Disruption of guinea pig urinary bladder permeability barrier in noninfectious cystitis

Lavelle, John P., Gerard Apodaca, Susan A. Meyers, Wily G. Ruiz, and Mark L. Zeidel. Disruption of guinea pig urinary bladder permeability barrier in noninfectious cystitis. Am. J. Physiol. 274 (Renal Physiol. 43): F205–F214, 1998.—Although most cell membranes permit rapid flux of water, small nonelectrolytes, and ammonia, the apical membranes of bladder epithelial umbrella cells, which form the bladder permeability barrier, exhibit strikingly low permeabilities to these substances. In cystitis, disruption of the bladder permeability barrier may irritate the bladder wall layers underlying the epithelium, causing or exacerbating inflammation, and increasing urinary frequency, urgency, and bladder pain. To determine the effects of inflammation on the integrity of the permeability barrier, guinea pigs were sensitized with ovalbumin, and the bladders were exposed subsequently to antigen by instillation on the urinary side. Inflammation of the bladder wall markedly reduced transepithelial resistance of dissected epithelium mounted in Ussing chambers and increased water and urea permeabilities modestly at 2 h and more strikingly at 24 h after induction of the inflammation. Transmission and scanning electron microscopy of bladders at 30 min and 24 h after antigen exposure revealed disruption of tight junctions, denuding of patches of epithelium, and occasional loss of apical membrane architecture. These permeability and structural effects did not occur in nonsensitized animals in which the bladders were exposed to antigen and in sensitized animals exposed to saline vehicle rather than antigen. These results demonstrate that inflammation of the underlying muscle and lamina propria can disrupt the bladder permeability barrier by damaging tight junctions and apical membranes and causing sloughing of epithelial cells. Leakage of urinary constituents through the damaged epithelium may then exacerbate the inflammation in the underlying muscle layers.

to permeation of these substances must exhibit exceptionally low permeabilities to prevent their leakage into the underlying layers, where they would be expected to cause irritation and inflammation (25). In agreement with this notion, water, urea, and ammonia permeated rabbit bladder urothelium extremely slowly in recent direct measurements (17). The permeability barrier in the bladder is located in the tight junctions and apical membranes of the most superficial layer of epithelial cells (13, 25). In addition to maintaining a tight barrier, the urothelial cells must expand and reduce the surface area of the apical membrane as the bladder fills and empties (25). These cells regulate their apical surface area by trafficking membrane-containing endosomes into and out of the apical surface (4, 14, 15). Any disease process that disrupts the bladder permeability barrier or the ability of the cells to expand or reduce the apical membrane surface area will lead to leakage of urine constituents into the underlying bladder layers and the blood.

Cystitis may be caused by infection or chemical or radiation damage to the epithelium and underlying layers (hemorrhagic cystitis) or by unknown causes (interstitial cystitis) (22, 25). It is presumed in cystitis that the urothelial permeability barrier fails, leading to leakage of potentially noxious urinary substituents across into the underlying layers of the bladder wall. In several experimental forms of cystitis, such an increase in permeability has been suggested by increased appearance of isotopic urea instilled in the bladder in the circulation or by the movement of vital dyes from the lumen into the bladder wall (8, 24). Although these semiquantitative studies suggested a failure of the bladder permeability barrier, they provided only indirect evidence and did not explore the mechanisms involved. These studies also did not determine whether the defect was specific to urea or was generalized to water and electrolytes. The present studies define the permeabilities and ultrastructure of the bladder permeability barrier in the absence and presence of inflammation.

We have recently demonstrated that the rabbit bladder epithelium exhibits exceptionally low permeabilities to water, urea, and ammonia (4, 17). The present studies were designed to explore the mechanisms leading to disruption of this barrier. Prior models of cystitis used chemicals such as acetone to damage the epithelium. However, these models do not resemble cystitis in vivo and involve direct toxicity to the epithelium and subepithelial structures. We have chosen instead a well-defined model of cystitis in which inflammation is set up in the underlying bladder musculature and...
The distention to 80 cmH\textsubscript{2}O occurred 24 h after the saline
infusion, respectively. This pressure is below the maximal
level achieved by rat bladder during micturition but was
sufficient to induce bladder distention and thereby to stimu-
late expansion of the apical membrane surface area of
umbrella cells via membrane trafficking mechanisms (20, 21).
Additional control animals included several that were sensi-
tized but exposed to saline without ovalbumin and animals
not sensitized but exposed to ovalbumin. Both of these control
groups gave results identical to the control animals listed
above. The cystitis subgroups were 1) with albumin infusion
alone, 2) with immediate distention to 80 cmH\textsubscript{2}O with 0.9%
saline for 5 min, and 3) where the distention was performed
24 h after the intravesical albumin infusion.

