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Pathobiology of the Intestinal Epithelial Barrier

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The major route of passive permeation across intestinal epithelia is paracellular. The intercellular tight junction lies in and serves as the rate-limiting barrier in this paracellular pathway. Once viewed as static, it is now clear that the structure and permeability of the tight junction is highly dynamic. Not only may inflammatory events (cytokines, neutrophil transmigration) reversibly effect the tight junction but this key barrier also is regulated by physiologic events such as activation of absorptive cell Na^+-nutrient cotransporters. Such physiologic regulation of the junction is of major importance to the absorption of nutrients* via *parcellular solvent drag. (Am J Pathol 1990, 137:1273–1281)

Although geometrically complex, the epithelial lining of the intestine offers some advantages for studies related to columnar epithelia: it remains viable for limited periods *in vitro,* the muscular layers of the intestinal wall can be removed from the mucosa without adversely affecting the epithelium, the resulting epithelium can be mounted as sheets in Ussing chambers for electrophysiologic studies, and the isolated epithelium *in vitro* performs a diverse array of functions characteristic of epithelia, including both absorption and secretion of fluid and electrolytes. In addition, this epithelium, like other epithelia, must restrict a variety of threatening noxious luminal elements from passively permeating into the underlying tissue—that is, the small intestinal epithelium also serves as a model for studies of epithelial 'barrier' function. While intuitively it would seem that barrier and active ion transport functions of an epithelium, like that of the small intestine, should be mutually distinctive and unrelated, the studies outlined below show this not to be the case. Rather this epithelium couples short-term modulation of barrier function with active transport events to conduct the symphony that is intestinal fluid, electrolyte, and nutrient absorption. Lastly it is now clear that intestinal epithelial barrier function can be abnormal in disease states—even when the epithelium remains confluent. Models of such states provide further insights into the dynamic nature of epithelial barrier function.

Tight Junctions: The Rate-limiting Barrier of the Major Permeation Pathway Across Intestinal Epithelia

As outlined in Figure 1, the multiple components of the intestinal epithelial barrier are separable into two broad groups: extrinsic barriers within the lumen, and the barrier intrinsic to the epithelium. The extrinsic barriers stabilize the microenvironment at the apex of epithelial cells and, in some instances, neutralize specific threats within the lumen (eg, H^+ buffering by HCO_3^- secreted by duodenal villus absorptive cells). These extrinsic barriers, however, although important, generally do not provide substantial physical restriction to passive transepithelial permeation. For example, while the so-called unstirred layer overlying the epithelium attenuates the convective movement of molecules (hence the name), diffusive equilibration of lumenal molecules within this layer proceeds according to Fick's law. It follows that the main physical barrier restricting passive molecular permeation resides within the epithelium. This intrinsic barrier has two components: the epithelial cells (transcellular pathway) and the spaces around the epithelium, termed the paracellular pathway. Considering the resistance of model ($\sim 10^6$ to 10^9 ohm · cm^2) or biologic ($\sim 10^3$ to 10^4 ohm · cm^2) membranes, which encase the transcellular pathway, and

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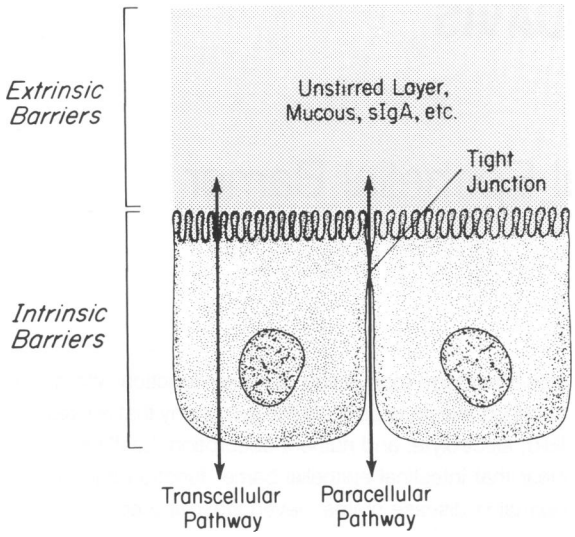

