THE ANATOMIC SITE OF THE TRANSEPITHELIAL PERMEABILITY BARRIERS OF TOAD BLADDER

DONALD R. DiBONA, MORTIMER M. CIVAN, and ALEXANDER LEAF

From the Departments of Medicine, the Massachusetts General Hospital, and Harvard Medical School, Boston, Massachusetts 02114

ABSTRACT

An examination of the mucosal epithelium of the urinary bladder of the toad reveals that the two major cell types which abut on the urinary surface, the granular and mitochondria-rich cells, also contact the basement membrane. Thus, the epithelium functions as a single cell layer. Although basal cells are interpolated between the granular cells and the basement membrane over a large portion of the epithelium, they do not constitute an additional continuous cell layer. This finding is consistent with extensive physiological data which had assumed that the major permeability barriers of this epithelium were the apical and basal-lateral plasma membranes of a single layer of cells.

INTRODUCTION

The urinary bladder of the toad has been intensively studied because of the interesting biological transport characteristics of this epithelium. The cells responsible for these transport phenomena are thought to reside in the mucosa since the serosal mesothelium constitutes a negligible resistance to either transepithelial current (7) or to ionic movement (8). The thinness of the mucosal layer of the toad bladder suggested to physiologists that this epithelium consisted of a single layer of cells. On the basis of this assumption, a considerable body of physiological data has been interpreted. Thus, the transport of water, sodium, and other solutes has usually been considered in terms of a three-compartment system, the substances crossing the apical and basal cell membranes with some distribution in the intracellular compartment of the mucosal epithelia cells.

The active transport of sodium has been described in terms of a double barrier hypothesis—a passive entry across the apical plasma membrane and an active extrusion across the basal cell membrane. The action of two of the important hormones affecting sodium transport has been investigated in terms of effects on these two sites (10).

The only evidence to date that has been convincingly in favor of a two-barrier model has been electrophysiological, i.e., penetration of this epithelial layer with Ling-Gerard micropipettes reveals a two-step potential profile generally ascribed to the high resistance of the two opposite cell membranes of a single layer of cells

(3, 7). But even here, the data could be consistent with the presence of two distinct and complete cell layers within the mucosal epithelium. It has seemed desirable to correlate these physiological data with the morphology of the tissue since previous anatomic studies present no consensus on this basic point (1, 2, 5, 13).

The present study demonstrates that all mucosal cells which abut on the mucosal surface have at least some contact with the basement membrane. Thus, the epithelium can be morphologically as well as functionally considered a single layer of cells. A preliminary report of these results has appeared elsewhere (6).

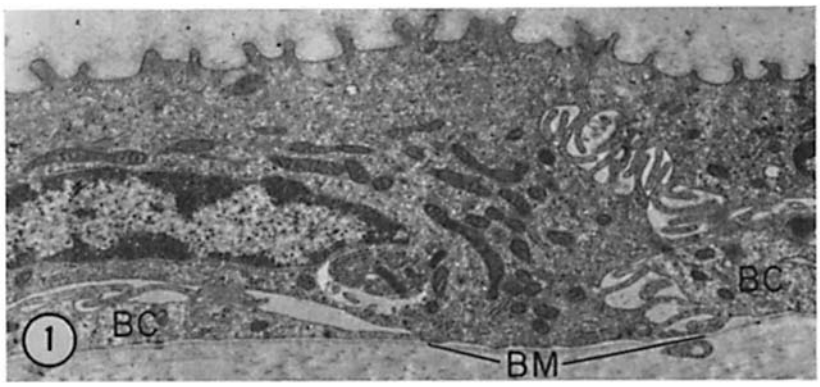

FIGURE 1 Granular cell in contact with the basement membrane (*BM*) over a broad region. The lack of convolution in the basement membrane (compare with Fig. 3 *a*) indicates a high degree of stretch in this preparation. Mitochondria (*m*) and basal cells (*BC*) are readily identified. × 11,500.