Experimental setup. The guinea pigs were sensitized as
outlined above, and on the day of study were anesthetized
with 5 mg/kg xylazine and 35 mg/kg ketamine. The intra-
vesical infusion was performed with or without distention as
outlined above. The urinary bladder was catheterized with a
3.5-French Tom Cat catheter (Sherwood Medical, St. Louis,
MO). The guinea pig was then killed with anesthetic overdose
for the immediate studies or allowed to recover for 24 h in the
24 h distention studies and then killed. The bladder was
opened and removed.

The bladder was placed immediately into Ringer solution
and then placed on a rack with the epithelium downward. The
muscle was dissected free from the epithelium in such a
way that the experimental epithelium was not injured. The ep-
ithelium was then stretched out on a 1-cm\textsuperscript{2} ring and held in place
that the experimental epithelium was not injured. The epithe-
lium was then stretched out on a 1-cm\textsuperscript{2} ring and held in place
by several pins away from the area through which the permeabil-
ty was to be measured. The epithelium was then
mounted between two halves of an Ussing chamber as
described (15), and the chamber was filled with Ringer
solution. Temperature was maintained at 37\textdegree C, and the
hemichambers were constantly stirred. Electrical measure-
ments of transmembrane resistance were performed to deter-
mine epithelial membrane integrity. The membranes were
allowed to stabilize for \(-1\) h before addition of isotope and
measurement of permeability.

Permeability measurements. These were performed as de-
scribed (17). [\textsuperscript{3}H\textsubscript{2}O (1 \muCi/ml) were added to the apical (luminal)
side of the membrane, and both hemichambers were then sampled (2 samples of 100 \mu l per
hemichamber) at 15-min intervals throughout the experi-
ment. After 1 h of baseline measurements, Nystatin (185 \mu M)
was added to the apical side to increase permeability, as
nystatin is a nondiscriminate pore former in the apical
membrane. To determine the contribution of unstirred layers
to the measured permeabilities, the apical membrane
was destroyed by the addition of 100 \mu l of Triton X-100 1 h after
nystatin had been added. In all experiments addition of nystatin
and Triton X-100 abolished transepithelial resistance. Correc-
tions were made for removing the sample volume by the addition
of replacement fresh Ringer solution, and the calculated fluxes
were corrected for these modest dilutional effects.

Calculation of permeabilities and statistical analysis. Re-
sults for each guinea pig are based on the mean of four flux
measurements during each experimental stage. Flux rates
were obtained before and after permeabilization of the ep-
ithelium with nystatin and Triton X-100. The values obtained
after permeabilization were used to estimate the permeabil-
ity of the unstirred layer. Diffusive water and urea permeabil-
ity coefficients \(P\text{\textsubscript{D}}\) were calculated from the isotopic fluxes
using the flux equation

\[
P\text{\textsubscript{D}} = \Phi/(A \cdot \Delta C)
\]

MATERIALS AND METHODS

Materials. Unless specified otherwise, all chemicals were
obtained from Sigma (St. Louis, MO) and were of reagent
grade. [\textsuperscript{14}C]Urea and [\textsuperscript{3}H\textsubscript{2}O were obtained from DuPont New
England Nuclear (Wilmington, DE). All animal studies were
carried out with the approval of the University of Pittsburgh
animal care and use committee and maintained according to
the standards set forth by the American Physiological
Society. The media used in the Ussing chambers was a modified
Ringer solution containing (in mM) 111.2 NaCl, 25 NaHCO\textsubscript{3},
5.8 KCl, 2 CaCl\textsubscript{2}, 1.2 MgSO\textsubscript{4}, 1.2 KH\textsubscript{2}PO\textsubscript{4}, and 11.1 glucose.
The solution was maintained at 37\textdegree C and at pH 7.5 with
constant bubbling with 95\% O\textsubscript{2}-5\% CO\textsubscript{2}.

