Transepithelial and transendothelial leak, often through the tight junction, appear to be responsible for morbidity and, perhaps, progression of various disease states ranging from allergies to cancer

Keynote review:

Epithelial and endothelial barriers in human disease

James M. Mullin, Nicole Agostino, Erika Rendon-Huerta and James J. Thornton

There is a spectrum of distinct disease states that have in common their effect of breaking down epithelial and/or endothelial barrier function. Fluid compartmentalization goes awry, with profound implications for epithelial and stromal homeostasis, fluid and/or electrolyte balance, generation of inflammatory states, and even tumor microenvironment. Specific effects on the tight junction are found to be integral to bacterial invasion and tumor progression.

In order not to denigrate the species unnecessarily (and is true in some more than others), we could view humans as a collection or a series of gas- and fluid-filled sacs. Lungs, bladders, colons, stomachs, mammary glands, prostate and thyroid glands are examples of such 'sacs'. Tissues, including the uterus, ovary, brain capillaries and even the epidermis, are all barriers – tissues that exist primarily to separate compartment A from compartment B. Apparently, the Creator (however indirect) was a believer in 'good fences make good neighbors' because we are designed in this way. This humble reality is starkly seen in both evolution and development. The coelenterate and the jellyfish, among the earliest forms of multicellular animal life, are somewhat like floating intestines and bladders. The blastocyst, one of the earliest multicellular forms of higher animal life developmentally, is a sac. Life appears to like this idea of sacs, bladders and tubes – in short, barriers separating two distinct fluids (many people of note, researchers among them, are living paradigms of sacs and bladders). This mirrors the compartmentalization one sees on a subcellular level, where intracellular space is compartmented, for example, by nuclei, mitochondria, Golgi and lysosomes.

The first and foremost characteristics of these tissue-level barriers are physiological. Barriers not only separate fluids, but they also perform thermodynamic work on them, by reabsorbing solutes from compartment A to B, or secreting others from B to A. In so doing, they establish gradients across themselves, similar to the gradients that exist

James M. Mullin*
Nicole Agostino
Lankenau Institute for Medical Research,
100 Lancaster Avenue,
Wynnewood,
PA 19096,
USA
Erika Rendon-Huerta
Department of Biochemistry,
Faculty of Medicine,
Autonomous National University of Mexico (UNAM),
Ap 70-159,
Mexico D.F. 04510
James J. Thornton
Department of Medicine,
Division of Gastroenterology,
Lankenau Hospital,
100 Lancaster Avenue,
Wynnewood,
PA 19096,
USA
*e-mail:mullinj@mlhs.org

JAMES MICHAEL MULLIN

James Michael Mullin was born in Philadelphia, Pennsylvania, where he received a BS degree in Biology from St Joseph's University in 1976, and a PhD in Physiology from the University of Pennsylvania School of Medicine in 1980 under the supervision of Leila Diamond and Arnost Kleinzeller, demonstrating for the first time active sugar transport in an epithelial cell culture model (LLC-PK1). After postdoctoral studies at Wistar Institute and Yale University School of Medicine, Mullin returned to the Wistar Institute in 1984, where he began his studies on the action of tumor-promoting secondary carcinogens on epithelial tight junction permeability. In 1986, he transferred these studies to the Lankenau Institute for Medical Research in Wynnewood, Pennsylvania, where he is now a Senior Investigator. These studies were developed into clinical applications for cancer screening, in addition to mechanistic studies for neoplastic progression by demonstrating epidermal growth factor leak across epithelial cell layers exposed to tumor-promoting agents. In 2003, Mullin became Director of Research for the Division of Gastroenterology, Lankenau Hospital.

