FLUID TRANSPORT BY GALLBLADDER EPITHELIUM

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SUMMARY

The absorption of fluid by epithelial tissues is thought to be due to the existence of hypertonic regions within the epithelium. The magnitude of the required hypertonicity as well as its localization have been the subject of considerable experimental and theoretical effort. Model calculations demonstrated the need for knowledge of the water permeability of the membranes of epithelial cells for the purpose of estimation of the osmotic gradients required for fluid absorption. We measured the hydraulic water permeability of the individual cell membranes of *Necturus* gallbladder by quantitative light microscopy. The water permeabilities were sufficiently high so that small osmotic gradients were required to achieve normal rates of fluid transport. The cell osmolality was calculated to exceed that of the mucosal bathing solution by about $2 \, \mathrm{mosmol \, kg^{-1}}$, and the basolateral interstitial osmolality was calculated to be about $1 \, \mathrm{mosmol \, kg^{-1}}$ greater than that of the cell. The fluid absorbed by the epithelium must be slightly hypertonic to the bathing solutions. Knowledge of the apical cell membrane water permeability and the relative area of the cell and tight junction allow a calculation of the relative flow of fluid across both pathways. It can be readily shown that osmotically induced flow across the epithelium occurs predominantly transcellularly because of the small area of the junctional pathway and the high water permeability of the cell membranes.

INTRODUCTION

The absorption of salt and water by epithelia has been a subject of interest for the last 80 years (Reid, 1902; Goldschmidt, 1921). Epithelia such as the gallbladder, intestine and renal proximal tubule absorb fluid isosmotically; the direction of fluid transport is from the apical (mucosal) surface toward the basolateral (serosal) surface of the epithelial cell. Fluid transport is secondary to solute movement, and the general scheme for the transport of NaCl by epithelia is shown in Fig. 1. NaCl enters the cell across the apical membrane; the mechanism by which this entry occurs has been recently reviewed (Frizzell, Field & Schultz, 1979). Active Na transport occurs across the basolateral cell membranes by means of the Na^+, K^+-ATPase common to most cells (DiBona & Mills, 1979; Zeuthen & Wright, 1978; Levitt, 1980; Wright, 1978). The mechanism of chloride exit from the epithelial cells is not well understood
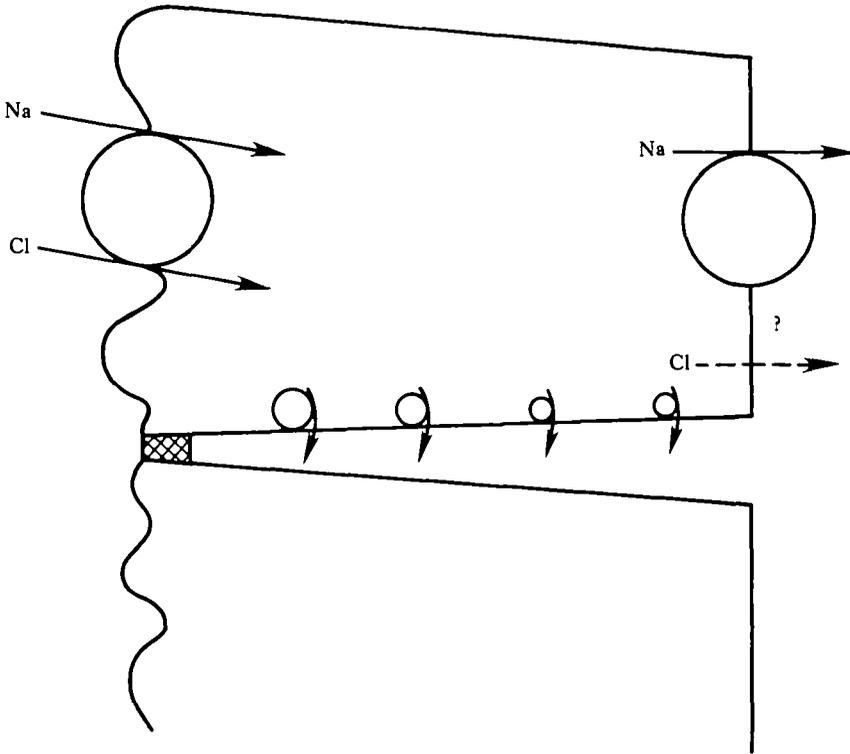
Fig. 1. A schematic diagram of an epithelium showing the entry and exit of NaCl. NaCl entry across the apical membrane (left) is shown as coupled process. Na exit is the result of active transport out of the cell across the basal and lateral membranes. Cl exits across the basolateral membrane by an unknown mechanism.

