invited review

Everything you wanted to know about the bladder epithelium but were afraid to ask

SIMON A. LEWIS
Department of Physiology and Biophysics, University of Texas Medical Branch,
Galveston Texas 77555-0641

Lewis, Simon A. Everything you wanted to know about the bladder epithelium but were afraid to ask. Am J Physiol Renal Physiol 278: F867–F874, 2000.—The mammalian urinary bladder epithelium (urothelium) performs the important function of storing urine for extended periods, while maintaining the urine composition similar to that delivered by the kidneys. The urothelium possesses four properties to perform this function. First, it offers a minimum epithelial surface area-to-urine volume; this reduces the surface area for passive movement of substances between lumen and blood. Second, the passive permeability of the apical membrane and tight junctions is very low to electrolytes and nonelectrolytes. Third, the urothelium has a hormonally regulated sodium absorptive system; thus passive movement of sodium from blood to urine is countered by active sodium reabsorption. Last, the permeability properties of the apical membrane and tight junctions of the urothelium are not altered by most substances found in the urine or blood. The importance of the barrier function of the urothelium is illustrated by infectious cystitis. The loss of the barrier function results in the movement of urinary constituents into the lamina propria and underlying muscle layers, resulting in suprapubic and lower back pain and frequent, urgent, and painful voiding.

ion transport; tight epithelium; cystitis; tissue culture

THE MAIN FUNCTION OF THE URINARY bladder is to act as a short-term storage site for urine, while maintaining the composition of the urine similar to that generated by the kidneys. This storage function increases the sanitary conditions of an animal's living area whereas controlled emptying plays a role in territorial identification. Structurally, the mammalian urinary bladder is a hollow sphere, with the wall of the sphere comprising (from outside to inside) the serosa, muscularis, submucosa, muscularis mucosa, and lamina propria (13). Embedded within the above structures is a circulatory system, sensory and motor neurons, and an immune system. A layer of epithelial cells (the urothelium) lies on top of the lamina propria and covers the inside surface of the sphere. The luminal surface of the urothelium is covered by an adhering glycosaminoglycan layer (GAG) (36). The urothelium allows the urinary bladder to minimize alterations in the composition of the urine during storage. Ideally, the urothelium should possess four properties to perform this function.

Storage
The bladder should be able to store a variable volume of urine while maintaining a minimum ratio of epithelial surface area to urine volume. The geometry of the bladder (a sphere) is the ideal geometry to have a minimum epithelial surface area to urine volume. The small surface area for movement of substances reduces the amount of movement of substances between the urine and blood.

Passive Permeability
With the exception of actively transported substances, the urothelium should be impermeable to all substances present in the urine or blood. Movement across the epithelium occurs via two parallel pathways, through the cells (a transcellular pathway) and through the tight junctions and lateral intercellular space (a paracellular pathway). Thus both tight junction and cell membrane should be impermeable to substances found in urine or blood. Alteration of either cellular or
tight junction permeability can increase or decrease the efficacy of the epithelium’s barrier properties.

**Active Transport**

The bladder might have a regulated active transport system(s) for the directed movement of physiologically important substances between the lumen and blood.

**Inertness**

Substances normally present in the urine or blood should not alter the passive permeability of the apical membrane and tight junctions.

Before a discussion of the urothelium in light of the above properties, it is useful to describe the structure of the urothelium. The urothelium is a transitional epithelium of endodermal origin and a turnover time of ~6 wk in mice (31). When viewed in cross section, the urothelium is composed of three cell layers (Fig. 1A), with each layer having a morphologically distinct cell type (40, 47). The basal cell layer is germinall in nature, having cells with a diameter of 5–10 µm, intermediate cells are 20 µm, and the superficial umbrella cells are hexagonal and, depending on the degree of bladder stretch, range from 50–120 µm across. Cell replacement is by fusion of the basal cell layer to form intermediate cells and fusion of intermediate cells to form umbrella cells (31).

Although the basal and intermediate cells are unremarkable in morphology (47), the superficial umbrella cells have two unique morphological features. First, the apical membrane is covered with scalloped-shaped plaques (Fig. 1B) that are separated by plasma membrane domains called the “hinge.” These plaques make the outer leaflet of the apical membrane appear thicker than the inner leaflet [the asymmetrical unit membrane (39)]. Second, the cell cytoplasm of the umbrella cells has a high density of cytoplasmic vesicles with an associated cytoskeletal network of fine fibrils (see Ref. 12 for a review).