1359-6446/04/$ – see front matter PII: S1359-6446(04)03379-9

BOX 1

Disease states that are known to target epithelial and/or endothelial barrier function

There are certain disease states that are known to target epithelial/endothelial barrier function, as follows:

Microorganisms
Vibrio cholera
Shiga toxin and verotoxin-producing *E. coli*
Bacteroides fragilis
Yersinia enterocolitica
Clostridium perfringens
Clostridium difficile
Chlamydia pneumonia
Cryptococcus neoformans
Helicobacter pylori
Rotavirus

Allergens
Dust mite proteases
Cotton and flax fibers

Immune system hyper-responsiveness
Neutrophils and macrophages
Cytokines

Inflammatory states
Ulcerative colitis and Crohn's disease
Psoriasis
Encephalitis

Diabetes
Glucose
Diacylglycerol
Protein kinase C

Cancer
Tumor tissue
Tumor-promoting chemicals
Transforming-growth factor
Oncogenes

Genetic diseases
Hypomagnesemia and claudin-16
Deafness and claudin-14

Miscellaneous
Celiac sprue
Menetrier's disease
Multiple sclerosis
Emotional stress
Ageing

across cell membranes. These gradients are then used to establish other gradients. The gradient of Na^+ that exists across the nephron, for example, is used to reabsorb a host of other solutes such as sugars and amino acids that the body would rather not part with. The very high concentration of protons in gastric juices, the extremely low concentration of glucose in normal urine and the high concentration of urea in mammalian urine are all important physiological realities that owe their existence to epithelial barriers. The ability of increasingly more-advanced animal life to migrate ever further from the tidal pools that they called home eons ago, without desiccating in the process, was all a result of the premium which evolution paid on water retention and, in the process, barriers. The physiological value of barriers is fundamental.

Barriers are no less important immunologically. With the exception of the central nervous system (CNS), nearly all luminal fluid compartments communicate directly or indirectly with the environment. The bloodstream functions as a common 'compartment B', protected from the environment by the epithelial barriers surrounding those various luminal fluids. Microorganisms, toxins and allergens are more often than not foiled from contacting the bloodstream by this simple defense wall. But, the second 'defensive line' is an active and vital immune system that waits like a reserve military division for that one in a 1000 infectious or noxious agent that will successfully cross the barrier and enter this privileged compartment.

Having considered how our immune system and our physiology are based on barriers, the theme of this review is that the breakdown of barriers, by whatever mechanism, is not a good thing. Indeed, a surprising variety of disease states owe their morbidity, and often their origins, to states of altered or uncontrolled leak across epithelial or endothelial barriers (Box 1). Ask yourself how often edema is associated with a given disease state, and then reflect what constitutes and gives rise to edema. Edema is just one of the more visible outcomes of barrier breakdown. This is not to say that diseases are not in the end cellular and molecular in origin. There are also diseases that have little to do with transcellular barriers. Cardiac arrhythmias, leukemias and platelet disorders are three prominent examples.

Nature has gone to a good deal of trouble to maintain barriers and compartmentalization. Tight junction (TJ) barriers are maintained even when epithelial cells divide or apoptose [1–3]. The TJ protein occludin is seen to concentrate in the midbody between daughter cells [4]. When epithelial cells in the gastrointestinal (GI) barrier are sloughed off in a normal course, a specialized scenario ensues, maintaining junctional barrier connections to the last moments of cell detachment [5]. Single-cell defects in an epithelial pavement have been shown to be temporally and spatially minimized by interplay between cytoskeleton and TJ in an actinomyosin 'purse string' closure mechanism [6]. Remodeling efforts and TJ retention by an epithelium undergoing scattered, isolated cytokine-induced apoptotic cell death also provides similar evidence [7]. Nature has been said to abhor vacuums, but it seems just as averse to leaks.

What constitutes a barrier?

An epithelial or endothelial tissue has several elements to its barrier function (Figure 1). There are luminal secretions such as mucus or unstirred layers on top of the apical membrane surface of tissue. There are the epithelial or endothelial cells *per se*, whose lipid plasma membranes and highly specific membrane transport systems pose a formidable obstacle to transepithelial and/or transendothelial