(Reuss, 1979; Shindo & Spring, 1981), but it is clear from a variety of measurements that there is a large transcellular chloride flux. The cells are joined at their apical borders by tight junctions which form a belt-like network of strands surrounding each cell. The space beneath the tight junction separating the epithelial cells, known as the lateral intracellular space, has assumed great importance in models of transepithelial fluid transport. In this chapter I will review models of epithelial fluid transport as well as measurements of the water permeability properties of epithelia. Finally the pathway and mechanism of transepithelial fluid transport in *Necturus* gallbladder epithelium will be considered.

HISTORICAL REVIEW

Investigations into the mechanism of transepithelial fluid transport by intestine (Curran & Solomon, 1957; Reid, 1902) and amphibian proximal tubule (Windhager *et al.* 1959) had established that water flux was secondary to solute transport. These studies demonstrated that the primary process in transepithelial fluid absorption was the transport of solute (primarily NaCl) out of the lumen. Diamond's work on the fish (Diamond, 1962) and rabbit gallbladder (Diamond, 1964a,b) showed that the osmolality of the transported fluid approximated that of the solution in the lumen of

██bladder. These results were in agreement with previous work on intestine by Curran & Solomon (1957) as well as that on *Necturus* proximal tubule (Windhager *et al.* 1959). In both of these studies the calculated osmolality of the transported fluid agreed, within experimental error, with that expected for isosmotic fluid transport. Thus there developed the concept of isosmotic fluid transport by epithelia. In the following 20 years there has been a succession of theoretical and practical attempts to establish the exact mechanism of fluid transport and routes taken by fluid as it crosses the epithelium. It has been generally agreed in these studies that water movement must be the result of osmosis. The perplexing problems arose from the fact that no transepithelial osmotic gradients could be detected (Diamond, 1964*b*, 1979; Schafer, Patlak, Troutman & Andreoli, 1978) and in most epithelia the transepithelial potential difference was also near zero (Diamond, 1964*b*, 1979; Hill, 1980). Therefore both simple osmosis as well as electro-osmosis seemed to be ruled out by the lack of detectable transepithelial driving forces (reviewed by Diamond, 1979; Hill, 1980). The most widely accepted solution to these problems was put forward by Diamond & Bossert (1967) in their 'standing gradient' model for fluid transport. This model was an outgrowth of an earlier three-compartment model for epithelial fluid transport. Curran & MacIntosh (1962) proposed that the osmotic driving force for transepithelial fluid transport arose within the epithelium. They developed a three-compartment model which resulted in vectorial fluid transport because of solute transport into the middle compartment. The membrane between the middle compartment and the lumen was assumed to be similar to a cell membrane while the membrane facing the serosal (blood side) bath was assumed to be similar to a basement membrane. The lumen-facing membrane was an effective semipermeable barrier while the blood-facing membrane was ineffective as an osmotic barrier. Thus as solute was injected into the middle compartment by active transport (or in the case of the physical model by one of the investigators) water was osmotically drawn into this compartment from the luminal bath. Water was not drawn into the middle compartment from the serosal bath because of the lack of solute reflection by the serosal membrane. As water entered the middle compartment the pressure within it rose and hydrostatically forced fluid out across the leaky serosal membrane. Fluid could not be forced out of the middle compartment across the luminal membrane because of solute reflection by this barrier. Any hydrostatically driven water flow across this membrane would result in solute accumulation and powerful opposing osmotic gradients. The exact location and geometry of the middle compartment was not defined by Curran & MacIntosh (1962) although the characteristics of the limiting membranes were described.

Whitlock & Wheeler (1964) suggested, on the basis of morphological studies of the rabbit gallbladder, that the middle compartment of the Curran & MacIntosh model was the lateral intercellular space separating epithelial cells. Tormey & Diamond (1967) confirmed and extended the work of Whitlock & Wheeler and provided the morphological basis for the mathematical model developed by Diamond & Bossert (1967). It is worthwhile recalling why Diamond & Bossert rejected simple osmotic water flow as the mechanism of fluid transport. They reviewed the literature values for epithelial hydraulic water permeability and concluded that the water permeability ██the cell membranes would be too low to enable water and solute to move out of the

cell with equal speed. In other words they concluded that salt actively transported ⬤ of the cell would rapidly diffuse away before exerting a significant osmotic effect across the basolateral membrane. They therefore proposed a region of restricted solute diffusion within the epithelium where water and solute could equilibrate and achieve isotonicity. The lateral intercellular space between the epithelial cells was the postulated site of this solute-solvent coupling. The space geometry and the location of the solute pumps figured prominently in this model. The spaces had to be sufficiently narrow and tortuous to restrict solute diffusion; the pumps had to be located near the blind, tight junctional end of the channel. The tight junction was assumed to be virtually impermeable to solutes and water. Alterations of interspace geometry, such as widening or shortening of the space resulted in the formation of a hypertonic absorbate. Thus a model was developed and widely accepted which assigned critical importance to the morphology of the epithelium.

