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REGULATION OF TIGHT-JUNCTION PERMEABILITY DURING NUTRIENT ABSORPTION ACROSS THE INTESTINAL EPITHELIUM

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CONTENTS

ABSTRACT

Tight junctions are located at the luminal aspect of adjacent epithelial cells and form a barrier that limits the paracellular diffusion of hydrophilic solutes. In recent years, evidence has accumulated to indicate that tight-junction permeability is regulated by the absorption of various nutrients. In this review,

we present the physiological basis and importance of tight-junction regulation in intestinal epithelium. The molecular structure of tight junctions and their interactions with the cell cytoskeleton as well as the physical and chemical forces that influence tight junction permeability are described. Much of this review addresses the controversial Pappenheimer hypothesis, which states that a major portion of intestinal glucose absorption occurs through tight junctions and not by saturable transcellular active transport. The absorption of a significant portion of glucose through tight junctions requires increased junctional permeability, a very high intralumenal glucose concentration, and a sufficient osmotic gradient to promote volume flow.

INTRODUCTION

Epithelial cells are characteristically joined together in vivo and in culture by structures called tight junctions or *zonula occludens*. Tight junctions are protein complexes that hold adjacent cells together at their luminal aspect and restrict the transepithelial diffusion of solutes between cells. In recent years, evidence has accumulated suggesting that the tight-junction complexes are dynamic structures that may be regulated by intracellular processes. In a series of reports published in 1987, Pappenheimer and associates (69, 78, 81) observed that the permeability of small intestinal epithelial cells was increased when glucose was added to the solution that bathed the gut lumen. They hypothesized that glucose taken up by the enterocytes served as an intracellular signal to decrease tight-junction integrity and thereby facilitate absorption of hydrophilic solutes by paracellular bulk flow. Because this hypothesis challenged conventional theories of intestinal solute and water absorption, these provocative studies have sparked much debate. In this review, we present the principal findings of the Pappenheimer group as well as relevant supportive and contradictory findings from other laboratories to help the reader understand the significance of these studies almost one decade after their initial publication.

Three aspects of tight junctions are discussed in this review: molecular organization, mechanisms of physical and chemical disruption, and mechanisms of cellular regulation (with an emphasis on the Pappenheimer hypothesis). This chapter is not intended to be an exhaustive review of tight-junction structure and function. We refer the reader to several excellent reviews on this subject (3, 14, 15, 36, 62, 63, 85, 88, 91). Because this review is principally concerned with the regulation of tight junctions in enterocytes, we focus on studies using intestinal epithelia or other epithelia with similar resistance properties, such as gallbladder or proximal tubular epithelium. However, we also refer to other tissues when appropriate.

STRUCTURE OF TIGHT JUNCTIONS

The Epithelial Barrier

Epithelia are sheets of closely apposed cells that form boundaries between various compartments of the body. These cells form the interface between the body and its environment, as seen in the skin, gastrointestinal tract, lungs, and urogenital system, or between different organ compartments, as does the choroid plexus that separates blood from cerebrospinal fluid. Epithelia vary greatly in their permeability to water, ions, and hydrophilic solutes (32). For instance, in tissues that transport large quantities of water and solutes, such as those in small intestine, gallbladder, and proximal tubules of the kidney, the epithelial cell barriers are "leaky" to ions and water. Conversely, the epithelia in other tissues, such as in the skin, urinary bladder, and stomach, are much more restrictive to water and solute permeation and are often termed "tight" epithelia.

The epithelial barrier is composed of a cellular component and a paracellular component (Figure 1). The cellular component is comprised of two membranes in series: the apical or luminal cell membrane, and the basolateral or abluminal cell membrane. Passage of substances across the cellular aspect of the barrier is controlled by epithelial cell membrane channels and transporters that provide for selective passive and active movement of physiologically important solutes. The paracellular component of the epithelial barrier is the pathway between adjacent epithelial cells. Transport across the paracellular pathway is restricted by the junctional complex and the lateral intercellular spaces. The junctional complex is composed of tight junctions, adherens or intermediate junctions, desmosomes, and gap junctions. Although the lateral intercellular space can contribute to overall paracellular resistance, the most important component of the junctional complex for restricting passage of small solutes through the paracellular pathway is the tight junction. This review focuses on the structure, function, and regulation of tight junctions.

In transmission electron micrographs, the tight junction or *zonula occludens* appears as a close juxtaposition of the membranes of adjacent epithelial cells (26, 66). Associated with the tight junction is a collection of electron-dense material that is part of the actin cytoskeleton (64, 67, 68, 72). In intestinal epithelial cells, an extensive actin network supports the apical villi (71, 73) and is linked to the tight junctions (23). Immediately below the tight junctions, the adherens junctions are tightly coupled to a circumferential actin–myosin II ring (61, 66, 69). This perijunctional actin ring is a dynamic structure and may transmit cytoskeletal changes to the junctional complex (68) (Figure 2).