Induction of cystitis. Guinea pigs were sensitized with 10
mg of chicken ovalbumin (grade V) delivered by three intra-
peritoneal injections, each 48 h apart, as described (5, 8–10,
12). At least 28 days following the final injection, cystitis was
induced by instilling ovalbumin dissolved in normal saline at
a concentration of 1 mg/ml via bladder catheter in anesthe-
tized animals. Ovalbumin was maintained in the bladder for
30 min. Because of the difficulty in maintaining the albumin
in the bladder for 30 min without ligating the urethra, the
albumin was infused at 0.2 ml/min to maintain constant
intravesicular exposure to the albumin and the animal was
allowed to void spontaneously around the catheter during
instillation. This modification induced cystitis in all but one
(animal that was excluded from analysis) animal in the cystitis
group. In all of the cystitis group animals studied further, the
bladder wall was thickened, inflamed, and hemorrhagic.

Experimental groups. The guinea pigs were divided into
the following groups: a group receiving ovalbumin and a
control group that received 0.9\% saline in the same manner
as the albumin group. The control group was subdivided into
nondistended, distended, and 24 h distention, where the
bladder was 1) infused with saline with minimal \(<5 \text{ cmH}_2\text{O}
baseline pressure, 2) the bladder was infused with saline at
minimal baseline pressure and then distended with infusion
of saline to maintain a pressure of 80 cmH\textsubscript{2}O for 5 min, and 3)
the distention to 80 cmH\textsubscript{2}O occurred 24 h after the saline
connection tissue without any direct damage to the
epithelium (5, 8, 9, 12). Guinea pigs are sensitized by
systemic exposure to ovalbumin, and the apical surface
of the bladder is exposed to ovalbumin. The antigen is
apparently transported across the epithelium, likely
via an endocytic pathway, and reaches the underlying
bladder wall, where it sets up an inflammatory reaction.

The present studies show that, like the rabbit blad-
er (17), the guinea pig bladder exhibits very high
transepithelial resistances and exceptionally low water
and urea permeabilities. Induction of inflammation
markedly reduced transepithelial resistance and in-
creased water and urea permeabilities. Permeabilities
rose markedly when inflamed bladders were subjected
to distention prior to excision and when the inflamma-
tion had been active for 24 h prior to the studies. These
permeability effects were accompanied by structural
damage to multiple cellular components of the bladder
permeability barrier. It is thought that leakage of urine
components of the urine may
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and then placed on a rack with the epithelium downward.

The muscle was dissected free from the epithelium in such a
way that the experimental epithelium was not injured. The epithe-
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by several pins away from the area through which the permeabil-
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mine epithelial membrane integrity. The membranes were
allowed to stabilize for \(-1\) h before addition of isotope and
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Permeability measurements. These were performed as de-
scribed (17). [\textsuperscript{14}C]Urea (0.25 \muCi/ml) and [\textsuperscript{3}H\textsubscript{2}O (1 \muCi/ml) were added to the apical (luminal)
side of the membrane, and both hemichambers were then sampled (2 samples of 100 \mu l per
hemichamber) at 15-min intervals throughout the experi-
ment. After 1 h of baseline measurements, Nystatin (185 \mu M)
was added to the apical side to increase permeability, as
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Calculation of permeabilities and statistical analysis. Re-
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ity coefficients \(P\text{\textsubscript{D}}\) were calculated from the isotopic fluxes
using the flux equation

\[
P\text{\textsubscript{D}} = \Phi/(A \cdot \Delta C)
\]
where $\Phi$ is the flux of the tracer across the membrane and is calculated from the net increase of the tracer in the basolateral side, $A$ is the area of the apical membrane and is calculated from the capacitance measurements, and $\Delta C$ is the concentration gradient for isotope across the membrane and is calculated from the mean concentration of the isotope in each chamber for the sampling period. In all flux measurements the flux rate was linear $(R > 0.98)$. Corrections for the dilution by the replacement of the previous sample with nonradioactive solution were made for the flux calculations as described (17).

