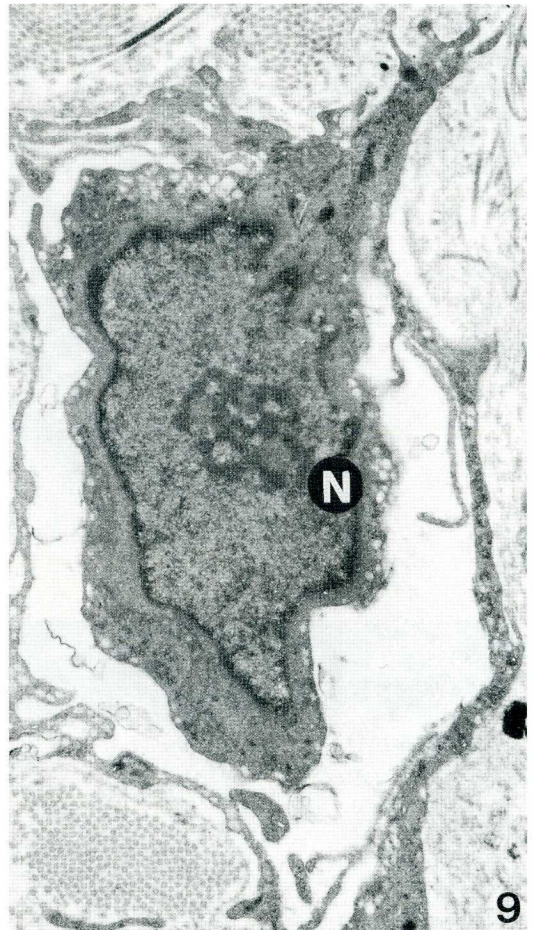


Fig. 9. An evident luminal protrusion of the nuclear region (N) of the endothelial wall of a lymphatic vessel. A discontinuous basement membrane and bundles of collagen fibers surround the vessel (Tumoral bladder). 13000x

displayed ultrastructurally (Fig. 12) a thin and non-indented endothelial wall, simple intercellular connections and sometimes open junctions. Rare anchoring filaments and a loose connective tissue with a scattered network of collagen and elastic fibers surrounded these lymphatic vessels.

DISCUSSION

Papillary urothelial tumors of the urinary bladder comprise ~90% of all primary bladder neoplasms (1). Classification of papillary

Fig. 10. Multiple protrusions (arrows) and one overlapping (arrowhead) between contiguous endothelial cells. (Tumoral bladder). 13000x

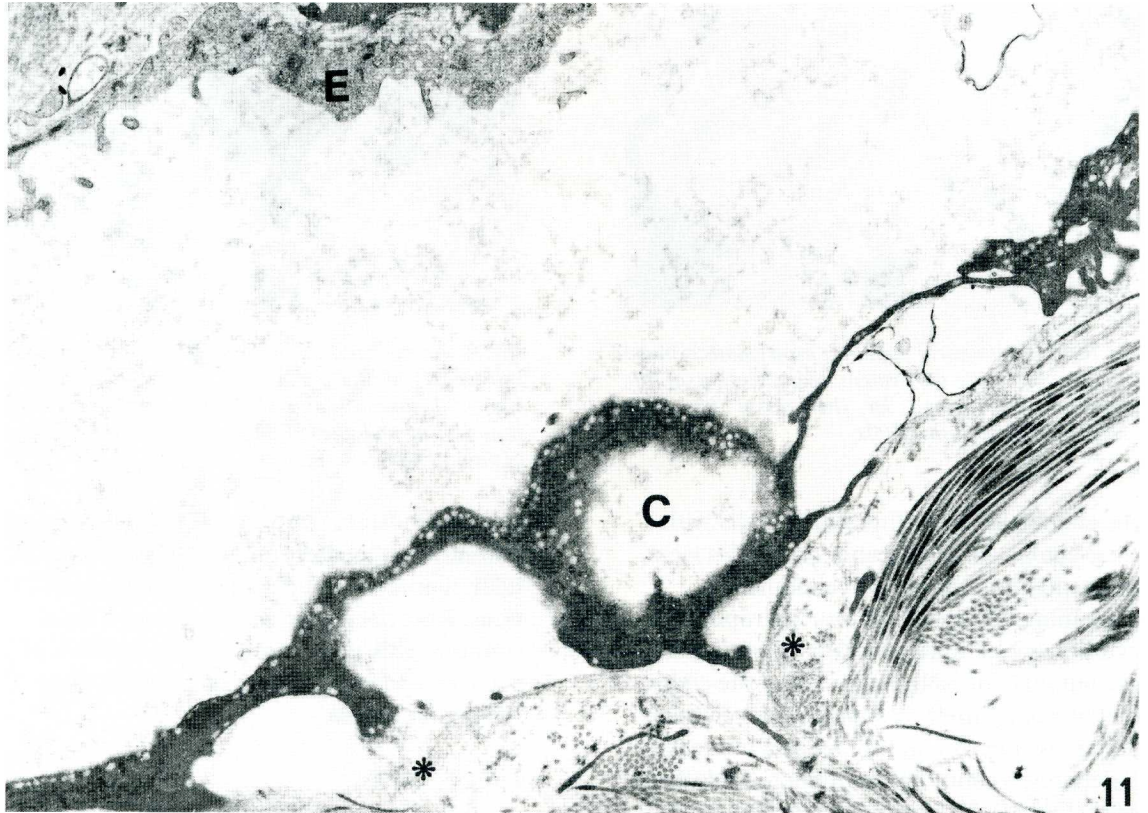
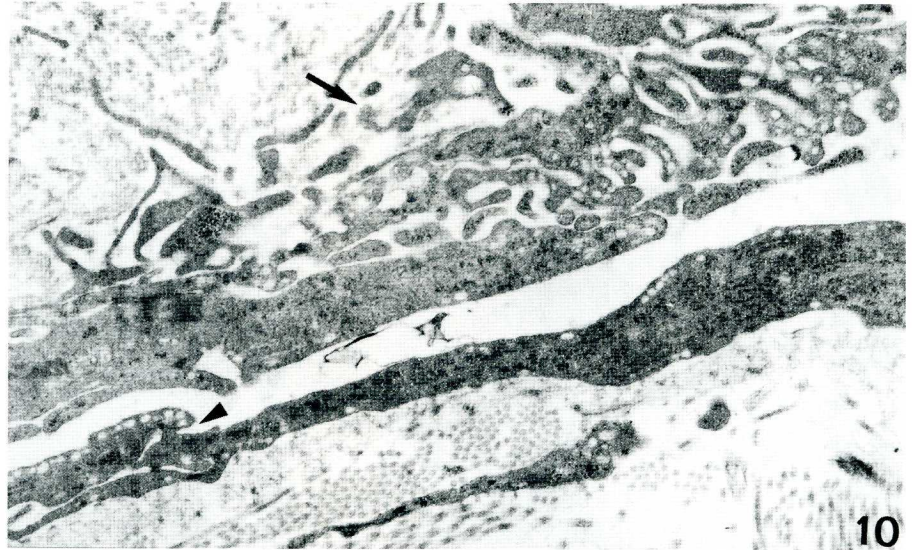


