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Epithelial Cell Electrolytes in Relation to Transepithelial Sodium Transport across Toad Urinary Bladder

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Summary. Aspects of the relationships between cellular composition and transepithelial sodium transport across toad urinary bladder are reviewed. Changes in cellular sodium produced by amiloride, vasopressin, aldosterone, hypoxia, ouabain, and sodium-free media are consistent with a cellular sodium transport pool. Metabolic studies suggest that this pool gains its sodium from the mucosal medium and that there is little recycling of sodium between cell and serosal medium. One-third of the cellular potassium equilibrates readily with serosal potassium. The rate of exchange of potassium is much less than the rate of sodium transport supporting the contention that sodium transport in this tissue is electrogenic. Studies with ^{36}Cl suggest that chloride does not cross the apical cellular membranes, but exchanges with serosal chloride. Possible relationships between transepithelial sodium transport and cellular volume regulation are discussed.

The pioneering studies of Ussing and his collaborators [23, 24] laid the foundations for our understanding of ion movements across epithelia. They explained the electrical activity across transporting epithelia in terms of specific ion fluxes and demonstrated the equality of the short-circuit current and net transepithelial sodium transport across frog skin [24]. Since this work, many electrophysiological investigations have similarly characterized a wide variety of epithelia in terms of their ion transport.

Koefoed-Johnsen and Ussing [7] described the frog skin in terms of three compartments: the two media bathing the outer and inner surface and a cellular compartment separated, respectively, from the outer medium by the apical cell membrane and from the inner medium by the basolateral membrane. Much important information about ion transport has been obtained from studies which essentially disregard the composition of the cellular compartment. However, a full description of transepi-

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Table 1. Summary of experimental methods

1. Toad pithed.
2. Heart perfused with Na Ringer's until hemibladders bloodless.
3. Each hemibladder removed and mounted as a sheet in Ussing-type chamber.
4. Open-circuit PD measured, then hemibladders short-circuited, and incubated under appropriate experimental conditions.
5. After incubation, hemibladders removed, and blotted.
6. Tissue placed on glass petri dish, mucosal surface uppermost, and epithelial cells removed by scraping with glass slide.
7. Scrapings transferred to pyrex tubes.
8. H_2O content determined gravimetrically.
9. Dried scrapings extracted with 0.1 M HNO_3.
10. Extract analyzed for Na, K, Cl, and radioactive inulin.
11. Tissue values corrected for extracellular medium and expressed in terms of cellular content/kg dry wt.

thelial ion transport requires an understanding of the relationships between this transport and cellular composition. We have been engaged in a study of these relationships, using the toad bladder as a model epithelium and analyzing the composition of the epithelial cells under a variety of conditions which modify transepithelial sodium transport. Four aspects of these studies will be discussed: (i) the sodium transport pool in the epithelial cells, (ii) the relationship of transepithelial sodium transport to potassium uptake by the cells, (iii) the contributions of mucosal and serosal medium chloride to cellular chloride, and (iv) relationships between transepithelial sodium transport and the regulation of cellular volume.

The methods used have been presented in detail elsewhere [12, 14] and are summarized in Table 1. It is important to note that the epithelial cells are removed from hemibladders incubated in chambers and are then immediately analyzed. They are not incubated further. Therefore, their composition will be essentially that attained in the chambers.

