

# Increased bladder permeability in interstitial cystitis/painful bladder syndrome

Robert E. Hurst<sup>1,2,3</sup>, Beverley Greenwood-Van Meerveld<sup>3,4</sup>, Amy B. Wisniewski<sup>1</sup>, Samuel VanGordon<sup>1</sup>, HsuehKung Lin<sup>1</sup>, Bradley P. Kropp<sup>1</sup>, Rheel A. Towner<sup>5</sup>

<sup>1</sup>Department of Urology, <sup>2</sup>Department of Biochemistry and Molecular Biology, <sup>3</sup>the Oklahoma Center for Neurosciences, <sup>4</sup>Department of Physiology, College of Medicine, Oklahoma University Health Sciences Center, Oklahoma City, OK 73104, USA; <sup>5</sup>the Advanced Magnetic Resonance Center, Oklahoma Medical Research Foundation, Oklahoma University Health Sciences Center, Oklahoma City, Ok 73104, USA

*Contributions:* (I) Conception and design: RE Hurst; (II) Administrative support: RE Hurst; (III) Provision of study materials or patients: RE Hurst, S VanGordon; (IV) Collection and assembly of data: RE Hurst; (V) Data analysis and interpretation: RE Hurst; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

*Correspondence to:* Dr. Robert E. Hurst, Department of Urology, College of Medicine, Oklahoma University Health Sciences Center, 940 S. L. Young Blvd, Oklahoma City, OK 73104, USA. Email: Robert-hurst@ouhsc.edu.

**Abstract:** The definition of interstitial cystitis (IC) has evolved over the years from being a well-defined entity characterized by diagnostic lesion (Hunner's ulcer) in the urothelium to a clinical diagnosis by exclusion [painful bladder syndrome (PBS)]. Although the etiology is unknown, a central theme has been an association with increased permeability of the bladder. This article reviews the evidence for increased permeability being important to the symptoms of interstitial cystitis/painful bladder syndrome (IC/PBS) and in treating the disorder. Recent work showing cross-communication among visceral organs is also reviewed to provide a basis for understanding IC/PBS as a systemic disorder of a complex, interconnected system consisting of the bladder, bowel and other organs, nerves, cytokine-responding cells and the nervous system.

**Keywords:** Cystitis; interstitial; irritable bowel syndrome (IBS); models; animal; permeability; urinary bladder

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## Introduction

The urinary bladder functions to collect urine and then to expel it under the voluntary control. It generally performs this function well until later life when problems often arise with voluntary control (incontinence), urgency/frequency [overactive bladder (OAB)] or urgency-frequency-pain [interstitial cystitis/painful bladder syndrome (IC/PBS)], although the latter can occur at any age (1). Urine contains a number of noxious substances that need to be kept out of the bladder tissue. Therefore, the bladder urothelium evolved to be the most impermeable membrane in the mammalian body (2). In this article we review the structure of the urothelium, particularly discussing how the structure

of the urothelium contributes to its unique function how it can fail in disease, and how loss of barrier function may be a major factor in bladder disorders. Our hypothesis is that the loss of impermeability of the bladder urothelium is not only responsible for the symptoms of pain and urgency but also is the trigger for degenerative changes often seen in the urothelium that may be irreversible. We also will show that the urothelium can become permeable both as the result of endogenous factors (i.e., neural modulation) as well as from failure of the bladder defenses against urine substances such as organic cations. Thus, instead of viewing the organs of the lower abdomen in isolation, they must be viewed as an interconnected system and that disorders of one organ may perturb the homeostasis of others.

## Structure of urothelium and its barrier function

### *Anatomical structure*

The urothelium consist of three layers of cells (2-4). Adjacent to the lamina propria is a layer of basal cells that likely contain stem cells with each stem cell being responsible for a monoclonal patch (5,6). Increasing knowledge of urothelial stem cells is identifying markers and the lineage of urothelium (7,8). Virtually all cell division is restricted to the basal layer. Above the basal cells is a layer of partially differentiated intermediate cells. Their function is to rapidly terminally differentiate when one of the highly specialized apical or umbrella cells is lost. This outer layer comprises the main protective barrier against urine. These cells are relatively long-lived (turnover more than 6 months) (9), so the normal level of mitosis in the healthy bladder is very low (10). In case of injury or infection, in which the apical cells typically slough off and carry infecting bacteria with them, the intermediate layer cells rapidly differentiate into apical layer cells and cell division is ramped up in the basal layer and replaces the intermediate layer cells that differentiated into apical layer cells (11). Even before the intermediate cell is fully differentiated into an umbrella cell, it forms tight junctions (12), which illustrates the importance of the urothelial barrier. When repairing damage, the normally quiescent urothelium expresses among the highest levels of cell division of any epithelium in the body (13).

