

DIRECT OBSERVATION OF TOAD BLADDER RESPONSE TO VASOPRESSIN

JARED GRANTHAM, F. E. CUPPAGE, and DARRELL FANESTIL. From the Departments of Medicine and Pathology, University of Kansas Medical Center, Kansas City, Kansas 66103

It has been suggested that the lateral intercellular space is a pathway for transepithelial water flow in several tissues. Fundamental to this postulate is the observation that coincident with accelerated net fluid transport, either coupled to active electrolyte transport as in the gallbladder (1, 2), or due to passive water flow along osmotic gradients as in the collecting tubule (3, 4) and anuran membranes (5, 6), the lateral intercellular spaces widen. To correlate morphologic changes with fluid transport the epithelium is usually fixed with aldehydes or osmium tetroxide in preparation for electron microscopy. There has been no uniformity in the manner of applying the different fixative solutions to the different tissues. Water translocations may occur during the period of primary fixation, resulting in distortions of the lateral intercellular spaces unrelated to the physiologic function of the viable tissue (7). The problem of fixation artifacts, specifically with regard to changes in the size of the intercellular channels, can be obviated by observing the effect of hormone on the living tissue directly (3, 4). In the present report we describe a simple method to observe directly the effect of accelerated hydrosmotic flow on the size of intercellular spaces in the isolated bladder of the toad, *Bufo marinus*.

METHODS AND DISCUSSION

Both lobes of a toad's urinary bladder were removed and washed in isotonic toad Ringer's solution. The composition of the medium is described elsewhere (8). To reduce muscular contraction during photography, atropine sulfate (0.05 mg/100 ml) was added to the serosal medium. In two studies atropine was not used and, although photographs could not be taken, the morphologic responses to experimental manipulations were identical to those when atropine was present. Each hemibladder (*T*) was everted over the end of a glass tube and secured with rubber bands as illustrated in Fig. 1. The glass tube (*G*) was 8 cm long, 0.7 cm outer diameter, 0.5 cm inner diameter. The mounted bladder was fastened above the stage (*MS*) of a Unitron inverted light microscope by attachment to an arrangement per-

mitting vertical adjustment of the specimen. The bladder was lowered into a cylindrical chamber (*C*) 2.7 cm in diameter with side walls extending 4.5 cm above the stage of the microscope. The chamber contained the mucosal bathing medium. The floor of the mucosal chamber was a glass cover slip (*CS*) 80 μ thick. The mucosal surface of the everted bladder was positioned approximately 20 μ above the glass cover slip. The light source (*L*) was a high-intensity lamp bulb 20 cm above the mucosal surface. An opalized glass diffusion disk (*D*) was placed between the light source and the bladder, 8 cm above the latter. Thus, diffused light was transmitted down the lumen of the supporting glass tubing to the specimen. With the oil immersion objective the depth of focus is only 1–2 μ with diffused light illumination. At the microscope we could adjust the focus and identify the outlines of individual cells in the mucosal layer in the control periods. However, owing to the thinness and uneven projection of cells from the mucosal surface, and close apposition of lateral cell membranes, it was not possible to obtain photographs in which the outlines of several individual cells could be readily identified in one field. As will be discussed later, after interventions which increased the size

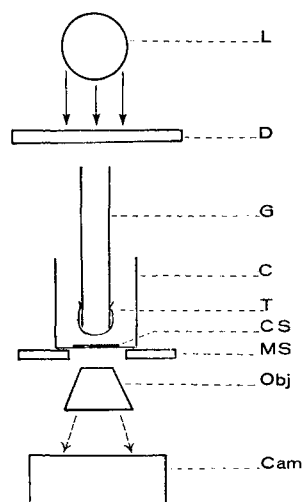


FIGURE 1 Arrangement for viewing mucosal surface of toad bladder. See text for explanation.

of both cells and lateral intercellular spaces, the cells and intercellular regions could be seen readily in photographs as well as at the microscope.