What followed in the next 15 years was a large number of morphological and theoretical studies concerned with the geometric changes associated with alterations in the rate of fluid transport. It became evident that several assumptions of the standing gradient model were incorrect. It was convincingly demonstrated that: (1) the tight junction was leaky to ions (Frömter, 1972; Sackin & Boulpaep, 1975), (2) solute pumps were uniformly distributed (Kyte, 1976; DiBona & Mills, 1979) and (3) it was mathematically impossible to achieve an isosmotic absorbate (Sackin & Boulpaep, 1975; Hill, 1980; Weinstein & Stephenson, 1981a,b). A number of serious questions were raised about the validity of the standing gradient theory by several investigators (reviewed by Hill, 1980). Diamond (1979) rebutted their criticisms and pointed out the need for direct measurement of the epithelial cell membrane water permeability. Diamond (1979) argued that all previous measurements of epithelial water permeability were underestimates of the true value because of solute polarization effects on the unstirred layers on each side of the tissue. The result of the conflicting publications of the 1970's was a disconcerting disarray of theoretical and experimental observations which cast the field of fluid transport adrift with no clear solution to the dilemma.

EPITHELIAL CELL MEMBRANE PERMEABILITY

If nothing else the debate of the last 10 years has emphasized the need for knowledge of the water permeabilities of the cell membranes and tight junction. Remarkably few measurements have been made of the water permeability of the membranes of the epithelial cell. Epithelial hydraulic water permeability (Lp) has been measured in a number of preparations by the imposition of transepithelial osmotic pressure gradients. Unstirred solution layers adjacent to or within the tissue cause substantial underestimates of Lp (Diamond, 1979; Pedley & Fischbarg, 1980). The extent of the unstirred layer dependent error has been discussed by a number of investigators (Pedley & Fischbarg, 1980; Whittembury, Verde de Martinez, Paz-Aliaga & Linares, 1981; Van Os, Wiedner & Wright, 1979; House, 1974) and it has generally been concluded that the Lp of most epithelia has been underestimated by at least one order of magnitude (Diamond, 1979). The exception to this conclusion is the renal tubule which, because of its small size, is essentially free of unstirred layer related erro ◤

rich, Rumrich & Fuchs, 1964; Schafer *et al.* 1978; Persson & Ulfendahl, 1970). Until recently epithelial cell membrane Lp had only been measured in the toad urinary bladder (Hays & Leaf, 1962; Finkelstein, 1976*a,b*), and there is still debate regarding the exact values of the water permeability of the apical and basolateral membranes (Levine & Kachadorian, 1981; Hardy & DiBona, 1982). As Diamond (1979) made clear in his review, there was a need for new approaches to the study of fluid transport by epithelia.

One such new approach has been developed in our laboratory over the last 7 years. We utilized quantitative light microscopy of *Necturus* gallbladder epithelium to measure cell volume (Spring & Hope, 1979; Persson & Spring, 1982). The tissue was mounted in a miniature Ussing chamber and placed on the stage of a microscope equipped with differential interference contrast optics and a video camera. Optical sections of the cells could be produced at different focal planes from the apical to basal ends of the cells. These images were recorded on video and stored on video disc for subsequent analysis. The area of each section was determined by planimetry and cell volume computed from the section area and displacement of focus. The acquisition of the images and control of the experiment was computerized; a complete set of sections were obtained in 1–1·5 s. Thus records of cell volume could be obtained rapidly (i.e. at 4 to 6 s intervals) and the stored images could then be analysed off-line. The Lp of the membranes of the *Necturus* gallbladder epithelium was measured by determining the rate of change of cell volume in response to a rapid change in the osmolality of the mucosal or serosal bath by the addition or removal of mannitol from the perfusion solution (Persson & Spring, 1982; Ericson & Spring, 1982). The initial rate of change of cell volume was used to estimate the water permeability of the appropriate cell membrane.