Fig. 11. One intercellular channel (C) in the thin and indented endothelial wall of a lymphatic vessel surrounded by a scanty basement membrane and rare anchoring filaments (). At the opposite side, an endothelial cell with a clear and organelle-poor cytoplasm (E) is seen in the vessel wall (Tumoral bladder). 10000x*

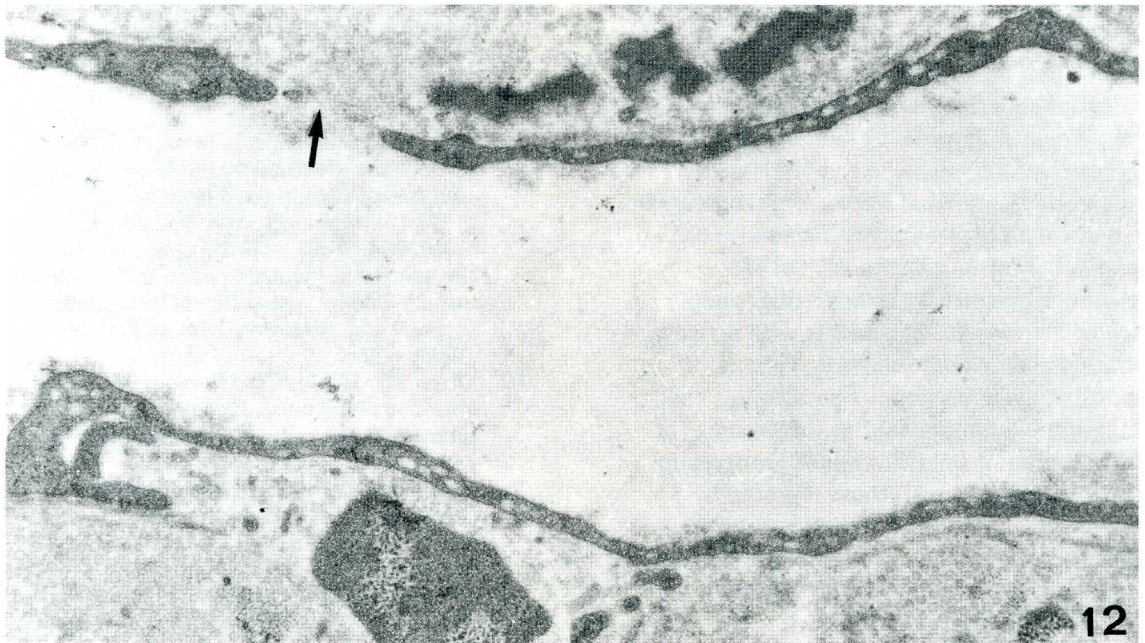


Fig. 12. Distended wall and one open junction (arrow) characterized this lymphatic capillary in the deeper region of the mucosa (Tumoral bladder). 16000x