Figure 1. *Schematic illustration of intestinal epithelial barrier.*

comparing this with the resistance of small intestinal epithelia ($\sim 10^2$ ohm · cm^2), it comes as no surprise that the major permeation pathway across the epithelium is paracellular.[1–3] As the above resistance data would indicate, more than 85% of passive permeation is paracellular even for molecules as small as ions.[4] This paracellular pathway consists of two components, the apical junctional complex (Figure 2), consisting of the tight junction (TJ), the intermediate junction, and the belt desmosome, and the subjunctional space. Because even macromolecules such as horseradish peroxidase can freely diffuse within the subjunctional paracellular space but are restricted by the tight junction,[1–3] it is clear that the TJ is the key barrier within this system—it is the rate-limiting barrier of the major permeation pathway. As has been the topic of several reviews,[1–3] and first described by Farquhar and Palade,[5] the TJ (or zonula occludens) is a narrow belt that circumferentially wraps the apical pole of epithelial cells (Figure 2). At the site of the TJ, lateral membranes of adjacent cells focally form fusions or 'kisses' that course around the cell in linear anastomosing fashion. These fusions are represented on freeze fracture replicas as netlike meshworks of strands and grooves. Using a model consisting of a cultured intestinal epithelial cell line (T84), which develops structurally and functionally defined TJ slowly after confluency, we were able to demonstrate that, as the number of strands within a TJ rose arithmetically, TJ resistance increases as a log function.[6] These observations supported the TJ structure–function hypothesis, based on theoretical grounds, of Claude.[7] By fitting structural data to an electrical circuit analog of the mammalian intestinal epithelium, we also showed that similar such structure–function correlates held in the natural epithelium.[8] As will be outlined below, there undoubtedly are several

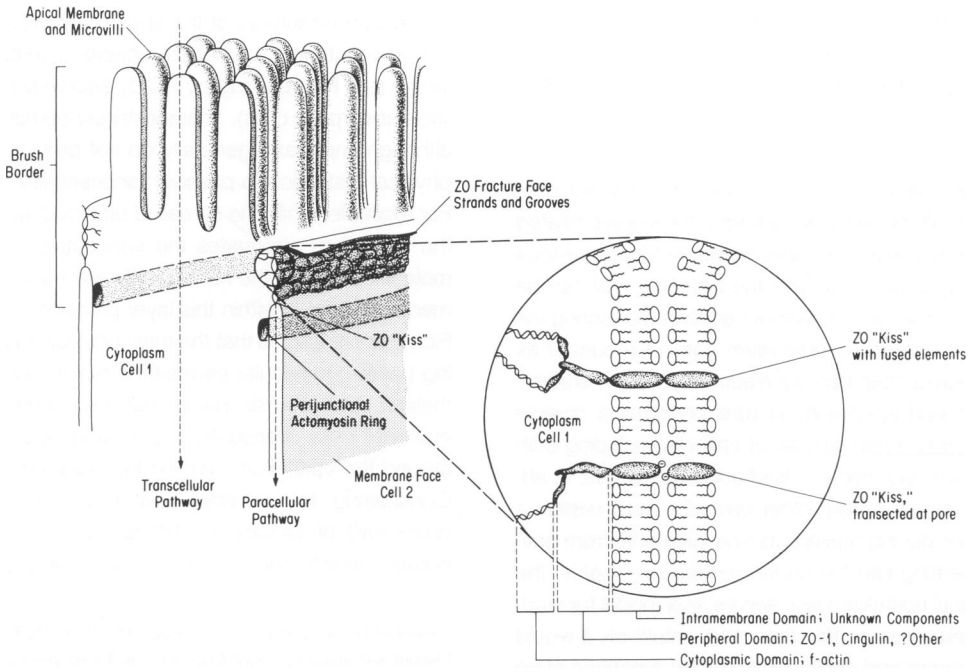

Figure 2. *Schematic illustrations depicting TJ location and structure in intestinal epithelial cells. Shown on the left is the brush-border region of a transected cell. Fragments of the lateral membranes of flanking, neighbor cells are shown. The membrane face of the neighboring cell in the foreground is shown to highlight the freeze-fracture appearance of the TJ. The inset displays a speculative model of the molecular substructure of the TJ (see text). Note that not only might the cytoskeleton modulate the TJ indirectly by tensile forces within a perijunctional actomyosin ring, which circumferentially wraps the cell and inserts on the lateral membrane just below the TJ (left) but direct cytoskeletal-TJ interactions also appear to occur (inset). From Madara[1] with permission.*

factors other than the number of strand subunits that determine TJ resistance.

