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Topical Review

Epithelial Cell Volume Modulation and Regulation

Kenneth R. Spring and Ann-Christin Ericson
Laboratory of Kidney and Electrolyte Metabolism, National Heart, Lung, and Blood Institute
National Institutes of Health, Bethesda, Maryland 20205

Summary. Epithelial cell volume is a sensitive indicator of the balance between solute entry into the cell and solute exit. Solute accumulation in the cell leads to cell swelling because the water permeability of the cell membranes is high. Similarly, solute depletion leads to cell shrinkage. The rate of volume change under a variety of experimental conditions may be utilized to study the rate and direction of solute transport by an epithelial cell. The pathways of water movement across an epithelium may also be deduced from the changes in cellular volume. A technique for the measurement of the volume of living epithelial cells is described, and a number of experiments are discussed in which cell volume determination provided significant new information about the dynamic behavior of epithelia. The mechanism of volume regulation of epithelial cells exposed to anisotonic bathing solution is discussed and shown to involve the transient stimulation of normally dormant ion exchangers in the cell membrane.

Key words water permeability · salt transport · quantitative light microscopy · transepithelial fluid transport · *Necturus* gallbladder

Introduction

The transport of large quantities of solute and water by epithelial cells necessitates substantial transcellular flows of these substances. In this review we will consider both the mechanism of these transcellular flows as well as the means by which the epithelial cells maintain their composition and volume in the face of a virtual torrential flood. Many investigators have concluded that the epithelial cell could not possibly withstand such rapid flows of salts and water. They proposed that transepithelial fluid movements are accomplished by an extracellular (paracellular) route [9, 10, 14, 25, 26, 38, 51, 52]. We will review evidence favoring a transcellular route for transported fluid in *Necturus* gallbladder epithelial cells. In addition, we will consider the mechanisms by which these cells maintain their integrity and control their volume during fluid transport.

Epithelial cells function to transport fluid from one surface to the other; this transepithelial transport is dependent on metabolism of the cells. The transport of a solute such as NaCl results in net fluid flow because the hydraulic conductivity of the epithelial cell is sufficiently great to preclude any substantial transepithelial gradient in osmotic pressure. Therefore transport of solute results in proportionate movement of water and the mucosal and serosal bathing solutions remain virtually equal in osmolality. Study of the significance of transcellular flows in the transepithelial movement of fluid requires a method for the rapid measurement of cell volume, so that water flow into or out of the cell may be quantitated. We developed an optical microscopic method for the repetitive determination of epithelial cell volume. We first describe the technique for the measurement of the size and shape of epithelial cells and then consider various applications of cell volume measurements.

Measurement of Epithelial Cell Volume

Quantitative determinations of epithelial cell volume have been made from electron micrographs of serial sections [2, 69]; however, studies of the dynamic behavior of the cells were not possible because of the need for fixation and staining. When *Necturus* gallbladders or other epithelia are mounted in a thin perfusion chamber the cell outlines may be readily visualized in the light microscope [12*a*, *b*, 44, 56, 58–60, 63]. The use of microscope optics with an extremely shallow depth of field permits the "optical sectioning" of a cell. This means that only a thin cross section of the cell is in focus at one time; the remainder of the cell above and below the focal plane is invisible. Examples of such optical sections of a *Necturus* gallbladder epithelial cell are shown in Fig. 1. The use of