To determine the water or urea permeability of the apical membrane, it is necessary to estimate not only the resistance to the flux exerted by the unstirred layer but also the resistance exerted by the postapical membrane structures (intracytosolic membranes, basolateral membranes, basement membranes, connective tissue, and the remnants of the muscle layer). The relationship of the measured value of $P_D$ ($P_{D(AM)}$) to the $P_D$ of the apical membrane ($P_{D(DAM)}$) and the $P_D$ of the total unstirred layer including the bulk-phase unstirred layer and the postapical membrane structures ($P_{D(TUL)}$) can be expressed in the following equation

$$\frac{1}{P_{D(DAM)}} = \frac{1}{P_{D(TUL)}} + \frac{1}{P_{D(DAM)}}$$

Equations 2 and 3 can be written for water, permitting calculation of $P_{D(DAM)}$ for water from $P_{D(TUL)}$ for water.

To determine the $P_{D(TUL)}$ for water in rabbit bladder epithelium, we used two approaches (2). The first approach consisted of measuring the permeability coefficient of the lipophilic molecule, butanol, and the second approach involved measuring fluxes following permeabilization of the epithelium with nystatin and Triton X-100. In these earlier studies, both methods gave identical values for the contribution of the unstirred layer to permeability. We have therefore used the permeabilization method in these studies. The electrical resistance values represent the means of four to five recordings taken during the course of each section of the experiment. Results are means ± SE, except where explicitly stated. Statistical significance is taken at less than 5% probability ($P < 0.05$) based on analysis using the Student’s $t$-test.

Preparation of samples for electron microscopy. Animals were anesthetized, a catheter was inserted into their bladders, and ovalbumin was instilled for 30 min as described above. The bladders were rinsed with buffer for 5 min and then dehydrated in absolute ethanol for 15 min. The samples were then washed three times over a 45-min period in absolute ethanol and then dehydrated for 15 min each in the following graded series of ethanol: 30% (vol/vol, in PBS), 50% (vol/vol, in PBS), 70% (vol/vol, in PBS), and 95% (vol/vol, in water). The samples were then incubated three times over a 45-min period in absolute ethanol. The dehydrated samples were critical point dried, sputter coated with gold-palladium, and viewed in a J EOL model JSM T300 scanning electron microscope at 20 kV. Images were captured on Kodak type 52 film, scanned, assembled, and output as described above.

**RESULTS**

Permeability barrier of the control guinea pig bladder epithelium. The results of electrical recordings for the guinea pig bladder epithelium are summarized in Table 1. These values ranged consistently from 1,300–2,900 $\Omega \cdot$ cm$^2$. Although these values are somewhat lower than those obtained in the rabbit bladder epithelium, studies in that tissue demonstrated that resistances in the range observed in the present study were associated with exceptional low water and urea permeabilities (17). Transepithelial resistance was examined in bladders that were distended in situ.

Table 1. Electrical resistances

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Nystatin</th>
<th>Triton</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, no distention</td>
<td>1.34 ± 0.27 (10)</td>
<td>0.126 ± 0.004 (8)</td>
<td>0.08 ± 0.001 (8)</td>
</tr>
<tr>
<td>Control + distention</td>
<td>2.38 ± 0.26 (35)</td>
<td>0.17 ± 0.01 (28)</td>
<td>0.08 ± 0.003 (28)</td>
</tr>
<tr>
<td>Control + 24-h distention</td>
<td>2.96 ± 0.41 (20)</td>
<td>0.147 ± 0.005 (16)</td>
<td>0.09 ± 0.001 (16)</td>
</tr>
<tr>
<td>Cystitis, no distention</td>
<td>0.435 ± 0.06 (25)</td>
<td>0.167 ± 0.01 (24)</td>
<td>0.103 ± 0.005 (24)</td>
</tr>
<tr>
<td>Cystitis + distention</td>
<td>0.422 ± 0.02 (40)</td>
<td>0.17 ± 0.02 (36)</td>
<td>0.09 ± 0.001 (36)</td>
</tr>
<tr>
<td>Cystitis + 24-h distention</td>
<td>0.255 ± 0.008 (20)</td>
<td>0.109 ± 0.004 (16)</td>
<td>0.08 ± 0.001 (16)</td>
</tr>
</tbody>
</table>

Values are means ± SE in $\Omega / \text{cm}^2$ epithelium; numbers of experiments are in parentheses.
prior to excision, to simulate the effects bladder filling in vivo and stimulate expansion of umbrella cell apical membrane surface area. Interestingly, transepithelial resistance following distention was nearly doubled (P < 0.001). This may result from trafficking of membrane into the apical surface or potentially a change in the tight junctions as a result of bladder stretching. Addition of nystatin promptly lowered resistance to 120–170 Ω·cm², and further addition of Triton X-100 lowered it to 80–90, indicating that these treatments permeabilized the barrier to ions.