METHODS

Female specimens of the toad, *Bufo marinus*, (obtained from the Dominican Republic (National Reagents Inc., Bridgeport, Conn.) were kept on moist earth at room temperature after forced-feeding of meal worms upon arrival. The urinary hemibladders were excised from the doubly-pithed toads, rinsed in Ringer solution, and mounted in a Lucite double chamber (14). Both surfaces were bathed with circulated, aerated Ringer solution of the following composition: Na, 113.4 mM; K, 3.5 mM; Ca, 0.9 mM; Cl, 116.3 mM; HCO_3, 2.4 mM; pH, 8.0; and tonicity, 220 mosm/kg H_2O. After monitoring of the spontaneous transepithelial potential difference and short-circuit current by techniques previously described (4), the preparations were fixed in vitro by adding 50% glutaraldehyde (Fisher Scientific Company, Pittsburgh, Pa.) to the serosal medium to a final concentration of 1%. After a 30-min. fixation, rectangular areas were excised from the bladder, excluding material within 3 mm from the compressed edges. These specimens were fixed for an additional 30 min at room temperature in 0.1 M potassium phosphate buffer, pH 7.4, containing 1% glutaraldehyde. The tissues were subsequently rinsed three times over a 1-hr period in 0.1 M potassium phosphate buffer (not containing glutaraldehyde) before immersion into a 1% osmium tetroxide solution of the same buffer. After a 30-min post fixation the tissue was dehydrated through a graded series of aqueous ethanol solutions. Propylene oxide was used for the tissue transfer to the Araldite-Epon 812-DER-732 embedding mixture, in which the tissue was infiltrated overnight. The embedding material consisted of Araldite 502, Epon 812, DER-732 (Dow Chemical Company, Midland, Mich.), DDSA, NMA, DMP-30, in the proportions, 4.5:4.5:2.0:10.0:2.0:0.5, respectively. Tissues were embedded with fresh resin in flat molds (13) to insure likelihood of nearly perfect cross-sections, and were hardened over an 8-hr period at 75°C. Sections were cut with an Om U2 ultramicrotome (C. Reichert Optische Werke A. G., Vienna, Austria) with diamond knives (E. I. duPont de Nemours and Company, Inc., Wilmington, Del.). Sections were chosen to exhibit gray-silver interference colors, corresponding to about 400–750 A, and were mounted on naked 300-mesh copper grids. Sections were stained with uranyl acetate, lead citrate, or both. Serial sections were obtained for study by mounting sets of six consecutive sections on 150-mesh formvar-coated copper grids of 0.58 fractional open area and were stained similarly. Since the serial sections gave a silver interference pattern (750–1000 A), each grid contained a sectioned length of 0.45–0.60 μ. Microscopy was per-

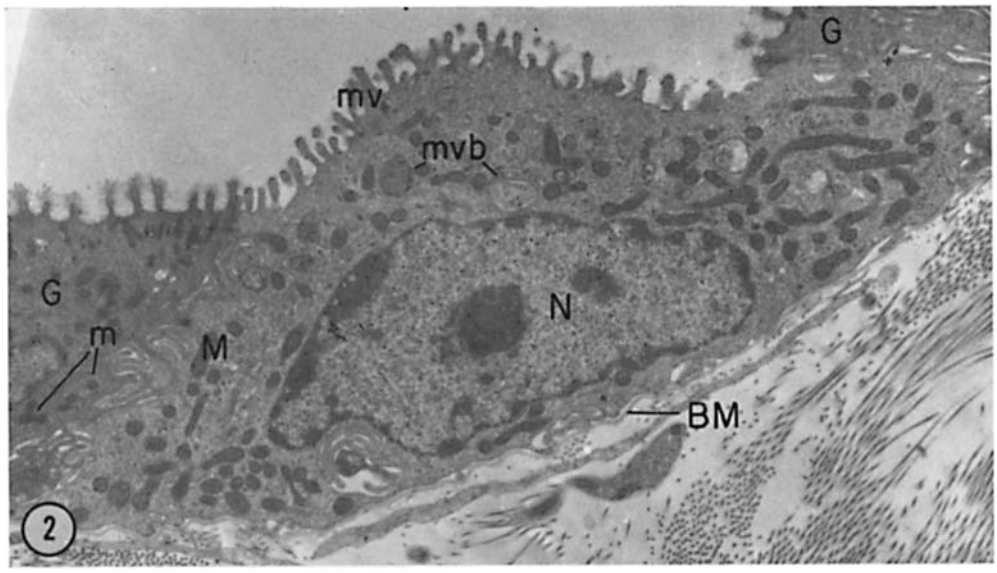

FIGURE 2 Mitochondria-rich cell (*M*). The irregular microvilli (*mv*) and the multivesicular bodies (*mvb*) typical for this species are evident. Note the very extensive region of contact between this cell and the basement membrane (*BM*). *G*, granular cells; *m*, mitochondria. × 8900.

formed with a Philips EM-200 electron microscope at 1,390–71,000 magnifications.

RESULTS

Examination of sections selected at random has confirmed the presence of four cell types in the mucosal epithelium. The names assigned to them by Choi (5), i.e. granular, mitochondria-rich, mucous (or goblet), and basal cells, will be retained here.