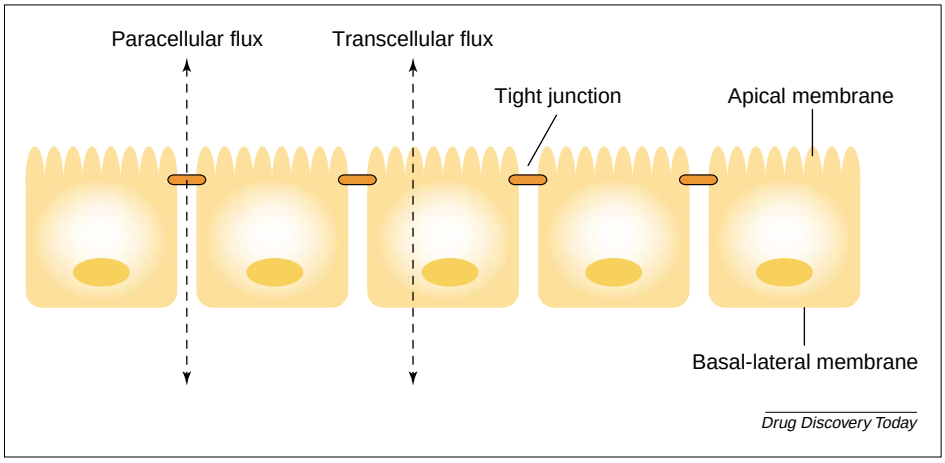

FIGURE 1

Basic epithelial architecture in a simple epithelium. The epithelial barrier is a combination of cells and tight junctions arranged in a manner similar to parallel resistors. A solute can cross a barrier either transcellularly or paracellularly. As a barrier, the epithelium separates two fluid compartments, one luminal and one antiluminal. Luminal fluids exist even in unlikely places such as the alveoli or the colon. Luminal fluids are unique to the specific tissue. In nearly all instances *in vivo*, the antiluminal fluid compartment comprises interstitial fluid (stromal) in series with the bloodstream.

passage of most molecules. There is the stromal compartment 'underneath' the epithelial or endothelial layer, whose sometimes-significant contribution is shown by bioelectrical impedance measurements [8,9]. In addition, there is the intercellular space between epithelial or endothelial cells, whose degree of dilatation can add or take away the resistance factor of a cell layer [10]. Finally, there is the TJ, which sits like a bottleneck at the apical end of the lateral intercellular space, and whose interweaving, threadlike proteinaceous strands can generate a barrier almost on the order of the cells *per se* in tissues such as the urinary bladder, or specific types of epidermis when the ion channels are open in cell membranes [11,12]. This review will concentrate on the TJ component and, to a lesser degree, on the cells themselves. Other barrier elements will also be considered briefly here [13–16].

There have been many reviews on the structure and regulation of TJ [17–21], and we ask that the reader here takes a 'broad view' of the TJ barrier discussed in those reviews and realize that the TJ is a continuum of sorts of the cell cytoskeleton (Figure 2). Actin filaments use TJ-associated molecules (e.g. ZO-1, ZO-2, ZO-3, cingulin, AF-6, 7H6) to connect with the proteins forming the actual strands of the barrier itself (occludin and a family of claudins). In addition, a host of signaling molecules has been implicated in the regulation (and deregulation) of these barrier structures. Protein kinase C (PKC) isoforms [22,23], protein kinase A [24,25], myosin light chain kinase [26–28], calcium [29–31] and G-proteins [21,32–36] are five examples of where the cell machinery can be 'hijacked' to produce altered transepithelial and transendothelial permeability states (Figure 3). Disease therefore has a plethora of molecular targets with which to wreak havoc on TJ barrier function. Examples of diseases that have their origins and/or owe their morbidity in large to impairment of the TJ barrier (for further details, please refer to Ref. [37]) are discussed.

Effects of disease states on barrier function

When one reviews the published literature dealing with disease and epithelial and/or endothelial barriers, one might skip over a simple paradigm: the effect of disease processes on epithelial and endothelial barriers is almost always to make them leakier. More often than not, this reflects a change in the TJ itself such that it either disappears entirely or its permeability increases significantly. This includes a diverse range of disease states such as chronic inflammatory diseases, diabetes, cancer and microbial infections. When one steps back to think, this situation is remarkable in its broadness and probably reflects the biological importance of barriers. A scenario that would appeal to any physical chemist is that the net result of these diverse disease states is then a massive increase in entropy as the barriers fall and the disease progress.