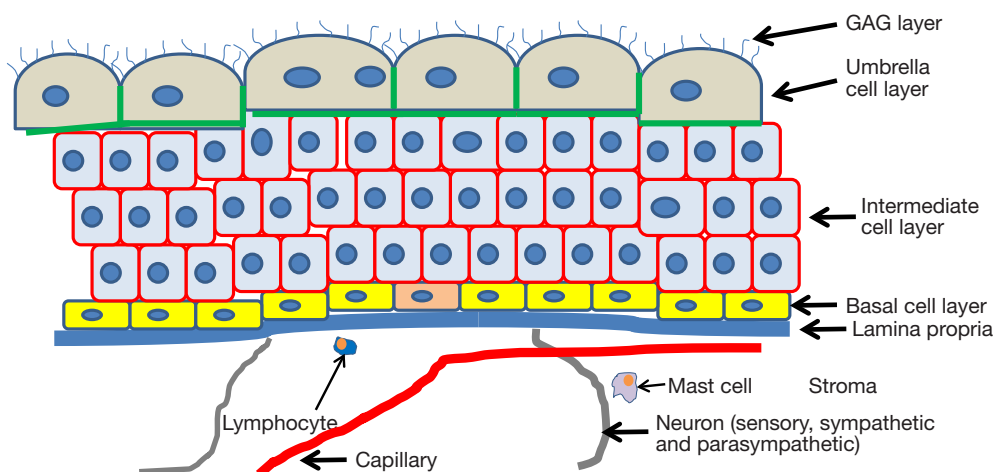
The apical cells are highly evolved for their function of providing an impermeable barrier to urine in the bladder lumen. The barrier function is comprised of multiple defensive molecules—tight junctions, uroplakin plaques, and a dense layer of glycosaminoglycan (GAG) on the apical surface (the “GAG layer”) The apical cells highly express tight junction proteins on their basolateral surfaces (12,14,15) that provide a barrier to urine passing between cells. The apical surface of the apical cells also is composed of plaques of uroplakins, which are tetraspannin proteins that form hydrophobic plaques on the cell surface (16,17). Loss of uroplakin in knockout mice increases the permeability about 2-fold (18). The urothelium also expresses a unique means for stretching and contracting. When contracted, small segments of membrane are removed from the cell surface and then stored in specialized vesicles, and when the bladder again is stretched, this stored membrane is reintegrated with the luminal surface (19).

*Figure 1* schematically illustrates the structure of

the bladder and illustrates the relationships among the urothelium and nerves and blood vessels. The urothelium itself is not enervated nor do capillaries penetrate within it. Therefore nutrients must diffuse across the lamina propria, a relatively large distance as compared to the vascularization observed in other tissues. Urothelial cells also have some very unique properties. They are both immune cells and also have characteristics of neurosensory cells in the form of receptors that are typically found in neural cells (20-22).

### *The GAG layer*

The apical surface is also densely coated with a layer of GAG that comprises a major component of the permeability barrier. This layer can be seen with Alcian blue staining (23) or by immunohistochemical staining for chondroitin sulfate (23,24). It has long been suggested that this GAG layer was substantially responsible for bladder impermeability (25,26). The GAG layer visualized by Alcian blue and immunochemical staining of bladder tissue for GAG layer components are illustrated in papers from our group (see figures in all three papers, all of which can be accessed from PubMed) (23,27,28). The presence of the GAG layer was demonstrated previously by Hurst and co-workers (28-32), who characterized its composition and demonstrated that its removal with dilute HCl, which causes loss of the apical cells within 24 hrs, leads to enhanced permeability to  $^{86}\text{Rb}^+$ , a  $\text{K}^+$  mimetic. Restoring the GAG with exogenous GAG (e.g., intravesical administration of chondroitin sulfate) restores impermeability to  $^{86}\text{Rb}^+$  to baseline levels (33) and also substantially inhibits the recruitment of inflammatory cells to permeabilized areas (23). The reason that excluding  $\text{K}^+$  is important is that one theory for the origin of pain in the bladder in interstitial cystitis (IC) is that penetration of bladder tissue by  $\text{K}^+$  depolarizes sensory nerves (34-36). This observation led to the development of the potassium sensitivity test (PST) in which 0.1 m KCl (but not 0.1 m NaCl) instilled into the bladder elicits a pain response in most IC patients (35) as well as about 40% of patients diagnosed with OAB (37). These earlier findings were completely confirmed recently by Janssen and co-workers, who also showed that without introducing any damage to the urothelium other than to digest the GAG layer with chondroitinase ABCase the permeability could be increased in a cell culture model (24). Recently our group has demonstrated that *in vivo* digestion of the GAG layer with chondroitinase ABC led to a decrease of the transepithelial