Tight junctions appear on freeze-fracture electron micrographs as complex strands or fibrils imbedded in the cell membrane (19, 35, 95). When different epithelia are compared, the complexity and number of tight-junction strands

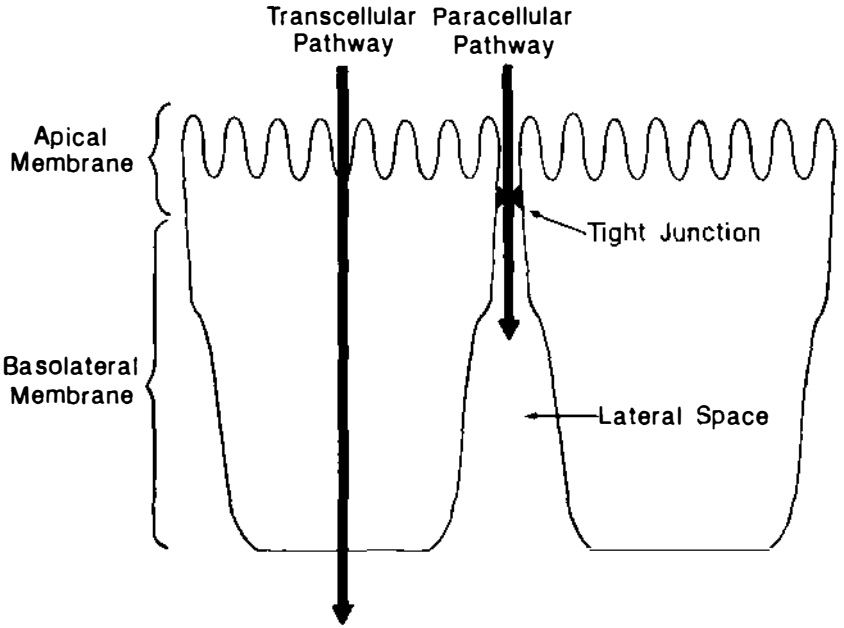

Figure 1 Schematic representation of the epithelial barrier showing transcellular and paracellular pathways. The lateral tight junctions restrict solute and water movement across the paracellular pathway and define the boundary between apical and basolateral membranes. Depending whether it is expanded or collapsed, the intercellular lateral space may also influence solute and water movement through the paracellular pathway.

generally correlate with the electrical resistance or "tightness" of the barrier (18, 19). In some cases, however, differences in permeability between strains of cultured cells do not correlate with the number of strands or with their complexity (98).

Molecular Anatomy of the Tight Junction

Several proteins have been identified that are found in close approximation to tight junctions. The first tight junction–associated protein to be characterized was named ZO-1 (*zonula occludens* 1) (4, 99). ZO-1 is a 220-kDa serine phosphoprotein (4) found only on the cytoplasmic side of the junctional complex and therefore does not form the interconnection between adjacent cells. Phosphorylation of ZO-1 may be important in regulation of permeability because ZO-1 phosphate content varies between kidney epithelial cell strains with different tight-junction permeabilities (97). The full-size isoform of ZO-1 is expressed in most types of epithelia, whereas a truncated form lacking an 80–amino acid domain is expressed in endothelial cells, Sertoli cells, and

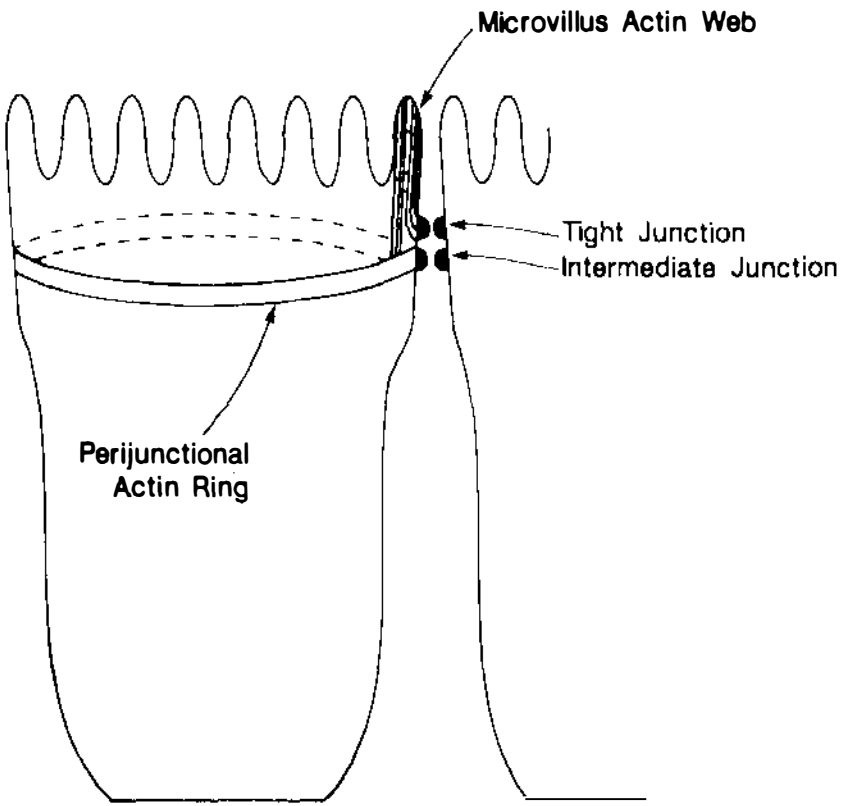

Figure 2 Interaction of tight and intermediate junctions with the actin cytoskeleton. In intestinal epithelial cells, microvillar actin filaments are connected to both tight and intermediate junctions. A contractile perijunctional actin ring extends circumferentially on the cytoplasmic aspect of the cell membrane in close association with the intermediate junctions.

glomerular epithelial cells (55, 102). In general, the cells expressing the shortened isoform have junctions that are more easily remodeled (7). Therefore, some aspect of the full-length ZO-1 molecule may be involved in the stabilization of tight junctions.