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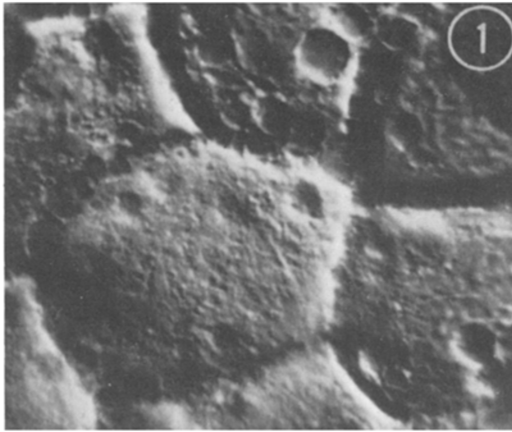
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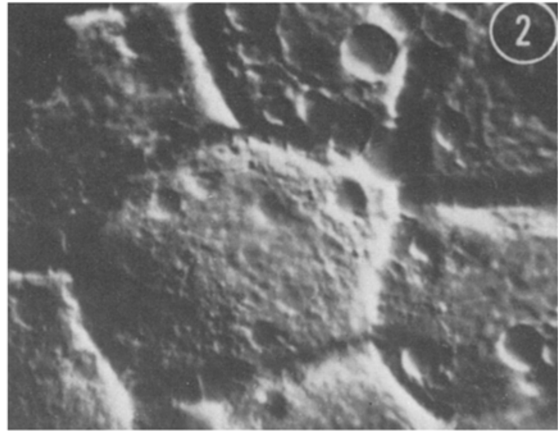
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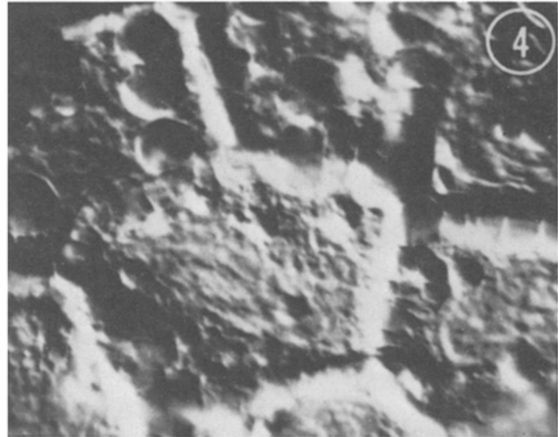
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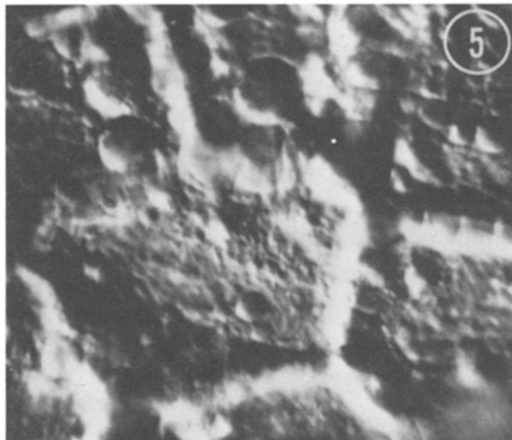
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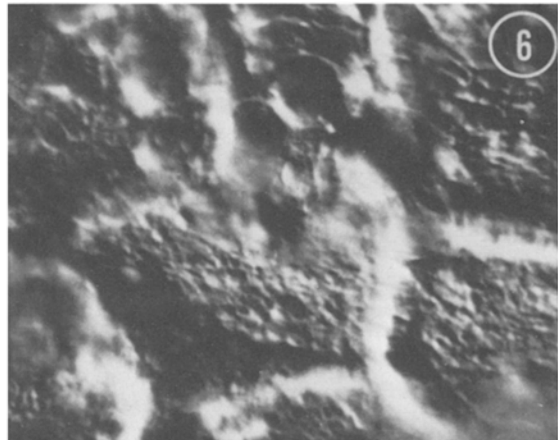
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high magnification (100 ×) differential interference contrast optics reduces the optical section thickness to 0.5 μm or less. The cells are visualized during an experiment by the use of a low light level television camera, and the video images of the optical sections are stored on a video disk. The size and shape of a cell may be adequately defined by 8 to 10 optical sections from the level of the apical surface to the level of the underlying basement membrane. The microscope focus and video recording are both under computer control. A series of records of the sections through a cell requires about 1 to 1.5 sec, and a complete series of sections can be repeated every 6 sec [44, 63]. At a later time the video images are replayed and the cell outlines traced electronically to determine the cross sectional area and perimeter of each optical section. Cell volume is calculated from the area of each section and the known displacements of focus between sections [44, 59]. This method enables the frequent determination of the volume of an epithelial cell during the course of a change in the composition of either bathing solution. In-as-much as the only parameter measured is the volume of an epithelial cell, care must be taken to design experiments that produce interpretable effects on that parameter.