**Figure 1** Schematic illustration of bladder anatomy. The stroma is composed of the detrusor muscle, connective tissue, nerves, and the veins, arteries and capillaries of the connective system. The urothelium sits atop the lamina propria. The nerves, and capillaries do not penetrate the lamina propria but do connect with it. Therefore the bladder urothelium is less well supplied than are other epithelia. Also found are neurons, including the sensory, sympathetic and parasympathetic systems. These generally are found adjacent to the lamina propria, indicating the lamina may respond to neural signals. The basal layer is the lowest layer of cells. These are generally the only cells that divide. The stem cells, here one is shown in pink, also exist within the basal cell layer. Atop the basal cells is a layer of 3-5 cells deep comprised of intermediate cells. Not shown is that these cells maintain a connection via a cytoplasmic process to the lamina propria (not shown). Most likely this connection serves a cell communication function. The outer layer of cells, or umbrella cells, are highly specialized and terminally differentiated. They may be multinucleated as the result of cell fusion. Their lifetime is as much as 6 months or more. Loss of an umbrella cell immediately leads to the underlying intermediate cell to differentiate and replace the lost umbrella cell. The umbrella cells are coated with a dense layer of GAG, mostly if not exclusively, chondroitin sulfate. They also have tight junctions here shown in red along their basolateral surfaces. Together the uroplakin plaques (not shown) and GAG on the apical surface plus the tight junctions combine to make the urothelium the least permeable mammalian epithelium. GAG, glycosaminoglycan.

electrical resistance (TEER) as measured in the Ussing chamber. In control and sham treated rat bladders, the TEER measurements were means of  $2,524 \pm 1,117$  vs.  $2,623 \pm 1,124$  vs.  $1,175 \pm 518 \Omega\text{cm}^2$  and  $1,080 \pm 687 \Omega\text{cm}^2$  in the protamine sulfate-treated and chondroitinase-treated rat bladders ( $P=0.0016$  and  $P=0.0039$  respectively). Similar differences were seen in dextran permeability. Thus, treatment with organic cations and specific removal of the GAG layer both produce permeability.

## Loss of barrier function in human bladder disorders

### *IC and loss of barrier function*

IC is the disorder most closely associated with loss of bladder permeability. Although IC can appear at any age, it becomes more common in women, during middle age and

does not seem to vary with race or ethnicity (38). IC was initially thought to be quite rare following its description by Hunner (39). At that time there was an objective diagnostic criterion, namely the presence of Hunner's lesion or ulcer upon cystoscopy. As time went by and the disorder was investigated in more detail came the realization that a great many more patients were very similar to the classic description, but had more diffuse symptoms. In 1978 Messing and Stamey (40) introduced a new diagnostic criterion, namely the observation of petechial bleeding on hydrodistention. This was incorporated into the research criteria promulgated by the NIDDK in 1987 (41), but these rapidly became the clinical definition of the disorder in spite of the specific intent of the NIDDK that this not be the case. This conflict between the strict research definition that was intended to improve the power of clinical trials and a looser definition that seemed to be emerging in practice led to a

re-examination of the diagnostic criteria (42) and a further broadening of the definition such that the disorder is no longer rare (1). It is diagnosed by the triad of pain, urgency and frequency and is a diagnosis of exclusion (43-45), and no objective diagnostic criteria have stood the test. Although some investigators claim that objective criteria for diagnosis can be derived from histology (46-48), these have not proven specific or sensitive enough for diagnostic use. As a consequence, the syndrome is undoubtedly heterogeneous, which greatly complicates clinical trials because non-responders are automatically included in any clinical trial (49).

