Chapter 1

Normal Anatomy and Histology

Bladder Pathology, Second Edition. Liang Cheng, Antonio Lopez-Beltran and Rodolfo Montironi.

Embryology

Early in fetal life, when cloacal dilation first appears and the hindgut ends in a blind sac, an ectodermal depression develops under the root of the tail.[1] This depression, known as the proctoderm, deepens until only a thin layer of tissue, the cloacal membrane, remains between the gut and the outside of the body. The division of the cloaca results from development of the urorectal fold that closes caudally toward the cloacal membrane. As the urorectal fold cuts progressively deeper into the cloaca, a wedge-shaped mass of mesenchyme accompanies it and forms a dense septum between the urogenital sinus anteriorly and the rectum posteriorly. This separation of the cloaca is completed before the cloacal membrane ruptures so that its two parts open independently. When it first opens to the outside, the urogenital sinus, that is the ventral division of the cloaca, is tubular and continuous with the allantois. At this stage, it can be divided into a ventral or pelvic portion, which will become the bladder proper, and a urethral portion, which receives the mesonephric and fused müllerian ducts and later becomes the prostatic and membranous urethra in the male and the entire urethra in the female.[2]

After eight weeks, the ventral part of the urogenital sinus expands to form an epithelial sac, the apex of which tapers into an elongate narrowed urachus. The splanchnic mesoderm surrounding both segments differentiates as interlacing bands of smooth muscle fibers and an outer fibroconnective tissue coat. By 12 weeks, the layers of the adult urethra and bladder can be recognized. This sequence of events indicates that the detrusor muscle and the urethral musculature have the same origin, constituting one uninterrupted structure.[2] This arrangement is easily observed in the female, in that the bladder and urethra form one tubular unit with expansion of the upper part. However, in the male, the structure is complicated by simultaneous development of the prostate gland. The developmental sequence is the same in both sexes, and the structural arrangement in the male is only slightly more complex than that in the female.[2]

Anatomy

Gross Anatomy

The bladder is a hollow muscular organ whose main function is that of a reservoir. When empty, the adult bladder lies behind the symphysis pubis, and is largely a pelvic organ. In infants and children, it is more cephalad than in adults. When full, the bladder rises above the symphysis and can readily be palpated or percussed. When overdistended, as in acute and chronic urinary retention, it may cause the lower abdomen to bulge visibly and is easily palpable in the suprapubic region. The empty bladder has an apex (superior surface), two infralateral or anterolateral surfaces, a base (posterior surface), and a neck. The apex extends a short distance above the pubic bone and ends as a fibrous cord derivative of the urachus. This fibrous cord extends from the apex (or dome) of the bladder to the umbilicus between the peritoneum and the transversalis fascia. It raises a ridge of peritoneum called the median umbilical ligament. There is a peritoneal covering at the apex in both sexes that also covers a small part of the base in men.[2,3]

The apex of the bladder is apposed to the uterus and ileum in the female, and the ileum and pelvic portion of the colon in the male. The base of the bladder faces posteriorly and is separated from the rectum by the uterus and vagina in the female, and by the vasa deferentia, seminal vesicles, and ureters in the male. The anterolateral surface on each side of the bladder is apposed to the pubic bone, levator ani, and obturator internus muscles but the central anterior bladder is separated from the pubic bone by the retropubic space that contains abundant fat and venous plexuses. The neck of the bladder, its most inferior part, connects with the urethra. When the bladder is distended with urine, the neck remains fixed and stationary, whereas the dome rises above the pelvic cavity into the lower abdomen, touching the posterior aspect of the lower anterior abdominal wall and the small and large bowels.[3]

Beneath the urothelial lining of the inner bladder, there is loose connective tissue that permits considerable stretching of the mucosa. As a result, the urothelial mucosal lining is wrinkled when the bladder is empty but smooth and flat when distended. This arrangement exists throughout the bladder except at the trigone, where the mucous membrane adheres firmly to the underlying muscle; consequently, the trigone is always smooth, regardless of the level of distension (**Figs. 1-1** and **1-2**). At the lateral points of the trigone, the ureters empty into the bladder cavity through the ureteral orifices. The muscle of the trigone is derived from the detrusor muscle of the bladder and the muscle of the ureters. Within the wall of the bladder, the ureters are surrounded by sheaths of muscle and fibrous tissue known as Waldeyer sheath. The ureters pass obliquely through the wall of the bladder in such a way that when the bladder fills, the pressure compresses and closes the ureters, preventing reflux.[4–9]

Blood Supply and Lymphatic Drainage

The bladder is supplied by the superior, middle, and inferior vesical arteries; all of which are branches of the anterior division of the hypogastric artery. Between the bladder wall proper and the outer adventitial layer, there is a rich plexus of veins that ultimately terminate in the hypogastric veins after converging in several main trunks. The bladder lymphatics drain into the external iliac, hypogastric, and

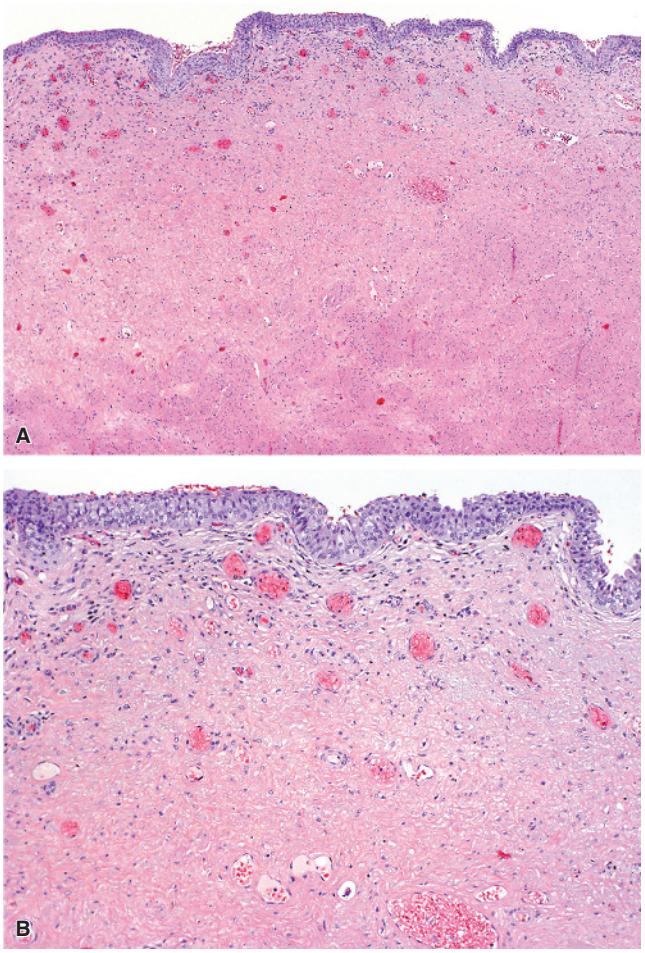


Figure 1-1 Normal trigone (A and B).

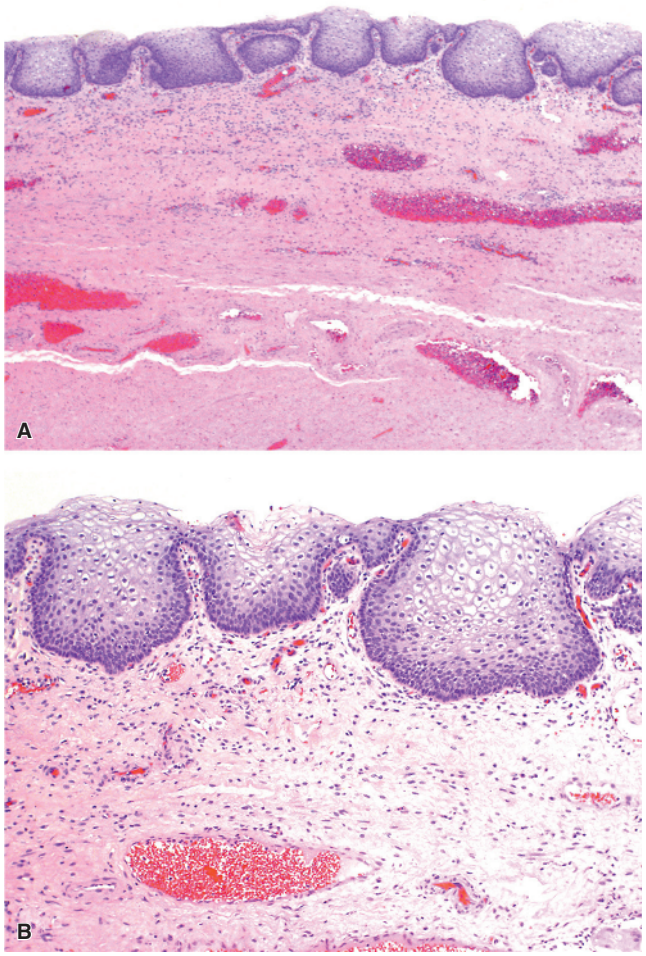


Figure 1-2 Normal trigone in a woman during the reproductive years. Note the squamous mucosa and closely packed underlying muscle (A and B).

common iliac lymph nodes. There are rich lymphatic anastomoses between the pelvic and genital organs.[10–12]

Nerve Supply

The bladder is richly innervated by divisions of the autonomic nervous system.[2,13] Sympathetic nerves originate from the lower thoracic and upper lumbar segments, mainly T11 to T12 and L1 to L2. These sympathetic fibers descend into the sympathetic trunk and the lumbar splanchnic nerves, connecting with the superior hypogastric plexus, an inferior extension of the aortic plexus. The latter separates into the right and left hypogastric nerves, and these extend inferiorly to join the pelvic plexus of the pelvic parasympathetic nerves. Parasympathetic nerves arise from sacral segments S2 to S4, and these form the rich pelvic parasympathetic plexus. This plexus joins the sympathetic hypogastric plexus, and vesical branches emerge from this plexus toward the bladder base, innervating the bladder and urethra.[13,14]

Normal Histology

Urothelium

The urothelium is a unique stratified epithelium of variable thickness (**Figs. 1-3** to **1-6**). The number of cell layers depends on the degree of distension of the bladder, usually varying from three to seven layers. When distended, the bladder is three to six cell layers thick, although the typical biopsy contains about five layers; in the contracted state, it consists of six to eight layers.[15] For practical purposes, urothelium composed of more than seven cell layers is considered abnormal unless this finding can be attributed to tangential cutting of tissue.[16,17] In addition, the urothelium is thought to be monoclonal in origin, with some features of mosaicism.[18]

The normal urothelium contains a layer of large superficial cells that are frequently binucleated or multinucleated,

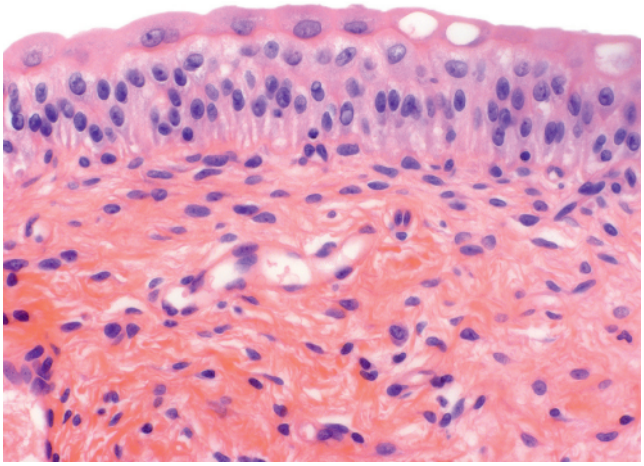
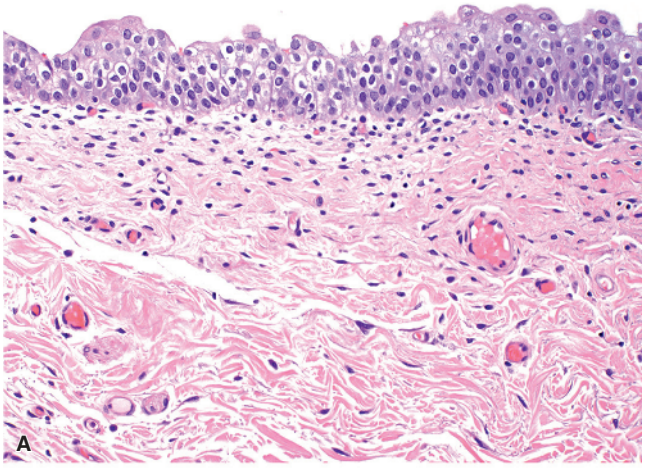


Figure 1-3 Normal urothelium. The thickness of urothelium is variable, up to seven cell layers in normal urothelium. Note the prominent superficial umbrella cells.