The polygonal-shaped plaques are ~0.5 µm in diameter, 12 nm in thickness, and occupy from 70 to 90% of the apical surface area (Fig. 1C). Hinge membrane surrounds the individual plaques, occupies the remaining 10–30% of the apical membrane surface area, and is ~8-nm thick. The plaques are composed of subunits with center-to-center spacing of ~16 nm (48). Each plaque contains ~1,000 subunits. The subunits have sixfold symmetry and are composed of an inner ring comprising six large particles and an outer ring comprising six smaller particles (Fig. 1D). A three-dimensional reconstruction of the surface architecture of the subunits suggests that each subunit resembles a twisted...
of the apical membrane allowed the urothelium to accommodate urine-volume changes. The measurement of surface area (by capacitance) as a function of increasing stretch (measured as tissue dry weight) suggested an unfolding of the apical membrane followed by an increase in apical membrane area (26). Morphometric analysis (33) confirmed that during the initial phase of bladder filling, the apical membrane unfolds. However, during the latter phase of filling, the apical membrane accommodates volume changes by insertion of cytoplasmic vesicles. The energy for the movement of vesicles into and out of the apical membrane is provided by expansion of the bladder during filling and collapse of the bladder during muscle contraction. The movement of vesicles into and out of the apical membrane requires an intact microfilament system but not an intact microtubule system (25). Sarikas and Chlapowski (42) showed that disruption of intermediate filaments in rat urinary bladder inhibited the insertion and withdrawal of cytoplasmic vesicles, implying a role of intermediate filaments in vesicle translocation. In addition, ATP seems to be necessary for vesicle insertion during stretch but not vesicle removal during collapse (41).

Table 1. Historical perspective on the physiology of the urothelium

| Date       | Event                                                                 
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1400s</td>
<td>Macroscopic structure of the human bladder described by Leonardo Da Vinci.</td>
</tr>
<tr>
<td>1780s–1920s</td>
<td>Conflicting evidence presented about bladder permeability. Toxic substances (e.g., turpentine) increase bladder permeability.</td>
</tr>
<tr>
<td>1920s–1960s</td>
<td>Bladder has a finite permeability. Movement of substances between urine and blood is passive.</td>
</tr>
<tr>
<td>1964</td>
<td>Wickham (49) demonstrated active sodium transport across the bladder epithelium. Aldosterone regulates the rate of transport.</td>
</tr>
<tr>
<td>1966/1967</td>
<td>Bladder surface area changes by the insertion/removal of cytoplasmic vesicles at the apical membrane demonstrated (11; 39).</td>
</tr>
<tr>
<td>1969/1972</td>
<td>That bladder epithelium accommodates an increase in urine volume by infolding of luminal membrane and not by vesicle insertion proposed (18; 44).</td>
</tr>
</tbody>
</table>

STORAGE

Ideally, the bladder should maintain a minimum ratio of surface area to urine volume. Hicks (11) and Porter and colleagues (39) proposed that vesicles found in the cytoplasm of umbrella cells insert into and exit from the apical membrane during expansion-contraction cycles of the bladder. During bladder filling, this insertion will minimize the ratio of surface area to urine volume. This hypothesis was challenged (18, 44), and an alternate hypothesis was offered. Instead of vesicle insertion and retrieval, Koss (18) and Staehelin and colleagues (44) proposed that unfolding and folding of the apical membrane allowed the urothelium to accommodate urine-volume changes. The measurement of surface area (by capacitance) as a function of increasing stretch (measured as tissue dry weight) suggested an unfolding of the apical membrane followed by an increase in apical membrane area (26). Morphometric analysis (33) confirmed that during the initial phase of bladder filling, the apical membrane unfolds. However, during the latter phase of filling, the apical membrane accommodates volume changes by insertion of cytoplasmic vesicles. The energy for the movement of vesicles into and out of the apical membrane is provided by expansion of the bladder during filling and collapse of the bladder during muscle contraction. The movement of vesicles into and out of the apical membrane requires an intact microfilament system but not an intact microtubule system (25). Sarikas and Chlapowski (42) showed that disruption of intermediate filaments in rat urinary bladder inhibited the insertion and withdrawal of cytoplasmic vesicles, implying a role of intermediate filaments in vesicle translocation. In addition, ATP seems to be necessary for vesicle insertion during stretch but not vesicle removal during collapse (41).