Hydraulic Conductivity (Lp) of Epithelial Cells

Although there has been considerable debate over the magnitude of epithelial permeability to water [8, 25], little effort has been directed towards measurement of the water permeability of epithelial cell membranes. Recently the hydraulic conductivity of *Necturus* gallbladder epithelial cells was measured by determining the rate of change of cell volume in response to a change in the osmolality of either bathing solution (44, 60, 63]. The osmotic water permeability of the cell membranes was very high (0.055 to 0.12 cm/sec) and only small transmembrane osmotic gradients (about 3.5 mOsm) would be required to accomplish normal rates of fluid absorption. Since the directional transport of fluid requires favorable gradients in water activity as well as adequate hydraulic conductivity, there must be differences in osmolality between the cell interior and the solutions bathing the cell surfaces. The entry of fluid into the gallbladder cell across the apical membrane would only occur because of lower water activity within the cell than in the mucosal medium. We calculated from our Lp and fluid absoprtion data that the osmolality of the epithelial cell must exceed that of the luminal fluid by approximately 2.4 mOsm to achieve a normal rate of fluid absorption [44, 60]. The exit of water from the cell must similarly be the result of an osmotic gradient across the basolateral membrane. We calculate from the Lp of the basolateral membrane that the cell must be bathed on its basolateral surface by a solution approximately 1.1 mOsm hypertonic to its interior or 3.5 mOsm hyperosmotic to the mucosal bathing solution. The absorbate would then be 3.5 mOsm hyperosmotic to the bathing solutions, a difference of 1.8% from isoosmolality. A deviation in osmolality of this magnitude is within the measurement error of current methods for osmolality determination and could not be detected with certainty. Similar conclusions have been made previously on the basis of mathematical models of epithelial fluid transport [36, 51, 65–68]. The high Lp of the cell membrane removes the need for consideration of standing osmotic gradients [8, 67] or other more exotic mechanisms [25] for isosmotic fluid absorption. The required osmotic gradients are miniscule, and the intercellular space solute concentration profile or interspace geometry are not significant factors during normal fluid absorption [43, 44, 51, 67]. A further consequence of the ready movement of water across epithelial cell membrane is that solute entry or exit from an epithelial cell is followed by water movement in about the same ratio of solute/solvent as in the bulk solution.

The Route of Fluid and Ion Flows – Transcellular or Paracellular

There has been considerable speculation about the routes taken by water and solutes as they cross the epithelial layer; most conclusions about the routes are heavily dependent on model calculations

Fig. 1. *(Facing page)* Optical sectioning is illustrated by light microscopic (differential interference contrast) images of *Necturus* gallbladder epithelium. Each image was recorded after the focus was adjusted 3 μm toward the base of the cell. Image *1* is approximately at the level of the tight junctions between the cells. *2* is 3 μm serosal to the tight junction and each subsequent image is 3 μm closer to the serosal side. Note that intercellular bridges are most prominent in *2* through *5* (3 to 12 μm beneath the tight junctions). Cell boundaries are indistinct in *8* and strands of connective tissue are visible. Magnification 630 × . Photographs by Harry G. Schaefer. *(Reprinted from Reference 63 with permission of Raven press)*