TJs Have the Potential to Be Regulated

Given the cell-type-specific variation that occurs in TJ structure or permeability,[9-12] in the intestine as well as in other organs, it has been suggested that TJ barrier function is integrated into specific functional roles of epithelial cells. For example, in the intestine, crypt cells are responsible for the active secretion of ions and water, which is the basis of secretory diarrhea.[13] Such secretion occurs by opening of a Cl^- conductance on the apical membrane, followed by passive paracellular movement of Na^+ down the voltage gradient created by the Cl^- secretory process[13] (the TJ is cation selective, thus favoring the passive outward movement of Na^+ over the passive inward movement of Cl^- in this voltage gradient). Thus, passive movement of Na^+ across the TJ is an integral part of the function of this cell type. Accordingly, crypt cells express TJ with structural irregularities and, because of the narrow apex of these cells, with very high density (80 m TJ per square centimeter crypt lumenal surface).[8] This contrasts with the TJ of absorptive cells from fasted animals, which display more structural subunits and approximately only one-fourth the TJ density that crypt secretory cells express.

The composition of the TJ strands is unknown.[1-3] Also, precisely how the anatomically defined subunits of the TJ relate to TJ barrier function is uncertain, although, as outlined above, a general relationship between strand number and resistance appears to exist. Taking into consideration a variety of indirect data such as TJ structure-function correlations,[6,7,14] TJ ion selectivity sequences,[3,7,15-18] TJ sieving characteristics,[6] and TJ charge selectivity,[19-22] we formulated the following hypothetical model of how the TJ might function: TJ kisses/strands could be viewed as relatively impermeable structures in which discontinuities, 'channels' or pores, reside (Figure 2). As with channels of biomembranes, it is proposed that these channels may open and close.[7] The interior of the channel would appear to be highly hydrated and contain fixed negative charges. Assuming this model is correct, it is evident that there exist numerous potential ways to modify TJ barrier function: the number of kisses/strands (ie, TJ subunits) could be changed, the probability of channels being in the open state could be altered, or physical characteristics of the pore interior might be changed. Evidence suggests that some of the above-outlined mechanisms by which TJ function could be altered do indeed occur. Examples include alteration in TJ subunit number (number of strands/kisses),[17,20,23,24] and alteration in surface charge within the pores of the TJ, as suggested by altered TJ charge selectivity.[18]

Recognition that TJs could potentially be regulated by intracellular events came from various observations. First, intracellular mediators can result in altered TJs. Using microelectrode impalement techniques, Duffey et al[21] showed that the TJs of gallbladder epithelium increase in resistance to passive ion flow as cAMP is elevated. Concurrently, TJs gained structurally subunits and TJ charge selectivity was altered. Cyclic adenosine monophosphate (cAMP) also substantially alters TJ function in goldfish[22] and in flounder[18] intestine. Additionally, exposure of amphibian gallbladder epithelium to Ca^{++} ionophore appears to enhance TJ resistance and induce alterations in TJ charge selectivity and structure.[17] Lastly, using a kidney epithelial cell line, it has been shown that phorbol esters diminished TJ resistance.[25,26] It is not known how such intracellular activation signals influence TJs and there is little direct data bearing on this issue. Indirect data suggest however that the cytoskeleton is anatomically and functionally tied to the TJ and may be involved in transducing signals that alter TJ permeability. This hypothesis, which links functional alterations in TJs to cytoskeletal rearrangement, is supported by the observation that structural changes occur in the cytoskeleton adjacent to the TJ during the above-described response to cAMP.[21]

Functional links between the cytoskeleton and the TJ were first described in cultured renal epithelium[27] and in gallbladder epithelium.[24] These seminal studies took the approach of pharmacologically manipulating the cytoskeleton and subsequently assessing the alterations that occur in TJ function. The intestinal epithelium has provided a useful model in which to further examine this putative TJ-cytoskeletal link, as the cytoskeleton of these cells has been so extensively characterized. As shown in Figure 2, one characteristic feature of intestinal (and other) epithelial cells is an apical circumferential ring of actin and myosin.[28-30] This perijunctional actomyosin ring appears to associate with the lateral plasma membrane just below the TJ (Figure 2). This ring is also termed a contractile ring, as studies of isolated brush borders show that, using divalent cations and adenosine triphosphate (ATP), morphologic alterations suggestive of ring contraction can be elicited[31,32] and, in parallel, myosin becomes phosphorylated.[33] What was unclear was whether ring contraction could be induced to occur in an intact epithelial sheet and, if so, whether such contraction would alter the TJ, as some had hypothesized.[34] This suggestion was certainly plausible, as mechanically applied lateral tension[35] (which presumably would be the result if rings contracted) by itself is capable of altering TJ structure. Using cytochalasin D, an agent that affects actin microfilaments, we were able to demonstrate that intestinal absorptive cell TJs became perturbed in structure and displayed diminished charge selectivity and resistance.[23] In parallel, the perijunctional ring became segmented and condensed and the brush borders became rounded—all features

suggesting that, in analogy to isolated brush borders, contraction of isolated segments of the ring had occurred. Supporting this view was the subsequent finding that the effects of cytochalasin D on TJ structure, TJ function, and ring condensation were energy dependent and appeared to be interrelated.[36] Similar data regarding pharmacologically stimulated ring contraction and enhanced TJ permeability have been generated using a model intestinal epithelium[37]—monolayers composed of the human intestinal epithelial cell line T_{84}.