Less well studied is the effect of stretch on the basolateral membrane area and tight junctional length. Does the basolateral membrane surface area change during an expansion-contraction cycle? A mechanism of vesicle insertion and retrieval during bladder distension and collapse was proposed for the basolateral membrane (1). Minsky and Chlapowski (33) reported that during expansion the surface cells change from a goblet shape to a flattened/squamous shape. To accommodate this change the lateral membrane folds and the basal membrane unfolds. During contraction, the lateral membrane unfolds and the basal membrane folds. The aforementioned authors did not report changes in lateral or basal membrane area. There are no reports on whether the length of the tight junction responds to expansion-contraction cycles of the bladder.
Initial filling of a collapsed bladder results in unfolding of a folded apical membrane and a reorientation of cytoplasmic filaments from being parallel with the lateral membrane to being parallel to the apical membrane. This reorientation of the filaments brings the basal membrane toward the apical membrane. Once the apical membrane is smooth, further filling causes the filaments to attach at the tight junction to stretch and pull vesicles toward, and finally into, the apical membrane. The insertion of the vesicle presumably occurs at the hinge region. During filling the shape of the umbrella cell has changed from goblet to squamous. Smooth muscle contraction causes the cells to change from squamous to goblet shaped. This shape change occurs in the movement of the basal membrane away from the apical membrane and an infolding of the apical membrane toward the cell interior. The infolding of the apical membrane is due to the attachment of the filaments to the apical membrane and basal membrane. The apical membrane plaques pinch off to reform cytoplasmic vesicles.

PASSIVE PERMEABILITY

Until the 1920s, there was conflicting evidence of whether the bladder had even a small but finite permeability (30). From the 1920s to the 1960s, improved measuring techniques demonstrated that the bladder had a small but finite passive permeability to most substances (electrolytes and nonelectrolytes) found in the urine and blood (see Ref. 12 for a review).

A very sensitive measure of the ion permeability of an epithelium is the transepithelial electrical resistance (see Ref. 22). Fromter and Diamond (8) divided epithelia into two categories, leaky and tight. This division was based on both the magnitude of the transepithelial resistance as well as the relative resistance of the transcellular pathway to the tight junction pathway. Leaky epithelia typically have a resistance $<500 \ \Omega \cdot \text{cm}^2$, whereas tight epithelia have resistances $>500 \ \Omega \cdot \text{cm}^2$. Given that the function of the urinary bladder is to store urine, one would predict that it would have a high transepithelial resistance. With the necessary precautions taken not to damage the epithelium during the isolation procedure or when it was placed in the Ussing chambers, the resistance of the rabbit urinary bladder was found to range from 10,000 to a high of 75,000 $\Omega \cdot \text{cm}^2$ (26). According to the criterion of Fromter and Diamond (8), the urinary bladder epithelium is a tight epithelium. This epithelium has the highest recorded transepithelial resistance of all epithelia measured to date. Thus the rabbit urothelium has a very low permeability to sodium and chloride. This low permeability to sodium and chloride is in agreement with radioactive tracer measurements for sodium and chloride (26). Cat and guinea pig urothelium have resistance values of $\sim3,500$ and $\sim1,300 \ \Omega \cdot \text{cm}^2$, respectively (20). It is not clear whether the lower resistance values for cat and guinea pig, compared with the rabbit urothelium, are due to technical differences or reflect a real difference among these species. The transepithelial resistance is due to the parallel arrangement of the cell resistance and the tight junction resistance. For the rabbit urothelium, it was found that the tight junction resistance was $>100,000 \ \Omega \cdot \text{cm}^2$, and the cell resistance varied from 10,000 to $>100,000 \ \Omega \cdot \text{cm}^2$. The cell resistance is the sum of the resistance of the apical (urine-facing) membrane and the basolateral (blood-facing) membrane. Variability of the cell resistance is due to variability of the apical membrane and not the basolateral membrane (see ACTIVE TRANSPORT). The basolateral membrane has a resistance of $\sim1,500 \ \Omega \cdot \text{cm}^2$ (5).