Goblet cells are irregularly distributed throughout the mucosa, occasionally in clusters of four or five. In view of the established function of these cells to secrete mucus onto the urinary surface, this cell type is unlikely to be involved in the transport of sodium and water from urinary to serosal surface and was, therefore, not studied in great detail.

Basal cells occupy the greater portion of the basement membrane face showing many points of attachment in the form of hemidesmosomes (12). It is this cell type that has been suggested as the second functional layer of cells (2).

Our findings are in general agreement with previous estimates (5, 9) that the mitochondria-rich cells constitute roughly 10% of the mucosal cell population. These cells are characteristically flask-shaped, and in random view are easily seen to extend from urinary surface to basement membrane (Fig. 2).

More than 90% of the urinary surface of the epithelium is occupied by granular cells while regions of contact[1] with basement membrane by these cells were not in all instances readily seen. However, in each of 13 preparations where evidence of contact was sought it was found to be present. In Fig. 1, there is a broad contact while in Figs. 3 *a* and *b* the contact is much more limited. It was for this reason that serial sectioning was undertaken, so as to determine if all cells bridged the space from urinary surface to basement membrane.

A region of bladder mucosa, approximately ten cells wide, was chosen for serial sectioning, on the basis that a random section demonstrated no granular cell contact with the basement membrane. The results of the serial sectioning are summarized in the schematic drawing (Fig. 4). Each of the six granular cells which could be adequately followed contacted both the urinary

[1] Contact in this sense means that no process of another cell type was interposed between the limiting plasma membrane of the granular cell and the basement membrane.

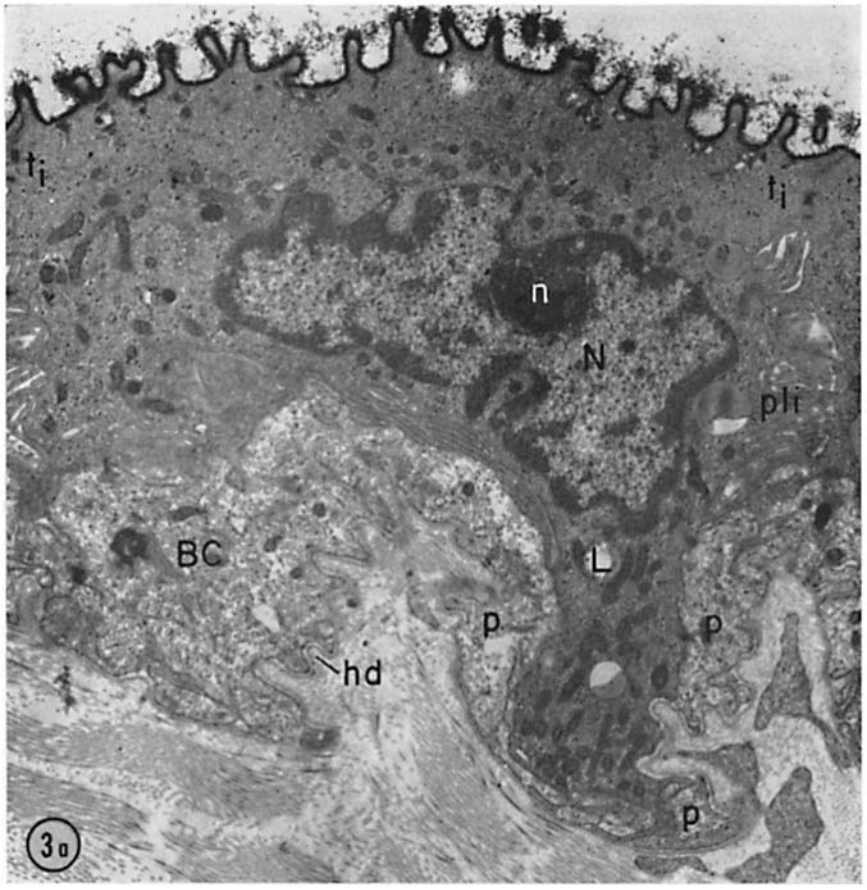

FIGURE 3 *a* Granular cell from region of slightly stretched mucosa. This figure illustrates many of the features that characterize this cell type: the highly lobed nucleus (*N*), the dense nucleolus (*n*), the plications of the lateral margins (*pli*), typical tight junctional complexes (*tj*), occasional lipid droplets (*L*). Basal cells (BC) and their tortuous processes (*p*) are indicated; here the close investment of the processes is demonstrated about a region in which the granular cell reaches the basement membrane. Half-desmosomes (*hd*) or hemi-desmosomes are readily seen and suggest an anchoring of the basal cell serosal surface to the basement membrane. × 11,000.

surface and the basement membrane. Consideration of the resulting profiles of those granular cells which do not show contact with the basement membrane suggests that each of them was initially sectioned beyond a region of contact or that contact occurred beyond the length of tissue sectioned. The serial sections indicate that the granular cells of the preparations studied were funnel-shaped with a broad mouth at the urinary surface and a comparatively small, nonplicated region of plasma membrane in contact with the basement membrane.