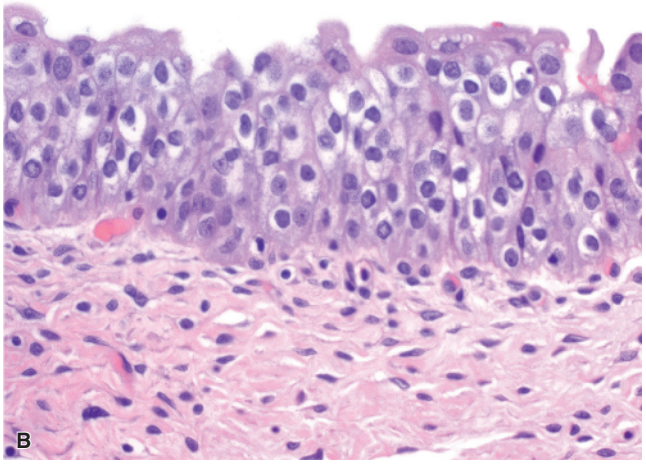


Figure 1-4 Normal urothelium (A and B). Cytoplasmic vacuolization is observed. In this preparation, the urothelium is up to seven cells in thickness (B).

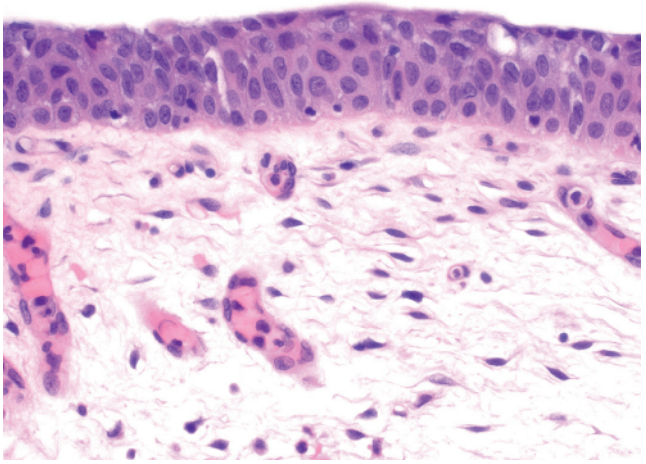


Figure 1-5 Normal urothelium. Note the orderly arrangement of the urothelial cells. The long axis of urothelial cells is often perpendicular to the mucosal surface. The superficial cells are less distinct. Prominent nuclear grooves are noted in some cells.

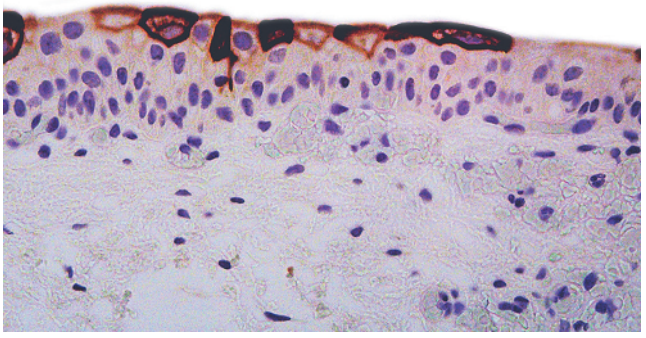


Figure 1-6 Cytokeratin 20 immunostains highlight superficial umbrella cell.

often referred to as umbrella cells (**Figs. 1-7** to **1-9**). These cells have abundant eosinophilic cytoplasm, with large nuclei whose long axes are perpendicular to those of the smaller cells of the underlying basal and intermediate cell layers. The superficial cells vary in size and configuration according to the degree of bladder distension and angle of tissue section; they may appear cuboidal in the distended bladder but are often flattened. In addition, superficial cells are loosely attached to the underlying cells despite being interconnected with each other by extensive junctional complexes, and may be absent from otherwise normal urothelium in routine biopsies.[19] Superficial umbrella cells express uroplakins, cytokeratin 20, and GATA3 (**Table 1-1**).

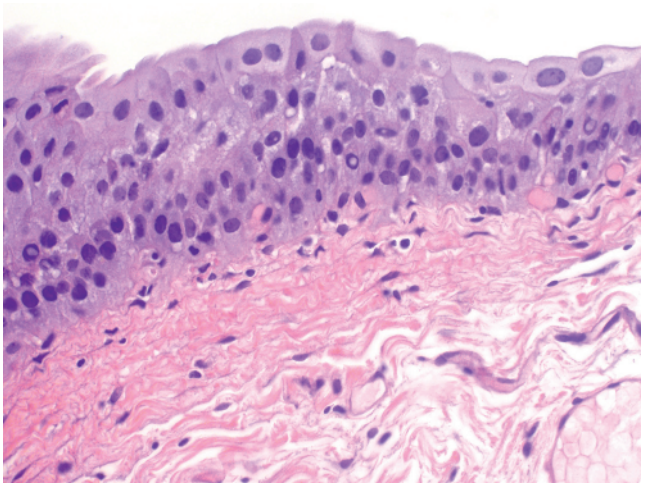


Figure 1-7 Normal urothelium. Note the prominent superficial cells. Nuclear vacuolization is occasionally seen in intermediate cells. Some variation of cell size and shape can be observed in normal urothelium and should not be interpreted as dysplasia.

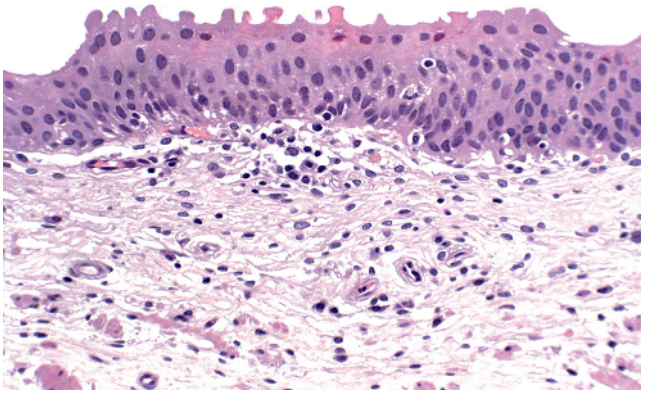


Figure 1-8 Normal urothelium. Note the prominent superficial cells.

The apical plasmalemma is thickened, with stiff plaques, unlike the short microvilli seen in the underlying intermediate cells.[19] However, the trigonal superficial cells of women during the reproductive years have a cobblestone pattern with long clubbed microvilli.[20] Superficial cells may persist on the surface of papillary urothelial carcinoma, particularly low-grade carcinoma; a finding of potential importance in pathologic grading of bladder cancer (also see Chapter 9).

Basal and intermediate cells are located between the basal lamina and the superficial cells (**Figs. 1-8** to **1-10**). These cells are morphologically identical to each other, and are distinguished only by their position in the mucosa.[19] They are regularly arranged, with distinct cell boundaries and oval, round, or fusiform nuclei with occasional prominent nuclear grooves. The nuclei are located centrally in the

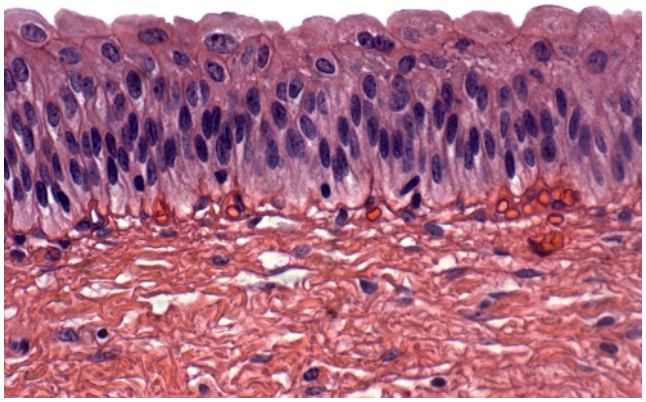


Figure 1-9 Normal urothelium. Basal and intermediate cells are located between the basal lamina and the superficial cells. Occasional prominent nuclear grooves may be seen. Note the binucleated superficial umbrella cells.

cells and contain finely granular chromatin that often accentuates the nuclear borders. Nucleoli are usually small and difficult to detect. Mitotic figures are rare in the normal urothelium. The basal layer of epithelial cells expresses Bcl-2 while the intermediate cells express RB1 and PTEN at varying intensities. HER2 and p53 are not expressed by normal urothelial cells. Ki67, indicating proliferation, may not be expressed in a single field. The long axis of the basal and intermediate cells is perpendicular to the basement membrane. The basement membrane is usually not visible in routine hematoxylin and eosin or periodic acid-Schiff-stained sections but appears as a razor-thin layer beneath the mucosa when present. Basement membrane markers, such as laminin and type IV collagen, may be useful diagnostically in select cases to define the basement membrane, but are not routinely employed.[21]

Delicate capillaries of the lamina propria (or subepithelial connective tissue) are in intimate association with the basement membrane, and invaginations or tangential cutting may create the factitious appearance of intraepithelial extension.[4] Frequently, the urothelium invaginates to the lamina propria forming von Brunn nests which may be solid or show a small central lumen. Occasionally, von Brunn nests may become numerous or hyperplastic, a finding that should not be misdiagnosed as cancer in frozen sections.[4,22–24]

The urothelium is able to respond to thermal, mechanical, and chemical stimuli ("sensor functions"), and has the ability to release chemicals ("transducer functions").[25] Urothelial basal cells express certain receptors and ion channels (e.g., vanilloid receptor-1), similar to afferent nerves.[25] The presence of afferent nerves adjacent to the urothelium suggests that these cells may be targets for transmitter release from bladder nerves or that chemicals released by urothelial cells may alter afferent excitability.