There are numerous studies of nonelectrolyte movement across the in vivo urothelium (see Ref. 12 for a review). Only recently have nonelectrolyte permeabilities been calculated for the in vivo urothelium. The advantage of the in vitro measurements is that the permeability of the urothelium to nonelectrolytes (measured by using radioisotopes) can be quantified. Quantification is possible because of the known surface area of the urothelium [measured by using capacitance (5)], the specific activity of the isotope in the efflux compartment, the volume and isotope activity of the influx compartment, and the ability to estimate the influence of unstirred layers on the permeability measurements. Urea, ammonia, water, and proton permeabilities were measured across the rabbit urothelium (3, 34). The values are all very low and suggest that the in vivo bladder is an excellent barrier to the movement of these substances from urine to blood.

At present there is a controversy as to whether the major permeability barrier across the urinary bladder is the GAG layer (which is associated with and lies on top of the apical membrane) or the tight junctions and epithelial cells (23). Although there is compelling evidence that the epithelium is the major barrier to the movement of electrolytes and nonelectrolytes (23), the relative contribution of the GAG and epithelium as a barrier to macromolecules and microscopic organisms has not been assessed.

ACTIVE TRANSPORT

A second property that the urothelium possesses is active transepithelial ion transport. In 1964, Wickham (49) demonstrated that the rabbit and dog urothelium generated a spontaneous transepithelial potential difference in vitro. It was shown (by using radioactive isotopes) that rabbit and dog urothelium actively absorbed sodium (but not calcium) under short-circuit conditions. In rabbit urinary bladder epithelium, aldosterone increased the rate of sodium absorption (49). In the same year, Henderson and Webber (9) demonstrated that in vitro rat urinary bladder (including muscle layers) increased the amino acid (lysine, aspartic acid, and leucine) concentration in the serosal compartment by 5–15% over 4 h compared with the mucosal compartment. These studies have not been repeated by using radioisotopes; thus it is possible that the increase in amino acid concentration in the serosal compartment might have come from the underlying muscle layers and not active transport from the mucosal compartment.
The observation of active sodium transport was confirmed by Lewis and Diamond (26). By comparing the measured short-circuit current to net sodium transport (under short-circuit conditions), these authors also demonstrated that the spontaneous transepithelial potential difference could be accounted for by the rate of active sodium transport. This sodium transport can be inhibited by mucosal amiloride and serosal ouabain. In addition, chloride movement across the rabbit urothelium is passive. Further studies led to the development of the following model for transepithelial sodium absorption by the urothelium. Sodium entered across the apical membrane through amiloride-sensitive sodium channels down a net electrochemical gradient. This gradient is due to an apical membrane voltage of ~55 mV (cell interior negative with respect to the mucosal solution) and a chemical gradient with cell sodium activity being ~7 mM with luminal sodium activity of ~120 mM. Once in the cell, the sodium exits across the basolateral membrane through the Na\(^+\)-K\(^+\) ATPase. As sodium is extruded, the pump brings potassium into the cell (21). This potassium exits across the basolateral membrane through potassium-selective channels (28). This outward movement of potassium across the basolateral membrane results in the generation of a membrane potential that is ~55 mV (cell interior negative with respect to the serosal solution).

Immunolocalization demonstrated that the sodium channel is present only in the apical membrane of the surface cells, with diffuse cytoplasmic staining in the cytoplasm of surface cells (43). This cytoplasmic staining agrees with previous reports that cytoplasmic vesicles contain sodium channels (24). These cytoplasmic vesicles are presumably the same population of vesicles that fuse on bladder expansion.

The apical membrane also contains an amiloride-insensitive, cation-selective channel and a cation channel that seems to partition between the apical membrane and the mucosal bathing solution. These two channels seem to be partially degraded, amiloride-sensitive sodium channels and not different gene products (23). The basolateral membrane contains potassium and chloride channels (28), Na\(^+\)/H\(^+\) exchangers, and Cl\(^-\)/HCO\(_3\)\(^-\) exchangers. The potassium and chloride channels and exchangers are important in cell volume recovery during an increase in serosal osmolality (6).