A number of investigators have reported changes in the urothelium that suggest loss of the barrier function may be a factor, at least in many patients. In the classic ulcer patients typically show a very thin urothelium that even erodes away in places, leaving ulcers that can be plainly seen by cystoscopy (43). In the more common non-ulcer form histopathologic changes, including loss of umbrella cells (28,46-48,50), which leads to loss of the GAG layer, have been reported. However, some patients show nearly normal-appearing urothelium (28,47,48). Whether this heterogeneity of loss of the GAG layer is a sampling difference, that is the loss of impermeability is focal and just was not sampled, or whether it is widespread in some bladders and those that do not show this loss represent a different class of patients is not known. However, a recent study in a feline model of IC by our group in which large sections of bladder were available for analysis showed considerable heterogeneity in the molecular pathology of the GAG layer, uroplakin and cell adhesion molecule distribution (51), suggesting that the changes reported in human IC biopsies may be focal. Wider sampling therefore might prove more useful diagnostically, but imposes a greater burden on patients. Several investigators have suggested an altered differentiation program in the IC urothelium could be responsible for these histopathologic changes (29,52-55). Although a number of papers have been published on urothelial differentiation (56-62), the flaw in IC is unknown. In recent years it has been recognized that IC may represent a manifestation of a wider problem called painful bladder syndrome (PBS) that likely is even more heterogeneous than IC itself (1,44,63,64). Evidence for a wider etiology is the observation that patients with IC have a roughly 70% comorbidity with irritable bowel syndrome (IBS). Recent work has shown a link between bowel and bladder such that dysfunction with one organ can lead to dysfunction in the other (65,66). This suggests that the

origins of the changes in the urothelium could be quite complex and could result from both neurally modulated mechanisms as well as from intravesical toxins.

Parsons and coworkers in 1983 suggested that a defect in the bladder barrier function was the root cause of IC symptoms (67). It certainly is an attractive theory because it both suggests a diagnostic criterion and therapy. Interestingly, although Parsons demonstrated in 1991 that IC patients absorbed a significantly higher amount of urea instilled into the bladder than did controls (68) the theory has remained controversial. Other, indirect evidence has supported the increased permeability of the bladders of IC patients. A high percentage of IC patients exhibit a positive potassium sensitivity test as compared to controls (43,69,70), and IC patients show a slower elimination of fluorescein administered intravenously, presumably due to resorption through the bladder (71). As discussed above considerable evidence from several laboratories suggests that the urothelium has adopted an aberrant differentiation program that could lead to loss of terminal differentiation of the apical cells or altered protein expression that could lead to loss of the barrier function with increased permeability. Clearly, this is an area for further research, given the heterogeneity within the IC/PBS population. The question of whether phenotypically these patients divide into "leakers" and "non-leakers" that may respond completely differently to various treatments is unresolved (72). In our laboratory we have recently shown (unpublished) that bladder permeability in rats can be measured directly by instilling fluorescein into the bladder and sampling a small volume every 2 minutes. This should be able to be performed on humans as well.

### *GAG replenishment therapy*

Given that we demonstrated a deficiency in the GAG layer in at least some IC patients and that exogenous GAG preferentially adheres to damaged urothelium (33,73), it is surprising that most clinical trials of GAG replacement show that although some patients clearly benefit and often have nearly complete remissions, other patients derive very little benefit (74-80). Clinical trials are often ambiguous because most are underpowered to detect an effect that occurs in only about 50% of the treated population. Similar observations apply to other therapies (81,82). GAG has been delivered orally as well as intravesically. The only GAG to be delivered orally is pentosan polysulfate (Elmiron), which is about

7,000 Da molecular weight and a few percent of the oral dose ends up in circulation, where it is apparently cleared into the urine. There are several possible explanations for the failure of GAG replenishment therapy to be more successful. One could be that the disorder is heterogeneous and comprised of “leakers” and “non-leakers”. A second is the disorder could be progressive, and the non-responders could represent an end stage where progressive damage has altered the bladder to the degree that it can no longer respond. Third, the optimal dosing regimen is incorrect and either the agent needs to be delivered more frequently or the dosing is too frequent and is producing side effects.

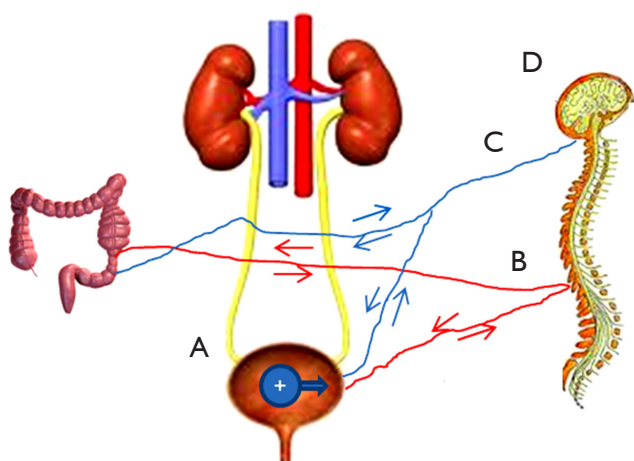
### ***Bladder-bowel intercommunication***