Table 1-1 Common Immunohistochemical Biomarkers to Identify the Different Compartments in the Urothelium

Antibody	Staining Pattern; Expression in Benign Urothelium	Expression in Other Tissues
CK7	Full thickness (C, M); 90% to 100%	Simple epithelia, gallbladder, esophagogastric junction, lung, salivary glands, thyroid, placenta, prostate, endometrium
CK20	Umbrella cells (C, M); 67% of cells	Gastrointestinal mucosa, particularly the colon and appendix, small intestine, gastric mucosa, Merkel cells
CD44	Full thickness (M); 90% to 100% of cells	Stem cell marker in the breast, colorectal, stomach, lung, ovary, liver, and prostate
High molecular weight cytokeratin (clone 34βE12)	Basal cells (C); 90% to 100% of cells	Basal cells of the prostatic glands, skin
P63	Basal cells (N); 90% to 100% of cells	Squamous epithelium of various organs, basal cells of the prostate, bronchiolar epithelium, adnexal structures, epithelial cells of the Bowman capsule, cytotrophoblast, basal epithelium of the breast
Uroplakins II and III	Apical surface of umbrella cells; 90% to 100% of cells	Urothelium
Thrombomodulin	Variable and patchy (C, M); 40% of cells	Stratified squamous mucosa of different organs, ureter, endothelium, mesothelium
GATA3	Full thickness (N); >95% cells	Breast gland epithelium, T-lymphocytes, thymocytes, adipose tissue, kidney, seminal vesicles, epididymis, sympathetic nervous system, ductal epithelium of salivary glands adnexal structures of the skin

C: Cytoplasmic staining; M: Membranous staining; CK: Cytokeratin; N: Nuclear.

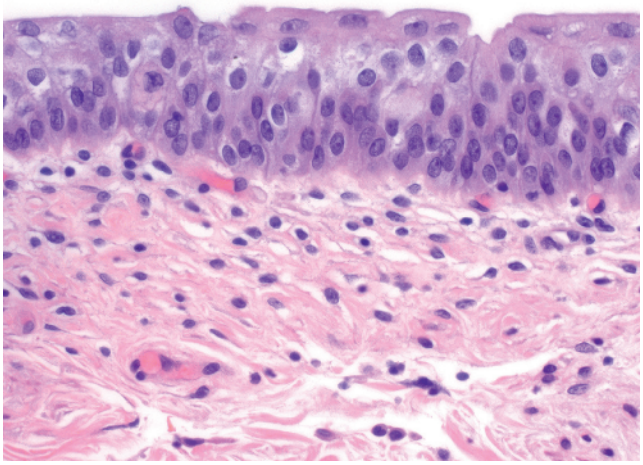


Figure 1-10 Normal urothelium. Basal and intermediate cells are more densely packed with higher nuclear: cytoplasmic ratio than superficial cells.

A recent advance related to the neoplastic process in the urothelium includes a hypothesis in which cells in normal appearing urothelium that line bladder and the urinary passages accumulate mutations caused by endogenous mutagenic processes (mostly APOBEC mediated) or by exogenous mutagens (mostly chemical compounds and tobacco derivatives).[24] Some of these mutations, observed in KMT2D and KDM6A chromatin modifying genes, confer competitive advantage and allow extending and colonizing of large regions of the urothelium.[24] Additional mutations, perhaps in commonly altered genes in bladder cancer, such as TP53, PIK3CA, FGFR3, or RB1, and other changes are needed to trigger malignant transformation as we see it in bladder cancer.[24]

Nonkeratinizing, glycogenated squamous epithelium seen in the adult female bladder trigone is currently regarded as a normal variation of the urothelium. This metaplastic epithelium expresses markers of squamous differentiation including desmoglein 3, MAC387, HMWCK, and CK14.

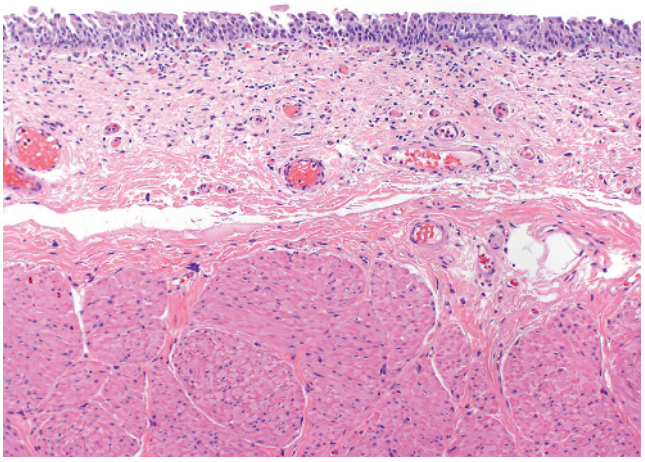


Figure 1-11 Normal lamina propria.

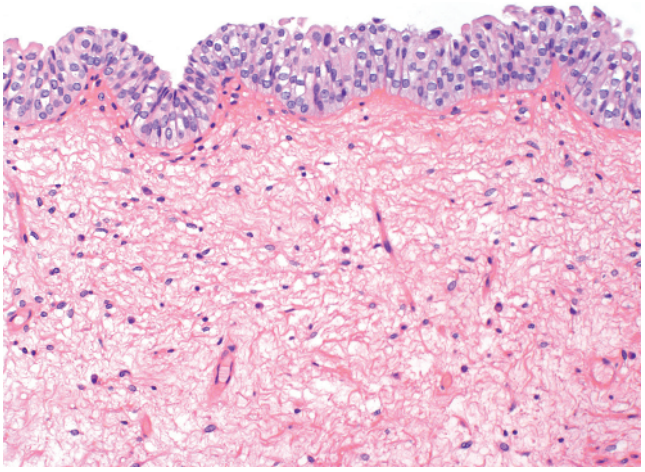


Figure 1-12 Normal lamina propria.

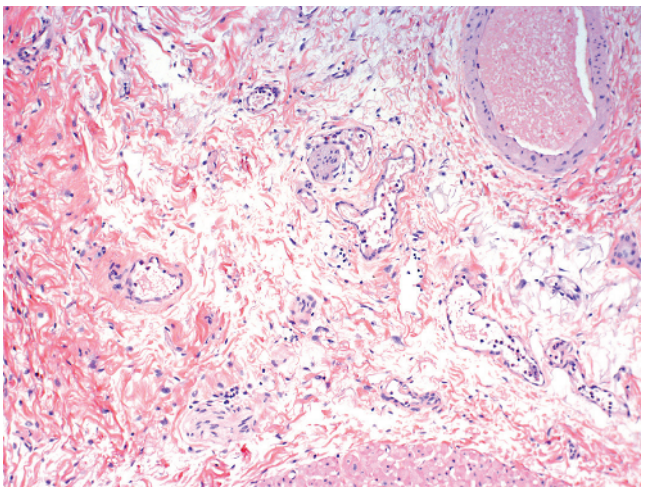


Figure 1-13 Normal lamina propria.

Bladder Wall

The lamina propria, located beneath the basement membrane, consists of a compact layer of fibrovascular connective tissue (**Figs. 1-11** to **1-13**). It may contain an incomplete muscularis mucosae composed of thin delicate smooth muscle fibers that may be mistaken for muscularis propria in biopsy specimens (**Figs. 1-14** to **1-16**; **Table 1-2**).[26–32] The muscularis mucosae is an important diagnostic pitfall in evaluating bladder carcinoma because the management of cancer invading the muscularis propria is different from that of tumors limited to the lamina propria and surrounding the muscularis mucosae. Therefore, it is important for pathologists to be aware of the existence of the delicate muscle bundles of the muscularis mucosae within the lamina propria.[30,31,33] In biopsy specimens, these smooth muscle fibers may appear as a continuous layer, a discontinuous or interrupted layer, or as scattered thin bundles of smooth muscle fibers that do not form an obvious layer (**Fig. 1-17**).[31,34] These thin muscle

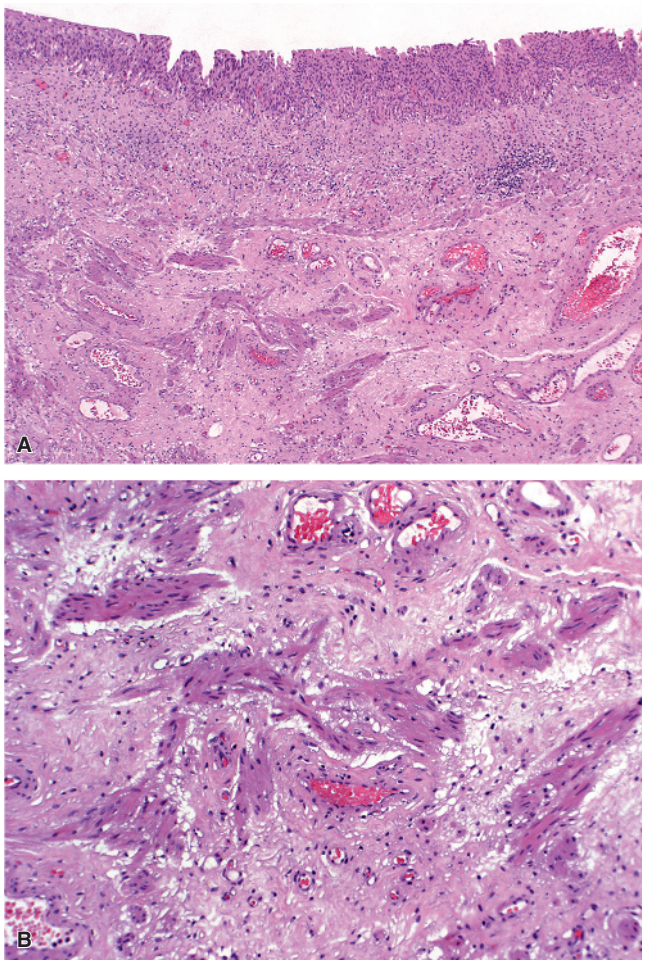


Figure 1-14 Muscularis mucosae in the lamina propria (A and B). The muscularis mucosae consists of scant delicate muscle bands interspersed with blood vessels and connective tissue stroma.

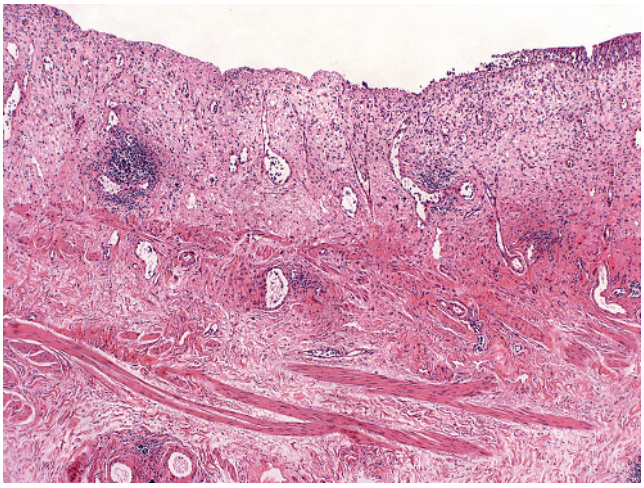


Figure 1-15 Muscularis mucosae.