The functional studies of the amiloride-sensitive sodium channel in urothelium (28) have recently been complemented with measurements of mRNA levels of the epithelial sodium channel subunits (\(\alpha\), \(\beta\), and \(\gamma\)ENaC) and immunolocalization of the channel in rats (43). Of interest is that the mRNA levels are not the same for the three subunits, with \(\gamma\)ENaC being 5- to 10-fold lower than the other subunits. In contrast, Kopp and colleagues (17) found a different mRNA profile in rat urothelium, with \(\beta\) and \(\gamma\)ENaC being at much higher levels than \(\alpha\) ENaC. The reason for this difference in subunit abundance between these two studies is not known, although differences in the animals’ diets were suggested.

Comparison of the selectivity, single-channel conductance, gating kinetics, and the relationship between channel conductance and sodium concentration for the urothelial channel to mixtures of subunits injected into oocytes might yield information about the significance of the differing mRNA levels. For example, if the level of mRNA reflects the level of protein subunit, then the sodium channel should show characteristics of a channel composed of \(\alpha\) and \(\beta\)-subunits. McNicholas and Canessa (32) demonstrated that the amiloride sensitivity and sodium concentration dependence of the short-circuit current of a sodium channel were dependent on the subunit composition. Sodium channels composed of \(\alpha\beta\) had an inhibition constant value (\(K_i\)) for amiloride of 4 \(\mu\)M and a Michaelis-Menten constant value (\(K_m\)) for external sodium of >180 mM. A channel composed of all three subunits or only the \(\alpha\)-and \(\gamma\)-subunits had a \(K_i\) for amiloride of 0.13 \(\mu\)M and a \(K_m\) for sodium of 30 mM. In the rabbit urothelium the amiloride \(K_i\) is 0.25 \(\mu\)M, and the sodium \(K_m\) is 40 mM (29). Thus the urothelial sodium channel is unlikely to be composed of only the \(\alpha\)- and \(\beta\)-subunits. This is in agreement with the observation that, even in the presence of different concentrations of messenger RNA, the sodium channel maintains a fixed subunit composition (7).

**INERTNESS**

An essential requirement for normal bladder function is that normal urinary constituents should not compromise the barrier properties of the bladder. Indeed, substances normally found in the urine do not alter the permeability properties of the urothelium. Thus changes within the physiological range for urine pH, calcium, or urea concentrations have minimal effects on the barrier function of the urothelium as determined from measurements of the transepithelial resistance (24). Nonphysiological concentrations of these substances, e.g., acid pH, low calcium, or high urea (4 M), cause an increase in the ion permeability of the urothelium. The sites of increased ion permeability are at the apical membrane and tight junction. After pH, calcium, or urea return to normal values, the permeability of the urothelium returns to control values.

The active ion transport properties of the urothelium are altered by urinary proteases. Normal urine contains a number of proteases, among them are three (trypsin-like) serine proteases. These are urokinase, plasmin, and kallikrein. The function of urokinase and plasmin is well known. Urokinase (a component of the fibrinolytic system) is released from distal nephron segments and hydrolyzes plasminogen into plasmin. Plasmin is then able to degrade blood clots. The function of kallikrein is not presently known, but it has been postulated to play an important role in hypertension. All three proteases were demonstrated to degrade the amiloride-sensitive sodium channel in the apical membrane of the urothelium (24). This degradation did not render the sodium channel nonconductive but rather altered its permeability properties. Thus an amiloride-sensitive sodium channel was converted into a channel that was cation selective and not inhibited by...
amiloride. This latter channel was then degraded into a channel or fragment that seemed to partition between the apical membrane and luminal solution. The effect of these proteases explains the origin of both the apical cation channel and the unstable cation channel. This effect of serine proteases on rabbit urinary bladder sodium channels is different from that reported for the Xenopus laevis sodium channels, where trypsin activated Xenopus sodium channels (46).

A number of nonphysiological factors can also alter the barrier function of the urothelium. These include bacterial products such as amphotericin B, nystatin, gramicidin, polymyxin B, and perhaps α-hemolysin as well as positively charged proteins released from eosinophils and found in sperm (histones and protamine; 23). All of these substances increase the ion permeability of the urothelium by interacting with the apical membrane and causing a nonselective increase in membrane ion permeability. If the increase in membrane permeability persists, cell swelling and lysis will occur. The loss of cells from the epithelial layer results in a loss of barrier function.