1. The Sodium Transport Pool

The sodium transport pool consists of cellular sodium involved in transepithelial sodium transport. In toad bladder epithelial cells sodium exchanges completely with medium sodium within 30 min. Of this sodium, 20–25% comes from the mucosal and 75–80% from the serosal medium [12] (Fig. 1). Cellular sodium behaves as would be predicted

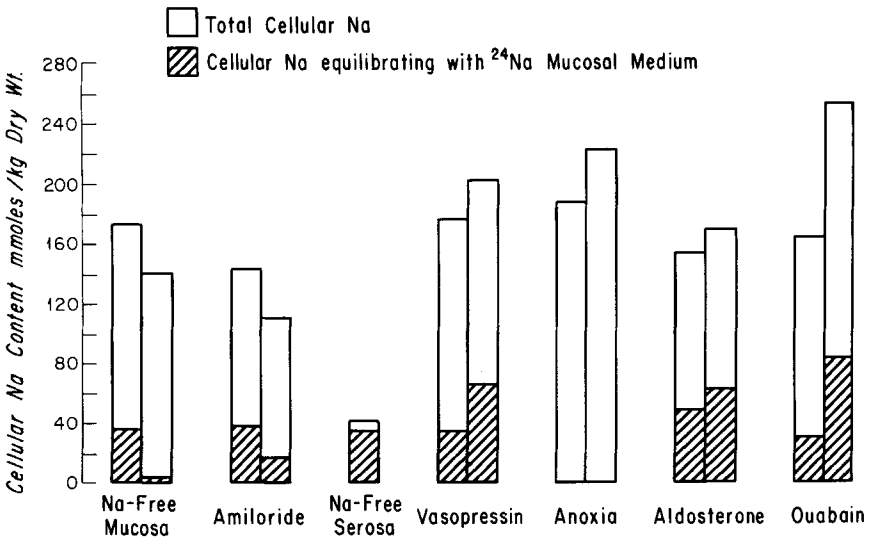

Fig. 1. Cellular sodium under different incubation conditions. The first column in each pair shows the control values with aerated sodium Ringer's bathing both surfaces of the hemibladder. The shaded portion of each column represents the amount of cellular sodium which equilibrated with ^{24}Na in the mucosal medium. The experiments in which tissue was incubated under anoxic conditions were performed without ^{24}Na

for a cellular transport pool. Anoxia [14] and ouabain [13], which inhibit transport by blocking the active extrusion of sodium from the cells to the serosal medium, increase cellular sodium. Of the increase in sodium after ouabain the major portion comes from the mucosal medium and varies from tissue to tissue, seemingly related to the initial transporting capacity of the preparation. A smaller, relatively constant amount (50 mmole/kg dry wt) comes from the serosal medium. The sodium gained is balanced by an equivalent loss of cellular potassium so that chloride and water content are unaffected [13, 14].

In the absence of mucosal sodium, cellular sodium decreases and that labeled from the mucosal medium almost disappears [12]. Amiloride (10^{-4}–10^{-3} M) in the mucosal medium blocks sodium entry to the cells [1, 3], and again cellular sodium decreases [12]. However, a fraction of sodium lying outside of the inulin (or mannitol) spaces on the mucosal surface remains (Fig. 2). This is true whether or not ^{24}Na is added before or after amiloride. Rapid washing of the mucosal surface with choline Ringer's, choline Ringer's with amiloride, or sodium Ringer's with amiloride removes this fraction of noninulin space sodium. Similarly, with labeling from the mucosal medium, a small amount of potassium

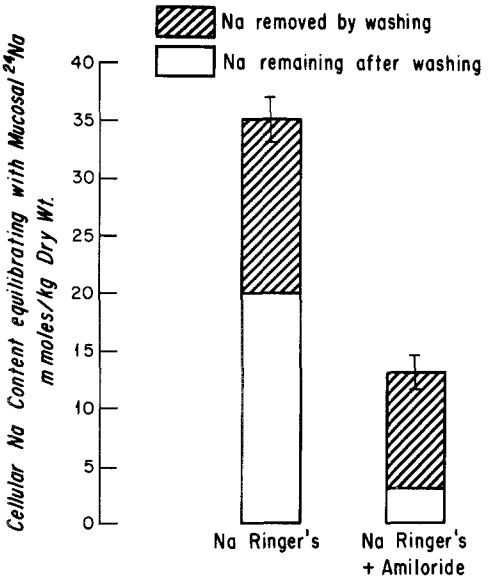

Fig. 2. The removal of noninulin space sodium from the mucosal surface of the epithelial cells by rapid washing with choline Ringer's, choline Ringer's with 10^{-4} or 10^{-3} M amiloride, or with sodium Ringer's containing 10^{-3} M amiloride

[22], choline (A.D.C. Macknight, *unpublished observation*) and chloride [11], none of which ions seems able to cross the mucosal membranes, is removed by rapid washing. The anatomical location of these ions remains to be established though it would not seem to be cellular.