based on the geometry and structure of the tight junction and extracellular shunt pathway [9, 10, 11, 14, 25, 26, 51]. Disputes about these parameters have led to a wide range of estimates of the relative flows of water and salts across the cellular and shunt pathways. General agreement does exist about the ratio of the relative areas of the cell and tight junction at the mucosal surface. In most epithelia the area of the apical cell membrane is approximately 10^4 times that of the cross sectional area of the tight junction [25, 51, 59]. Significant rates of transepithelial flow of water and solutes across the tight junction occur when the permeability of that pathway equals or exceeds that of the cellular path, after correction for the relative areas of the two routes. The driving force for fluid movement across the tight junctions is the difference in osmotic pressure between the basolateral interstitum and the mucosal bathing solution. The same osmotic pressure difference exists across the tight junction as that across the entire cell, therefore equal-fluid flows across the two pathways (junctional and cellular) would require equal hydraulic conductivities. The available area of the tight junction is 10^{-4} times that of the apical membrane. In *Necturus* gallbladder the apical cell membrane has an osmotic water permeability of 0.055 cm/sec; significant transjunctional water flow would then require water permeability values of the tight junction in the range of 5 m/sec, a physically unrealistic value. We conclude that the cell membrane water permeability is sufficiently high that all transported fluid takes a transcellular route driven by small osmotic gradients.

When a similar calculation is made about the routes of ion flow across the epithelium, a different conclusion is reached. In most epithelia, the apical membrane permeability to ions is about 10^{-5} to 10^{-7} cm/sec. Equal diffusional ion flows through the cellular and shunt paths would occur when the junctional ion permeability is in the range of 10^{-1} to 10^{-3} cm/sec. Transjuctional flows would predominate when the junctional permeability exceeds that of the cell membrane. The maximum diffusional permeability to NaCl of the tight junction is about 1.5 cm/sec based on a junctional height of 0.1 μm. The permeability properties of the shunt pathway would then predominantly determine the characteristics of transepithelial ionic diffusion when the junctional ion permeability is in a physically reasonable range. The difference in the paths taken by water and salts in response to transepithelial gradients arises not because the junctions are selectively permeable but because the cell membrane restricts salt markedly compared to water.

Transcellular NaCl Transport

Necturus gallbladder epithelial cells transport NaCl from their apical to basolateral surfaces. The bile facing the apical surface is thereby concentrated by the removal of NaCl and water. The mechanism of NaCl transport by these cells has been studied by several groups of investigators [12*a*, 19, 20, 22, 42, 47–50]. In most epithelia the rate-limiting step for the transcellular transport of Na or NaCl lies at the apical membrane of the cell [4, 6, 17–20, 30, 57, 62]. Sodium entry has been shown to be a saturable function of the Na concentration in the mucosal bathing solution, suggesting that the entry step could be carrier-mediated. There is a consensus that NaCl enters *Necturus* gallbladder cells across the apical membrane driven by the sodium concentration gradient across the membrane [12*a*, 20, 47, 48]. Electroneutral, mediated entry of NaCl into epithelial cells across the apical membrane has been reported in a number of low-resistance epithelia [16–18, 24, 30, 53, 57, 62]. Indeed, this may be a general mechanism for the entry of salt into epithelial cells, inasmuch as most epithelial cells have chloride activities far above the equilibrium value [16]. A high intracellular chloride is the consequence of the coupled nature of the entry step for NaCl into the epithelial cell. The intracellular chloride activity is maintained above equilibrium by the continuing entry of NaCl across the apical membrane as a result of the favorable electrochemical gradient for Na [16, 18, 20]. This entry of NaCl maintains the cell osmolality above that of the apical bathing solution. Normally the entry of NaCl across the apical membrane is exactly balanced by its exit across the basolateral cell membrane; the exit is due to active sodium transport by the epithelial cell. The relationship between the rate of Na entry into epithelial cells and the rate of Na exit has been the subject of considerable research [4, 34, 64]. A negative feedback hypothesis has been put forth which suggests that increased Na entry elevates intracellular Na concentration and that this elevated Na concentration results in a reduction of Na entry and the restoration of the original intracellular Na concentration [34, 64].