When the osmolality of either bath was altered, the cell rapidly changed to a new volume appropriate for ideal osmometric behaviour. This initial osmotic response was followed by a volume regulatory phase during which cell volume returned to near control value despite the altered bathing solution osmolality. Both the osmotic shrinkage and the subsequent volume regulation have been studied in detail (Ericson & Spring, 1982; Persson & Spring, 1982) and are not the topic under consideration here. I would like to concentrate on the estimates of the water permeabilities of the cell membranes calculated from the initial rate of change of cell volume after the change in osmolality. The water permeability of the apical membrane was estimated to be $0·055\,\mathrm{cm\,s^{-1}}$ and that of the basolateral membrane to be $0·12\,\mathrm{cm\,s^{-1}}$ (Persson & Spring, 1982). These water permeabilities are expressed per unit surface area of the cell neglecting the amplification of the membrane due to microvilli or infoldings. Recent measurements of membrane capacitance of *Necturus* gallbladder indicate that apical membrane area is increased eight times by surface amplifications and the basolateral membrane area is increased 26·3 times (Suzuki, Kottra, Kampmann & Frömter, 1982). Thus the area-corrected water permeabilities of the two membranes would be $6·8 \times 10^{-3}\,\mathrm{cm\,s^{-1}}$ for the apical membrane and $4·6 \times 10^{-3}\,\mathrm{cm\,s^{-1}}$ for the basolateral membrane. The water permeabilities of the two membranes are similar and are comparable to those of other membranes such as red blood cell (Terwilleger & Solomon, 1981; House, 1974).

Recent measurements of cell membrane water permeabilities in epithelia by other

methods have resulted in values comparable to those measured in *Necturus* g[...]
bladder epithelium (Welling, Welling, Ochs & Bliss, 1982; Fischbarg, 1982; Gon-
zalez, Medina & Whittembury, 1982). The water permeability of epithelial cell mem-
branes does not seem to be remarkably different from that of other non-polar cells. It
is worthy of note that the cell membrane water permeabilities measured in most
epithelia far exceed the published values of transepithelial water permeability. This
discrepancy is obviously due to errors in the estimates of epithelial Lp because of the
presence of unstirred layers. The one exception to this disparity is the rabbit proximal
tubule where transepithelial water permeability has been estimated at about $0\cdot5$ cm s^{-1}
(Schafer *et al.* 1978). The cell membrane permeability, on the other hand, was
initially reported to be only about 50 % of the epithelial value (Gonzalez *et al.* 1982).
The cell membrane permeability measurements were made on tubules with collapsed
lumens by direct visual determination of tubular diameter changes subsequent to
alterations in the osmolality of the bathing solution (Gonzalez *et al.* 1982). A recent
report (Welling *et al.* 1982), utilizing video records of the changes in tubule dimen-
sions, has resulted in considerably higher values of permeability approaching those of
the entire epithelium. These membrane values may still be underestimates of the
water permeability of the proximal tubule cell because the preparation is unphysio-
logical, due to the absence of a luminal perfusate. Knowledge of epithelial cell mem-
brane water permeability as well as transepithelial permeability permits some con-
clusions regarding the mechanism of fluid transport as well as the route taken by the
transported fluid.

<center>ROUTE OF TRANSEPITHELIAL FLUID FLOW</center>

The possible routes of transepithelial water flow are outlined in Fig. 2. The trans-
cellular movement of water may not be the sole pathway for transepithelial flow because
the tight junction is known to be leaky to ions. There is no reason to believe that the
junctions are not permeable to water, and the possibility must be entertained that
transepithelial fluid transport could be 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
based on the geometry and structure of the tight junction and extracellular shunt
pathway. 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 (Blom &
Helander, 1977; Bentzel, Parsa & Hare, 1969; Spring & Hope, 1979). Significant
rates of transepithelial flow of water and solutes across the tight junction occur when
the area-adjusted permeability of the pathway approaches or exceeds that of the
cellular path. The driving force for fluid movement across the tight junctions is the
difference in osmotic pressure between the basolateral interstitium and the mucosal
bathing solution. Let us assume that the same osmotic pressure difference exists
across the tight junction as across the entire cell. The basis for this assumption is that
any large difference in osmolality between the interspace and the cell interior wo[...]

lt in water flow across the relatively permeable basolateral cell membrane. Thus because the water permeability of the basolateral membrane is high, it is not possible to establish an osmotic gradient across the tight junction which differs substantially from that across the epithelial cell. If the same osmotic pressure difference is exerted across both the cellular and paracellular pathways, the relative water flow through each path will depend on the area-adjusted water permeability of each. It is instructive to calculate the water permeability of the tight junction required to give a significant flow across that structure in *Necturus* gallbladder. The water permeability of the apical membrane of the *Necturus* gallbladder epithelium is $0\cdot055$ cm s^{-1}. To achieve equal flows across the junctional and cellular routes, the junction water permeability required is 10^4 times greater than that of the apical cell membrane, or 5 m s^{-1}. This is a physically unreasonable value, representing a flow velocity which cannot be achieved in biological systems. Similar calculations have been made by a number of previous investigators (reviewed by Diamond, 1979; Weinstein & Stephenson, 1981*a,b*), who have rejected transjunctional water flow as a significant component of transepithelial fluid movement.