Vasopressin [16] and, to a considerably smaller extent, aldosterone [9] increase cellular sodium (Fig. 1) in association with their stimulatory effect on transepithelial sodium transport.

Cellular sodium,therefore, behaves as predicted for a cellular transport pool, increasing when the active transport step is inhibited, and decreasing when sodium entry from the mucosal medium is prevented. The direction of these changes in cellular sodium need not reflect the direction of the change in transepithelial sodium transport. For example, vasopressin increases both cellular sodium and transport [16], but ouabain inhibits transport yet cellular sodium increases [13], and restoration of metabolism to hypoxic tissue stimulates transport but decreases cellular sodium [14]. These observations preclude the possibility that the sodium ascribed to the transport pool has in reality already been transported from this pool to the subepithelial tissues as seems to occur when whole tissues, rather than epithelial cells themselves, are analyzed [8, 26].

The observation that the noninulin space (cellular) sodium exchanges 20–25% with the mucosal and 75–80% with the serosal medium, raises the question of the relative contributions to the active transport pool. The observation is consistent with the possibility that a single cellular sodium pool exists with the rate of entry from the mucosal side being only one-quarter that of the rate from the serosa. Alternatively, there might be two distinct pools which do not mix, with only the mucosal pool representing the active transport compartment (Fig. 3). That the second interpretation is correct is supported most directly by metabolic studies. Canessa, Labarca and Leaf [2] and Macknight and McLaughlin [17] have found that the rate of production of CO_2 by toad hemibladders whose transepithelial transport is virtually abolished by amiloride (10^{-4} M) falls by about 45%. However, it is then little affected when serosal sodium is replaced by choline or by Tris (Table 2). If a large fraction of cellular sodium in a common pool recycled between the cells and serosal medium, removal of serosal sodium should have had a marked inhibitory effect upon the rate of production of CO_2. Similarly, when sodium transport was not inhibited, removal of serosal sodium did not affect the ratio of sodium transported to CO_2 produced [2, 17], providing further evidence that serosal sodium does not enter the transport pool.

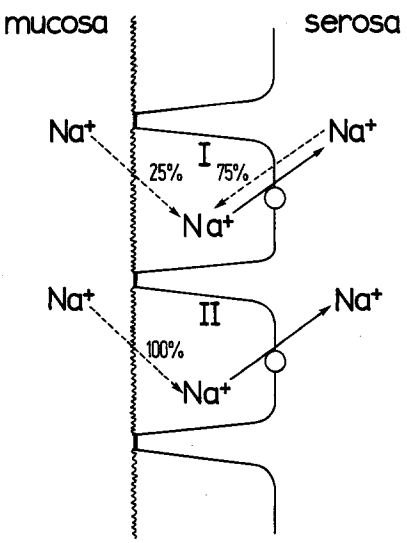

Fig. 3. Two possible interpretations of the relationships between cellular sodium and transepithelial sodium transport: *I* shows a common pool with the rate of entry from the serosal medium being much greater than from the mucosal medium; *II* shows only sodium from the mucosal medium entering the active transport pool

Table 2. Effects of sodium-free medium on the rate of production of CO_2

a) Effect of removal of sodium from the serosal medium on the coupling of transepithelial sodium transport (J_{Na}) to the associated production of CO_2 ($J^{sb}_{CO_2}$)