Recently it has been recognized that subtle but direct anatomic associations appear to exist between the cytoskeleton and the TJ (Figure 3).[28,29] Detergent-extracted preparations of intestinal absorptive cells display plaquelike condensations of electron-dense material immediately adjacent to the cytoplasmic face of the TJ.[28] This material often specifically localizes at the sites of kiss/strands within the TJ. It is possible that this material in part represents ZO-1[30,38]—a TJ-specific peripheral membrane phosphoprotein that is a candidate molecule for linking the cytoskeleton with the TJ. As determined using both immunoelectron microscopic[29] and detergent extraction[28] techniques, actin microfilaments intimately associate with these plaquelike condensations that flank the TJ. These data raise the speculative possibility that not only may the TJ be indirectly affected by tension within the perijunctional actomyosin ring, but perhaps elements of the TJ could be directly manipulated through cytoskeletal interactions mediated by TJ-specific proteins such as ZO-1. The functional significance of TJ-specific proteins (including the more recently described cingulin[38,39]) is unclear, but their existence provides additional clues that cytoplasmic signals might influence TJ structure and function. On the basis of such data, a tentative working model of the direct structural relationships between the cytoskeleton and the TJ is presented as an enlargement in Figure 2.

TJs Are Physiologically Regulated

Although TJs are readily viewed as barriers, what follows will indicate that intestinal absorptive cell TJs also constitute a major absorptive transport pathway in the intestinal epithelium. Such observations show how intimately intertwined absorptive and barrier function of epithelia can be.

As expressed in major reviews and texts until very recently,[40,41] the predominant view of how uptake of hydrophilic nutrients such as glucose and amino acids occurs can be summarized as follows: glucose is cotransported across the apical membrane with Na^+, and, via the Na^+-K^+-ATPase pump and by basolateral facilitated glucose transport, these solutes are subsequently deposited into the paracellular space. Absorption of water across the TJ is driven by the deposition of these osmotically active solutes in the paracellular space (or by the creation of a hypertonic subepithelial compartment by other means such as the putative countercurrent exchanger in the villus core[42]). In summary, this view held that nutrient uptake is largely a transcellular event. We have shown that luminal glucose exposure results in alteration of the TJ[43,44] and perturbation of TJ structure (Figure 4).[44] Recently, we have shown that the trigger for this response is turnover of Na^+-nutrient cotransporters present on the apical membrane of absorptive cells.[45] Because apparent condensation of the perijunctional actomyosin ring accompanies these changes, it is suggested that, in analogy to the data previously described, enhanced ring tension underlies this TJ response. Furthermore activation of these cotransporters alter the sieving characteristics of TJs such that there is enhanced clearance of nutrient-sized molecules.[45,46] Last, it appears that this nutrient-induced change in TJ resistance is maximal at nutrient concentrations that saturate the transcellular uptake pathway. The following alternative theory of nutrient absorption arises from these observations and can be summarized as follows: exposure of the intestinal epithelium to lumenal glucose (or amino acids) results in the same events outlined above under the existing dogma of transcellular nutrient absorption but, in addition, TJs become leaky to nutrient-sized molecules because of enhanced cytoskeletal tension. Thus, as water flows across the TJ, substantial nutrient absorption occurs by solvent drag. This view suggests that as luminal nutrient concentration rises above that at which the capacity of the transcellular uptake mechanism is saturated, an increasingly large percentage of nutrient will be absorbed by solvent drag across the TJ. Because the cotransporters of the apical membrane are saturated at low nutrient concentrations (below 25 mmol/l [millimolar] for glucose) but, after a meal, nutrient concentrations in the proximal intestine may be substantially higher,[47,48] it follows that the TJ may be a major pathway of nutrient uptake. The amount of nutrient absorbed by solvent drag would necessarily depend on the concentration of nutrient within the lumen. Thus, one should expect increasing nutrient absorption from the intestine as lumenal solute concentration increases—even the transcellular transport pathway is saturated. Also predicted by this view is that, at high luminal concentrations of Na^+ cotransported lumenal nutrients, the precise composition (glucose *vs.* amino acids) of the lumenal solute would not substantially influence net absorption as long as one of the cotransporters was saturated. These predictions, based on the above trans-TJ solvent drag theory of absorption are being realized. For example, it is clear that glucose uptake by the intestinal epithelium (in the presence of an intact vasculature) continues to increase with increased luminal nutrient concentration past the point of saturation of the transcellular pathway.[49,50] Further, it now appears that oral rehydration solutions combining