Because it is found in cells that do not form tight junctions, ZO-1 may have a more generalized function than first thought (47, 48). In those cells, ZO-1 associates with cadherins, the cell-adhesion molecules essential for formation of cell-to-cell contacts (38). ZO-1 was shown to bind spectrin in these nonepithelial cells (47, 48), suggesting that ZO-1 may link both tight junctions and cadherins to the actin cytoskeleton. In this regard, ZO-1 has physicochemical properties similar to ankyrin, the protein that links the actin cytoskeleton to membrane-anchoring proteins in erythrocytes (4, 15, 25). ZO-1 is the largest of the known tight

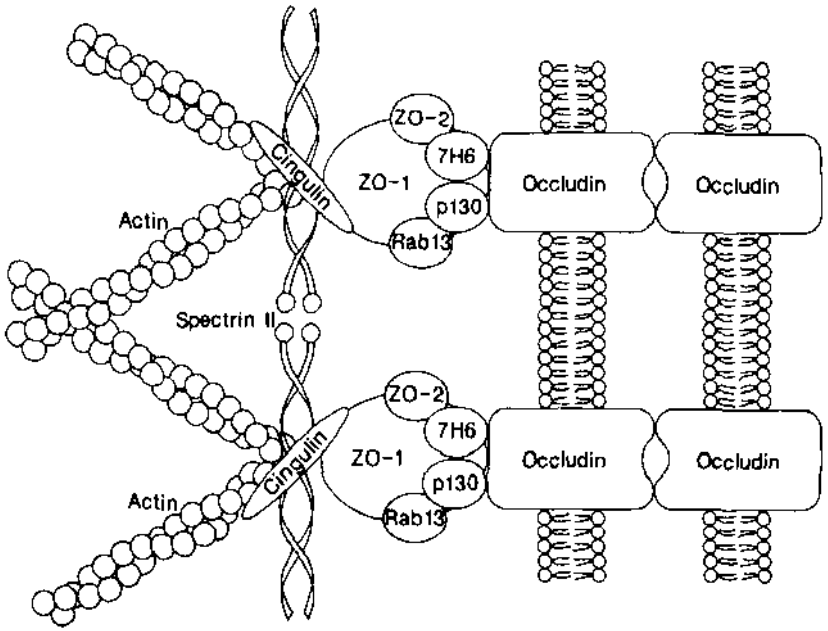

Figure 3 Proposed molecular organization of tight junctions. The transmembrane-spanning protein occludin is shown binding complementary domains on occludin molecules in an adjacent cell. The cytoplasmic domains of occludin are bound to ZO-1 and its associated multiprotein complex. This ZO-1 complex is linked to the actin cytoskeleton through spectrin II and cingulin. Contraction of the actin cytoskeleton or changes in phosphorylation of proteins in the ZO-1 complex could alter the tight-junction barrier formed by occludin.

junction–associated proteins and is located close to the cell membrane (96). If ZO-1 proves to be the key protein linking the actin cytoskeleton to a membrane-spanning tight-junction protein, then changes in the conformation of ZO-1 as well as in the actin cytoskeleton could modulate tight-junction function. Figure 3 shows ZO-1 connecting the membrane-spanning protein, occludin, to actin through nonerythroid spectrin (spectrin II) and cingulin (33).

Since the discovery of ZO-1, several additional tight junction–associated proteins have been found, including ZO-2 (37, 44, 49), cingulin (16, 96), p130 (8), 7H6 (105), and Rab13 (104). These proteins are located on the cytoplasmic side of tight junctions and probably perform bridging or regulatory functions. Figure 3 depicts these proteins as components of a speculative multimeric protein complex, with each member bound to ZO-1.

ZO-2 is a 160-kDa phosphoprotein that coimmunoprecipitates with ZO-1 and shares some sequence similarity with ZO-1 (8, 37, 49). The sequence homology includes a guanylate kinase–like domain that may interact with regulatory G proteins. Unlike ZO-1, ZO-2 has only been found in association

with tight junctions (49). Another phosphoprotein of unknown function that immunoprecipitates with ZO-1 is p130 (8). Cingulin, a 140-kDa protein found in many but not all cells that form tight junctions (15–17), is thought to interact with actin via a myosin-like domain (17) (Figure 3). Rab13 is a small GTP-binding protein that may provide a *ras*-related pathway for tight-junction membrane targeting or regulation (3, 104).

Recently, the proposed transcellular component of the tight junction was cloned and named occludin, after *zonula occludens* (33). Occludin contains four transmembrane-spanning domains and two extracellular loops, as predicted from its hydrophobicity plot. The extracellular domains likely interact with complementary domains on occludin molecules from adjacent cells, thereby forming the barrier portion of the tight junction (33). The predicted structure of occludin is similar to that of the gap junctions, another class of membrane proteins that form complexes with complementary molecules in apposing cells. Figure 3 depicts occludin as the central transmembrane component of the tight-junction complex. The size of the tight-junction strands seen on freeze-fracture electron micrographs suggests that each strand of the tight junction is composed of large aggregates of occludin molecules.