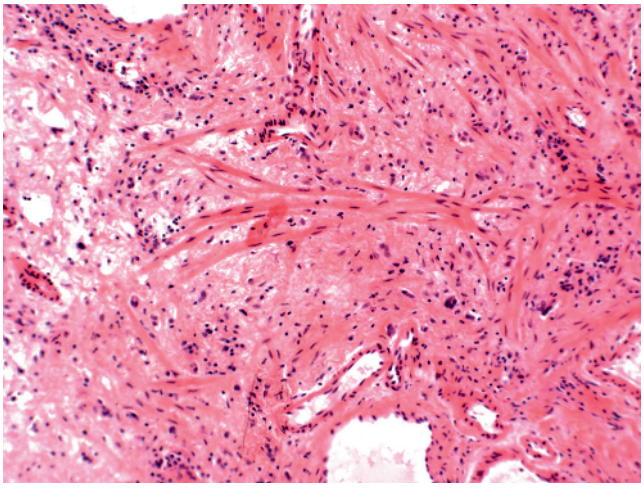


Figure 1-16 Muscularis mucosae.

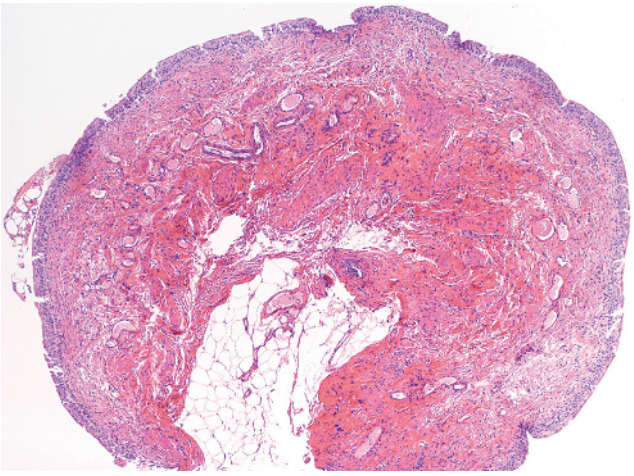


Figure 1-17 Muscularis mucosae in the biopsy specimen.

fibers lie parallel to the mucosal surface, midway between the urothelium and the underlying muscularis propria.

Moderate-sized or large thick-walled blood vessels are a constant feature of the lamina propria, running parallel to the surface urothelium in close association with the smooth muscle fibers of the muscularis mucosae (**Fig. 1-18**). However, these vessels are variable in distribution and may be close to the superficial lamina propria (**Figs. 1-19** and **1-20**). Therefore, large vessels cannot be used as a substitute for muscularis mucosae as some studies have done. It may be difficult to distinguish muscularis mucosae from muscularis propria (detrusor muscle), and trichome staining may be useful in resolving difficult cases.[35] Smoothelin can also differentiate muscularis mucosae (negative or weak) from muscularis propria (positive and intense) (**Fig. 1-21**; **Table 1-2**).[36–38] To avoid overstaging bladder cancer, it is also important for the pathologist to be aware of the existence

Table 1-2 Microscopic Characteristics of the Smooth Muscle Present in the Wall of the Urinary Bladder

Muscularis Mucosae (MM)	Muscularis Propria (MP) (detrusor muscle)
A continuous MM is only rarely seen. Thin, disorganized, and discontinuous wispy fascicles of smooth muscle of variable caliber separated by fibrovascular stroma are the usual appearance.	Compact and dense smooth muscle bundles forming large solid aggregates separated by variable interspersed fibroblasts and collagen fibers and frequent adipose tissue.
In the trigone or bladder dome, MM is frequently seen as small individual round to oval dense bundles of smooth muscle separated by fibrovascular stroma.	In some cases, there may be dispersion of superficial MP bundles into the subepithelial connective tissue, a situation in which it is difficult to establish it from hypertrophic MM.
In cases of bladder outlet obstruction (i.e., benign prostatic hyperplasia), there may be marked increased numbers of MM fascicles, but they remain disorganized and arrayed in multiple directions. This is frequently described as compensatory hyperplasia of the MM.	Some authors suggest using the solid compact layer of smooth muscle as the line of demarcation between MP and MM. It is acceptable to report the specimen as indeterminate for type of muscle and ask for a repeat transurethral resection in difficult cases.

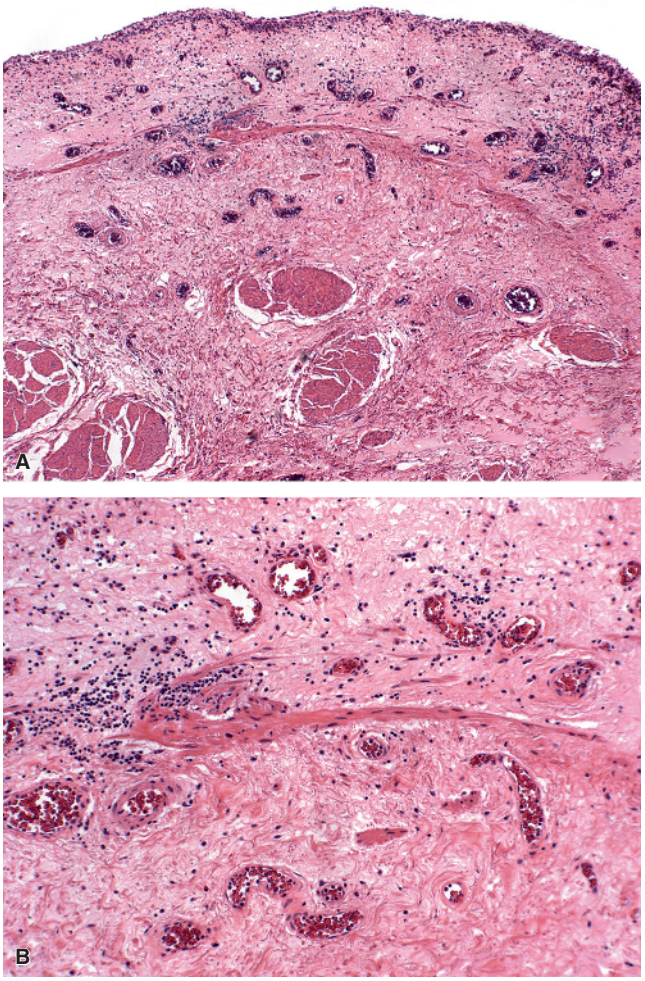


Figure 1-18 Muscularis mucosae in close proximity of large vessels (A and B).

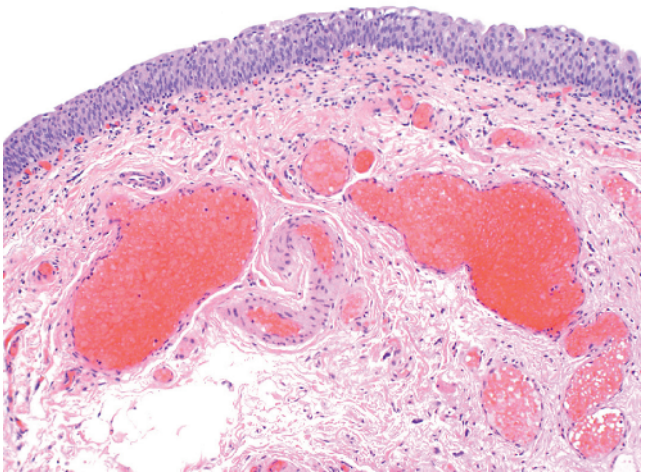


Figure 1-19 Large vessels may be seen in superficial lamina propria and may or may not be associated with muscularis mucosae. Therefore, large vessels cannot be used as substitute for muscularis mucosae.

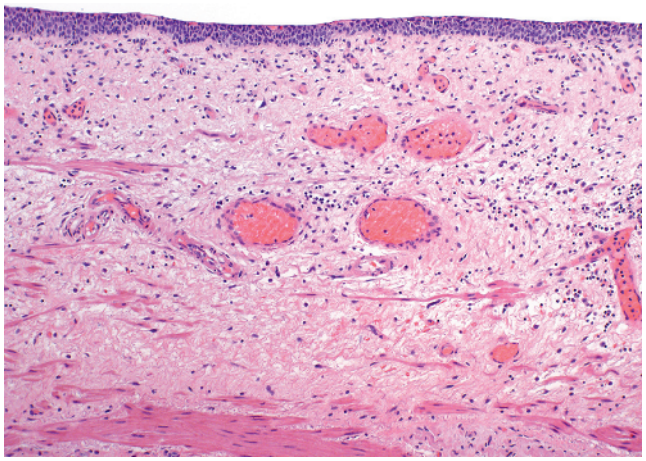


Figure 1-20 Variable distribution of large vessels and variable sized muscularis mucosae bundles.

of fat within the lamina propria and the muscularis propria (**Figs. 1-22** and **1-23**).[39]

Occasional bizarre stroma cells may be seen in the lamina propria and can be mistaken for invasive cancer cells. In difficult cases, immunostains for cytokeratin reveal that these bizarre stroma cells are negative, unlike cancer cells (**Figs. 1-24** and **1-25**). An important pitfall to be avoided is related to the positivity of myofibroblasts with pan-cytokeratin and smooth muscle actin. Awareness of this immunoprofile will prevent misclassification of few cytokeratin-positive or smooth muscle actin-positive cells as malignant cells infiltrating lamina propria.[4,23]

The muscle proper (or detrusor muscle) of the bladder, the muscularis propria, is moderately thick and consists of an inner longitudinal layer, middle circular layer, and outer longitudinal layer (**Fig. 1-26**). It spirals around each ureteral orifice and increases in thickness around the internal urethral orifice, forming the internal sphincter of the bladder. The muscularis is surrounded by a coat of fibroelastic connective tissue, adventitia, and perivesical fat (**Table 1-2**).

Paraganglionic Tissue

Paraganglia are rarely found in routine sections of the urinary bladder.[40] Their presence in a bladder biopsy may be confused with neoplasm. Distinguishing features that are useful include the distinctive arrangement of cell nests, sinusoidal vascular pattern, monotonous benign cytology of the cells, and absence of a stromal reaction.[41] Paraganglionic tissue typically demonstrates immunoreactivity with neuroendocrine markers, such as chromogranin, synaptophysin, and neuron specific enolase. The reported expression of GATA3 in paraganglionic tissue and paraganglioma should not be mistaken as malignancy in small biopsies of the bladder. The sustentacular cells exhibit immunostaining for S100 protein.