DISCUSSION

The purpose of this study was to learn if the assumption of a single layer of cells comprising a double permeability barrier, a basic tenet for many physiological studies, did, in fact, have morphological substance. The presence of basal cells, noted by others clearly not to extend to the urinary surface, raised the question of a multilayered epithelium. Physiological interpretations of data in terms of a three-compartment system would have required revision if there existed in fact a multilayered structure.

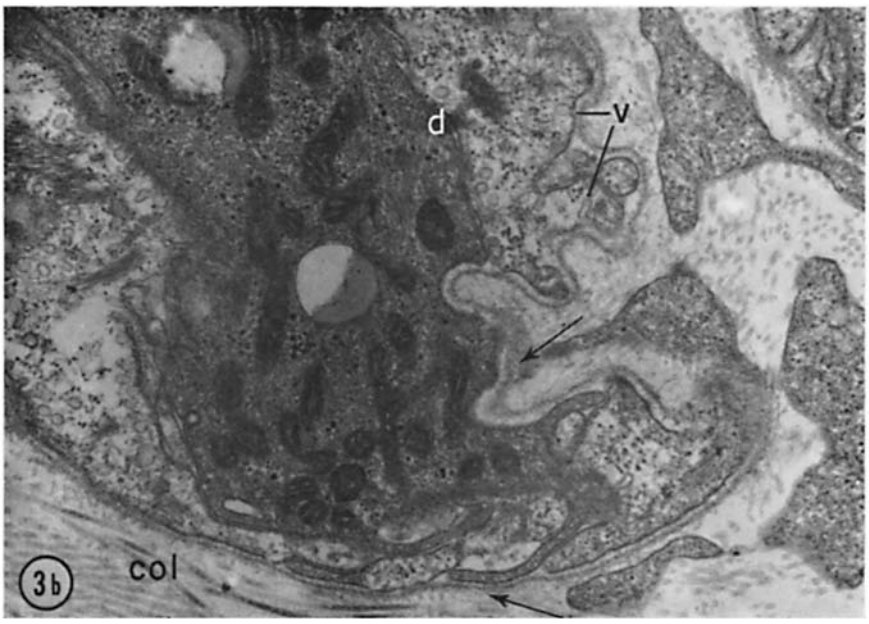

FIGURE 3 *b* Enlargement of the lower region of Fig. 3 *a*, showing the region of contact between granular cell and basement membrane. Large arrowheads point to the two regions in which this contact is visible. Note that the granular cell processes contacting the basement membrane are closely apposed by processes from the neighboring basal cell. The desmosomal connection (*d*) between granular and basal cell is demonstrated as are the vesicles (*v*) of the basal cell processes. Collagen fibers with typical striations (*col*) are seen in the submucosa. × 18,000.

The present study supports the view that the major cell types abutting on the urinary surface (granular and mitochondria-rich) make contact with the basement membrane.

The basal cells interpolated between the mucosal cells and basement membrane in many areas would appear to be developing cells not yet in contact with the urinary surface. Since they comprise a discontinuous layer, their presence does not vitiate the conclusion from this study that the epithelium in effect is a single cell layer.

Prior to this study, the most convincing argument for a single cell layer was the two-step potential and resistance profiles usually observed as glass micropipettes were advanced through the tissue. Since the cytoplasm of cells in general and these cells in particular (11) is known to be of low electrical resistance, it had been assumed that the anatomic sites of these two resistance barriers were the apical and basal plasma membranes of a single cell layer. The fact that the mucosal cells often appear cone-shaped with the apex at the basement membrane is consistent with a two-step potential profile. If the junction between adjacent cells is of relatively high resistance, the apical membrane and the combined lateral and basal membranes would constitute a double resistance barrier

These morphological observations are consistent with a "passive" barrier localized at the apical surface and an active transport mechanism for sodium transfer located at the lateral and basal cell membranes. This model permits transepithelial transport to be regulated by agents which modify one or both barriers. The current evidence strongly supports an action of vasopressin localized largely or entirely at the apical membrane (3).

Separate functions have been attributed to the granular and mitochondria-rich cells, but present evidence dose not permit specific functions to be definitely attributed to either cell type.