The high hydraulic conductivity of the cell membranes of leaky epithelia leads to the flow of water into or out of the cell in response to a change in the solute content of the cell [44]. The maintenance of a stable volume by an epithelial cell would then seem to be a consequence of the balance between solute entry and exit across the two faces of the cell. Indeed, this has been demonstrated in

a number of different ways in *Necturus* gallbladder epithelial cells [12*a*, 59, 60] as well as in isolated rabbit proximal tubule segments [37]. Because two processes compete with one another for the solute content of the cell, the volume of the cell may be predictably altered by cessation of solute entry or exit. For example: (a) removal of mucosal Na or Cl stops entry of both solutes and abolishes fluid absorption by *Necturus* gallbladder epithelium [59]. Cessation of NaCl entry leads to cell shrinkage because NaCl exit continues as long as the intracellular Na concentration is sufficiently high to maintain the Na, K-ATPase activity. Cell volume predictably decreases under these circumstances as the intracellular NaCl pool is depleted and the solute content of the cell decreases [59]. The magnitude and rate of this decrease in cell volume have been used to estimate the NaCl transport pool in the cell as well as the rate of NaCl exit from the cell due to active sodium transport [59]. (b) NaCl exit may be blocked by the inhibitor ouabain [12*a*, 44]. Addition of 10^{-4} M ouabain to the serosal bathing solution of *Necturus* gallbladder results in cell swelling because NaCl exit has been blocked but entry continues. The rate of cell swelling and the magnitude of the maximum volume increase are a function of the ionic composition of the apical bathing solution. The NaCl entry step in *Necturus* gallbladder epithelium may be characterized by analysis of the rate of cell swelling in the presence of ouabain [12*a*]. The kinetic characteristics and inhibitor sensitivity of this process are consistent with the coupled, neutral transport of NaCl (*see* Table).

In summary, our view is that fluid absorption by *Necturus* gallbladder (and possibly other leaky epithelia) may be explained as follows:

1) NaCl enters the epithelial cell across the apical membrane by a carrier-mediated process in which the co-transport of chloride is driven by the gradient for sodium. This entry process is the principal mode of NaCl movement associated with transepithelial transport and is rate limiting for transepithelial fluid transport.

2) The entry of NaCl into the cell maintains the cell osmolality slightly higher than that of the mucosal bath (required gradient is about 2 mOsm). Water flows from the mucosal bathing solution into the cell driven by the gradient in activity from mucosal bath to cell.

3) Na is transported out of the cell across the basolateral membrane by the Na, K-ATPase. The mode of chloride exit from the cell is uncertain [45, 48, 53]. The transport of NaCl into the basolateral interstitial space increases its osmolality above that of the cell (required gradient about 1 mOsm). Water moves from cell to basolateral interstitium driven by the gradient in its activity.

4) A small hydrostatic pressure (about 3 cm H_2O) [58] develops in the basolateral intercellular space and drives the fluid across the submucosal connective tissue.

Transepithelial fluid flow is transcellular with fluid entering the cell across the apical membrane and exiting across the basolateral membrane. The lateral intercellular spaces constitute the final common path for transported fluid but do not play an important role in solute-solvent coupling. A specific role for the leaky tight junction is not apparent to us.

Table 1. Characteristics of the two modes of NaCl entry in *Necturus* gallbladder epithelial cells

Coupled NaCl transport associated with transepithelial fluid absorption	Na-H and Cl-HCO_3 exchange associated with volume regulatory increase
Present in apical membrane of cell	Present in apical membrane of cell after exposure to hypertonic bathing solution
Requires Na in mucosal solution	Requires Na in mucosal solution
Requires Cl in mucosal solution	Requires Cl in mucosal solution
No requirement for HCO_3	Requires HCO_3 (sidedness not determined)
Blocked by bumetanide in mucosal solution	No effect of bumetanide
No effect of SITS	Blocked by SITS (sidedness not determined)
No effect of amiloride in mucosal solution	Blocked by amiloride in mucosal solution
Na kinetics:	Na kinetics:
$K_{1/2}=26.6$ mM	$K_{1/2}=2.8$ mM
$V_{max}=1.73\times10^{-6}$ cm/sec	$V_{max}>10\times10^{-6}$ cm/sec
Cl kinetics:	Cl kinetics:
$K_{1/2}=19.5$ mM	$K_{1/2}=1.9$ mM
$V_{max}=1.56\times10^{-6}$ cm/sec	$V_{max}>10\times10^{-6}$ cm/sec
Functions constantly	Functions transiently, stimulated by hypertonicity