As shown in Table 2, the measured water permeability of the control epithelium was 5.0–7.1 × 10⁻⁵ cm/s under all conditions. Correction for unstirred layer effects gave P(AM) values of 4.6–8.6 × 10⁻⁵ cm/s. The measured urea permeability of the control epithelium was 1.6–2.2 × 10⁻⁶ cm/s. Because of the very small contribution of the unstirred layers, the P(AM) for urea was 1.7–2.2 × 10⁻⁶. Both the water and urea apical membrane permeabilities for the guinea pig epithelium are exceptionally low and resemble those obtained in the rabbit bladder epithelium (17). These results indicate that the basal electrical and permeability characteristics of the guinea pig bladder epithelium resemble closely those of the rabbit epithelium.

Effect of inflammation on electrical and permeability properties of the guinea pig bladder permeability barrier. When bladders from nonsensitized animals were exposed to apical ovalbumin or when bladders in sensitized animals were exposed to luminal saline, the transepithelial resistances and permeability values were similar to those of control animals (data not shown). By contrast, when bladders in sensitized animals were exposed to ovalbumin, there were marked changes in both transepithelial resistance and permeability. Transepithelial resistance for all cystitis protocols ranged between 255 and 420 Ω·cm², significantly below all of the values for the respective controls (P < 0.001). These results indicate that inflammation of the bladder wall markedly increased the permeability of the barrier to ions. Following exposure to nystatin and Triton X-100, transepithelial resistance values fell to levels similar to those observed in control bladders under the same conditions.

Apical membrane water permeability values rose modestly immediately following induction of inflammation (P < 0.05). Distention appeared to increase permeability still further, although the difference between control and cystitis did not reach statistical significance. At 24 h following induction of inflammation, distended bladders with cystitis exhibited markedly increased apical membrane water permeabilities compared with controls (P < 0.01). Indeed, following 24 h of inflammation, apical membrane permeability approached that of the unstirred layers, so that the apical membrane ceased to function as a barrier to water flux. Similarly, apical membrane urea permeabilities increased significantly immediately after induction of inflammation, whether the bladder was distended (P < 0.02) or not (P < 0.01). After 24 h of inflammation, apical membrane urea permeability rose markedly, approximating that of the unstirred layer.

Ultrastructure of normal and inflamed bladder epithelium. To identify the morphological consequences of ovalbumin-induced cystitis, we examined the bladder from unsensitized animals infused with ovalbumin for 30 min, sensitized animals infused with ovalbumin for 30 min, or sensitized animals infused with ovalbumin for 30 min and then allowed to recover for 24 h. As noted above, in some of the permeability studies, the underlying muscle was stripped from the urothelium. Because we were concerned that stripping may artifactually induce morphological changes in the bladder epithelium, we instead fixed the tissue in situ after ovalbumin addition and then processed the samples for TEM and SEM.

Initially, we analyzed the urothelium of unsensitized, control animals whose bladders were exposed to ovalbumin. Identical results are observed in control animals that have not been exposed to ovalbumin and are not shown. The border between two adjacent umbrella cells is shown in Fig. 1A. Prominent features of these cells included a plasma membrane composed of raised microvilli (hinge regions, marked by arrows in Fig. 1A) and intervening plaque regions that have been shown previously to contain uropilaks arranged in a paracrystalline array. The cytoplasm of these cells was filled with abundant subapical vesicles (marked with asterisks in Fig. 1A), which also contained plaques and hinges and are thought to fuse with the apical plasma membrane in response to stretch or changes in basolateral osmolality. The other hallmark of these cells was the presence of junctional complexes (Fig. 1B), composed of the tight junctions (zonula occludens, ZO), adherens junctions (zonula adherens, ZA), and desmosomes (macula adherens, MA).