Since it is known that the junctions are leaky to ions the question arises whether water could be restricted in its movement across such a pathway. The calculation above does not indicate that the junctions are water impermeable, but that their contribution to transepithelial water flow is negligible. Other investigators have concluded that transjunctional flow of water exceeds the transcellular flow (Bentzel *et al.* 1969; Sackin & Boulpaep, 1975; Gonzalez *et al.* 1982; Whittembury *et al.* 1981;
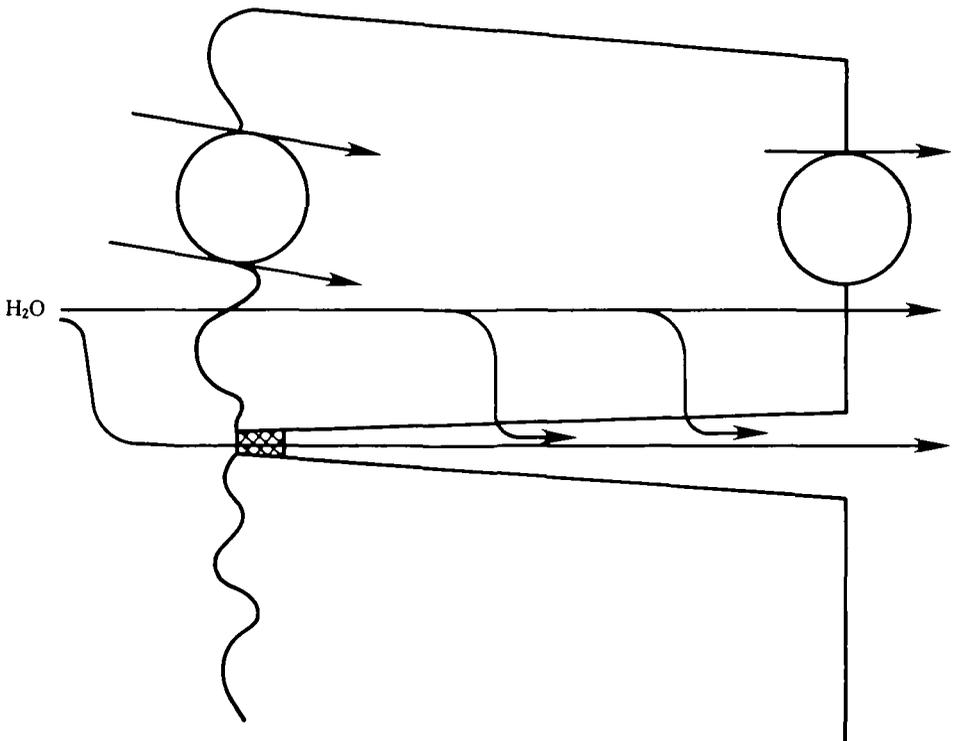

Fig. 2. Possible pathways of transepithelial water flow are shown.

Fischbarg, Warshavsky & Lim, 1977). This conclusion was based either on calc tions of junctional geometry (Bentzel et al. 1969; Sackin & Boulpaep, 1975) or comparison of epithelial and membrane water permeabilities (Gonzalez et al. 1982; Sackin & Boulpaep, 1975). The geometry arguments are not supported by morphological data on the size and shape of openings through the junctions and do not therefore provide convincing evidence for significant flow of fluid across the tight junctions. The comparison of the water permeabilities of the entire epithelium and of the apical cell membrane are only meaningful when both permeabilities are correctly measured; satisfactory comparative measurements have not been reported to date.

The water permeability measurements and calculations for *Necturus* gallbladder lead to the conclusion that virtually all transported fluid takes a transcellular route. Is the same conclusion warranted for all leaky epithelia? The relative flow through the junctional route would increase as the junctional water permeability or area increased or as the cellular water permeability decreased. The apical membrane water permeability could be far less than that of the *Necturus* gallbladder epithelium, for example, equal to that of the toad bladder epithelium in the absence of antidiuretic hormone (ADH) (Hays & Leaf, 1962; Finkelstein, 1976a,b). Such a low water permeability, $2 \times 10^{-4} \mathrm{cm\,s^{-1}}$, is at the lower limit of unmodified lipid bilayers (Fettiplace & Haydon, 1980; Finkelstein, 1976a). An estimate of the maximum water permeability of the tight junction may be calculated from the junctional thickness of $0 \cdot 1 \mu\mathrm{m}$ and by assuming free diffusion of water across the junction. This permeability, $2 \cdot 5 \mathrm{cm\,s^{-1}}$, is about 10^4 times that of the apical cell membrane. Thus after area adjustment the two pathways would have equal flows; these flows would be extremely small ($<2\%$) compared to those typically observed in leaky epithelia. Thus I conclude that junctional fluid flow is not significant in any leaky epithelia.