Acetate, propionate, butyrate, or succinate at pH 4.4 also alters the transepithelial permeability of the rabbit urothelium, but not at pH 5.0 (14). The increase in transepithelial permeability due to volatile fatty acids is rapid (minutes) and is due in part to an increase in the apical membrane permeability to sodium and chloride. However, neither the mechanism by which these volatile fatty acids increase the apical membrane ion permeability at low pH nor the long-term effect of these agents on the barrier function of the urothelium is known.

Less is known about the effect that plasma or interstitial substances (such as those released from mast cells) have on the transport properties and barrier function of the urothelium. Birder and colleagues (2) demonstrated that nitric oxide (NO) can alter the barrier function of tissue cultured urothelial cells. The increase in transepithelial permeability due to NO required prolonged exposure of cultured urothelium to 5 mM S-nitroso-N-acetylpenicillamine, a NO donor, and resulted in a 90% reduction in the transepithelial resistance (2). For the in vitro urothelium, 1–2 h after 100–200 µM spermine nonate (a NO donor) was added to the mucosal and serosal solutions there was a 60% decrease in transepithelial resistance and a small increase in ion transport. In approximately one-half of the bladders studied, the transepithelial resistance returned to control after removal of spermine nonate (unpublished observations). Because afferent nerves in the bladder and the urothelium generate NO, this second messenger might serve a role in regulation of urothelial barrier function.

In addition to the barrier function being disrupted by bacterial infection (bacterial cystitis), toxic chemicals (e.g., cyclophosphamide, which produces a hemorrhagic cystitis), or mechanical damage, the barrier function can be altered during a nonbacterial, nonchemical inflammatory response. The experimental approach was to sensitize guinea pigs by intraperitoneal (ip) injection of ovalbumin (16). Four weeks after the last ip injection, ovalbumin was instilled into the bladder for 1 h and then replaced with a saline solution containing radiolabeled urea. Thirty minutes after radiolabeled urea was placed into the bladder, there was a significant increase in radiolabeled urea in the blood compared with nonsensitized guinea pigs. This antigen challenge after sensitization resulted in a large increase in urea permeability of the guinea pig urothelium. Christensen and colleagues (4) demonstrated that antigen challenge after sensitization resulted in degranulation of mast cells. Using a similar sensitization protocol, Lavelle and colleagues (19) demonstrated that the ion permeability of the urothelium from guinea pigs was dramatically increased, as were the urea and water permeabilities. Transmission and electron microscopy of the sensitized and challenged urothelium demonstrated loss of tight junctions, focal loss of umbrella cells, and alteration of the structure of the apical membrane.

The above-mentioned cystitis is used as a model for the study of interstitial cystitis. Interstitial cystitis is a cystitis (bladder inflammation) of unknown etiology that affects mostly women. Symptoms of interstitial cystitis include diminished urinary capacity, hematuria, and frequent and painful urination (15). Histologically, the bladder has an inflammatory infiltration, with possible mucosal ulceration (Hunner’s ulcer). In some patients, there is a loss of barrier function, as evidenced by an increase in urea movement across the urothelium (36). Cats suffer from feline cystitis, which might be similar to interstitial cystitis. In studies of bladders from cats diagnosed with feline cystitis, there is a decrease in the transepithelial resistance of the urothelium (20) and an increase in urea flux. This suggests that the symptoms of feline cystitis are due in part to a loss of urothelial barrier function and resemble interstitial cystitis.

Infection, radiation, and toxic chemicals can lead to loss of urothelial barrier function either by a direct effect on the urothelial cells or as a secondary effect of inflammation. This loss of barrier function and inflammation leads to the movement of urine constituents (e.g., urea and high potassium concentrations) into the underlying connective tissue and muscle layers, exacerbating the resulting cystitis. A recent in vitro study demonstrated that serosal urea (0.5 M) caused an increase in apical membrane permeability of the umbrella cells over a 5-min period, followed by an irreversible and rapid increase in paracellular permeability after ~25 min. Of interest is that the effect of 0.5 M serosal urea was slowed by the presence of 0.5 M mucosal urea (unpublished observations). Thus the increase in apical membrane permeability occurred over a 20-min period, and irreversible increase of paracellular permeability occurred after ~65 min. Although serosal urea is detrimental to the urothelium, the presence of mucosal urea offers a level of protection.
TISSUE CULTURE OF THE UROTHELIUM