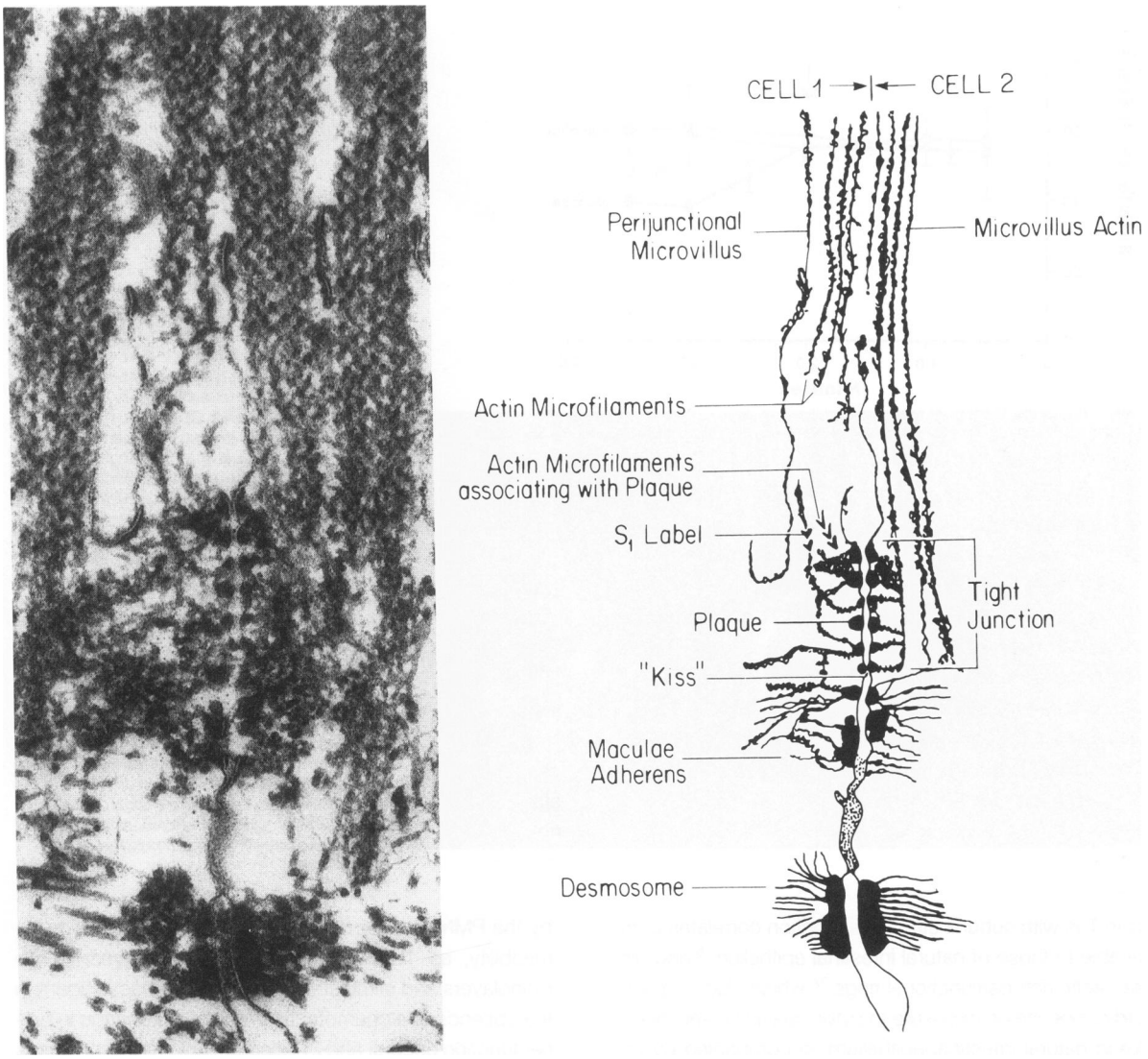

Figure 3. *Electron micrograph (left) and labeled sketch (right) of naked cytoskeleton in zone of ideally sectioned absorptive cell TJ. Electron-dense plaques intimately associate with intrajunctional 'kisses' on one side and with cytoskeletal elements on the other. Specifically, in sections unlabeled with S_1-actin probe, such cytoskeletal elements appear to be microfilaments (not shown) and in sections labeled with S_1 (shown) such microfilaments are shown to be actin microfilaments by characteristic arrowhead label due to S_1-actin association. ~×115,000. From Madara[28] with permission.*

glucose with glycine (attempting to enhance absorption by using two transcellular absorption pathways instead of one) are no more efficacious in providing volume absorption in patients with secretory diarrhea than are solutions containing high concentrations of glucose alone.[51]