	$J_{Na}/J^{sb}_{CO_2}$
Na Ringer's mucosa and serosa	26.3 ± 4.1
Na Ringer's mucosa, Tris Ringer's serosa	22.0 ± 1.5
Δ	4.4 ± 3.7
P	$> 0.20 \quad (n=6)$

b) Effect of amiloride, 10^{-4} M mucosal medium, on CO_2 production

	J_{CO_2} (nmole/mg/dry wt/min)
Na Ringer's mucosa and serosa	0.580 ± 0.064 (J_{CO_2})
+amiloride mucosa	$0.342 + 0.045$ ($J^{b}_{CO_2}$)
Δ	0.238 ± 0.033 ($J^{sb}_{CO_2}$)
P	$< 0.001 \quad (n=6)$

41% of total CO_2 production was associated with transepithelial sodium transport

c) Effects of serosal Tris Ringer's and sodium Ringer's on CO_2 production with transepithelial sodium transport inhibited by amiloride, 10^{-4} M in the mucosal medium

	$J^{b}_{CO_2}$ (nmole/mg dry wt/min)
Tris Ringer's serosa	0.289 ± 0.037
Sodium Ringer's serosa	0.342 ± 0.045
Δ	0.053 ± 0.011
P	$< 0.01 \quad (n=6)$

production of CO_2 increased by only 18% when sodium was returned to the serosal medium

In the table J_{CO_2} is the rate of production of all CO_2, $J^{b}_{CO_2}$ is the rate of production of CO_2 unrelated to transepithelial sodium transported, assessed by eliminating this transport with amiloride 10^{-4} M in the mucosal medium, $J^{sb}_{CO_2}$, which is $J_{CO_2}-J^{b}_{CO_2}$, is the CO_2 produced in association with transepithelial sodium transport. (Data from Macknight and McLaughlin [17]).

The contention that the cellular transport pool consists only of sodium derived from the mucosal medium is further supported by the results of some recent electron microprobe experiments [19*a*]. The cellular sodium concentration of 13 mmole/kg wet wt measured in the epithelial cells and unaffected by the removal of serosal sodium is approximately equivalent to 40–50 mmole/kg dry wt and is therefore similar in magnitude to the sodium of mucosal origin in chemical analyses.

Furthermore, ouabain increased cellular sodium and decreased cellular potassium only when sodium was available to the cells from the mucosal medium, a finding consistent with the hypothesis that the basolateral cellular membranes are relatively sodium impermeable.

The nature and anatomical localization of the noninulin space sodium which equilibrates with sodium in the serosal medium remains conjectural. Inulin and polyethylene glycol, mol wt 1,000, occupy the same serosal space, and even sucrose which, since it is metabolized by the tissue, must enter cells, occupies a space only 20% greater than the inulin space [18]. If sodium in the space is at the same concentration as in the serosal medium, the total space would need to be twice that of the inulin space for the noninulin space sodium to be extracellular. Alternatively, the sodium concentration throughout the serosal inulin space would need to be 80 mM higher than in the medium to account for this sodium [12]. Neither of these possibilities seems likely. Though there may be some exchange of serosal sodium for sodium in the basal cells, as suggested by the effects of ouabain on ^{24}Na exchange [13], this would not account for all the noninulin space sodium. Further work is required to resolve this problem, but our inability to characterize this sodium adequately does not affect our conclusion as to the nature and properties of the cellular sodium transport pool.

We conclude, therefore, that the cells contain a sodium pool which gains this ion only from the mucosal medium. The electrochemical activity of the sodium within this pool is not known, and even its concentration can not rigorously be derived by dividing sodium in the pool by noninulin space water content since we do not know either how uniformly the sodium is distributed through the cellular water or how much of this water is associated with the noninulin space sodium, which exchanges readily with sodium in the serosal medium but is not part of the transport pool.