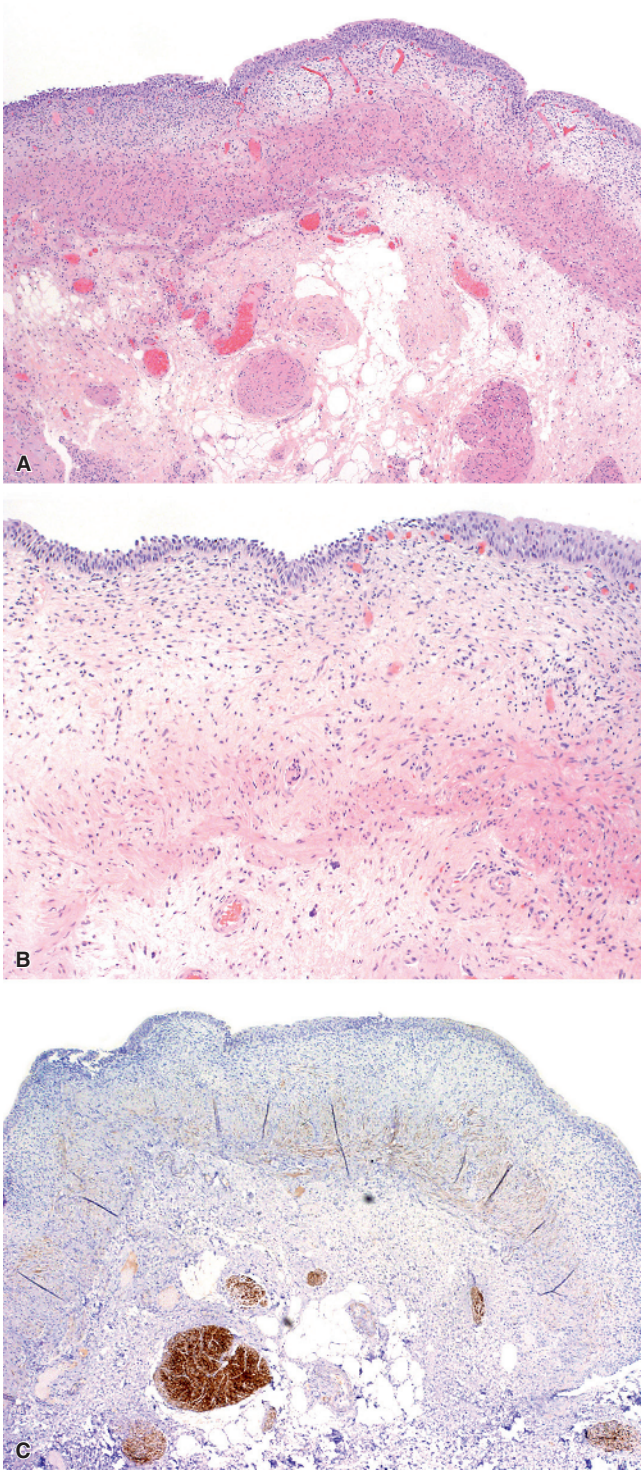


Figure 1-21 Muscularis mucosae is negative or shows weaker staining as compared to muscularis propria (A-C). Smoothelin usually stains strongly in muscularis propria (detrusor muscle) (C).

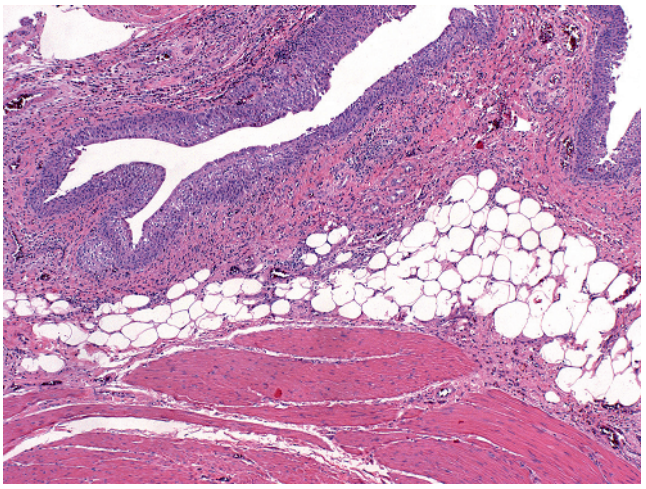


Figure 1-22 Adipose tissue can be seen in the lamina propria.

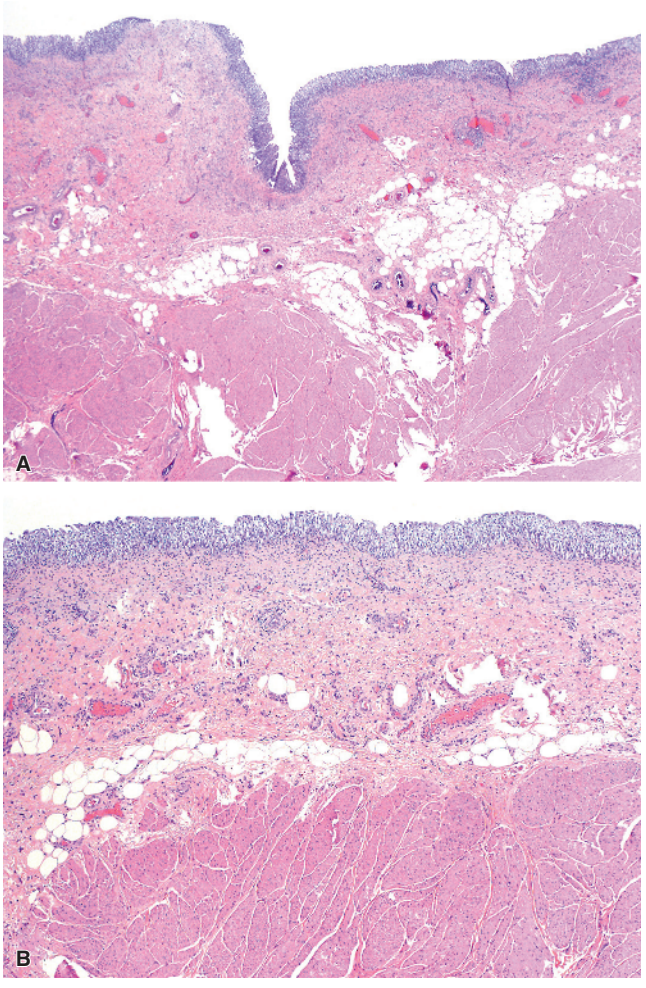


Figure 1-23 Adipose tissue is present in both the lamina propria and muscularis propria (A and B). The presence of fat invasion in transurethral resection specimens does not indicate extravesical extension.

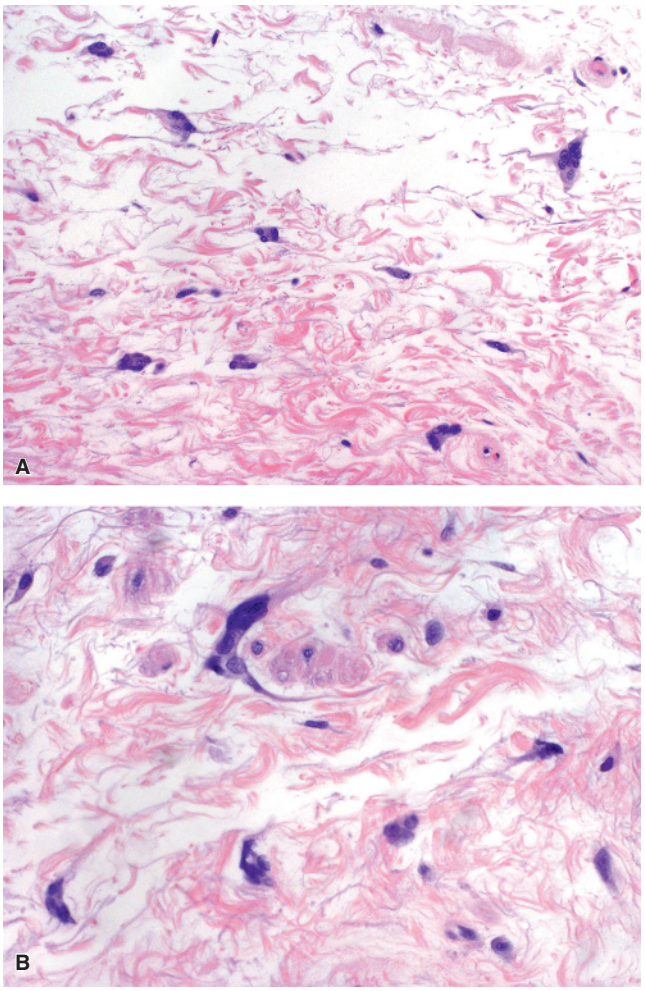


Figure 1-24 Bizarre stromal cells in the lamina propria (A and B).

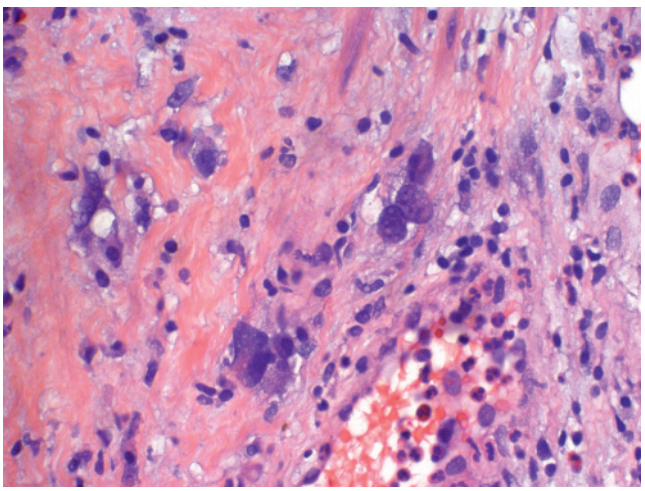


Figure 1-25 Bizarre stromal cells in the lamina propria.

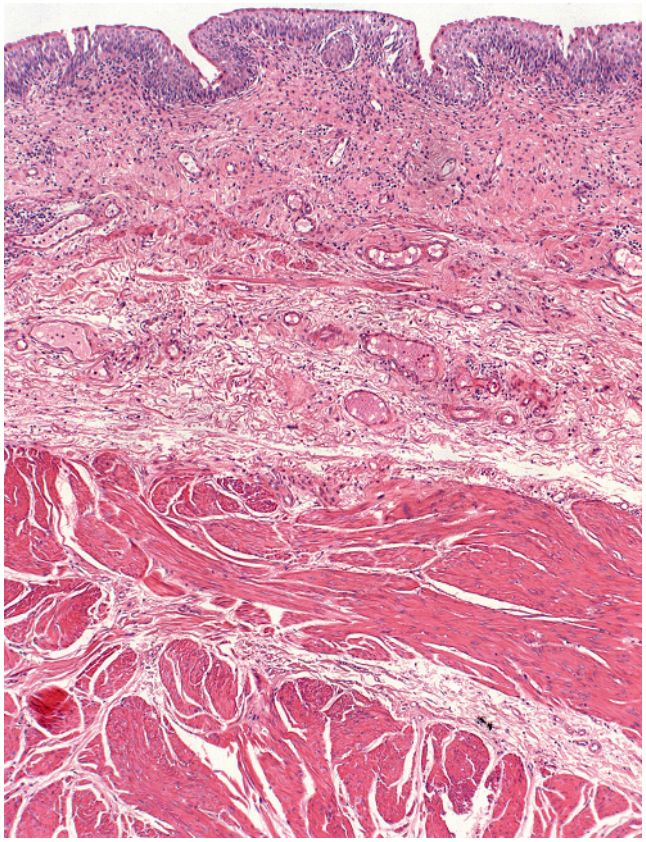


Figure 1-26 Muscularis propria (detrusor muscle). Note the contrast between detrusor muscle and muscularis mucosae in the lamina propria.

The Urachus

The urachus is an intraabdominal embryonic remnant. It contains the allantois, connecting the apex of the urinary bladder to the body wall at the umbilicus. The allantois originates in the portion of the yolk sac that gives rise to the cloacal portion of the hindgut. As the embryo grows, the urachus elongates to maintain its connection with the bladder dome and the body wall. At birth, the dome of the bladder and the umbilicus are closely opposed, and the urachus is only 2.5 to 3 mm long, with a diameter of 1 mm throughout most of its course and 3 mm where it joins the bladder.[42] The urachus lies in a space anterior to the peritoneum, bounded anteriorly and posteriorly by the umbilicovesical fascia.[43] Laterally, it is bounded by the two umbilical arteries, which, in turn, are surrounded by umbilicovesical fascia. Inferiorly, the umbilicovesical fascial layers cover the surface of the dome of the bladder. This space, the space of Retzius, is roughly pyramidal, and fascial planes separate it from the peritoneum and other structures. At the junction with the urinary bladder, the adult urachus is 4 to 8 mm wide, narrowing to about 2 mm at its superior end.