Although TEM gives a high-resolution view of the urothelium, it is only possible to view a thin cross section of a small area of bladder at any one time. To

**Table 2.** Summary of permeability results for water and urea in guinea pig noninfectious cystitis

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Observed Water, ×10⁻⁵ cm/s</th>
<th>Triton Water, ×10⁻⁵ cm/s</th>
<th>P(AM)</th>
<th>Observed Urea, ×10⁻⁵ cm/s</th>
<th>Triton Urea, ×10⁻⁵ cm/s</th>
<th>P(AM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3</td>
<td>5.0 ± 0.3</td>
<td>39.0 ± 0.9</td>
<td>5.7 ± 0.4</td>
<td>1.6 ± 1.1</td>
<td>226 ± 57</td>
<td>1.7 ± 1.1</td>
</tr>
<tr>
<td>Control + distention</td>
<td>6</td>
<td>7.1 ± 0.9</td>
<td>39.0 ± 0.9</td>
<td>8.6 ± 1.1</td>
<td>2.2 ± 0.7</td>
<td>226 ± 57</td>
<td>2.2 ± 0.7</td>
</tr>
<tr>
<td>Control + 24 h distention</td>
<td>3</td>
<td>4.1 ± 1.1</td>
<td>39.0 ± 0.9</td>
<td>4.6 ± 1.2</td>
<td>1.7 ± 0.2</td>
<td>226 ± 57</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>Cystitis</td>
<td>6</td>
<td>6.9 ± 1.0</td>
<td>26.6 ± 7.0</td>
<td>9.4 ± 1.4</td>
<td>10.0 ± 2.5</td>
<td>166 ± 49</td>
<td>11.8 ± 3.0</td>
</tr>
<tr>
<td>Cystitis + distention</td>
<td>8</td>
<td>9.4 ± 3.0</td>
<td>21.8 ± 6.4</td>
<td>16.5 ± 5.3</td>
<td>13.7 ± 4.2</td>
<td>106 ± 39</td>
<td>15.7 ± 4.8</td>
</tr>
<tr>
<td>Cystitis + 24 h distention</td>
<td>4</td>
<td>16.7 ± 3.8</td>
<td>27.4 ± 6.6</td>
<td>42.8 ± 9.7</td>
<td>86.7 ± 30.6</td>
<td>187 ± 48.3</td>
<td>162 ± 51.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of experiments.
obtain a general overview of the urothelium, we used SEM, which allowed us to view relatively large areas (5 × 5 mm) of bladder tissue at high resolution. A low-power overview of urothelium is shown in Fig. 2A, and a higher power view of individual umbrella cells is shown in Fig. 2B. There were several noteworthy features of these cells. Each cell (the border of one cell is marked with arrowheads in Fig. 2B) was surrounded by a junctional complex. The surface of each cell contained numerous microscopic folds, previously described in TEM studies, which gave the cells a raisin-like appearance. In addition, each cell was covered in an apparently random fashion with microplicae (see inset of Fig. 2B) that formed a series of anastamosing raised ridges and individual ring-shaped structures (see asterisk in inset of Fig. 2B).

Dramatic defects in the ultrastructure of the urothelium were observed in the bladders of sensitized animals after infusion of ovalbumin for 30 min (Fig. 3). These defects were most easily visualized by SEM (Fig. 3). Two types of lesions were observed. The first was a disruption of the tight junctions and separation of cell-cell contacts. One such region is shown to the right of the small box 1 in Fig. 3. The area within box 1 is...
shown at higher magnification in Fig. 3B. In these areas the adjacent umbrella cells were no longer joined, the tight junctions were disrupted, and the underlying intermediate cell layer could be seen. The second type of lesion was not observed in all samples but was seen in bladders that were particularly inflamed. In these lesions the overlying umbrella cells were denuded from the underlying tissue. One such area is marked with a dashed line in Fig. 3A. At higher magnification, the cells abutting these lesions (e.g., the cells in box 2 in Fig. 3A) had one free edge (demarcated by the broken line) unattached to other cells, but otherwise appeared normal (Fig. 3C). However, with these morphological techniques, it was not possible to assess the viability of these cells or their functional integrity. It is also important to note that areas of apparent injury were surrounded by areas that appeared normal. Lesions in the integrity of the urothelium were also observed in TEM cross sections. Figure 4A shows an example of a tight junction between two adjacent umbrella cells that has broken apart, and in Fig. 4B is an area where underlying epithelial cells (intermediate cells, IC) are exposed to the surface, either because adjacent umbrella cells have pulled apart or because an umbrella cell has sloughed off. The loss of tight junctions and defects in the structural integrity of the urothelium are entirely consistent with the decreases in transepithelial resistance we have observed in the Ussing chamber studies. Similar changes were observed in the bladders of sensitized animals that were exposed to ovalbumin for 30 min and then allowed to recover for a 24 h period. Figure 5A is an example of a region where many of the cells have lost their junctional contacts and appear pulled apart. A higher magnification view of these cells is shown in Fig. 5B. The separation between cells was clearly obvious in these regions. Grossly, the frequency
of areas where the tight junctions were clearly disrupted and where umbrella cells had sloughed off was similar between 30 min and 24 h bladders.