2. The Relationship of Transepithelial Sodium Transport to Potassium Uptake

The Koefoed-Johnsen and Ussing [7] model suggested that the operation of a tightly coupled Na-K pump at the basolateral membrane was responsible for sodium extrusion from the cell to the serosal medium and that the potential difference at this membrane arose as a consequence of its greater permeability to potassium than to sodium. The potential

was therefore a potassium diffusion potential, and the algebraic sum of this and a sodium diffusion potential at the apical membrane constituted the transepithelial potential difference. Frazier and Leaf [5], however, were able to demonstrate that complete substitution of serosal sodium by potassium, which should have abolished a potassium diffusion potential, had little effect on the potential measured across the basolateral

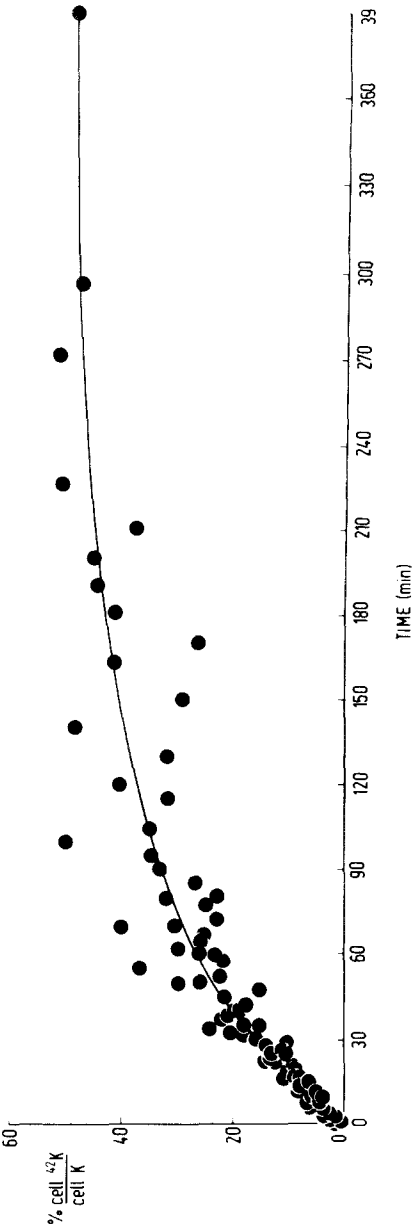

Fig. 4. Labeling of cellular potassium by medium ^{42}K with time. The percentage of cellular potassium labeled by medium ^{42}K is shown on the ordinate, with time on the abscissa (*from* Robinson&Macknight [22])

plasma membrane with an intracellular electrode. They therefore proposed that the toad bladder cells possessed an electrogenic sodium pump.

Robinson and Macknight have examined the exchangeability of cellular potassium in toad bladder epithelial cells [22] as part of a study of the relationship between medium potassium and sodium transport in toad bladder [20, 22]. There was virtually no potassium exchange