Although speculative, this model allows us to consider several potential mechanisms for the regulation of existing tight junctions. A change in the actin cytoskeleton could induce a change in tight-junction function by altering the tension applied to the junctional complex. A severe stimulus could disrupt the cytoskeleton, destabilize the tight-junction complex, and thereby increase tight-junction permeability. In addition, changes in the phosphorylation state of ZO-1 or any other tight junction–associated protein could cause conformational changes in these proteins that would affect their interactions with occludin. In this regard, the ZO-1 multiprotein complex could be a target for second messengers, including cyclic nucleotides, *ras*-related G proteins, protein kinases A and C, and calcium/calmodulin-dependent kinases. These mediators could induce conformational changes in occludin through their effect on tight junction–associated cytoplasmic proteins and the actin cytoskeleton that would alter solute permeation through the tight junctions. Site-directed mutagenesis studies of occludin and the other tight junction–associated proteins should clarify their respective roles in the regulation of tight-junction permeability.

PHYSICAL AND CHEMICAL ALTERATION OF TIGHT JUNCTIONS

Hydrostatic and Osmotic Pressure Effects

Under conditions where interstitial pressure increases to levels greater than approximately 4 cm H_2O, a level which is reached during volume loading,

the lateral intercellular spaces become dilated and net secretion of small solutes and liquids occurs (40, 46). This phenomenon is termed filtration secretion to distinguish it from active secretion processes, which are probably not greatly affected by increases in interstitial pressure (40, 46). Several investigators report that saline volume loading not only increases serosal to mucosal flux of solutes and water, but also increases paracellular permeability (13, 45, 46).

Small intestinal epithelium is very permeable to water (32). Therefore, water moves freely across the intestinal epithelium to render the luminal contents isosmotic with interstitial liquid. In small intestinal and gallbladder epithelium, serosal hypertonicity dilates the lateral intercellular spaces and decreases transepithelial resistance (R_t), whereas luminal hypertonicity collapses lateral spaces and increases R_t (11, 60, 86, 101). Changes in resistance associated with osmotic gradients have been attributed to the changing geometry of the lateral spaces (103). However, some investigators report that alteration of tight-junction morphology occurs as well. In isolated small intestine, luminal hypertonicity is accompanied by increases in the depth and number of tight-junction strands (60). A recent study with gallbladder epithelium demonstrated that mucosa-to-serosa raffinose gradients dilated lateral spaces and caused focal separations of tight-junction strands (56).

This finding raises an important question: Can physiologically derived osmotic gradients alter the tight-junction morphology of enterocytes? Liquid absorption across intestinal epithelium in response to active transport of electrolytes (e.g. Na^+ and Cl^-), monosaccharides (e.g. glucose and galactose), and amino acids is associated with dilation of intercellular lateral spaces and physical disruption of tight-junction strands (66, 69). However, these researchers contend that tight-junction strands are altered by contraction or rearrangement of the perijunctional cytoskeleton, not by increased interstitial hydrostatic pressure.

Chemical Effects

TITRATION OF CHARGE Tight junctions, although often modeled as simple transcellular pores, do not behave as such. As in most epithelia, the tight junctions of intestinal and gallbladder epithelium are selectively permeable to cations over anions (9, 75, 91). Paracellular cation selectivity of gallbladder and intestinal epithelium is abolished by treatment with polycations such as 2,4,6-triaminopyrimidine (43, 74) and thorium (Th^{4+}) (57), suggesting that the tight junctions are lined with titratable negative charges. Lanthanum (La^{3+}) increases R_t as well as NaCl diffusion potentials and deposits within the tight junctions and lateral spaces of gallbladder and intestinal epithelial cells (58). However, the actions of polyamines and polyvalent cations on tight junctions

may produce effects more complex than those of mere charge since protamine administration increases R_t as well as the number and depth of tight-junction strands and reduces osmotically induced mucosa-to-serosa liquid flow (10, 56).

EXTRACELLULAR CALCIUM Formation of tight junctions in cultured cells requires the presence of extracellular Ca^{2+} (3). Extracellular Ca^{2+} is thought to facilitate tight-junction formation by promoting the binding of complementary cadherins between adjacent cells and alignment of tight junction–forming proteins (38). Tight-junction formation is accompanied by transient increases in intracellular Ca^{2+}, which augments the development of transepithelial resistance and relocation of ZO-1 from intracellular sites to the plasma membrane (100). If extracellular Ca^{2+} concentrations are reduced to very low levels, tight junctions are disrupted (34, 83, 92). The results of these studies are consistent with the concept that physiologic concentrations of extracellular Ca^{2+} are necessary for the formation and maintenance of the paracellular pathway.