In addition, the surface of many of the umbrella cells appeared more smooth than cells from control animals and either lacked or had diminished numbers of microvilli. This decrease in cell surface microvilli was observed even in areas where the tight junctions appeared intact. The smooth appearance of the umbrella cells in these samples suggested that they may be functionally altered as a result of ovalbumin-induced inflammation. However, there were areas of the tissue where normal looking umbrella cells were observed that contained microvilli (data not shown). Breaks in tight junctions, similar to those observed in Fig. 4A, were observed in these samples when analyzed by TEM cross sections (data not shown). Again, the ultrastructural changes observed in these samples correlated well with the loss of transepithelial resistance observed in our functional analysis of these tissues.

DISCUSSION

Because the urine has a composition that is quite different from that of plasma, and because it contains many potentially noxious substances such as urea, ammonia, and excreted toxins, the bladder permeability barrier plays a crucial role in preventing escape of these substances into the bladder wall and the circulation (25). The bladder wall underlying the epithelium contains the smooth muscle of the bladder detrusor, as well as numerous sensory neurons. Leakage of urinary constituents may irritate the bladder musculature directly, leading to contraction at relatively low volumes of stored urine (25). Furthermore, leakage into the wall may stimulate nociceptive bladder afferents, leading to firing of micturition reflexes when the bladder is only partially filled. The combination of these effects may cause the frequency, urgency, and bladder pain that define the symptoms of cystitis.

Although the bladder permeability barrier appears to play a crucial role in renal function and in the symptoms of cystitis, until recently, its function had not been measured quantitatively. The apical membranes of the epithelium have recently been isolated and shown to exhibit low permeabilities to water, urea, and ammonia (4). Moreover, rabbit bladder epithelium has been isolated and shown in Ussing chamber studies to exhibit extremely high transepithelial resistances and strikingly low permeabilities to water, urea, and ammonia (17). The development of quantitative methods for measuring barrier function led directly to the current studies, which begin the process of examining how disease states might injure bladder epithelial cells and disrupt the permeability barrier.

As an initial model of epithelial damage, we chose to induce cystitis in sensitized guinea pig bladders by exposure to luminal ovalbumin. This model has been well characterized previously and shown to cause increased irritability of the bladder smooth muscle (5, 8, 9, 11, 12). Moreover, in the first 2 h after exposure of bladders in sensitized animals to ovalbumin, luminal applied [14C]urea was found to reach the blood and the bladder wall more rapidly in inflamed than in control bladders (8). Although these studies suggested that the cystitis induced in this model might damage the bladder epithelial barrier, they provided no information as to whether the defect was selective for urea or what
components of the barrier (e.g., apical membrane or tight junctions) were damaged.

Before studies of inflamed bladders could be undertaken, it was necessary to define the electrical and permeability properties of the guinea pig bladder epithelium. As is apparent from Table 1, this epithelium, under a variety of conditions, exhibits a high transepithelial resistance and low permeabilities to water and urea. These values are strikingly similar to those of the rabbit bladder epithelium, and are also similar to values we have recently obtained in preliminary studies of cat bladder epithelium (J. P. Lavelle, S. A. Meyers, and M. L. Zeidel, unpublished observations). It is therefore likely that all mammalian bladder epithelia exhibit similar high resistances and low permeabilities, which make the mammalian bladder epithelium the most effective barrier epithelium yet studied (17, 25, 26).

A major feature of the passive inflammation model is the lack of direct injury to the epithelium (8, 9). The ovalbumin applied to the luminal surface does not affect epithelial function unless the animal has previously been sensitized. In addition, this model may resemble several human diseases, such as interstitial cystitis, where inflammatory mediators and mast cells appear to be more abundant in the bladder wall (5, 19), and infectious cystitis, where inflammation of the bladder wall follows bacterial infection. In these respects, this model is superior to the models of direct chemical injury to the epithelium which have been studied previously.