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 of ions is about 10^{-5} to $10^{-7} \mathrm{cm\,s^{-1}}$. 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 $10^{-3} \mathrm{cm\,s^{-1}}$. Transjunctional 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 $1 \cdot 5 \mathrm{cm\,s^{-1}}$ based on a junctional height of $0 \cdot 1 \mu\mathrm{m}$. The ionic permeability properties of the shunt pathway therefore can predominantly determine the characteristics of transepithelial ionic diffusion. The difference in the paths taken by water and salts in response to transepithelial gradients arises not because the junctions are selectively permeable to salt but because the cell membrane restricts the flow of salt markedly compared to that of water.

DRIVING FORCES FOR FLUID TRANSPORT

Knowledge of the cell membrane water permeabilities permits calculations to be made of the osmotic driving forces required to transport water across each membrane. The rate of transepithelial fluid transport has been measured in *Necturus* gallbladder as $1 \cdot 3 \times 10^{-6}$ to $3 \cdot 4 \times 10^{-6} \mathrm{cm\,s^{-1}}$ (Persson & Spring, 1982). Our most recent estimate of the rate of fluid flow into the cell across the apical membrane during normal fluid transport is about $1 \cdot 6 \times 10^{-6} \mathrm{cm\,s^{-1}}$ (Larson & Spring, 1983). The osmotic gradi
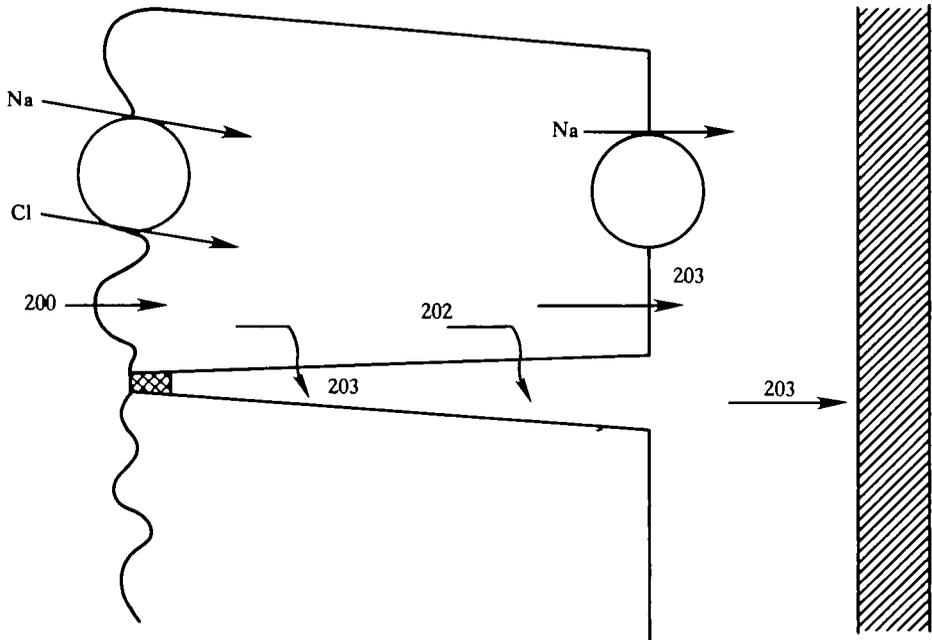
Fig. 3. Calculated osmolalities (in mosmol kg⁻¹) of the cell and basolateral interstitium are shown during normal fluid transport. The underlying basement membrane and connective tissue is shown on the right side of the figure as a shaded bar.

required across the apical membrane to achieve a flow of $1 \cdot 6 \times 10^{-6}$ is about $1 \cdot 6$ mosmol kg⁻¹. Vectorial water flow across epithelia must be the result of a gradient in water activity across the epithelial cell layer. The water activity in the interstitial fluid surrounding the basolateral membrane of the cell must be lower than that in the cell for the movement of water across that membrane. The osmotic gradient required across the basolateral membrane to achieve a fluid transport rate of $1 \cdot 6 \times 10^{-6}$ cm s⁻¹ is about $0 \cdot 7$ mosmol kg⁻¹. Thus the basolateral interstitial space must be about 3 mosmol kg⁻¹ hypertonic to the luminal bathing solution to achieve normal rates of fluid transport. The distribution of approximate osmolalities is shown in Fig. 3. Such hyperosmolality of the interstitial fluid would result in a reabsorbate which is about $1 \cdot 5 \%$ hyperosmotic to the bathing solutions. Such a small difference in osmolality would be difficult to detect by present techniques involving either cryoscopic determination of osmolality, or by direct measurements with ion-sensitive microelectrodes or an electron microprobe. Recent attempts to measure the osmolality of the solution on the lateral intercellular spaces of *Necturus* gallbladder with ion-sensitive electrodes (Curci & Frömter, 1979; Simon, Curci, Gebler & Fromter, 1981) or by interferometry (Coble, Leader & Spring, 1982) have not demonstrated significant differences between the fluid in the spaces and in the bathing solutions. The measurement errors in both techniques were sufficiently large to preclude any definitive conclusions.