TJ Modulation in Disease States

Epithelial barrier function can be severely altered in intestinal diseases. Many such diseases are often characterized histologically by small erosions or even macroscopic ulcers. It is intuitively obvious that when epithelial cells are grossly separated, the site at which the 'TJ' should reside—the ulcer—will not significantly impede transepithelial diffusion of noxious molecules. Diseases do exist, however, in which the epithelium remains morphologically confluent but TJs leak relatively large molecules. Celiac-sprue is an example of such a disorder.[52] Recent studies of modulation of intestinal TJs in disease states have been carried out in hopes not only of gaining insights into what may go wrong with these barriers, but also in hopes of gaining further insights into the mechanisms whereby individual intestinal epithelial cells regulate TJs. Given the uncertainties and complexity of many of the animal models of intestinal disease, these studies have relied heavily on the model human intestinal epithelium, consisting of monolayers of T84 cells.[53] The T_{84} cells grow as confluent monolayers, display high baseline resistance (Figure 5b),

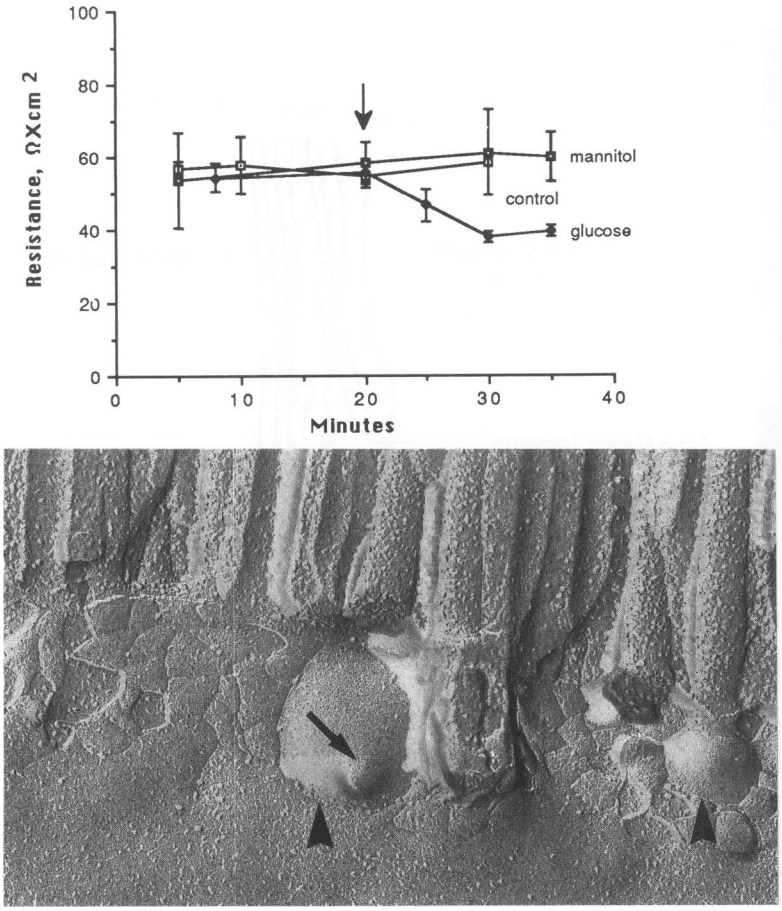

Figure 4. *Effects of luminal glucose on absorptive cell TJ.* **Top:** *Mucosal glucose elicits a decrease ($P < 0.01$) in small intestinal transepithelial resistance that can readily be detected using traditional Ussing chamber, direct-current techniques. Arrow signifies time at which 20 mmol/l glucose or 20 mmol/l mannitol was added to mucosal bath. From Atisook et al[45] with permission.* **Bottom:** *Perfusion of an isolated intestinal loop with glucose elicits focal dilatations of interstrand compartments (arrowheads), which often have concave surfaces and correspond to the intrajunctional dilatations seen in thin sections. Such dilated interstrand compartments also distort the anatomy of the junction. For example, at sites where large dilatations exist, only two junctional strands separate the luminal from the paracellular space, whereas in glucose-free preparation several junctional strands are always encountered separating these two compartments. Presumably this is the structural manifestation of the increased permeability to hydrophilic solutes and the decreased junctional resistance induced by glucose. From Madara and Pappenheimer[44] with permission.*

have TJs with subunit structure–function correlates comparable to those of natural intestinal epithelium,[6] and display actin-rich perijunctional rings,[37] which also segment and condense on exposure to cytochalasin D—an event, like in natural intestinal epithelium, accompanied by enhanced TJ permeability.[37]