Epithelial Cell Volume Regulation in Anisotonic Media

When *Necturus* gallbladder epithelial cells are exposed to anisotonic media they undergo an initial osmotically-induced volume change followed by a recovery to their original volume [12*b*, 44, 60, 63]. The recovery occurs despite the fact that the bathing solution osmolality differs from that of control

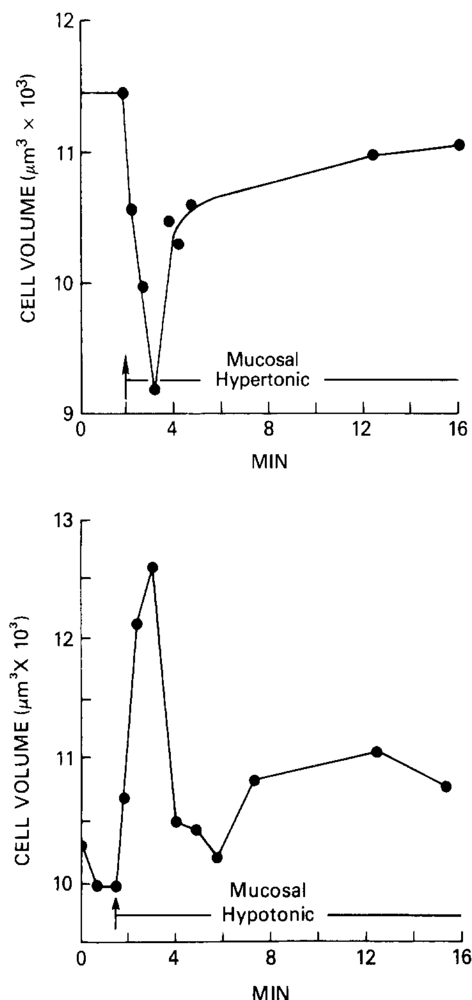

Fig. 2. *Top:* Cell volume is plotted on the ordinate as a function of time on the abscissa. After control measurements of volume, the mucosal perfusate was made hypertonic by 36 mOsm (arrow at 2 min). The cell quickly shrank and then recovered although the mucosal perfusate remained hypertonic for the duration of this record. *Bottom:* Cell volume is plotted for an experiment in which the mucosal perfusate was made hypotonic by 36 mOsm by the removal of mannitol from the control solution. The solution change occurred at the arrow and the mucosal perfusate remained hypotonic for the duration of this record

Ringer. Examples of this behavior are shown in Fig. 2. The return of the cell to its original volume has been termed "volume regulation," similar behavior has been described in red blood cells [31, 33], ascites tumor cells [27], isolated rabbit proximal tubules [7, 23], and frog skin epithelium [40]. In all of these tissues the mechanism of regulatory cell volume increase following hypertonicity differs from volume regulatory decrease following hypotonicity. The regulatory increase of cell volume which occurs after a hypertonic challenge is typically due to the gain of solute by the cell in the form of NaCl [33, 39]. On the other hand, the regulatory decrease of cell volume which follows hypotonicity is generally attributed to the loss of solute from the cell as KCl or NaCl [33, 39]. Since these volume regulatory responses appear to be independent, we will discuss them separately.

Volume Regulatory Decrease

Epithelia exposed to hypotonic media initially swell as water flows into the cell along its activity gradient. Then cell volume is reduced toward its original control value. An example of the response of *Necturus* gallbladder epithelium to an 18% reduction in osmolality is shown in Fig. 2 (bottom). The initial osmotically induced swelling is followed by volume regulatory shrinkage [44]. The ionic basis of this volume regulatory decrease has not been studied in *Necturus* gallbladder. Grantham and coworkers [7, 23] studied the volume regulatory decrease of isolated rabbit proximal tubules; they showed that the response involved the loss of intracellular KCl. As in the red blood cell [31, 33] and in the ascites tumor cell [21, 27], the solute content of the rabbit proximal tubule cells decreased when the cells underwent volume regulatory decrease. Loss of the intracellular solute leads to loss of cell water because of the high water permeability of the cell membranes.