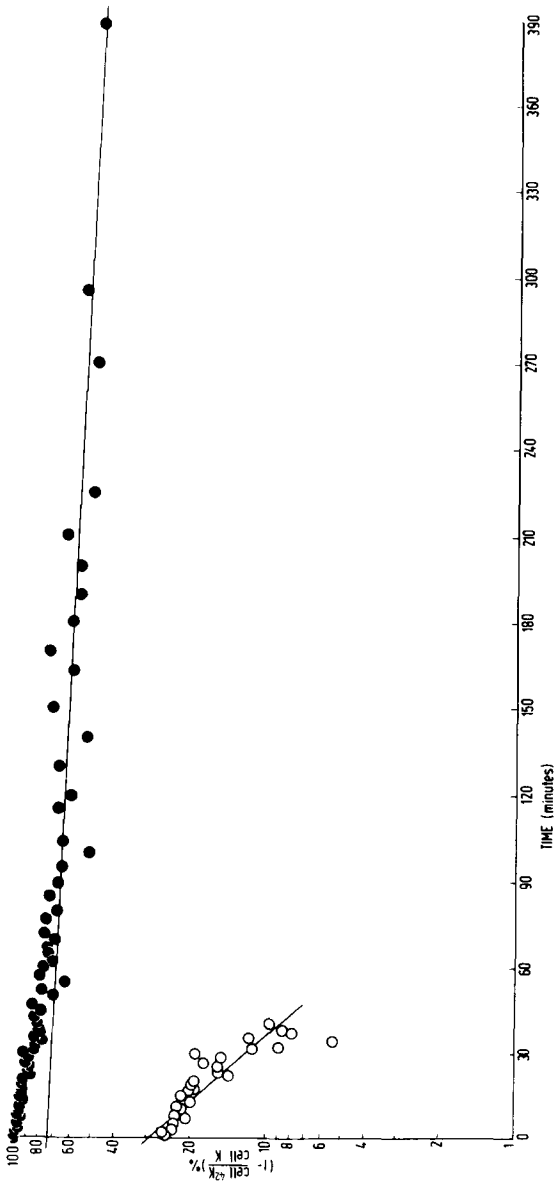

Fig. 5. Data shown in Fig. 4 replotted to demonstrate the presence of at least two cellular potassium pools. The ordinate shows (1 − cellular K labeled %) on a log scale while the abscissa shows time. The upper line is the regression line for all data for 80 min or longer ($y = e^{-0.0013t} + e^{4.30}$; $r = 0.69$; $P < 0.001$; $n = 21$). This regression line was subtracted from data for 0–40 min (0) and a second line drawn ($y^{-0.0264t} + e^{3.31}$; $r = 0.86$, $P < 0.001$; $n = 39$), (*from* Robinson&Macknight [22])

across the apical cell membrane. Of the total cellular potassium, one-quarter to one-third exchanged relatively rapidly with ^{42}K in the serosal medium, the half time being about 30 min. The remaining potassium exchanged much more slowly (Figs. 4 and 5), and it was not possible even after 19-hr incubation to label all cellular potassium. If this slowly exchanging potassium occupies a single pool it can be calculated that 56 hr of incubation would be required for complete labeling. The size of the fast pool measured isotopically is very similar to the amount of potassium lost from cells incubated with ouabain or in a low potassium medium, and to the amount exchanging with rubidium when this ion replaces potassium in the serosal medium. Recent work by Nellans and Schultz [19] with rabbit ileum reveals that in these epithelial cells also, only a fraction of cellular potassium readily exchanges.

There are therefore at least two potassium pools in toad bladder epithelial cells, only the more rapidly exchanging of which would be expected to be related to transepithelial sodium transport. From the size of this pool and its rate coefficient, the unidirectional flux of the rapidly exchanging potassium across the membrane can be calculated to be 3.04 mmole/min/kg dry wt. In a separate series of experiments in which short circuit current was measured active sodium transport ranged between 8.7 and 120 mmole/min/kg dry wt with a mean of 36.1 mmole/min/kg dry wt. Since even the lowest rate represents a movement of sodium which is about three times greater than the rate of potassium uptake, it would seem most unlikely that there is a tight 1:1 coupling of sodium extrusion to potassium uptake across the basolateral cell membrane. A similar conclusion was reached by Essig and Leaf [4] who studied the relationship between isotopic potassium uptake and active sodium transport using the complete tissue rather than epithelial cells alone.

These results therefore favor electrogenic sodium transport rather than an electrically neutral coupled cation exchange at the basolateral membrane. We are unable at present to localize the two potassium pools anatomically or to comment upon the relationships between cellular potassium and cellular water.