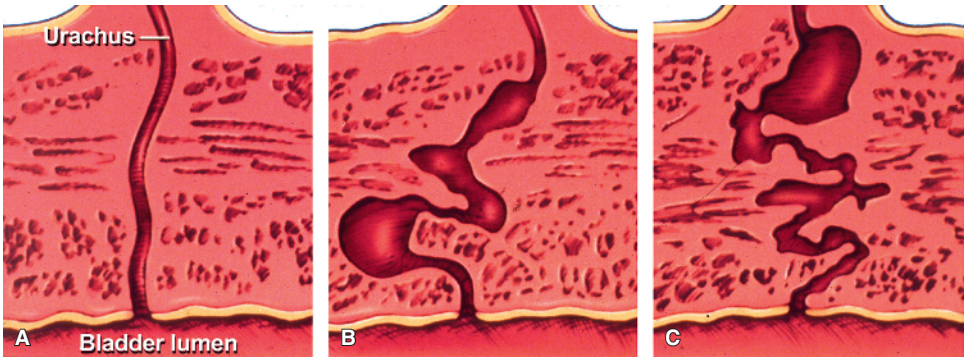


Figure 1-27 Patterns of intramural urachal canals. A, Tubular canal without complexity. B, Type II: Tubular canal with marked segmental dilatation and variable curvature. C, Type III: Tubular canal with marked tortuosity and distortion, including segmental dilatation.

The urachus has three segments: supravesical, intramural, and intramucosal.[42] Tubular urachal remnants are found within the wall of the urinary bladder in approximately one-third of adults and are evenly distributed between men and women. There are three architectural patterns of intramural urachal canals, varying from simple tubular canals to complex branching canals (**Fig. 1-27**).[44] The mucosal portion of the urachus may have a wide diverticular opening, papilla, or a small opening flush with the mucosal surface. The majority (70%) of intramural urachal remnants are lined by urothelium; the remainder are lined by columnar epithelium, occasionally with small papillae or, rarely, mucous goblet cells or mucus-secreting columnar epithelium in women (**Figs. 1-28** and **1-29**).[44–46] In rare cases, urachal remnants are lined by flattened epithelium and some may lack a covering epithelium.

The Renal Pelvis and Ureters

The ureter and renal pelvis develop from the ampullary bud, which arises from the distal mesonephric duct during the fourth week of development. As the ureter elongates, there is a period of luminal obliteration followed by recanalization in the fifth week. Recanalization begins in the middle of the ureter and extends proximally and distally with the ureteropelvic and ureterovesical junctions; these are the last segments to recanalize. The mesonephric duct distal to the ampullary bud (the common nephric duct) is incorporated into the developing urogenital sinus, while the ureteral orifice migrates to the trigone and contributes to the prostatic urethra in the male. Concomitant development of the male and female reproductive tract forms the mesonephric (Wolffian) and müllerian ducts, respectively; division of the cloaca into bladder and hindgut occurs as the ureter and kidney develop. As a consequence, multiple malformations in these areas often occur together.

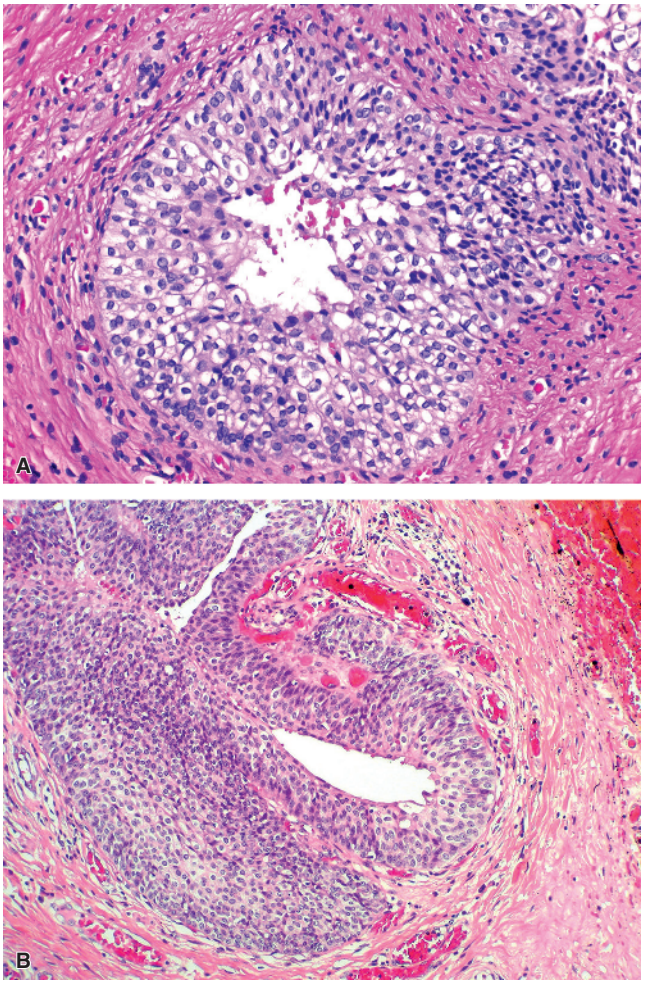


Figure 1-28 Normal urachus lined by stratified urothelium (A and B).

The lumen of the renal pelvis and ureter is lined by urothelium that rests on a basement membrane (**Fig. 1-30**). The urothelium is composed of three to five layers of cells in the pelvis and four to seven layers of cells in the ureter.

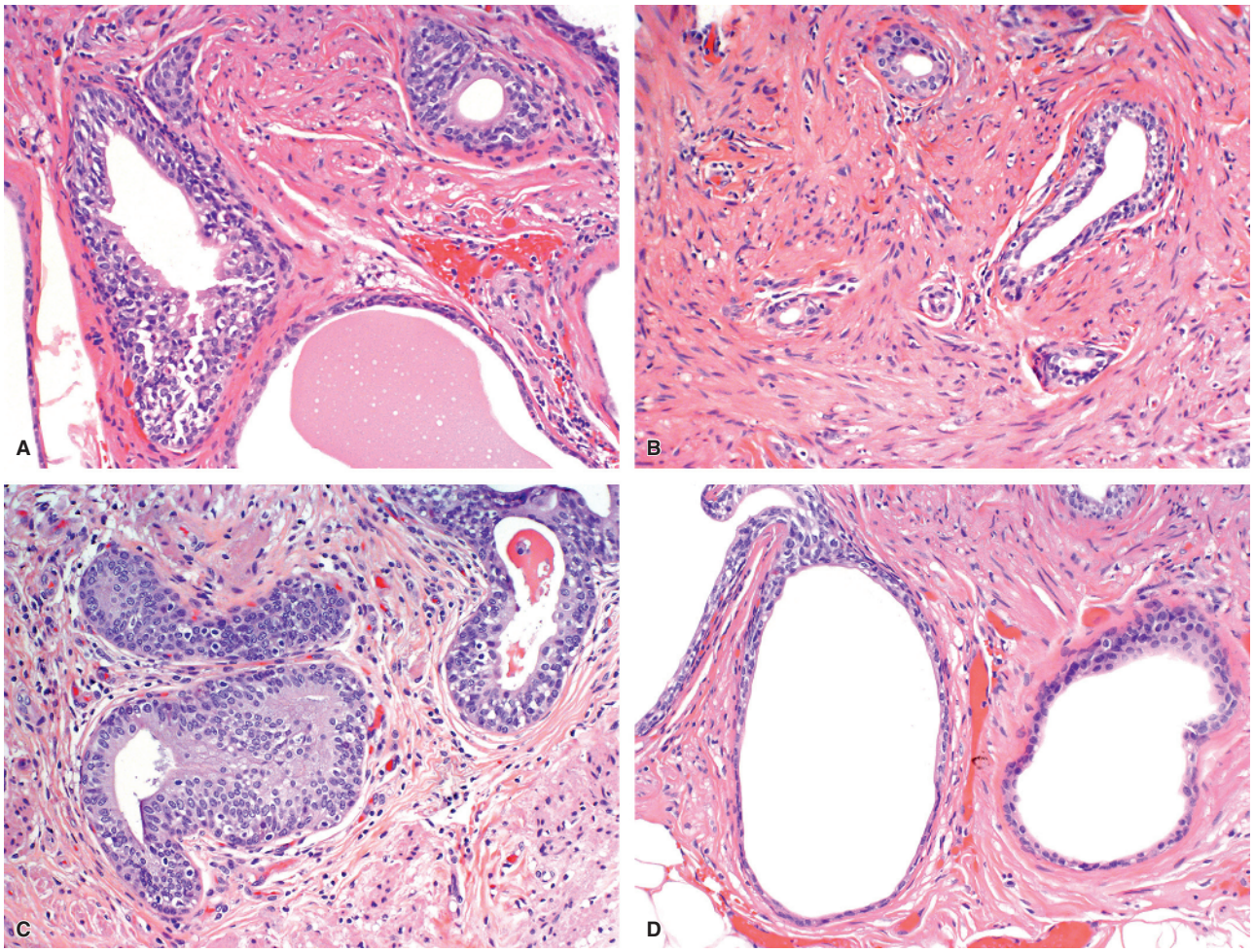


Figure 1-29 Urachal remnants (A to D).

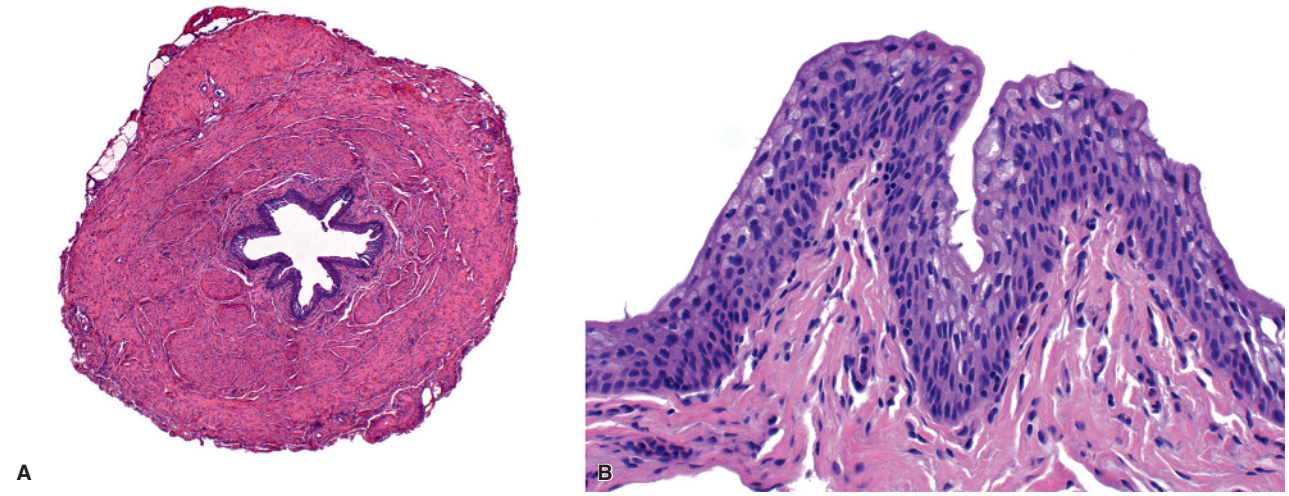


Figure 1-30 Normal ureter (A and B).