tumors has been based on histological grading, that is expression of the degree of histologic and cytologic changes according to a coding system of I to IV (9). In spite of this classification, the natural history of an individual papillary tumor is unpredictable; in fact, some patients show rapid progression of the disease whereas in others recurrent disease is not observed within their life span (1). Peripheral epithelial changes may be an important factor of this behavior but localization and structure of terminal lymphatics in the mucosa of the urinary bladder may also be important. In fact, lymphatic permeation by infiltrating urothelial carcinomas suggests a poor prognosis in relation to the likelihood of metastatic involvement of regional lymph nodes, an important parameter in the staging and prognosis of these neoplasms (2,10).

Under normal conditions, the human urinary bladder exhibits lymphatic vessels in the inner mucosal areas and in the muscular and subserosal sheaths. They increase in

number and diameter from the mucosal to the subserosal layer (6,11,12). Whereas numerous blood capillaries are observed at the interface between epithelium and lamina propria and in the subepithelial areas of the mucosa, lymphatics are not present at these levels in the human or in the rat urinary bladder (6,13).

In biopsies from neoplasms, initial lymphatics were again absent on histopathology from the subepithelial areas of the mucosa and in the stroma of the papillary protrusions. This feature may explain the rarity of metastasis via lymphatics in initially invasive urothelial tumors, in which only the subepithelial areas of the mucosa are involved. In contrast, permeation of initial lymphatics is demonstrable in the invasive urothelial carcinomas with involvement of the deeper mucosal layers where the number of initial lymphatics is normally greater.

In our patients, the number and diameter of initial lymphatics in the mucosa showing invasion by urothelial carcinomas seemed higher than in the uninvolved (normal)

vesical mucosa. This finding may derive from the constant presence of an inflammatory infiltration by lymphocytes, plasma cells and macrophages around and into neoplastic invasive proliferation. In fact, in these conditions, the inflammatory infiltrate is often associated with edema, with consequent enlargement and apparent increase in the number of initial lymphatics. On 120 specimens studied by transurethral endoscopic biopsy, 12% of patients with invasive urothelial carcinoma showed evidence of morphological patterns of permeation and invasion of lymphatics with the presence of intraluminal neoplastic emboli. Our results are in agreement with those reported by others which described metastases via invasion of lymphatics in the regional lymph nodes of 14% of patients with invasive bladder neoplasms (3).

REFERENCES

1. Murphy, WM, JB Beckwith, GM Farrow: Tumors of the kidney, bladder and related urinary structures. In: *Atlas of Tumor Pathology*, Third Series Fascicle 11. Armed Forces Institute of Pathology (ed.), Washington, 1994, 196-198.
2. Wood Jr, DP, JE Montie, JE Pontes, et al: Identification of transitional cell carcinoma of the prostate in bladder cancer patients: A prospective study. *J. Urol.* 141 (1989), 83-85.
3. Wishnow, KI, DE Jhonson, JY Ro, et al: Incidence, extent and localization of unsuspected pelvic lymph node metastasis in patients undergoing radical cystectomy for bladder cancer. *J. Urol.* 137 (1987), 408-410.
4. Nicolson, GL: Generation of phenotypic diversity and progression in metastatic tumor cells. *Canc. Metast. Rev.* 3 (1984), 25-42.
5. Bevacqua, SJ, DR Welch, SM Diez de Pinos, et al: Quantitation of human melanoma, carcinoma and sarcoma tumor cell adhesion to lymphatic endothelium. *Lymphology* 23 (1990), 4-14.
6. 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.
7. Scelsi, R, L Scelsi, A Gritti, et al: Structure of the lymphatic microcirculation in the human urinary bladder with different intraluminal pressure and distension. *Lymphology* 29 (1996), 60-66.
8. Dixon, JS, JA Gosling: Histology and fine structure of the muscularis mucosae of the human urinary bladder. *J. Anat.* 136 (1983), 265-271.
9. Bergkvist, A, A Ljungqvist, G Moberger: Classification of bladder tumours based on the cellular pattern. *Acta Chir. Scand.* 130 (1965), 371-378.
10. Friedell, GH, RL McAuley: Untreated bladder cancer: 31 autopsy cases. *J. Urol.* 100 (1968), 293-296.
11. Powell, TO: Studies on the lymphatics of the female urinary bladder. *Surg. Gynec. Obstet.* 78 (1944), 605-609.
12. Azzali, G, G Romita, R Gatti: Ultrastruttura dei vasi linfatici nella vescica urinaria. *Acta Biomed. Ateneo Parmense* 54 (1983), 105-115.
13. Inoue, T, G Gabella: The interface between epithelium and lamina propria in the rat urinary bladder. *Arch. Histol. Cytol.* 55 (1992), 157-163.

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