3. The Contributions of Mucosal and Serosal Medium Chloride to Cellular Chloride

Cellular chloride exchanges completely with medium chloride within 60 min [11]. Experiments with ^{36}Cl in only the mucosal medium, sum-

marized in Fig. 6, suggest that virtually no chloride crosses the apical membrane. Thus, only 7% of total cellular chloride labeled from the mucosal medium, and this was removed by rapid washing of the mucosal surface with isosmotic K_2SO_4-sucrose solution. Furthermore, replacement of medium sodium by choline containing 10^{-4} M amiloride, to prevent sodium uptake from the mucosal medium to the cells, though it decreased cellular sodium and chloride, did not change chloride labeling from the mucosal medium, a finding consistent with an extracellular localization of this chloride. Vasopressin increases cellular sodium by increasing sodium entry from the mucosal medium as shown by ^{24}Na labeling [16] (Fig. 1). The concomitant increase in cellular chloride, however, occurred with no change in ^{36}Cl labeling from the mucosal medium. Therefore, this chloride must have come from the serosal medium.

From these observations we conclude that the apical cellular membrane is virtually impermeable to chloride.

4. Relationships between Transepithelial Sodium Transport and the Regulation of Cellular Volume

Transepithelial transport of sodium and the regulation of cellular volume may both utilize the Na-K-ATPase. However, it has been suggested

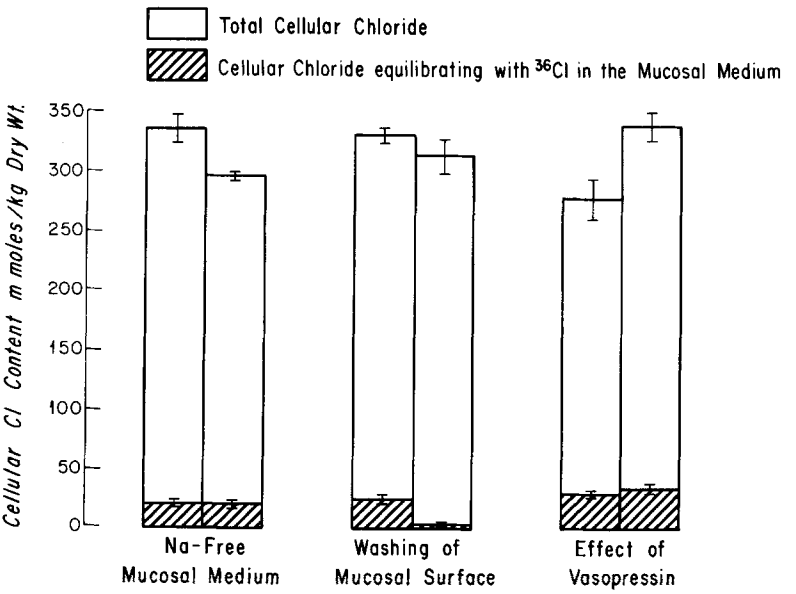

Fig. 6. Cellular chloride, and ^{36}Cl equilibrating with the mucosal medium (shown by the shaded portion of each column), under some different incubation conditions

that other mechanisms may be involved in both processes (*see* Macknight and Leaf, [15] for a review). In the toad urinary bladder, ouabain 10^{-2} M inhibits transepithelial sodium transport completely, yet no cellular swelling is detected after 50–120 min incubation in spite of loss of most of the readily exchangeable cellular potassium, which is replaced by medium sodium [13]. These observations might be interpreted as evidence favoring a ouabain-insensitive cellular volume regulating mechanism as