Volume Regulatory Increase

Exposure of *Necturus* gallbladder epithelial cells to hypertonic solution in either mucosal or serosal bath results in rapid cell shrinkage followed by volume regulatory increase (Fig. 2, top). The initial volume decrease is of the magnitude predicted from the water content of the cell and behavior of the cell as an osmometer [44]. The subsequent volume regulatory increase has been studied in detail [12*b*, 44]. Shrinkage of the gallbladder cell by as little as 6% results in volume regulatory swelling. Volume regulatory increase occurs because the cells take up NaCl rapidly from the mucosal bathing solution. As the solute content of the cells increases, cell swelling occurs. The mechanism of NaCl entry during volume regulatory increase is totally different from that during fluid absorption (*see* Table). Volume regulatory swelling has been shown to require Na and Cl in the mucosal medium [12*b*, 44], as well as HCO_3 in the bathing solutions [15]. In contrast to the coupled movement of NaCl during transepithelial transport, volume regulatory NaCl entry is inhibited by 10^{-3} M amiloride in the mucosal bath or by 10^{-4} M SITS in both bathing solutions. Volume regulatory swell-

ing is unaffected by 10^{-4} M bumetanide in the mucosal bath [12*b*] or by ouabain in the serosal bath [44]. The kinetics of volume regulatory increase also differ dramatically from those associated with transepithelial NaCl transport. The rate of volume regulatory increase is a saturable function of the mucosal Na or Cl concentration with a $K_{1/2}$ for Na of 2.8 mM and a $K_{1/2}$ for Cl of 1.9 mM and a maximum velocity approximately five times greater than that of transepithelial fluid transport [12*b*]. The mechanism of NaCl entry during volume regulatory increase is the parallel operation of Na-H and Cl-HCO_3 exchangers in the apical membrane [12*b*, 15]. While Na-H and Cl-HCO_3 exchangers are not measurably active under control conditions, significant stimulation of these exchangers occurs in the presence of a hypertonic solution. The mechanism by which the osmotic stimulus is transduced by the cell to result in the appearance of a new mode of NaCl entry is not known. A summary of the essential features of the two modes of NaCl entry into *Necturus* gallbladder epithelial cells is given in the table.

The mechanism of volume regulatory increase by *Necturus* gallbladder epithelial cells is remarkably similar to that previously reported in red blood cells of *Amphiuma* [3, 54]. An amiloride-sensitive Na-H exchange is stimulated by exposure of *Amphiuma* red blood cells to hypertonicity; a Cl-HCO_3 exchanger apparently is already active in these cells. Ascites tumor cells respond to hypertonicity by the stimulation of the co-transport of Na or K and Cl, a different mechanism from that in the gallbladder epithelium [21, 27]. It is important to note that the ion exchangers are only transiently stimulated by hypertonicity and the process ceases once cell volume has returned to control [12*b*, 33, 54].

Implications for Studies with Isolated Membrane Vesicles

The presence of powerful ion exchangers in the apical cell membrane which can be transiently stimulated by anisotonic bathing solutions has important implications for work done with isolated membrane vesicles. The demonstration in these vesicles of ion exchange processes, such as Na-H or Cl-HCO_3 exchange, has been taken as evidence that the mechanism of transmembrane NaCl movement involves such exchanges [1, 28, 41]. However, the procedures utilized for isolation of the membrane vesicles may lead to the stimulation of the normally dormant ion exchangers or to the isolation of intracellular vesicles containing these exchangers. Thus, it remains to be proved that ion exchangers demonstrated in vesicles preparations play a significant role in the transepithelial transport of NaCl and water.