The pelvis and ureter have a continuous muscular wall that originates in the fornices of the minor calyces as small interlacing fascicles of the smooth muscle cells. The muscularis propria is not divided into distinct layers. Near the bladder, the ureter acquires an external sheath from the detrusor muscle, and the muscle fascicles become oriented longitudinally. The longitudinal fibers continue through the wall of the bladder and into the submucosa, where they surround the ureteral orifice and contribute to the trigone muscle.

The Urethra

The epithelium of the urethra is derived from the urogenital sinus, which is formed when the endodermal cloaca divides into the rectum dorsally and the urogenital sinus ventrally, separated by the urorectal septum. In females, the epithelium of the urethra is derived from endoderm of the urogenital sinus, while the surrounding connective tissue and smooth muscle arise from splanchnic mesenchyme. In males, the epithelium is also derived from the urogenital sinus except in the fossa navicularis where it is derived from ectodermal cells migrating from the glans penis. As in females, the connective tissue and smooth muscle surrounding the male urethra is derived from splanchnic mesenchyme.

The male urethra is 15 to 20 cm long and is divided into three anatomical segments (**Figs. 1-31** and **1-32**). The prostatic urethra begins at the internal urethral orifice at the bladder neck and extends through the prostate to the prostatic apex. In the central part of the urethral crest is an eminence called the verumontanum. The verumontanum contains a slit-like opening that leads to an epithelium-lined sac called the prostatic utricle, a müllerian vestige. The ejaculatory ducts empty into the urethra on either side of the prostatic utricle. The membranous urethra extends from the prostatic apex to the bulb of the penis. Cowper glands are located on the left and right sides of the membranous urethra and their ducts empty into it. The penile urethra extends from the lower surface of the urogenital diaphragm to the urethral meatus in the glans penis. Bulbourethral glands are located in the proximal (bulbous) portion of the penile urethra. In addition, scattered mucus-secreting periurethral glands (Littre glands) are present at the periphery of the penile urethra except anteriorly (**Fig. 1-33**). The majority of unmyelinated nerve fibers penetrate the smooth muscle layers at 5 o'clock and at 7 o'clock, whereas the majority of myelinated nerve fibers penetrate the striated muscles of the prostatic capsule and of the urethral sphincter at 9 o'clock and at 3 o'clock.

The type of epithelium lining the urethra varies along its length (**Fig. 1-34**). In general, urothelium lines the prostatic urethra, pseudostratified columnar epithelium lines the membranous segment and most of the penile urethra, and nonkeratinized stratified squamous epithelium lines the fossa navicularis and external urethral orifice. In females, the proximal one-third of the urethra is lined by urothelium and the distal two-thirds by nonkeratinized stratified squamous epithelium. The proximal one-third consists of a circular smooth muscle sphincter, the middle one-third of two circular layers of smooth and striated muscle fibers, and the distal one-third of a circular layer of smooth muscle fibers surrounded by an omega-shaped layer of striated muscle fibers. In the proximal one-third of the urethral sphincter, myelinated fibers run with unmyelinated fibers from the pelvic plexus. These fibers are closely related to the lateral and anterior aspects of the vagina. Unmyelinated fibers enter the smooth muscle part of the sphincter at 4 o'clock and at 8 o'clock, whereas most myelinated fibers enter the sphincter at 3 o'clock and at 9 o'clock. The female urethra is approximately 4 cm long and, at its periphery, contains paraurethral Skene glands.

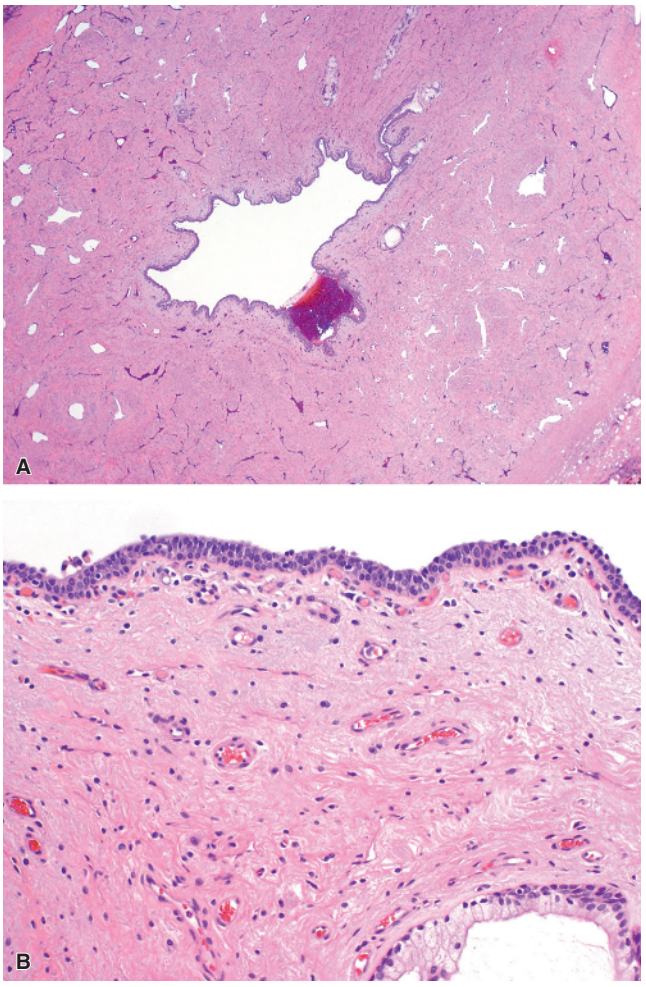


Figure 1-31 Normal urethra in male patient (A and B).

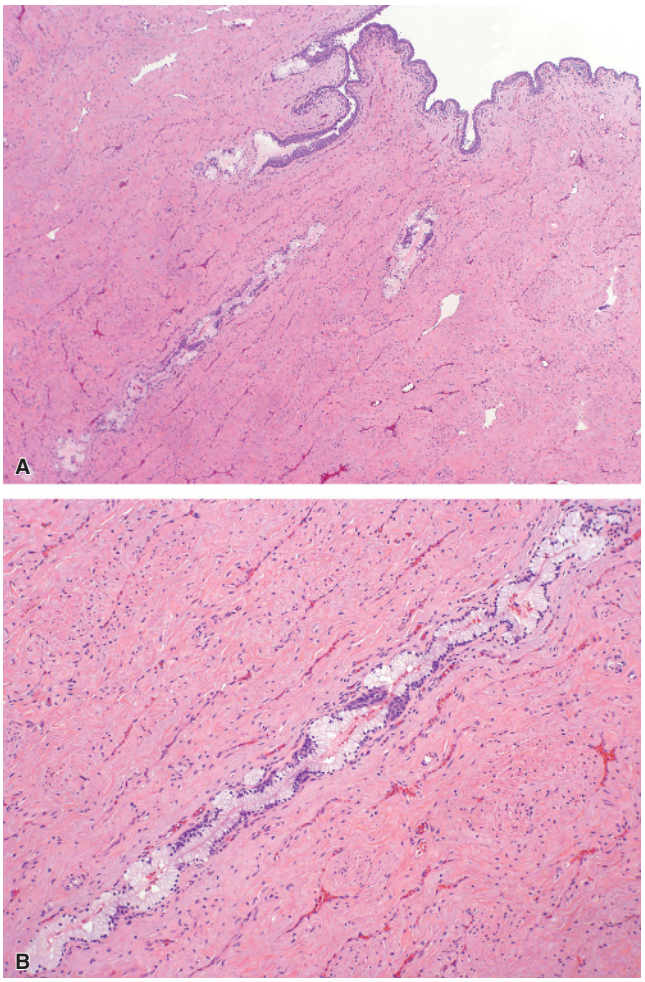


Figure 1-32 Normal urethra and periurethral glands (A and B).

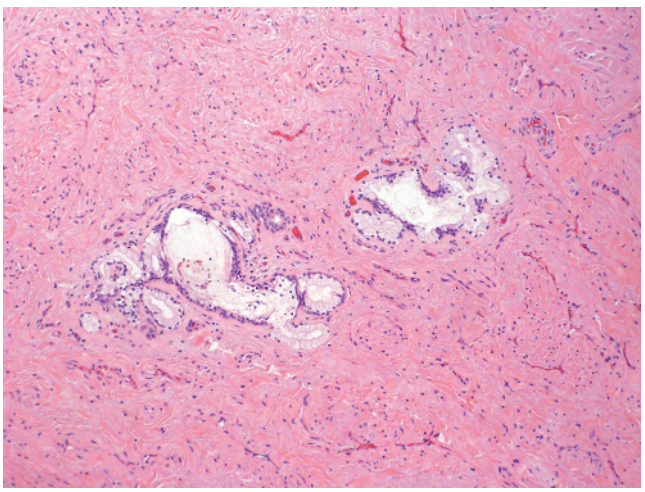


Figure 1-33 Littre glands are lined by mucus secreting columnar cells.

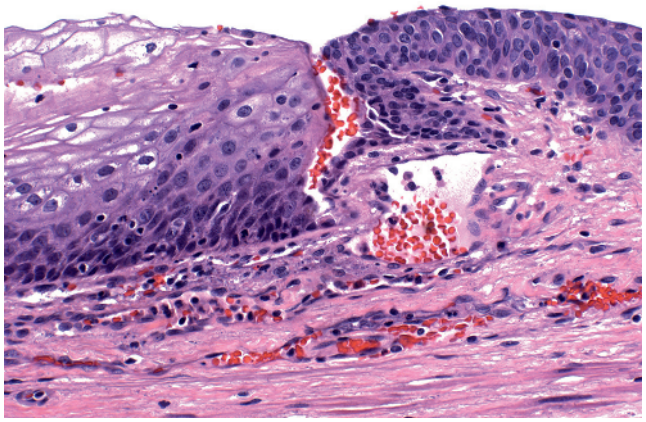


Figure 1-34 Transition from urothelium to squamous epithelium in the urethra.