The osmotic driving force for fluid transport has been estimated in other preparations by a variety of approaches including electron probe (Gupta & Hall, 1981), direct collection with pipettes (Wall, Oschman & Schmidt-Nielsen, 1970), measurement of

diffusion potentials (Smyth & Wright, 1966; Diamond, 1979), estimation of ▇▇ osmotic gradient required to stop net fluid transport (Whitlock & Wheeler, 1964; Smyth & Wright, 1966; Diamond, 1979). Electron microprobe measurements of the fluid composition of the lateral intercellular spaces of mammalian intestinal epithelium, insect salivary gland, and insect rectal papilla (Gupta & Hall, 1981) all indicated that the fluid in the intercellular spaces was substantially hypertonic to the bathing solutions. The technical difficulties with specimen preservation, as well as errors associated with calibration and measurements of water content, weaken these results and render suspect the conclusion that there are large differences in osmolality between the lateral intercellular space and the bathing solutions in mammalian intestine (see discussion following Gupta & Hall, 1981). The insects may indeed have evolved a fluid transport mechanism which involves large concentrations of organic solutes in the lateral intercellular spaces as indicated by the electron microprobe (Gupta & Hall, 1981) and direct sample collection with micropipettes (Wall *et al.* 1970). Obviously from the preceding considerations, the lateral intercellular spaces must be hypertonic to the mucosal bathing solution in an absorptive epithelium. What is in question is the magnitude of this osmotic pressure difference. The osmotic gradient required is inversely proportional to water permeabilities of the cell membranes. Such gradients will be very small if other fluid-transporting epithelia have cell membrane water permeabilities similar to those of *Necturus* gallbladder.

THE STRENGTH OF TRANSPORT

If the osmotic gradient required for fluid absorption by *Necturus* gallbladder and other tissues is in the range of 2–3 mosmol kg^{-1}, it seems surprising that the imposition of an opposing osmotic gradient of similar magnitude does not stop fluid absorption. Fluid transport was blocked only when the osmolality of the mucosal solution was increased by 20 mosmol kg^{-1} (Persson & Spring, 1982). Increasing the mucosal osmolality until fluid transport stops has been a standard technique for estimating the 'strength of transport' of an epithelium (see Weinstein, Stephenson & Spring, 1981). Rabbit gallbladder can transport fluid against osmotic gradients of up to 80 mosmol kg^{-1} (Diamond, 1964*a*,*b*; Whitlock & Wheeler, 1964), leading investigators to conclude that the interspace osmolality must equal or exceed this value (Diamond & Bossert, 1967; Whitlock & Wheeler, 1964; Smyth & Wright, 1966). Recently it was shown from mathematical models of a fluid-transporting epithelium that the strength of transport experiment is not a measure of the osmolality of the fluid in the lateral intercellular spaces but is instead proportional to the ratio of the active transport rate and the solute permeability of the basement membrane and underlying connective tissue (Weinstein & Stephenson, 1981*a*,*b*; Weinstein *et al.* 1981). Since this conclusion may not be intuitively obvious from consideration of the somewhat complex mathematical model, I will illustrate the point by depicting the sequence of events in a strength of transport experiment in *Necturus* gallbladder epithelium.