Initially, T_{84} cells were used as a model for studies of TJ barrier function in the state of acute inflammation. Acute inflammation of the intestine is characterized by movement of polymorphonuclear leukocytes out of subepithelial microvasculature and into and across the epithelium.[54] This process may be modeled by the placement of isolated PMN on one side of T_{84}[55] or other[56,57] monolayers and of a chemotactic factor such as a n-formylated-peptide on the opposite side. Under such conditions, polymorphonuclear neutrophils (PMN) move across T_{84} monolayers by crossing the TJ (Figure 6).[55,58] With large numbers of transmigrating PMN, TJ function is dramatically impaired (Figure 7). In T_{84} monolayers, impairment takes the form of diminished transepithelial resistance, enhanced transepithelial flux of inert tracers such as mannitol and inulin, and, during the phase in which TJs are actively being impaled by PMN, leaks to macromolecules.[55] On ablation of chemotactic conditions, these barrier alterations are readily reversed. It does not appear that products released by the PMN are responsible for these defects in TJ permeability, as 1) when PMN are densely layered onto monolayers and stimulated with the chemotactic agent in the absence of a chemotactic gradient, no change in barrier function occurs, and 2) selected inhibitors of products released by PMN under chemotactic conditions do not prevent altered barrier function.[58] It does appear that an adhesion plaque that forms before transmigration between the PMN and epithelial cells may be the 'foothold' from which the PMN is able to generate the force required to open the TJ.[58] Thus, we speculate that the opening of the TJ that occurs during PMN transmigration is produced by mechanical force, just as mechanical force may underlie the TJ perturbation induced by the above-outlined pharmacologic[23,36] and physiologic[44,45] manipulations. The difference being that, in these latter instances, the mechanical force putatively is generated by the cytoskeleton within the epithelial cell, whereas with PMN transmigration, the mechanical force is generated externally by the PMN pseudopod.

Unexpectedly *in vitro* models of intestinal disease also promise to yield insights into cytoskeletal–TJ relationships. For example, toxin A, a protein exotoxin of *Clostridium difficile,* causes a severe enterocolitis that in part may be due to its effects on inflammatory cells.[59] To determine

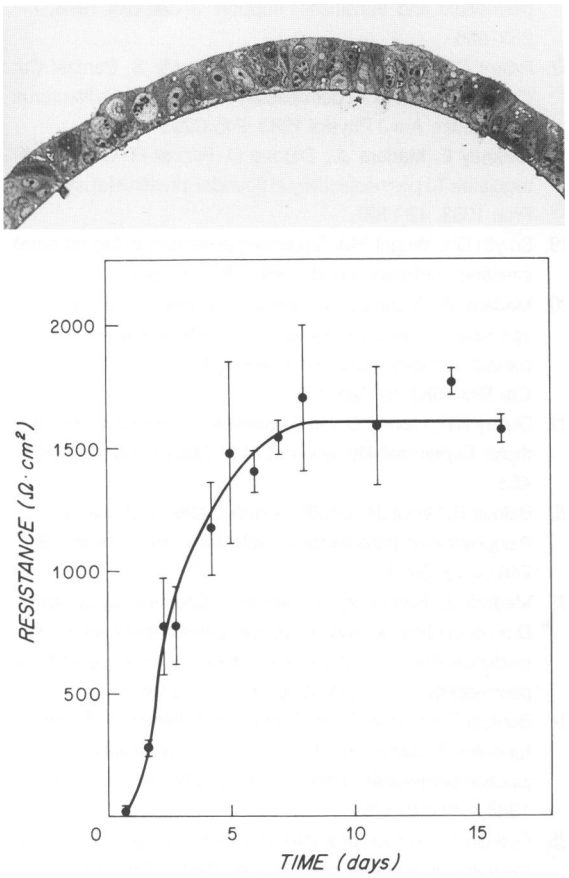

Figure 5. *T84 cells, a human intestinal epithelial cell line* **(left)** *grow as confluent monolayers of columnar polarized epithelia on permeable supports.* **Right:** *A progressive rise in T84 monolayers resistance to passive ion flow occurs in the 5-day period after plating. The high resistance values obtained in the steady state make this an invaluable model for TJ barrier studies. From Madara and Dharmsathaphorn[6] with permission.*