CELLULAR REGULATION OF TIGHT JUNCTIONS

cAMP

In addition to producing well-documented effects on cellular transport, intracellular cAMP alters paracellular permeability. Cholera toxin, cAMP analogues, and phosphodiesterase inhibitors reduce NaCl diffusion potentials and increase passive permeability to Cl^- as well as Cl^-:Na^+ permeability ratios in intestinal and gallbladder epithelium (6, 24, 43, 84). These effects suggest that this second messenger increases paracellular permeability. Ouabain treatment, which also increases intracellular cAMP concentrations, produces a similar spectrum of responses (6). However, increases in R_t have also been reported following stimulation with cAMP (24, 84). Although this response in intestinal and gallbladder epithelium has been attributed to collapse of the lateral intercellular spaces secondary to stimulation of liquid secretion (43, 53, 54), cAMP analogues have been shown to increase the number and depth of junctional strands in gallbladder epithelium (24). Therefore, the exact role of cAMP in the regulation of tight junctions is not yet clear. cAMP may decrease tight-junction resistance, but this effect may be masked by the increased resistance that accompanies collapse of the lateral spaces.

Intracellular Calcium and Protein Kinase C

Whereas extracellular Ca^{2+} is required for formation and maintenance of tight junctions, intracellular Ca^{2+} may be involved in regulation of tight-junction permeability. In *Necturus* gallbladder, the Ca^{2+} ionophore A23187 increased transepithelial resistance, NaCl diffusion potential, and the number of tight-

junction strands. This finding suggests that increased intracellular Ca^{2+} decreases tight-junction permeability (77). However, more recent studies dispute the notion that Ca^{2+} regulates tight junctions in gallbladder epithelium and attribute the observed changes in transepithelial resistance entirely to collapse of lateral spaces secondary to stimulation of liquid secretion (53, 54). Very different Ca^{2+} responses are reported for isolated cells. When intracellular Ca^{2+} increased in hepatocyte couplets following vasopressin administration, tight-junction permeability was increased (76). Permeability changes induced by vasopressin are inhibited by Ni^{2+}, which blocks Ca^{2+} channels. These results suggest that influx of extracellular Ca^{2+} was required for the response. Blood-to-bile transport of horseradish peroxidase in isolated perfused rat liver was increased by A23187, indicating that an increase in paracellular permeability occurred (51). Phorbol dibutyrate, a potent stimulator of protein kinase C, increases tight-junction permeability in hepatocyte couplets, suggesting that a phosphorylation pathway may be involved in the regulation of tight junctions (76). Because the response to phorbol dibutyrate was not accompanied by an increase in intracellular Ca^{2+} and because the protein kinase C inhibitor H7 blocked vasopressin-mediated effects, intracellular Ca^{2+} effects are likely mediated through protein kinase C in this system. The physiologic relevance of Ca^{2+} and protein kinase C pathways to the regulation of intestinal epithelial tight junctions remains to be determined.

Sodium-Glucose Cotransport

THE PAPPENHEIMER HYPOTHESIS In a series of papers published in 1987, Pappenheimer and coworkers proposed that the absorption of hydrophilic solutes across small intestinal epithelium was regulated by glucose uptake into enterocytes (69, 78, 81). They hypothesized that glucose transported across the luminal brush-border membrane of enterocytes effected a specific alteration of the cellular cytoskeleton, resulting in a rearrangement of tight-junction complexes and an increased paracellular permeability to water and hydrophillic solutes. They also suggested that at high luminal concentrations of glucose (>250 mM), more of the monosaccharide was absorbed by solvent drag through paracellular tight junctions than was transported by carrier-mediated transcellular transport. In the following sections, the principal conclusions from these studies as well as appropriate confirmatory and contradictory findings from other studies are presented.

Luminal glucose stimulates liquid absorption Using an in vivo perfused rat intestine preparation, Pappenheimer & Reiss (81) demonstrated that the addition of 25 mM glucose to the perfusion solution doubled the rate of liquid absorption. Numerous studies have reported this effect of glucose or amino acids on intestinal liquid transport (2, 30, 87, 94). Active transepithelial trans-