The bladder epithelial barrier consists of the apical membrane, the tight junctions, and the trafficking mechanism, which inserts apical membrane endosomes into the apical membrane and removes them in response to bladder filling and emptying, respectively. As determined in other models of epithelial injury, these components of the barrier are likely dependent on the integrity of cellular energy generating mechanisms and cytoskeleton (2, 3, 16). Our results provide some clues as to the mechanisms of epithelial damage during inflammation of the underlying bladder wall.

Fig. 4. TEM analysis of sensitized animals exposed to ovalbumin for 30 min. A: example of a broken junctional complex (arrow). B: discontinuity in the umbrella cell layer (UC) overlying the intermediate cells (IC). Bars = 1 μm (A and B).
As shown in Tables 1 and 2, induction of inflammation rapidly reduced transepithelial resistance to values near to those observed in epithelial permeabilized with nystatin and Triton X-100. These results indicate that the inflammation was causing a marked increase in permeability of the epithelium to ions. In addition, there was a progressive increase in water and urea permeabilities, so that permeabilities were increased some within the first hour of the inflammation and became indistinguishable from the unstirred layer values by 24 h. The early fall in transepithelial resistance and modest increase in urea and water permeabilities suggest that inflammation in the underlying bladder wall first damages the tight junctions, leading to leakage of ions, a fall in transepithelial resistance, and some leakage of water and urea. At this early time point, distention of the bladder prior to its study does not further compromise barrier function, indicating that the cells are capable of shuttling new membrane to the apical surface in response to bladder filling. By 24 h, all barrier function is lost, and there is a further reduction in transepithelial resistance and lack of ability to restrict the fluxes.

The ultrastructural studies reveal early damage to tight junctions and sloughing of whole patches of epithelial cells. These changes were also present at 24 h, at which point loss of apical membrane structure could also be found. It is notable that the changes in transepithelial resistance appear far more dramatic than those in water and urea permeabilities. It appears likely that the patchy nature of the lesions plays a critical role in limiting the permeability responses. As shown in Fig. 4, some areas of the epithelium remained normal, whereas other areas exhibited disruption of the tight junctions. Because the rate of permeation of water through the unstirred layer is relatively close to that of permeation through the membrane, leakage of water through the tight junctions in patchy areas of damage will not lead to large increases in transepithelial water flow. By contrast, urea permeation through the unstirred layer was over 100-fold higher than that through the apical membrane, so that leakage through
tight junctions does result in a larger effect on transepithelial fluxes.

The extent of denudation and damage to tight junctions appears similar at 30 min and 24 h of inflammation, yet the permeabilities to water and urea increase between these time points. It is possible that tight junctions become even more leaky during this period without obvious structural changes. It is also possible that the changes in apical membrane structure shown in Fig. 5 indicate increased leakiness across the apical membrane itself as a result of damage to this component of the barrier.

Epithelial damage and failure of the bladder epithelial barrier occur quite promptly following exposure of the apical surface of the cells to antigen. Unlike other epithelia such as that of the bowel or airway, the bladder surface is composed of a single cell type, the umbrella transitional epithelial cell, and lacks specialized surface cells such as M cells that participate actively in processing of antigens (18). Under some circumstances it is possible that the umbrella cells may ingest and process antigens for presentation to immune cells, likely in the underlying muscular and lamina propria layers. Alternatively, the antigen may be transported across the umbrella cell layer and released for processing by immune cells in the underlying cell layers. Although the mechanisms underlying the processing of antigen in this model remain unclear, these mechanisms may play a prominent role in the symptomatology of interstitial and infectious cystitis.

In summary, the guinea pig urinary bladder exhibits strikingly low basal permeabilities to water and small nonelectrolytes such as urea. Exposure of the apical surface of this epithelium to an antigen to which the animal has been sensitized leads to rapid structural damage to apical umbrella cells and failure of the bladder epithelial permeability barrier. Future studies will define the process by which the barrier fails in this model and how it can recover.

These studies were supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant RO-1-DK-48217. Address for reprint requests: M. L. Zeidel, Laboratory of Epithelial Cell Biology, Renal-Electrolyte Division, Dept. of Medicine, Univ. of Pittsburgh Medical Center, Rm. 937 Scaife Hall, 3550 Terrace St., Pittsburgh, PA 15213.

Received 10 J July 1997; accepted in final form 4 September 1997.

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