The well known mystery writer Arthur Conan Doyle always had his protagonist, Sherlock Holmes, emphasize that one must be alert to what does not happen as well as to what does happen in a given situation. What does not happen in 99% of disease situations is that the TJ barriers do not become tighter. Recent experimental approaches that have (by transfection) increased the expression of certain TJ proteins such as occludin have shown that decreasing TJ permeability is possible. [38]. Tighter junctions also result during, for example, exposure of cell layers to epidermal growth factor 3 (EGF3). However, in disease situations, tighter junctions do not happen. Instead, TJs become leakier.

Microorganisms: 'we want in'

Certain disease organisms seem intent on getting into the stromal and/or vascular fluid compartment from the luminal compartment they are situated in. Conditions in luminal compartments are often harsh and the nutrient 'pickings' are slim in relation to the well-regulated 'promised land' on the other side of the barrier. Microorganisms appear to be adept at breaching the barrier either by targeting junctions or cells. The model used here for describing this particular phenomenon is based on various diarrhea-generating organisms and their effect on the GI barrier. It can be assumed that the prokaryotic kingdom makes a general policy of frontal assaults against a variety of our barriers. Single-cell organisms equate to single mindedness in this case.

There are examples in the physiological literature as far back as the 1970s pointing to bacterial toxins that target TJs [39]. It was the work of Fasano on the second

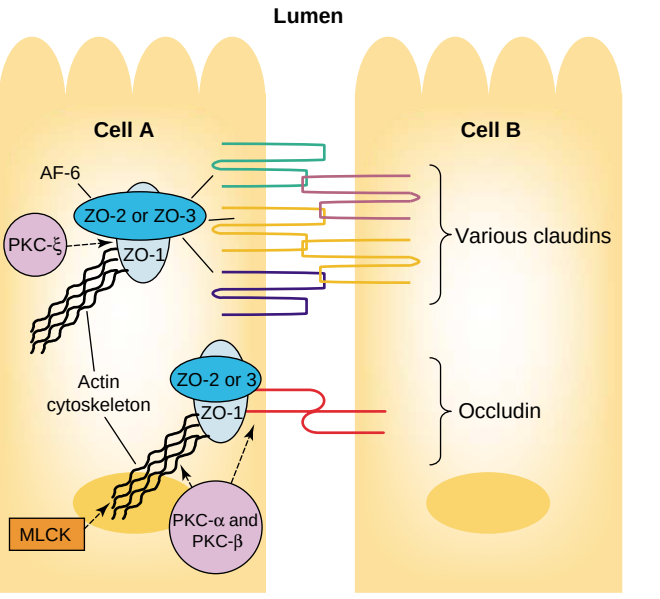

FIGURE 2

The tight junction barrier at a molecular level. Although occludin and a family of claudins form the cell-membrane-spanning, cell–cell bridging portion of the barrier, the tight junction is a complex forming a continuum with the cytoskeleton, using proteins such as ZO-1, ZO-2 and ZO-3 as linkers, and signaling proteins such as the protein kinase C (PKC) family and myosin light chain kinase (MLCK) as modifiers.

Vibrio cholera enterotoxin, ZOT, which pushed the concept to the forefront [40]. The later discovery of a human protein analogue to ZOT, termed zonulin [41], underwrote the importance of the phenomenon. The list of bacterial pathogen toxins targeting the TJ has since grown and has been reviewed elsewhere [42–46].

Diarrhea illustrates how epithelial barrier dysfunction leads to disease pathology. Diarrhea can be classified into two main types: inflammatory and noninflammatory. Inflammatory diarrhea causes cell death, with pus and/or blood present in the stool. Noninflammatory diarrhea falls into two main categories: secretory and osmotic. Secretory diarrhea occurs when, generally, the small intestine (and to a lesser extent the large intestine) secretes large amounts of electrolytes and water in excess of their reabsorptive capacity. Secretory diarrhea is caused by bacterial toxins and enteropathogenic viruses. Osmotic diarrhea occurs when solutes that are water-soluble and unabsorbable remain in the bowel and retain water, for example, in the case of lactose deficiency and lactose malabsorption. This review focuses on pathogens involved in secretory diarrhea. Sometimes, these organisms alter TJs directly and, in other cases, they produce a toxin that alters TJs.