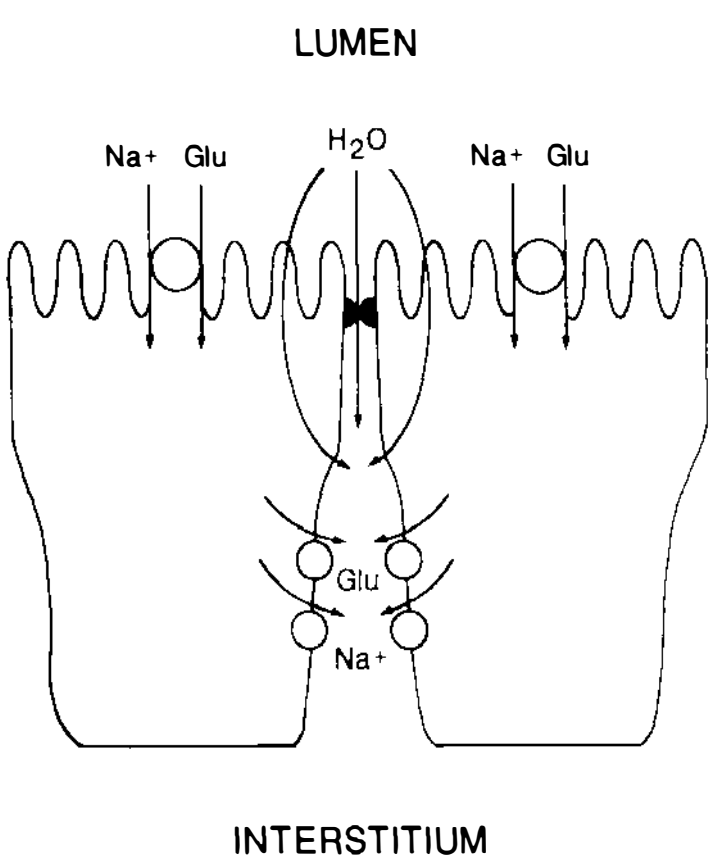

Figure 4 The standing osmotic gradient theory for solute and liquid absorption (22). Sodium (Na^+) and glucose (Glu) are transported across the luminal membrane by cotransport and delivered to the lateral intercellular space by active transport and facilitated diffusion, respectively. Water then flows through the cells and/or tight junctions and into the lateral spaces in response to the osmotic gradient created by the transport of sodium and glucose.

port of glucose and Na^+ is thought to drive liquid absorption by establishing a standing solute osmotic gradient across the enterocytes (22). As illustrated in Figure 4, glucose is first transported across the apical membrane of the enterocytes via a substrate-specific Na^+-dependent cotransporter and then moved across the basolateral membrane by facilitated diffusion. Na^+, which enters the cell with glucose, is actively transported across the basolateral membrane by the Na^+/K^+ ATPase. Active extrusion of Na^+ by the Na^+/K^+ ATPase maintains the Na^+ gradient, which drives the cotransport of glucose. This model of active Na^+-glucose absorption is supported by observations that the addition of glucose to the luminal solution causes significant increases in transepithelial short-circuit current, in net active Na^+ absorption, and in liquid absorption across intestinal epithelia (5, 81). Furthermore, addition of phlo-

ridzin (39), a selective inhibitor of Na^+-glucose cotransport, or replacement of Na^+ with other cations (21) blocks glucose-stimulated Na^+ and liquid absorption. Solute is thereby actively removed from the pericellular apical solution and deposited in the basolateral extracellular space, thus establishing an osmotic gradient for water flow. Whether water moves predominantly across the cell membranes or through the tight junctions remains under debate and is relevant to the Pappenheimer hypothesis. A recent study with *Necturus* gallbladder concluded that at least 30% of osmotically driven water absorption passes transjunctionally (56).

Glucose-stimulated liquid absorption is accompanied by dilation of the lateral intercellular spaces Pappenheimer, Madara, and coworkers used two approaches to document glucose-induced dilation of lateral intercellular spaces. First, Pappenheimer (78) applied an impedance analysis to define epithelial lateral-space resistance (R_l), tight-junction resistance (R_j), and membrane capacitance (C_m) in isolated segments of rat and hamster intestine. He observed that R_l decreased by more than 50% following addition of 25 mM glucose, a response consistent with the dilation or widening of the lateral intercellular spaces. Increased C_m, which accompanied the decrease in R_l, was interpreted to represent an increase in basolateral membrane surface area. Second, light and electron microscopic examination of hamster intestine revealed that the lateral intercellular spaces expanded following luminal glucose (as well as leucine and alanine) treatment (69). Under conditions favoring liquid absorption, dilation of the lateral spaces is also seen in renal proximal tubular (90) and gallbladder (56) epithelium and is generally attributed to expansion of the extracellular space by the absorbate. Because they could not halt dilation of lateral spaces with luminal ferrocyanide, an impermeant osmolyte, Madara & Pappenheimer (69) suggested that the morphological changes they observed resulted from the concomitant contraction of cellular cytoskeletal elements and not from liquid absorption. However, this conclusion may be unjustified because, according to their data, the concentration of ferrocyanide used in these studies (10 mM) reduced liquid-volume absorption (J_v) by only 20% (81).

Intracellular glucose and/or Na^+ mediate increased tight-junction permeability through specific changes in cell cytoskeleton Pappenheimer and coworkers demonstrated that glucose-stimulated liquid absorption is accompanied by reduced tight-junction integrity (69, 78). Their impedance analysis of isolated rat and hamster intestine indicated that tight-junction resistance (R_j) decreases by more than 50% following the addition of 25 mM glucose to the luminal solution (78). Similar reductions in R_j occurred when either 10 mM leucine or 10 mM alanine were added to the perfusion. Because amino acids, which are also transported by Na^+-coupled processes, exert effects on impedance

similar to those of glucose, Pappenheimer & Volpp (82) later proposed that intracellular Na^+, not glucose, triggers the changes in the cytoskeleton and in paracellular permeability. Morphological analysis revealed that luminal administration of either glucose, alanine, leucine, or L-tryptophan produced dilations within the tight junctions and condensation of microfilaments in the zone of the perijunctional actomyosin ring (65, 69). The authors postulated that tight junctions are actively regulated and that contraction of the actin cytoskeleton mediated the increase in paracellular permeability. The finding that cytochalasin D, which contracts the brush-border cytoskeleton of enterocytes, increases tight-junction permeability to a level comparable to that observed following glucose-stimulated liquid absorption supports the theory that the cytoskeleton dynamically influences tight-junction integrity (68). Other researchers also report that intestinal tight-junction permeability to hydrophilic solutes increases following L-alanine and D-glucose perfusion (89). However, some authors challenge the notion that glucose-stimulated liquid absorption increases tight-junction permeability to hydrophilic solutes. Fine and coworkers (28) found that D-glucose-stimulated liquid absorption in human jejunum was not accompanied by altered diffusion ratios of hydrophilic solutes of various radii, indicating that tight-junction permeability was unchanged. Whether these conflicting results can be attributed to species or experimental differences remains to be determined.