With this arrangement the media bathing the serosal and mucosal surfaces could be oxygenated and the hydrostatic pressure difference across the tissue could be regulated. An experiment was begun with isotonic toad Ringer's solution bathing both sides of the bladder. The fluid level of the serosal medium was kept 0.2–0.4 cm higher than the level of the medium on the mucosal side to cause the bladder to bulge slightly below the rim of the supporting glass tubing and, thereby, to facilitate use of the short working distance oil-immersion objective (Obj). After 30 min in isotonic Ringer's solution the hydrostatic pressure of the serosal side was raised 5 cm of water above that of the mucosal side. In obviously unhealthy bladders, e.g. those infested with parasites or adherent

to the peritoneal cavity by scar tissue, raising the serosal pressure 5 cm of water frequently caused the mucosal cells to become separated from the underlying basement membrane; thus, only bladders were used in which 5 cm of water pressure in the serosal side had no detectable effect on morphology.

We first observed and photographed the epithelial surface in the absence of vasopressin with isotonic media on both sides. The intercellular spaces were not expanded and the individual mucosal cells were faintly outlined (Fig. 2 [control] and Fig. 3, *a* and *d*). In eight experiments vasopressin (Pitressin [Park Davis and Company, Detroit, Mich.], final concentration 100 milliunits/ml) was added to the serosal medium. Within 30 min, in some of the studies, the granular cells appeared swollen; however, this impression could not be quantified. There was no change in the intercellular markings in response to vasopressin in isotonic media even when the serosal pressure was raised 5 cm of water (Fig. 3, *b* and *e*). However, when the mucosal medium of hormone-treated bladders was diluted one-fifth with distilled water, striking changes in tissue morphology could be seen (Figs. 2 and 3). Individual cells were identified more easily and there was widening of the intercellular spaces. The size of the intercellular spaces increased greatly within a few seconds after the serosal pressure was raised 5 cm of water (Fig. 2 and Fig. 3, *c* and *f*). In some tissues (Fig. 2, upper diagram, and Fig. 3 *c*) there was enormous distention of the lateral intercellular spaces, the width of the channels often exceeding the diameter of the cells. In other bladders, the dilation of intercellular spaces was less pronounced but could easily be detected as shown in the lower diagram of Fig. 2 and in Fig. 3 *f*.

As shown previously in isolated collecting tubules (3, 4) widened intercellular spaces viewed from the apical surface appear as clear vacuoles between the cells. It is not possible to identify the apical tight junctions owing to the fact that the apical attachments are thinned and transparent. Thus, in the toad bladder we assume that the swollen lateral channels do not communicate directly with the mucosal bathing medium.

When the isotonic mucosal medium was diluted one-fifth in the absence of hormone there was no visible effect on the tissue; in the latter instance the intercellular spaces widened only after vasopressin was added. When the mucosal hydrostatic pressure

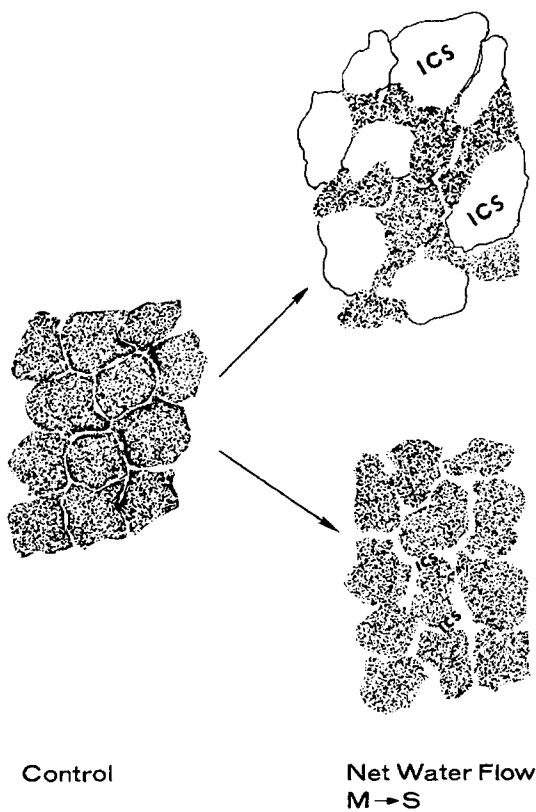


FIGURE 2 Schematic representation of effect of increased net water flow (mucosa to serosa) on morphology of toad bladders viewed from the apical side. ICS, intercellular space. The cells are stippled. See text for explanation.