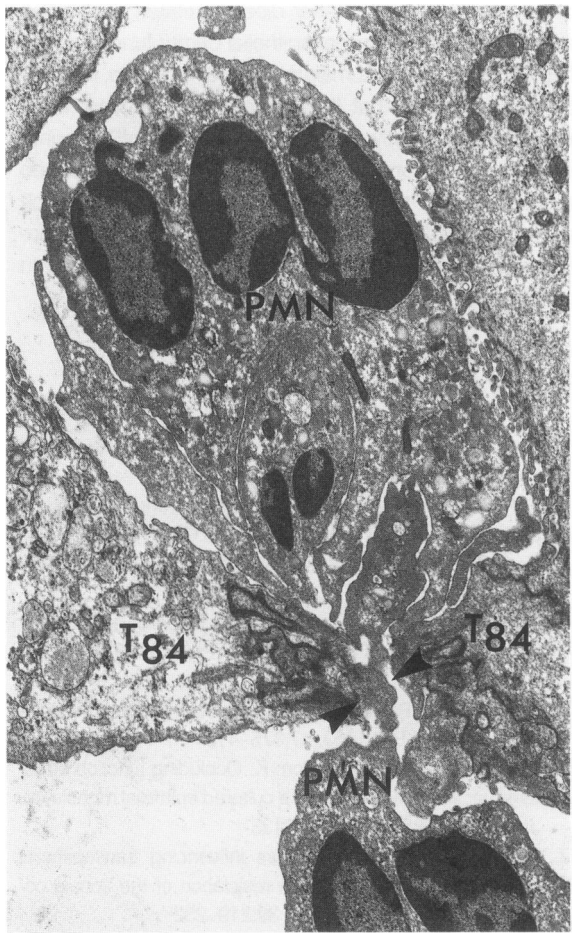

Figure 6. *Electron micrograph of PMN indenting a T84 monolayer and passing single file through a site of TJ impalement. Transmigration occurs by extension of pseudopodia through the site of epithelial discontinuity (arrowheads) (×11,000). From Nash et al.[55] with permission.*

whether this toxin also could exert direct effects on intestinal epithelial cells, T_{84} monolayers were exposed to this agent. Toxin A disrupted barrier function of T_{84} monolayers such that transepithelial resistance was nearly abolished within 6 to 8 hours.[60] The way in which this toxin abolished resistance in this early phase of epithelial perturbation was remarkable. The monolayers remained confluent, cells remained abutted to their neighbors, and no biochemically or morphologically discernible evidence of cytotoxicity was seen.[60] Flux data indicated that this toxin-elicited increase in permeability was restricted to molecules less than 5A in Stokes radius. Because toxin A did not increase the permeability of T_{84} cell plasma membrane to a hydrophilic solute 3.6 Å in radius, the above alterations in permeability were attributed to the toxin-elicited alterations in intercellular TJ. Analysis of the cytoskeleton showed that a prominent effect of toxin A was diminution of F-actin staining in the perijunctional ring. Such findings define a useful model to study cytoskeletal–TJ interactions and suggest that TJ–perijunctional ring interaction, even in the baseline state, may subtly influence the sieving characteristics of TJs.

Given the apparent plasticity of TJs and the putative relationships between TJs and the cytoskeleton, it will not

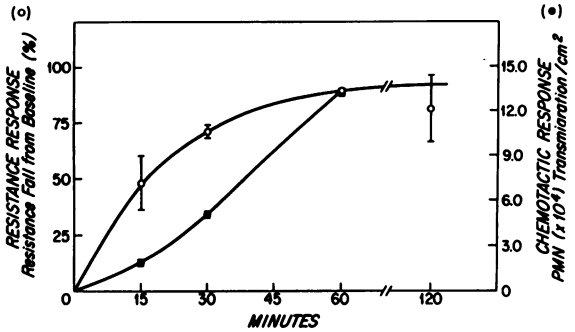

Figure 7. *Time course of chemotactic and resistances responses across T84 monolayers. PMN migrated across monolayers in response to an N-formylated peptide. The transmigration of PMN results in impaired monolayer resistance. From Nash et al.[55] with permission.*

be surprising if various other disease-related challenges substantially alter intestinal epithelial barrier function even if epithelial continuity is maintained. For example, recently it was recognized that the inflammatory mediator interferon-gamma directly effects barrier function of T_{84} monolayers in the absence of cytotoxicity and appears to do so by altering TJ permeability.[61] Further analyses of such systems should enhance understanding of the factors involved in modulating epithelial barrier function, both in disease states and in health.

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