We must also consider whether the increased permeability of tight junctions seen following glucose administration results simply from increased intercellular hydrostatic pressure occurring secondary to liquid absorption. If this were the case, the imposition of osmotic gradients that favor absorption across *Necturus* gallbladder epithelium would cause the lateral spaces to dilate, causing focal separations of the tight junctions comparable to those reported by Pappenheimer (56). Pappenheimer (78) rejected this possibility because 10 mM luminal ferrocyanide, an impermeable osmolyte, failed to block the glucose-induced decreases in R_l and R_j and the changes in tight-junction morphology (69). As discussed above, however, this conclusion is tenuous because 10 mM ferrocyanide blocks only a small fraction of the total liquid absorption in this preparation (81). The problems involved in separating the direct effects of glucose and Na^+ transport from the subsequent effect of the osmotic water flow make it difficult to determine whether changes in tight-junction morphology occur prior to or as a consequence of liquid transport. This question will perhaps be answered when the molecular details of tight-junction structure and regulation are elucidated.

A substantial fraction of glucose is absorbed across the small intestine by solvent drag through tight junctions According to Pappenheimer (78, 81), glucose and Na^+ transport creates a transepithelial osmotic gradient for liquid

flow and increases tight-junction permeability to hydrophilic solutes. The increased tight-junction permeability enables solutes such as glucose and amino acids to diffuse through tight junctions down their concentration gradients or to be carried through the junctions by solvent drag. Pappenheimer & Reiss (81) show that clearance of hydrophilic solutes such as glucose, creatinine, polyethylene glycol, and inulin increases linearly with liquid-volume absorption (J_v). This finding suggests that these solutes traverse the epithelial barrier by bulk flow through the tight junctions. Because glucose clearance from the intestinal lumen continues to increase as concentrations are raised well beyond the saturation of Na^+-coupled cotransport, the authors concluded that bulk flow through tight junctions must provide a significant route for absorption of glucose when this solute is present at high luminal concentrations. According to their calculations, absorption of glucose by bulk flow would exceed absorption by transcellular active transport at luminal glucose concentrations in excess of 250 mM. Pappenheimer emphasizes the importance of paracellular glucose transport by comparing the daily carbohydrate intake of several species with the maximum predicted rates of transcellular Na^+ cotransport (79). He estimates that total ingestion of sugars exceeds the capacity for active transcellular transport by threefold in rats and by fivefold in rabbits and concludes that paracellular transport of glucose must represent an important physiological pathway for uptake of this nutrient (79).

The above conclusion was based on an analysis using the linear flux equation developed by Kedem & Katchalsky (50). Pappenheimer & Reiss (81) studied the clearances of solutes that were only transported paracellularly and calculated the contribution of paracellular transport of glucose relative to total glucose transport in the intestine. According to this analysis, the total glucose ($J_{G,T}$) absorptive flux can be described as

$$J_{G,T} = \underbrace{J_{G,C} + J_{G,D}}_{\text{Paracellular}} + \underbrace{J_{G,A}}_{\text{Transcellular}}, \quad 1.$$

where C, D, and A refer to convective, diffusion, and active net transport fluxes, respectively.

The paracellular component of Equation 1 is

$$\begin{aligned} J_{G,P} &= J_{G,C} + J_{G,D} \\ &= (1-\sigma_G)\, J_V P_{LG} + PS_{PG}\Delta C_G, \end{aligned} \quad 2.$$

where C_{LG} is the concentration of glucose in the intestinal lumen; PS_{PG} is the permeability surface area product of glucose in the paracellular pathway; and ΔC_G is the concentration gradient of glucose acting across the paracellular barrier. σ_G, the reflection coefficient for glucose, is 1 when the paracellular pathway is impermeable to glucose and zero if the pathway offers no restriction to glucose. A functional pore radius of 50 Å for the paracellular pathway (81)

would yield a reflection coefficient of 0.03 for glucose. J_{VP} is the net volume flow across the paracellular junction and is defined as

$$J_{VP} = K_{FP}\,\sigma_T\Delta\pi_T, \qquad 3.$$

where K_{FP} is the volume conductance of the paracellular pathway, σ_T and $\Delta\pi_T$ refer to the aggregate reflection coefficient of all solutes and the lumen-to-interstitium osmotic pressure gradient, respectively, across the paracellular pathway.

Thus, the Pappenheimer hypothesis presents a paradox. Consider Equation 4, which describes the total glucose transport across the paracellular pathway:

$$\underline{\text{Convective}} \qquad \underline{\text{Diffusive}}$$
$$J_{GP} = (1-\sigma_G)C_{LG} \times K_{FP}\sigma_T\Delta\pi_T + PS_{PG}\Delta C_G. \qquad 4.$$

First, the driving force necessary to produce volume flow across the paracellular pathway is $\sigma_T\Delta\pi_T$, yet the amount of glucose going through the paracellular pathway by convection is a function of C_{LG} that also decreases the total effect of $\Delta\pi_T$. Because σ_T is small, either the driving force for volume flow, $\Delta\pi_T$, must be very large or K_{FP} must increase in the presence of glucose in order for significant paracellular convective flow to occur. Interestingly, if the luminal glucose concentration, C_{LG}, is increased, the diffusional flux ($PS_{PG}\Delta C_G$) can increase by a like amount, especially if the permeability–surface area product (PS) of the paracellular junctions is also increased and convective flux is small.