Immunohistochemistry

The urothelium has a characteristic immunophenotype. It expresses cytokeratins of both low and high molecular weights, including cytokeratins 7, 8, 13, and 19; cytokeratins 18 and 20 are present in the superficial cells.[47–50] This pattern of expression differs from that of normal stratified squamous epithelium, which shows predominantly high molecular weight keratin immunoreactivity and from endometrium, endocervix, colorectum, and prostate, which demonstrate a preponderance of low molecular weight keratin. High molecular weight keratin immunoreactivity is restricted to the basal cell layer of the urothelium and squamous mucosa of the trigone. This can also be readily stained with antikeratin MAC387, which is not present in basal cells but in squamous cells of the trigone.[51] Other epithelial markers, such as epithelial membrane antigen, carcinoembryonic antigen, and LeuM1, are found on the surface of the urothelium. The normal urothelium synthesizes blood groups isoantigens A, B, and H (O), as well as Lewis blood group antigens.[52] As mentioned above, the basal layer of urothelial cells expresses Bcl-2 while the intermediate cells express RB1 and phosphatase and tensin homolog deleted on chromosome ten (PTEN gene) at varying intensities. HER2 and p53 are not expressed by normal urothelial cells. Ki67, indicating proliferation, is uncommon in the normal urothelium. Prostate-specific antigen (or human glandular kallikrein 3), prostatic acid phosphatase, prostate-specific membrane antigen, and human glandular kallikrein 2 are not produced by the urothelium. **Table 1-1** shows a summary of staining patterns and expressions of commonly used antibodies in diagnostic immunohistochemistry of the urothelium.[4,22]

REFERENCES

1. Cheng L, MacLennan GT, Bostwick DG. Urologic Surgical Pathology, 5th ed. Philadelphia, PA: Elsevier, 2025.
2. Eble JN, Argani P, Grignon DJ, Cheng L. Tumors of the kidney, bladder, and related urinary structures. In: Armed Forces Institute of Pathology (AFIP Fascicle), Atlas of Tumor Pathology, 5th Series. Washington, D.C.: ARP Press, 2022.
3. Lopez-Beltran A, Montironi R, Cheng L. Pathology of the Urinary Bladder. Cambridge, UK: Cambridge University Press, 2016.
4. Lopez-Beltran A, Henriques V, Montironi R, Cimadamore A, Raspollini MR, Cheng L. Variants and new entities of bladder cancer. *Histopathology* 2019;74:77–96.
5. Gulinac M, Dikov D, Lichev S, Velikova T. Current concept for tertiary lymphoid structures in urothelial carcinoma of the bladder: A literature review and our experience. *Am J Clin Exp Immunol* 2020;9:64–72.
6. Hinata N, Hussein AA, Bando Y, et al. Histologic investigation of the female vesicourethral junction and adjacent tissues for nerve-sparing radical cystectomy. *Urology* 2021;149:161–7.
7. Kato MK, Muro S, Kato T, Miyasaka N, Akita K. Spatial distribution of smooth muscle tissue in the female pelvic floor and surrounding the urethra and vagina. *Anat Sci Int* 2020;95:516–22.
8. Muro S, Suriyut J, Akita K. Anatomy of Cowper's gland in humans suggesting a secretion and emission mechanism facilitated by cooperation of striated and smooth muscles. *Sci Rep* 2021;11:16705.
9. Zhou L, Xu B, Liu Y, Wang Z. Tertiary lymphoid structure signatures are associated with survival and immunotherapy response in muscle-invasive bladder cancer. *Onco Targets Ther* 2021;10:1915574.
10. Poggi P, Marchetti C, Tazzi A, Scelsi R. The lymphatic vessels and their relationship to lymph formation in the human urinary bladder. *Lymphology* 1995;28:35–40.
11. Ravery V, Chopin DK, Abbou CC. Surgical anatomy of the lymphatic drainage of the bladder. *Ann Urol (Paris)* 1993;27:9–11.
12. Scelsi R, Scelsi L, Gritti A, Gozo M, Reguzzoni M, Marchetti C. Structure of the lymphatic microcirculation in the human urinary bladder with different intraluminal pressure and distension. *Lymphology* 1996;29:60–6.
13. de Groat WC. Anatomy and physiology of the lower urinary tract. *Urol Clin North Am* 1993;20:383–401.
14. Takenaka A, Kawada M, Murakami G, Hisasue S, Tsukamoto T, Fujisawa M. Interindividual variation in distribution of extramural ganglion cells in the male pelvis: A semi-quantitative and immunohistochemical study concerning nerve-sparing pelvic surgery. *Eur Urol* 2005;48:46–52.
15. Konishi T. Architectural ultrastructure of the urinary bladder epithelium. II. Changes in the urine-blood barrier in the contracted and distended state in the normal and inflammatory bladder. *Hinyokika Kiyo* 1988;34:23–31.
16. Montironi R, Mazzucchelli R, Scarpelli M, Lopez-Beltran A, Cheng L. Morphological diagnosis of urothelial neoplasms. *J Clin Pathol* 2008; 61:3–10.
17. Montironi R, Lopez-Beltran A, Scarpelli M, Mazzucchelli R, Cheng L. Morphological classification and definition of benign, preneoplastic and non-invasive neoplastic lesions of the urinary bladder. *Histopathology* 2008;53:621–33.
18. Tsai YC, Simoneau AR, Spruck CH 3rd, et al. Mosaicism in human epithelium: Macroscopic monoclonal patches cover the urothelium. *J Urol* 1995;153:1697–700.
19. Congiu T, Radice R, Raspanti M, Reguzzoni M. The 3D structure of the human urinary bladder mucosa: A scanning electron microscopy study. *J Submicrosc Cytol Pathol* 2004;36:45–53.
20. Davies R, Hunt AC. Surface topography of the female bladder trigone. *J Clin Pathol* 1981;34:308–13.
21. Wilson CB, Leopard J, Nakamura RM, Cheresh DA, Stein PC, Parsons CL. Selective type IV collagen defects in the urothelial basement membrane in interstitial cystitis. *J Urol* 1995;154:1222–6.
22. Akgul M, MacLennan GT, Cheng L. The applicability and utility of immunohistochemical biomarkers in bladder pathology. *Hum Pathol* 2020;98:32–55.
23. Cheng L, Lopez-Beltran A, Wang M, Montironi R, Kaimakliotis HZ, Zhang S. Telomerase reverse transcriptase (TERT) promoter mutations in primary adenocarcinoma of bladder and urothelial carcinoma with glandular differentiation: Pathogenesis and diagnostic implications. *Mod Pathol* 2021;34:1384–91.
24. Rozen SG. Mutational selection in normal urothelium. *Science* 2020;370:34–5.
25. Birder LA, Kanai AJ, de Groat WC, et al. Vanilloid receptor expression suggests a sensory role for urinary bladder epithelial cells. *Proc Natl Acad Sci USA* 2001;98:13396–401.
26. Anderstrom C, Johansson S, Nilsson S. The significance of lamina propria invasion on the prognosis of patients with bladder tumors. *J Urol* 1980;124:23–6.
27. Dixon JS, Gosling JA. Histology and fine structure of the muscularis mucosae of the human urinary bladder. *J Anat* 1983;136:265–71.
28. Keep JC, Piehl M, Miller A, Oyasu R. Invasive carcinomas of the urinary bladder. Evaluation of tunica muscularis mucosae involvement. *Am J Clin Pathol* 1989;91:575–9.
29. Cheng L, Weaver AL, Neumann RM, Scherer BG, Bostwick DG. Substaging of T1 bladder carcinoma based on the depth of invasion as measured by micrometer. A new proposal. *Cancer* 1999;86:1035–43.
30. Cheng L, Montironi R, Davidson DD, Lopez-Beltran A. Staging and reporting of urothelial carcinoma of the urinary bladder. *Mod Pathol* 2009;22 (Suppl 2): S70–95.
31. Ro JY, Ayala AG, el-Naggar A. Muscularis mucosa of urinary bladder. Importance for staging and treatment. *Am J Surg Pathol* 1987;11:668–73.
32. Younes M, Sussman J, True LD. The usefulness of the level of the muscularis mucosae in the staging of invasive transitional cell carcinoma of the urinary bladder. *Cancer* 1990;66:543–8.

33. Cheng L, Bostwick DG. Progression of T1 bladder tumors: Better staging or better biology?: reply. *Cancer* 1999;86: 910–2.
34. Paner GP, Ro JY, Wojcik EM, Venkataraman G, Datta MW, Amin MB. Further characterization of the muscle layers and lamina propria of the urinary bladder by systematic histologic mapping: implications for pathologic staging of invasive urothelial carcinoma. *Am J Surg Pathol* 2007;31:1420–9.
35. Aydin A, Uçak R, Karakök M, Güldür ME, Koçer NE. Vascular plexus is a differentiation criterion for muscularis mucosa from muscularis propria in small biopsies and transurethral resection materials of urinary bladder? *Int Urol Nephrol* 2002;34:315–9.
36. Paner GP, Shen SS, Lapetino S, et al. Diagnostic utility of antibody to smoothelin in the distinction of muscularis propria from muscularis mucosae of the urinary bladder: A potential ancillary tool in the pathologic staging of invasive urothelial carcinoma. *Am J Surg Pathol* 2009;33:91–8.
37. Council L, Hameed O. Differential expression of immunohistochemical markers in bladder smooth muscle and myofibroblasts, and the potential utility of desmin, smoothelin, and vimentin in staging of bladder carcinoma. *Mod Pathol* 2009;22:639–50.
38. Miyamoto H, Sharma RB, Illei PB, Epstein JI. Pitfalls in the use of smoothelin to identify muscularis propria invasion by urothelial carcinoma. *Am J Surg Pathol* 2010;34:418–22.
39. Bochner BH, Nichols PW, Skinner DG. Overstaging of transitional cell carcinoma: Clinical significance of lamina propria fat within the urinary bladder. *Urology* 1995;45:528–31.
40. Honma K. Paraganglia of the urinary bladder. An autopsy study. *Zentralbl Pathol* 1994;139:465–9.
41. Young RH. Non-neoplastic Epithelial Abnormalities and Tumor-like Lesions. Pathology of the Urinary Bladder. New York: Churchill Livingstone, 1989;1–63.
42. Begg RC. The urachus: Its anatomy, histology and development. *J Anat* 1930;64:170–83.
43. Gearhart JP, Jeffs RD. Urachal abnormalities. In: Walsh PC, Retik AB, Stamey TA, Vaughan ED, eds. Campbell's Urology. Philadelphia: W.B. Saunders, 1992;1815–21.
44. Schubert GE, Pavkovic MB, Bethke-Bedurftig BA. Tubular urachal remnants in adult bladders. *J Urol* 1982;127:40–2.
45. Eble JN. Abnormalities of the urachus. In: Young RH, ed. Pathology of the Urinary Bladder. New York: Churchill Livingstone, 1989.
46. Tyler DE. Epithelium of intestinal type in the normal urachus: A new theory of Vesical embryology. *J Urol* 1964;92: 505–7.
47. Alonso A, Ikinger U, Kartenbeck J. Staining patterns of keratins in the human urinary tract. *Histol Histopathol* 2009;24:1425–37.
48. Hodges KB, Lopez-Beltran A, Emerson RE, Montironi R, Cheng L. Clinical utility of immunohistochemistry in the diagnoses of urinary bladder neoplasia. *Appl Immunohistochem Mol Morphol* 2010;18:401–10.
49. Lopez-Beltran A. Immunohistochemical markers in evaluation of urinary and bladder tumors. *Anal Quant Cytol Histol* 2007;29:121–2.
50. Yildiz IZ, Recavarren R, Armah HB, Bastacky S, Dhir R, Parwani AV. Utility of a dual immunostain cocktail comprising of p53 and CK20 to aid in the diagnosis of non-neoplastic and neoplastic bladder biopsies. *Diagn Pathol* 2009;4:35.
51. Lopez-Beltran A, Requena MJ, Alvarez-Kindelan J, Quintero A, Blanca A, Montironi R. Squamous differentiation in primary urothelial carcinoma of the urinary tract as seen by MAC387 immunohistochemistry. *J Clin Pathol* 2007;60:332–5.
52. Witjes JA, Umbas R, Debruyne FM, Schalken JA. Expression of markers for transitional cell carcinoma in normal bladder mucosa of patients with bladder cancer. *J Urol* 1995;154:2185–9.