Shiga toxin-producing *Escherichia coli* (STEC) or verotoxin-producing *E. coli* (VTEC) can cause diarrhea and hemorrhagic colitis. In the USA, O157:H7, a common strain of STEC, can lead to systemic illnesses such as hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). STEC does not cause gross injury to intestinal epithelial cells like its enteropathogenic *E. coli* counterparts. However, O157:H7 has been found to decrease transepithelial electrical resistance (Rt) in infected T84 cell monolayers. PKC, calmodulin and myosin light chain kinase have a role in these phenomena [47]. There is also an increased intercellular permeability in these monolayers as a result of an alteration in the TJ protein ZO-1 [48]. However, *E. coli* strains that are negative for shiga toxin produce these same effects, implying that other mechanisms of action for disease pathogenesis by these organisms have yet to be determined [47].

Bacteroides fragilis is a common anaerobe in human colonic flora. There are certain strains of this anaerobe that produce a zinc-metalloproteinase toxin, called fragilysin, which can cause diarrhea. Fragilysin causes a decrease in Rt and an increase in transepithelial mannitol permeability across HT-29 intestinal cell sheets [49].

Although the outcome in humans is different, *Yersinia enterocolitica*-induced diarrhea in mice did not alter Na^+–glucose cotransport or electrogenic chloride secretion, and did not produce erosions or ulcerations of the intestines. However, the Rt decreased by 33%, suggesting possible TJ alterations [50]. *Clostridium perfringens* enterotoxin also causes major increases in permeability, and this toxin physically associates with occludin, claudins 3 and 4 [51,52]. Dissolution of normal TJ strand pattern was demonstrated in freeze-fracture electron micrographs. *Vibrio cholera* has been also found to produce a lesser-studied cytotoxin, which can function as a protease and has been shown to digest occludin bands in Western immunoblots [53].

Clostridium difficile is an anaerobe that causes antibiotic-associated diarrhea and colitis. Common antibiotic culprits resulting in this infection are clindamycin, ampicillin and cephalosporins. *Clostridium difficile* secretes two toxins, toxins A and B, both with substrate specificity for the Rho family GTPases. Rho glycosylation by toxins causes disassembly of actin filaments and cell rounding. Before *C. difficile* causes the disassembly of perijunctional actin filaments, the bacterium induces changes in TJs [54]. Toxin A can elevate PKC activity, causing an increase in paracellular permeability and a reduction in Rt of intestinal T84 monolayers [54]. Toxin A also selectively disrupts ZO-1 from its regular, peripheral, ring-like pattern in T84 monolayers to irregular, scattered clumps of staining seen in immunohistochemistry, a process dependent on PKC [54]. *Clostridium difficile* appears to cause a general dissociation of integral TJ proteins, TJ-associated proteins and cytoskeletal proteins [55].

Expanding the consideration of microbial pathogens from diarrhea-causing organisms, *Chlamydia pneumoniae* exposure to brain capillary endothelia decreases occludin expression while increasing expression of cell-adhesion proteins [56]. The fungal pathogen *Cryptococcus neoformans*, a cause of meningitis, alters subcellular occludin localization