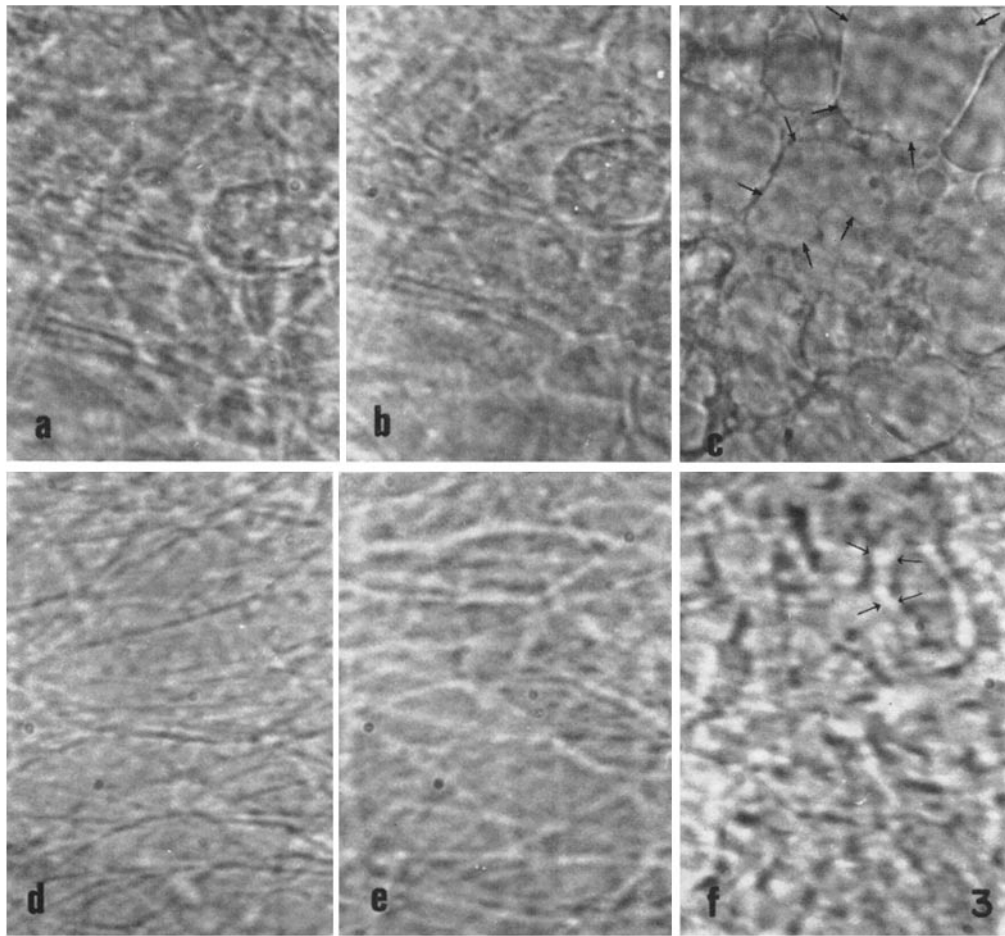


FIGURE 3 Effect of vasopressin on morphology of urinary surface viewed from the apical side. Results from two bladders are shown. All photographs were taken with the serosal pressure 5 cm. H_2O greater than the mucosal side. Focus is on the apical surface in each case. (*a* and *d*) Appearance of each bladder in the absence of hormone. Serosal and mucosal media, isotonic toad Ringer's solution. Individual cells are difficult to identify in these photographs owing to long strands of collagen and muscle fibers in the submucosa. At the microscope, individual cells were readily seen and the intercellular spaces were not dilated. (*b* and *e*) Appearance 30 min after treatment with vasopressin. There is no change. (*c* and *f*) Appearance of hormone-treated tissue a few minutes after diluting the mucosal solution one-fifth with distilled water. The intercellular spaces are widely dilated (arrows). The cells are crowded by the dilated spaces and frequently appear as irregularly shaped islands surrounded by dilated intercellular spaces (note *f*, the body of a cell surrounded by dilated spaces lies under the two arrows on the right). $\times 813$.

was increased 4 cm greater than that on the serosal side, the width of the intercellular spaces decreased but the channel never disappeared completely.