One of the more interesting recent developments in research into the clinical disorder of PBS/IC is the link between the bladder and the bowel. It has long been known that IBS shows a high comorbidity with IC/PBS, and a recent meta-analysis confirmed this finding, albeit with significant criticisms of experimental design (83). Other disorders with significant comorbidity include fibromyalgia and other generalized lower abdomen pain syndromes. Buffington attempted to identify a stress-related subset of patients (66) because stress was shown to be a major factor in feline IC (84). Whether the stress experienced by these patients is a product of the disorder or a causative factor in the disorder remains to be resolved. As is discussed above these findings have led some to suspect that IC actually represents a urologic manifestation of a more generalized pelvic pain syndrome and possibly whether there is a causative relationship between bowel and bladder symptoms. Supporting the latter hypothesis have been a number of recent papers demonstrating visceral organ crosstalk, as summarized in a recent review (85). The main question is whether the intercommunication arises from cellular communication by migratory cells such as mast cells or whether information is transmitted through neural communication and release of neurosecretory proteins that can alter one organ according to the status of another. There is evidence for both theories. Mast cells were implicated in a recent study that showed the cross-communication was not observed in *Kit*<sup>-/-</sup> mice lacking mast cells, but whether this was an effect of the loss of kit or of mast cells is unclear. Recent exciting work by Kevin Tracey and colleagues has shown that inflammatory

cells such as mast cells and macrophage respond to neural signals in a kind of neuroinflammation, the so-called inflammatory reflex (86-90). Interestingly, an anti-inflammatory network also has been identified (90,91). Up-regulation of innervation has been reported in IC (92), as upregulation of neuropilins and VEGF receptors.(93). This intercommunication is not restricted to IC and IBS. Constipation can worsen symptoms of OAB, and treatment of OAB with antimuscarinics can worsen constipation (85). Our group has recently shown that in rats, induction of bowel inflammation with trinitrobenzenesulfonic acid produces increased bladder permeability within 24 hrs as measured *ex vivo* in the Ussing chamber, and conversely, induction of bladder permeability with dilute protamine sulfate (which did not produce overt physical damage) resulted in increased bowel permeability (94). Moreover, the increased permeability was also detectable by magnetic resonance imaging (MRI), suggesting the technique could be used clinically to stratify patients according to permeability and to monitor response to therapy (95).

### ***Directions of future research and summary***

*Figure 2* summarizes the mechanisms that could produce bladder permeability. It should be obvious from the figure and discussion that the bladder, bowel (and possibly other organs as well), cytokine-responding and secreting cells, and the neuroendocrine system form a complex and interacting system that can no longer be considered as individual parts in isolation. Clearly one of the most pressing clinical needs is to better be able to classify patients. Finding effective treatments would be greatly improved if patients could be stratified into more homogeneous groups for clinical trials. An effective method of measuring bladder permeability could also represent an important step forward in this regard because it could offer an improved diagnostic as well as an objective measure of response to therapy that could be used to optimize therapies. Our group has recently shown that MRI can clearly demonstrate increased bladder permeability that correlates with patient condition (96). The mechanisms by which information concerning the status of one pelvic organ is communicated to another, and how this information affects the other organ is critical to know and is being actively investigated by a number of groups. Finally, understanding the role of the brain, stress, and past life experiences also needs to be investigated.



**Figure 2** Schematic of mechanisms producing increased bladder permeability. (A) Organic cations in the urine neutralize the GAG layer producing increased permeability. This occurs because they are present in abnormally high amounts or Tamm-Horsfall protein, the anionic cation scavenger in urine is defective with missing glycosylation. The increased permeability allows potassium ions and other molecules to pass into the urothelium or deeper, causing irritation, triggering inflammatory cells, and sending sensory signals. Upregulation of sensory fibers occurs; (B) the sensory signaling from the bladder affects other tissues as well as the bladder to cause increased bowel permeability. Increased bowel permeability from causes within the bowel (e.g., IBS) also signals back to the bladder causing increased bladder permeability by unknown mechanisms. Communication is through the dorsal root ganglia. The pain signal is also transmitted to the brain where chronic pain can rewire the brain; (C) signaling through the vagus nerve releases neurotransmitters and cytokines that activate mast cells and macrophages that then increase bladder permeability; (D) long-term effects on the brain, such as early life stress may potentiate some individuals toward IC/PBS by mechanisms that are not well understood. GAG, glycosaminoglycan; IBS, irritable bowel syndrome; IC/PBS, interstitial cystitis/painful bladder syndrome.

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### Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

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