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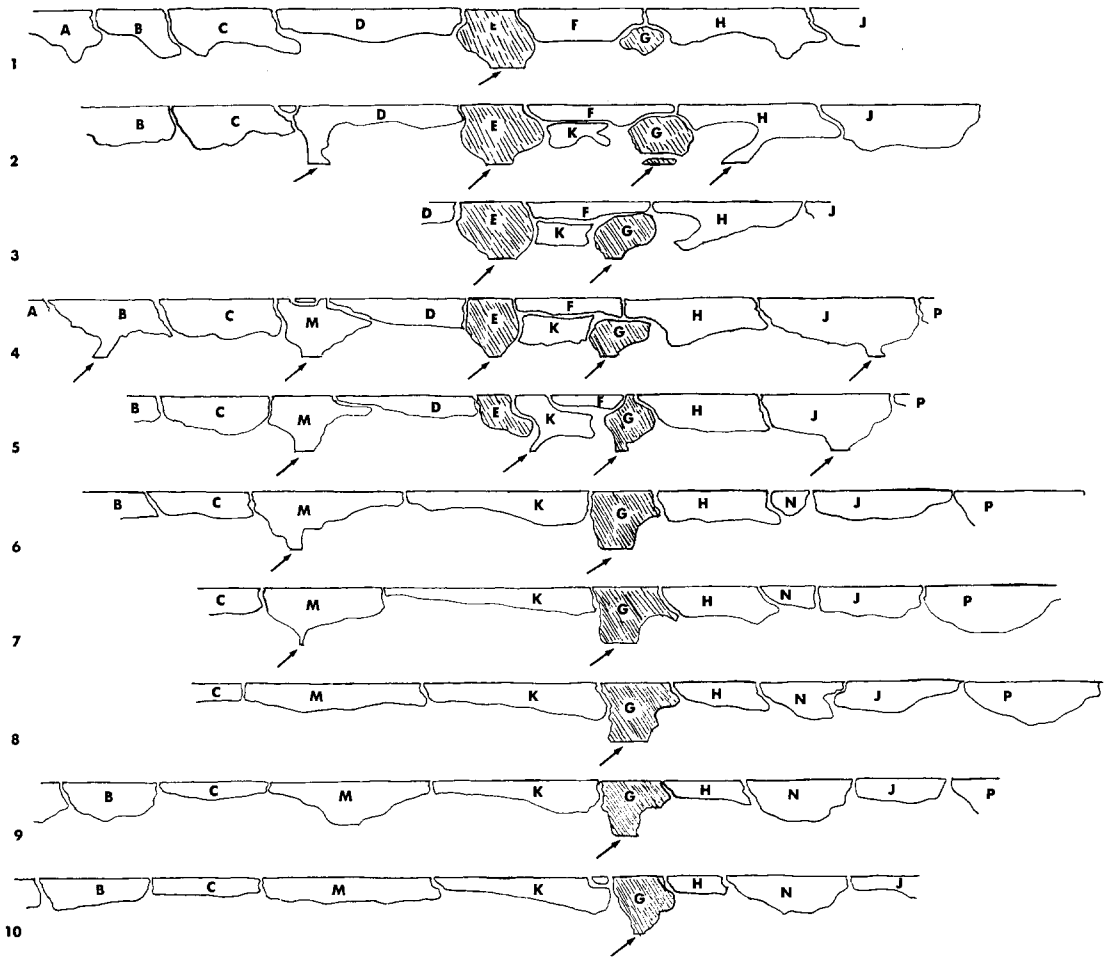

FIGURE 4 Schematic drawing to illustrate the results of serial sectioning. Only the outlines of granular (clear) and mitochondria-rich cells (cross-hatched) are shown; basal cells omitted for clarity, occupied space between granular cells and basement membrane. The basement membrane is indicated by arrows at those places at which a cell makes contact. (Contact regions are drawn as flattened margins). The natural convolutions of the bladder are not included in the figure which is, therefore, only approximately accurate to scale. The mucosal margin of cell *E* (in the first view) measures about 5 μ and the average separation between adjacent views is of the order of $\frac{1}{2}$ μ. Note that cells *E* and *G* (mitochondria-rich) make contact with the basement membrane over a fairly broad region. This is as expected from the frequency with which basement membrane contacts are found in randomly selected sections. Of the granular cells shown, cells *B, D, H, J, K,* and *M* are seen to make contact. The other granular cells are incompletely followed. Cells *A* and *P* are seen only rarely and are included simply to delineate the field; cell *C* was missed, unfortunately, in sections *3, 6, 7*; cells *F* and *N* are seen only at their peripheries; cell *F* is terminating as the sequence begins; cell *N* is only beginning to appear as the sequence ends. ca. × 6,000.

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