Previously, only bladders fixed in aldehydes and osmium tetroxide had been used to study the relation between osmotic water flow and the width of the lateral channels. We evaluated the effects of fixation on morphology. The serosal and mucosal

media were initially isotonic. Vasopressin was added to the serosal fluid, and 30 min later glutaraldehyde (Fisher Scientific Company, Pittsburgh, Pa., final concentration 1%) was added to the serosal medium only. Immediately, the individual cells could be seen more distinctly and, when the serosal hydrostatic pressure had been raised 5 cm of water coincident with fixation, the

intercellular spaces were dilated. Apparently glutaraldehyde, by virtue of its osmotic effect (1% approximately equal to 110 milliosmols/kg), induces sufficient mucosal to serosal water flow in hormone-treated, permeable bladders to cause the intercellular spaces to widen before the lateral membranes actually become rigid.

The effect on net water absorption of adding glutaraldehyde to the serosal medium in hormone-treated bladders was also tested. Net water absorption in hemibladder sacs was determined gravimetrically. In the absence of fixative vasopressin caused the expected increase in water absorption when the mucosal medium was one-fifth toad Ringer's solution, mean change $25.6 \mu\text{l}/\text{min}$. The mucosal solution was then changed to isotonic Ringer's solution and net absorption decreased to low levels ($1.6 \pm 1.2 \text{ SD } \mu\text{l}/\text{min}$). Addition of glutaraldehyde to the serosal medium of the hormone-treated bladder caused a sustained increase in mucosal to serosal water flux ($5.2 \mu\text{l}/\text{min} \pm 0.9 \text{ SE}$, in six bladders). With 2% glutaraldehyde the mean change was $10.3 \pm 1.0 \mu\text{l}/\text{min}$. When 1% glutaraldehyde was added to both sides of the permeable bladder simultaneously, there was no change in net water flux (-1.3 ± 1.1 , three bladders). It is notable that hormone-treated bladders fixed in glutaraldehyde were still highly permeable to water 24 hr later, thus confirming the studies of Jard et al. (9) who showed that the permeability to water of the viable tissue was essentially unchanged after fixation in glutaraldehyde.

In view of the present results, it is clear that in phase-contrast and electron micrograph studies of fixed toad bladder the presence and size of dilated intercellular spaces may be due to the method of fixation and transbladder hydrostatic pressure differences, rather than to the magnitude of transepithelial water flow in the viable tissue. Further, DiBona and Civan (10, 11) report that the degree of relaxation or stretch of toad bladders mounted in chambers may influence the change in size of intercellular spaces in response to smooth muscle relaxants or stimulants, including vasopressin. The conclusions of DiBona and Civan lack certainty, however, since they were adduced from phase-contrast and electron micrographs of bladders fixed by adding glutaraldehyde to the serosal side only. Pertinent in this regard is the report by Parisi et al. (6) that mucosal epithelial layers, isolated from frog bladders, develop widened

intercellular spaces in response to the hydrosmotic effect of oxytocin, an effect that could not be due to submucosal muscles.

In the present studies, when the stretch and transbladder pressure were controlled and no fixatives were used, the size of the intercellular spaces increased in response to vasopressin only when mucosal to serosal water flow was increased, indicating that the lateral channel is a transport path for transepithelial water flow. In the context of the present direct observations, it is suggested that in permeable bladders water flows into the cell across the apical membrane, and from the cell into the lateral channels by osmosis. Fluid is expressed from the lateral channels owing to a difference in hydrostatic pressure between the intercellular space and the serosal (blood side) medium. When the hydrostatic pressure of the serosal medium is raised, as in the present study, the osmotically transported fluid accumulates in the intercellular space. The intercellular space enlarges until the intercellular pressure is greater than that on the serosal side of the tissue. Thus, under the present conditions of study, the steady state volume of the intercellular spaces is dependent on the amount of osmotically transported fluid, the difference in hydrostatic pressure between the cells and the intercellular spaces, and the mechanical properties of the lateral plasma membranes (12).

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