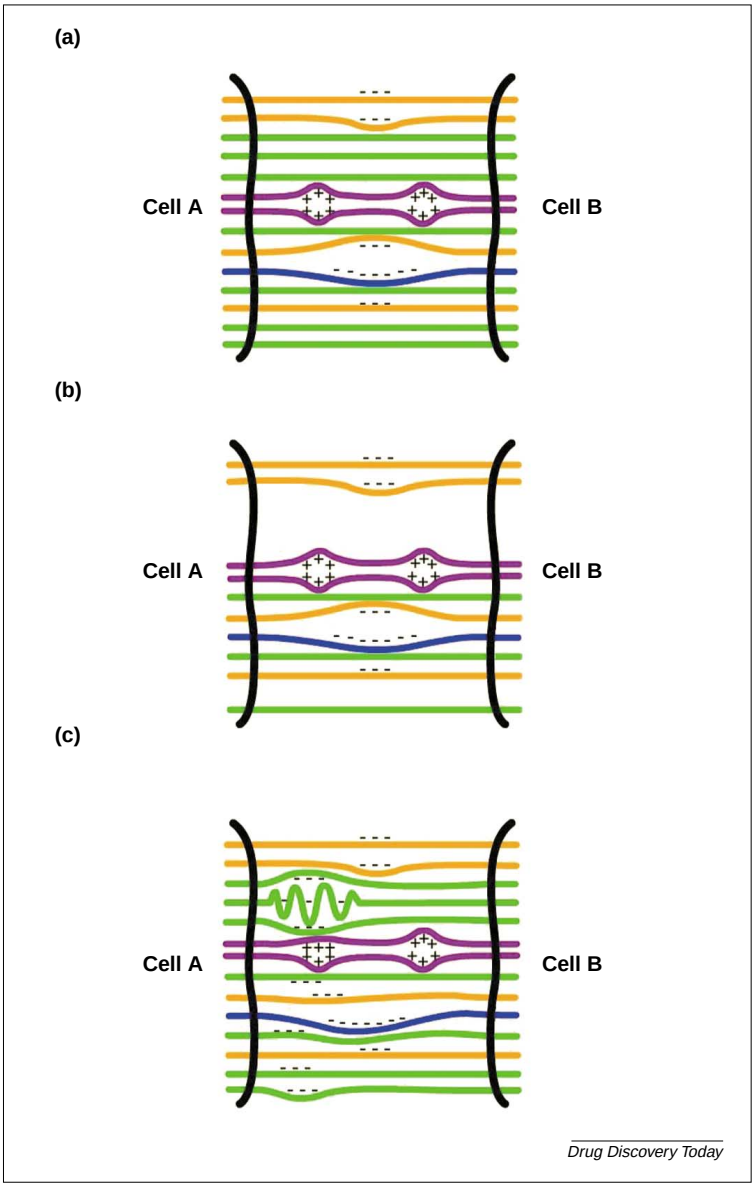

FIGURE 3

The culvert-like nature of the tight junction in a face-on view. A paracellular solute's view of a hypothetical tight junction comprising various claudins in homotypic and heterotypic interactions is indicated. Claudin protein alignment in pairs can create aqueous pores at points where similar charge alignment results in strand repulsion **(a)**. Pores created by such charge repulsion would, by definition, favor passage of certain solutes based on their charge and size. This model oversimplifies, but serves to highlight two types of changes of permeability. In the event of downregulation of a specific claudin **(b)**, the decreased expression of neutrally charged claudin-X creates opportunities for increased paracellular flow of moderately large electrolytes and nonelectrolytes between wider separations of claudin strands. Another possibility **(c)** is that events such as the phosphorylation of intracellular carboxyl tail in claudin-Y creates steric changes in the intercellular loops, resulting in misalignment of claudin-Y. The claudin-Y homotypic interactions then produce, in turn, pores created by interstrand repulsion of similar charges. The net result, as in **(b)**, is decreased transepithelial resistance or increased nonelectrolyte flux, or both. Please note that this is a static model that does not take into account the known dynamic nature of these TJ proteins, but it does provide an insight into at least one dimension of the barrier.