When the mucosal osmolality is increased by 20 mosmol kg^{-1} by the addition of an impermeant solute, the cell osmolality must also increase to the same value. This conclusion comes from the high water permeability of the apical membrane. When the cell osmolality increases by 20 mosmol kg^{-1} there must be water flow into the cell fro▇
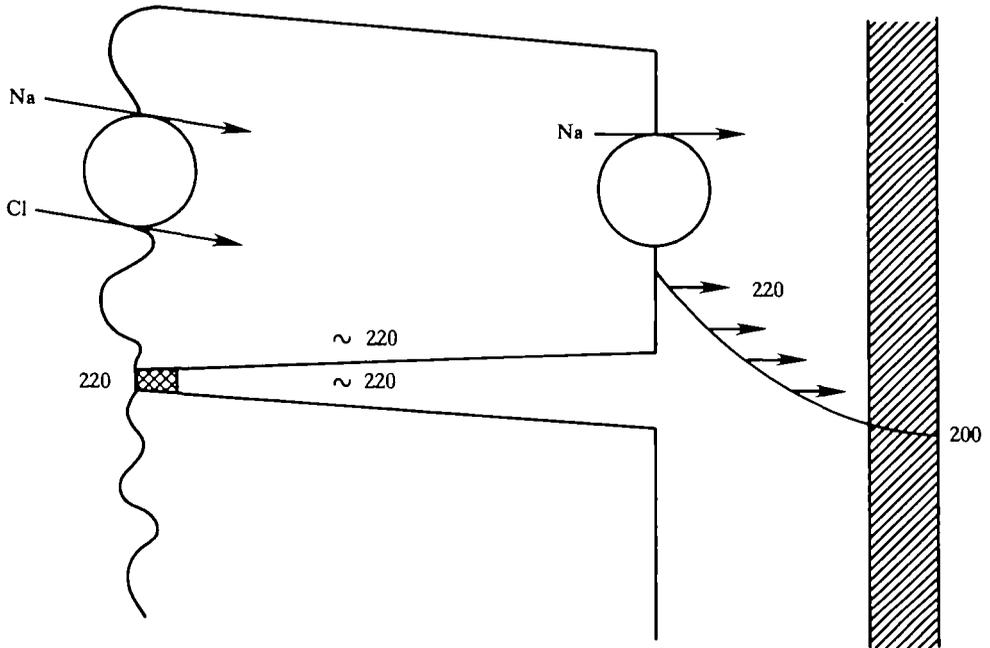
Fig. 4. Probable osmolalities (in mosmol kg⁻¹) in the cells and basolateral interstitium are shown for a strength of transport experiment. The curve connecting the basal cell membrane and the serosal connective tissue barrier illustrates the NaCl concentration gradient which should develop under these conditions. The underlying basement membrane and connective tissue is shown on the right side of the Figure as a shaded bar.

the basolateral interstitium because the area-adjusted water permeability of the basolateral cell membrane exceeds that of the apical membrane. By 30–40 s after imposition of the transepithelial osmotic gradient, the cell and interstitial osmolalities rise to a value approximately equal that of the mucosal bath. I assume that the impermeant solute used to increase the mucosal bath osmolality does not enter the basolateral interstitium and thereby increase its osmolality. What happens instead is that water leaves the interstitium and enters the cells; the primary solute of the basolateral interstitium, NaCl, is left behind. The osmolality of the interstitium is increased by water removal and the salt concentration is elevated in these spaces. An osmotic pressure does not develop between the basolateral interstitium and the serosal bathing solution because the basement membrane and connective tissue are freely permeable to both salt and water (Welling & Grantham, 1972; Weinstein & Stephenson, 1981a,b). Instead a NaCl concentration gradient exists across the connective tissue and basement membrane as shown in Fig. 4. The basement membrane connective tissue layer then constitutes an unstirred layer across which the concentration of NaCl falls until it equals that of the serosal bathing solution.

Why does fluid transport cease under these conditions? It is apparent that the driving force for fluid transport is the increased NaCl concentration in the lateral intercellular spaces which is elevated by active salt transport. Under control conditions the diffusion of NaCl out of the space is balanced by the salt input from active transport. However, when the osmolality has been increased during a strength of

transport experiment, solute diffusion out of the space is increased because of ▄▄▄ increased gradient across the connective tissue. The magnitude of the osmolality difference caused by active transport is diminished by this increased diffusional exit until the point is reached at which no net transport occurs. Thus, as shown by the mathematical model (Weinstein & Stephenson, 1981a,b), the strength of transport experiment is a measure of the rate of active solute transport into the basolateral spaces relative to the rate of solute exit due to diffusion across the basement membrane and connective tissue.

CONCLUSIONS

In summary, the importance of determinations of the water permeability of the cell membrane of fluid-transporting epithelia is illustrated by recent work on the *Necturus* gallbladder. Knowledge of the cell membrane water permeabilities permits estimation of the osmotic gradients required to transport water across the tissue. The relative magnitude of the transcellular and transjunctional flows of fluid can also be estimated from the membrane water permeability and geometry of the epithelium. The driving force for fluid transport by *Necturus* gallbladder is the very small difference in osmotic pressure which exists across the apical and basolateral cell membranes. Transepithelial fluid flow in this tissue is transcellular, with fluid entering the cell across the apical membrane and exiting across the basolateral membrane. Isosmotic fluid transport is then the consequence of the high water permeability of the cell membranes and is not a function of tissue geometry. The lateral intercellular spaces constitute the final common path for transported fluid but do not play an important role in solute-solvent coupling.

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