The induction of a new transmembrane transport system by hypertonic bathing solutions has a parallel in another biological system, the fertilized egg. Fertilization of the sea urchin egg leads to a rapid increase in intracellular calcium followed by the induction of amiloride-sensitive Na-H exchange [13]. As a result of the transient Na-H exchange intracellular pH rises leading to further development of the oocyte [13]. It is not known in the epithelial cell or the oocyte whether the induction of ion exchange involves the activation of preexisting carriers in the cell membrane, insertion of new carriers by the fusion of intracellular vesicles with the cell membrane, or the enzymatic cleavage of precursors to the carriers. It is also unknown whether any alteration in intracellular calcium occurs in epithelial cells following alteration of solution osmolality.

Implications for Studies with Ion-sensitive Electrodes

Ion-sensitive microelectrodes have become a popular and valuable tool for the determination of the cellular response to changes in ionic composition of the bathing solutions as well as to the presence of drugs or inhibitors. Many investigators have utilized the time course of changes in intracellular ionic activity to calculate ionic fluxes and permeabilities [5, 20, 22, 29, 35, 47–50, 53, 61, 70]. Cellular volume was not measured in these studies. We have shown that a combination of ion-selective electrode and volume data was required to determine the magnitude and direction of the movement of chloride ions in *Necturus* gallbladder epithelial cells [15]. Our experience is that the ion-sensitive electrode records alone may be misleading. We found, for example, that when the solution bathing the mucosal surface of *Necturus* gallbladder cells was made hypertonic, chloride ions were actually leaving the cell at a time when intracellular chloride activity was increasing because of osmotic water loss [15]. Also, at a time when intracellular chloride activity was unchanged, cell volume was rapidly increasing and there was actually a large net influx of chloride ions. In the same way it is easy to misinterpret the results of experiments involving ouabain inhibition of the Na pump [12*a*]. In *Necturus* gallbladder epithelium, as in other epithelial cells, ouabain has been reported to decrease intracellular K concentration [46, 49]. We have

shown that, if the cell swelling caused by ouabain is taken into account, there is no change in the K content of the *Necturus* gallbladder epithelial cell [12*a*]. Cell swelling and consequent dilution of intracellular K leads to the gradual depolarization of the cell membrane potential observed after exposure to ouabain [46, 49]. Therefore, brief exposure to ouabain does not cause the loss of a significant quantity of intracellular K from *Necturus* gallbladder epithelial cells, but this could not be determined from the ion-selective electrode data alone.

Physiologic Significance of Cell Volume Regulation

It is regularly stated in reviews of the control of cellular volume that cell volume regulation is an important process, crucial to the maintenance of the integrity of the cell [33, 39]. While this observation would seem unarguably true, we are not convinced that the sole purpose of the machinery involved is the regulation of cellular volume. It is superfluous for an epithelial cell to respond to a change in osmolality of only a few percent by a complete readjustment in volume within 90 sec, since the tissue probably never experiences such a rapid change in osmolality. The transporters involved in volume regulation have a remarkably low $K_{1/2}$ and high maximum velocity. Are they there only to compensate for unlikely changes in plasma osmolality? We think not. The importance of these exchange processes may lie in their link to hormonally induced alterations in epithelial transport. Anisotonicity may incidentally trigger a sequence of events usually involved in hormone action. Along this line, it is suggestive that both epinephrine and hypertonicity have similar effects on red blood cell volume and transmembrane transport [32]. It is unclear whether the factor that triggers a volume regulatory response is a change in cell pH [3], cell calcium, or some other parameter.

Concluding Remarks

Cell volume mirrors the solute balance of the epithelial cell and can be utilized as an indicator of the net flow of solute into or out of the cell. The experimental exploration of the responsiveness of epithelial cell volume to transmembrane solute flows has enabled the determination of a number of the cellular transport parameters. The importance of cell volume measurements for characterizing nonsteady-state conditions cannot be overemphasized. Neglect of the cell volume changes under these circumstances can lead to misinterpretation of not only the magnitude of the ion fluxes but even their direction. The advent of new techniques for the accurate determination of rapid changes in epithelial cell volume now permits investigation of the pathways of solute-linked fluid movements as well as the cell membrane transport parameters in a wide variety of epithelia.

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