The analysis and data presented by Pappenheimer and coworkers indicate that a considerable percentage of the glucose transport occurs in the paracellular pathways if the following conditions are met. First, if C_{LG} is high (250 mM), then 50% of the total glucose transport could pass through the paracellular pathway by convection (i.e. solvent drag). Second, $\Delta\pi$, the driving force across the paracellular pathways exerted by all solutes, must be sufficient to produce flow in the paracellular junctions since the reflection coefficients of Na^+ and Cl^- would be very small (0.03). If the K_{FP} is not 50% of the total membrane conductance but rather 25%, then the osmotic gradient would need to be doubled in order for 50% of the glucose to be transported via the paracellular pathway.

Although the equations used by Pappenheimer to distinguish solvent drag from diffusion have been criticized for their failure to account for the importance of pericellular unstirred layers (27), other researchers report that hydrophilic solute uptake by solvent drag is increased when liquid absorption is stimulated by glucose (29, 31). However, in order for passive paracellular transport of glucose to represent more than 25% of the total amount absorbed, luminal glucose concentrations must exceed 100 mM (81). Prior to 1970, numerous investigators reported that glucose concentrations in the intestinal

lumen ranged from 100 to 800 mM (1, 12, 20, 42, 52, 59, 70, 93). However, a more recent study (27) questioned the validity of these earlier studies, which used nonspecific assays, ommitted adequate controls, and directly intubated unphysiologically high concentrations of glucose into the stomach. When experiments were carefully designed to control for these factors, Ferraris and coworkers (27) found that glucose concentrations in intestinal chyme of rats, rabbits, and dogs ranged from 0.2 to 48 mM when animals were fed physiological diets. Even when animals were fed a 65% glucose diet, intestinal glucose concentrations never exceeded 100 mM. These data suggest that intestinal glucose concentrations in vivo do not reach levels at which paracellular transport could account for more than approximately 30% of the total glucose absorbed. Pappenheimer (80) claims that the Ferraris study is misleading since most glucose present in the small intestine is liberated from disaccharides cleaved by enzymes bound to the luminal membrane of enterocytes. Consequently, the glucose concentration in the pericellular unstirred layer may be far greater than that measured in the intestinal chyme and may, according to Pappenheimer, exceed 300 mM in the close vicinity of the enterocytes. Ferraris and associates found that monovalent cation concentrations ranged from 150 to 170 mM in intestinal chyme (27). Assuming an equivalent concentration of monovalent anions, the osmolality of electrolytes alone in luminal liquid totals more than 300 mOsm/kg. If 300 mM glucose were present in the pericellular layer, this liquid layer would presumably contain up to 600 mOsm/kg of solute, approximately twice the amount found in plasma or chyme (27). Whether such a large osmotic gradient could be maintained in vivo remains questionable owing to the high osmotic water permeability of the intestinal epithelial barrier (32).

Pappenheimer's contention that the absorptive capacity of the gastrointestinal tract far exceeds the capacity for carrier-mediated transport has also been questioned. Ferraris and coworkers (27) estimated that carrier-mediated glucose uptake capacity exceeds glucose intake by approximately twofold in rats fed normal diets. They admit, however, that in animals fed high-glucose diets, intake must exceed carrier-mediated absorption and conceded that the excess glucose could be absorbed paracellularly or fermented in the cecum.

CONCLUSIONS

What conclusions can we reach concerning the Pappenheimer hypothesis? It is difficult to dispute that glucose or amino acid absorption by enterocytes is accompanied by increased liquid absorption and dilation of intercellular lateral spaces. However, considerable disagreement exists as to whether glucose-stimulated liquid absorption is accompanied by increased tight-junction permeability. Even if tight-junction permeability is increased with glucose, it is

not possible at this time to distinguish between active regulation of the tight junction through cytoskeletal contraction and increased tight-junction permeability secondary to dilation of the intercellular lateral spaces by transported fluid. Finally, the notion that significant quantities of glucose are absorbed paracellularly by solvent drag requires that luminal glucose concentrations exceed 250 mM. Although intestinal chyme clearly never exceeds 100 mM glucose under physiologic conditions, higher glucose concentrations could be reached in the unstirred layers at the enterocyte luminal membrane, where the disaccharidases are located. Therefore, the importance of solvent drag for physiologic absorption of glucose, amino acids, and small solutes remains unresolved. In any event, transcellular active Na^+-glucose and Na^+-amino acid transport is of paramount importance for fluid absorption because these active processes establish the required osmotic gradients to produce both transcellular and paracellular liquid flow.

Although the Pappenheimer hypothesis cannot be completely proved or disproved at present, this provocative work has undeniably generated intense interest and study in the field of intestinal nutrient transport. Advances in our understanding of the molecular pathways involved in tight-junction regulation will be crucial to resolving many of these outstanding issues.

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