in human brain capillaries [57]. *Helicobacter pylori* sonicates are known to increase gastric mucosal permeability (decrease Rt and increase transepithelial mannitol flux levels) in a PKC-mediated process [58], with linear expression of occludin changing to a punctuate pattern [59]. Certain strains of *H. pylori* express a cytotoxin, VacA, which gives this pathogen a unique survival advantage of acting like a parasite to its host. VacA has the ability to decrease Rt in monolayers of MDCK, T84 and epH4 cells [60]. In MDCK cells, there is evidence that VacA increases the permeability of the monolayers to Fe^{3+} and Ni^{2+}. Fe^{3+} is required for *H. pylori* to grow and Ni^{2+} is a component of urease. Urease hydrolyzes urea to produce ammonia, which buffers the acidic microenvironment of the gastric mucosa for the bacteria. Ammonia is also a source of nitrogen for the bacteria. VacA can increase paracellular permeability to molecules with a molecular weight <350–440 in T84 and MDCK monolayers [60]. *Helicobacter pylori* can also cause these changes without altering ZO-1, E-cadherin or occludin. This suggests that there is a selective modulation instead of a permanent alteration of the TJ. Because many of the nutrients that *H. pylori* require are on the basal-lateral side of tissue, it is tempting to speculate that this bacterium causes leakiness of TJs to gain access to the required nutrients such as Fe^{3+} and Ni^{2+} (because *H. pylori* does not invade the mucosa).

Rotavirus causes severe secretory diarrhea in infants and children. The virus infects columnar epithelial cells and inhibits the absorptive capacity of these cells, causing a net secretion of water and salts, which results in watery diarrhea. This disease can kill its victim by dehydration. Rotavirus has been shown to decrease Rt and increase paracellular permeability in rotavirus-treated Caco-2 monolayers. The paracellular permeability increased in a size-selective manner, permitting charged and uncharged particles of up to 4000 Daltons. Immunohistochemistry studies showed that rotavirus infection decreases the levels of claudin-1, occludin and ZO-1 proteins in the perijunctional region. In addition to the loss of claudin-1 in the perijunctional region of rotavirus-infected Caco-2 monolayers, there was also a shift in the immunfluorescent signal to the cytosol [61]. This effect of rotavirus on epithelial barriers could be mediated by one of its nonstructural proteins, NSP-4, that contacts the apical surface [62]. An outer capsid rotavirus protein, VP4, is cleaved by host trypsin to a fragment, VP8, which also perturbs the TJ barrier [63].

These examples of TJ alteration caused by microorganisms lead to the question of why such alterations occur. It is likely that the ability of pathogens to alter TJs must produce an adaptive and/or evolutionary advantage for the microbes. This might be obvious in the case of *H. pylori* requiring Fe^{+3} and Ni^{+2}. What do other bacteria and viruses stand to gain? If a microorganism can gain access to systemic circulation, there is a wealth of nutrients and an ideal environment for many to proliferate. It has been shown that Na^{+}-coupled solute transport in the small intestine triggers contraction of perijunctional actinomyosin, which then leads to increased permeability of TJ [64]. This increased permeability, a result of normal

small intestine physiology, could cause small amounts of nutrients in interstitial fluid to leak through the TJ into the GI lumen. One wonders if microorganisms might be attracted to this nutrient leak by chemoreceptor-mediated chemotaxis, a well-known phenomenon in many bacteria [65,66], and have eventually evolved to target the region of TJ proteins as a survival advantage.

Allergens – not alive, yet they have a plan

Inert entities such as allergens have also been shown to affect barrier function. If one considers the immune system is normally in an abluminal compartment (the fluid compartment opposite the lumen which, in nearly all cases for epithelial tissues, is the interstitial fluid compartment in series with the bloodstream) and environmental allergens contact an organism through its luminal compartments (e.g. nasal airways, bronchi and gastric lumen), allergens will not cause an inflammatory response, the source of their bad reputation, unless they gain access to the interstitial compartment on the other side of the barrier.

In instances of chronic allergic rhinitis, nasal mucosa has exhibited not only desquamation of the epithelium, but also increased permeability of epithelial junctions [67]. Dust mite allergens have been found to possess several proteolytic enzymes capable of causing progressive cleavage of TJs (including the occludin molecule itself) and increased transepithelial permeability [68]. These proteases are both cysteine- and serine-specific. Specific cleavage sites have been found on the extracellular loops of occludin [69]. Although such proteases have also been linked to induction of epithelial apoptosis, this action was independent of the effect on TJs [70]. Dusts of cotton, hemp and flax fibers, thought to be the active agents in the occupational lung disease byssinosis, were able to increase paracellular permeability of alveolar epithelia [71]. This surprising topic of allergens targeting the TJs has been reviewed elsewhere [72].