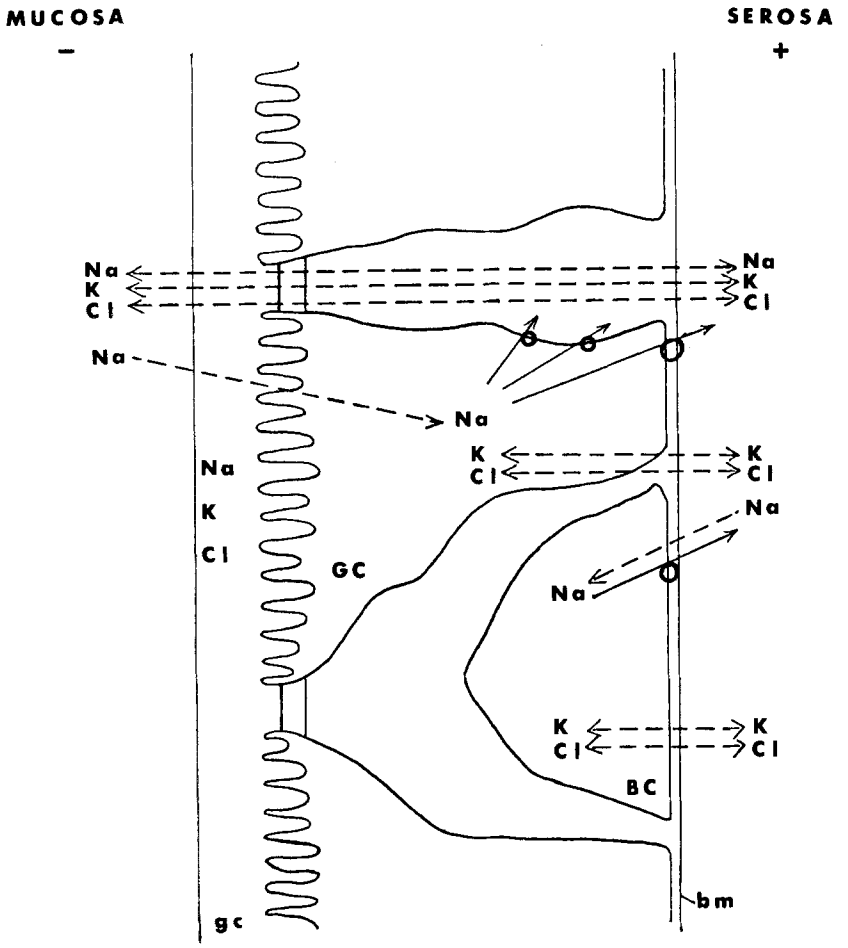

Fig. 7. A schematic summary of the relationships between cellular and medium sodium, chloride and potassium and transepithelial sodium transport. GC represents a granular cell which is probably the cell type responsible for transepithelial sodium transport in the toad bladder. BC represents a basal cell, *gc* is the glycocalyx and *bm* the basement membrane. The solid arrows represent active transport and the broken arrows passive ion movements. Though K and Cl movements between cells and serosal medium are shown as passive in both directions, the true nature of these movements remains to be firmly established

has been proposed for mammalian tissues [6, 10, 25]. However, significant cellular swelling in isosmotic medium could only occur if extracellular ions could readily enter the cells. If sodium can only enter cells across the apical membrane down its electrochemical gradient which would fall rapidly as sodium replaced cellular potassium, cellular swelling could be a very slow process which might take hours to manifest itself.

Conclusions

Some of the relationships between cellular composition and transepithelial sodium transport have been investigated. These are illustrated in Fig. 7. Though diffusible ions can equilibrate in a noninulin space on the mucosal side (shown in the diagram as the glycocalyx, though there is no direct experimental evidence for this), only sodium can enter the transporting cells across the apical membrane. This is thought to be a passive process though it does not involve simple diffusion. All the diffusible ions can cross the epithelium in both directions through the tight junctions. Sodium is actively transported from the cells across the basolateral membranes. It cannot diffuse passively across this membrane. Potassium and chloride do move in both directions across this membrane, the movement of potassium not being tightly coupled to sodium extrusion. Though cellular sodium and chloride are completely exchangeable with medium ions, only about one-third of cellular potassium equilibrates rapidly with serosal potassium. Experiments with ouabain suggest that the basal cells may gain sodium from the serosal medium, but further work is required to clarify the relationship of ion transport across the basal cells to that across the cells responsible for transepithelial transport.

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