Although in vitro studies of dissected bladder can be used to address many questions concerning urothelial properties, there are numerous questions that can be best studied by using a tissue culture system. Numerous laboratories, with varying levels of success, have attempted tissue culture of the urothelium. For the tissue culture of the urothelium to be considered successful, the fingerprint of the cultured urothelium should resemble that of the intact urothelium; i.e., it should look the same and act the same as the native epithelium. Thus the fingerprint should consist of (but not limited to) 1) high transepithelial resistances: native tissues have a resistance of 20,000 Ω · cm² or greater; 2) amiloride-sensitive transport system, typically 2–3 µA/cm²; 3) a stretch-sensitive movement of cytoplasmic vesicles into and out of the apical membrane; 4) low diffusive water and urea permeabilities; 4) differentiation markers of the umbrella cells such as the UP; 5) composition of three cell layers, with the umbrella cells containing an apical membrane with an asymmetrical unit membrane and a scalloped appearance and the cell cytoplasm containing vesicles; and 6) presence of tight junctions.

In only two studies of urothelial tissue culture were some or most of the above criteria filled. Perrone and colleagues (37) immortalized and grew human urothelial cells on permeable supports. These tissue culture cells had a transepithelial resistance of 500–1,000 Ω · cm², an amiloride-sensitive current of 2 µA/cm², and were four cell layers thick and characterized by the presence of tight junctions. The apical membrane was not scalloped and did not appear to have an asymmetrical unit membrane. In addition, the umbrella cells did not have cytoplasmic vesicles. Thus, although this tissue culture system had the appropriate transport system and a reasonable resistance, some of the morphological features were not present, whereas others were not determined. In contrast, a recent article by Truschel and colleagues (45) has generated a rabbit urothelial tissue culture system that has a fingerprint similar to the native urothelium. The only noted difference between the native urothelium and tissue culture urothelium was the lack of a scalloped appearance of the apical membrane of the tissue culture cells. This suggests a difference in the synthesis and processing of the membrane plaques. Such a tissue culture system will prove invaluable for the study of both the physiology and pathophysiology of the urothelium.

A goal of urothelial tissue culture is to provide cells for seeding sections of bladder lacking the urothelium. In this regard, Oberpenning and colleagues (35) have used tissue engineering to create, de novo, a functional canine urinary bladder. In brief, these investigators cultured urothelial and smooth muscle cells from canine bladder biopsies. The urothelial and muscle cells were expanded and passaged in tissue culture. The urothelial cells were seeded onto the inside of a bladder-shaped polymer whereas the smooth muscle cells were seeded onto the opposing side, generating a neorgan. The native bladders, with the exception of the trigone region (a triangular area at the base of the bladder between openings of the ureters and the urethra), were excised from the donor animals and replaced with the neorgan (essentially an autologous graft). After 1 mo, the histological appearance and physiological properties (urine capacity and compliance) of the neorgans were found to be identical to the native tissue. The advantage of this neobladder is that it circumvents the problems of tissue compatibility and organ rejection inherent in donor tissue.

CONCLUSIONS

The objective of this minireview was to act as an introduction and an update on the physiology of the mammalian urinary bladder epithelium. In the study of the urothelium, more questions were generated than were answered. What is the function of the asymmetrical unit membrane and the UP? What are the biosynthetic pathway and trafficking of the cytoplasmic vesicles? Are there changes in the urothelial cells as they migrate from the basal germinal layer to the intermediate layer to the fully differentiated umbrella cells? What biochemical machinery is needed for fusion and removal of cytoplasmic vesicles at the apical membrane? Are sodium channels from the urothelium similar in subunit composition to other sodium-absorbing epithelia? Are the channels trafficked during expansion-contraction of the bladder? Is the change in the barrier function of the bladder a cause or an effect of the factors that lead to interstitial cystitis? These are only a few examples of the questions about the urothelium that remain unanswered.

I thank Drs. Henry Sun and Gerald Apodaca for providing unpublished manuscripts. I also thank Jamie Rudy Lewis and Christian Spilker for constructive comments on this paper. This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-51382. Address for reprint requests and other correspondence: S. A. Lewis, Dept. of Physiology and Biophysics, Univ. of Texas Medical Branch, Galveston TX 77555-0641 (E-mail: Slewis@UTMB.edu).

REFERENCES