The immune response and inflammation: getting to where the action is

When one thinks about the immune system from a compartmental and/or barrier point of view, it is striking that the immune system is often an endothelial and an epithelial barrier away from the action. Microorganisms normally first enter the body by colonizing luminal compartments simply because these compartments have the most contact with the environment. The vast majority of immune system functions, however, start life in the vasculature, the opposite fluid compartment. 'Engaging the enemy' means that those components must on many occasions cross two junctional barriers, one endothelial and one epithelial. It is therefore not surprising that the immune system developed agents and mechanisms adept at making those barriers penetrable.

We discussed the issue of microbial invaders trying to get 'in'. Now the opposite issue exists, the immune system components trying to get 'out'. This physiological perturbation can be prolonged or transient in duration, while either pronounced or moderate in the intensity of the leak, considering the magnitude of what is transpiring. If these processes were understood in greater detail, it might be possible to manage chronic inflammatory diseases more effectively, such as inflammatory bowel disease (IBD), prostatitis, cystitis, rheumatoid arthritis and asthma.

The processes by which white blood cells traverse an epithelial barrier have been the subject of numerous investigations for >15 years [73–75]. Madara was among the first to show that this process does not proceed with cell death in the epithelium, nor does the process require oxygen radicals or proteases, suggesting that something more controlled is ongoing [76]. This transmigration process can occur without ultrastructural disruption of the TJ [77] and, indeed, at times without disturbance of Rt [77,78]. Although some researchers have observed an increase of permeability to small solutes before polymorphonuclear leukocyte transmigration (but after epithelial contact), there is still no concurrent downregulation or redistribution of TJ proteins [79]. This transmigration process could in part originate in the secretion of an eicosanoid termed HepA(3) across the apical surface of an epithelium followed by HepA3 diffusion back across the TJ into the lateral intercellular space, creating a chemoattractant gradient for the white blood cells [80]. Specific domains of the occludin molecule are thought to mediate the transepithelial migration of neutrophils across the TJ [75]. In addition, neutrophil transmigration might occur preferentially at the sites of three cells contacting each other, suggesting TJ connections are different at these sites than those between only two cells [81]. In general, such neutrophil migration occurs with surprisingly little impairment of barrier function.

Likewise, the processes by which proinflammatory cytokines such as interferon-? (IFN-?) or tumor necrosis factor-α (TNF-α) perturb a barrier can occur with remarkably transient and mild disruptions to the barrier, even to the point where epithelial apoptosis is induced [7,82–84]. Minimization of barrier disruption appears to be contingent on cell motility and cytoskeletal rearrangement [7]. In some cell systems, effects of proinflammatory cytokines on TJ barrier function can be longer lasting and more pronounced [85,86]. In other systems, as in IFN-? action on T84 cell sheets, the effect on permeability is prolonged (days), but is reversible and permits paracellular passage to only certain-sized molecules [87]. Effects of proinflammatory cytokines on junctional strand number and conformation have been observed in IFN-? and/or TNF-α action on HT29 cell layers visualized in freeze-fracture electron microscopy [88]. Anti-inflammatory cytokines such as interleukin 10 (IL-10) can block the barrier-disruptive effects of proinflammatory cytokines [89].

Some of the most elegant studies to date on cytokine effects on epithelial layers are from Fromm and Schulzke's

laboratories in Berlin, Germany. These groups along with others have shown that the actions of proinflammatory cytokines on transepithelial permeability involve not only the induction of apoptotic cells in the epithelial pavement, but also an apoptosis-independent direct effect on TJs of (non-apoptotic) cells. TNF-α was found to decrease the resistance of HT29 intestinal epithelial cell layer by >80% and, simultaneously, alter TJ strand number in freeze-fracture electron micrographs [90]. Later, quantitative analysis revealed that TNF-α–induced apoptosis accounted for only half of the transepithelial conductance increases, the other half being TJ alterations between non-apoptotic cells [91]. Single-cell apoptosis did cause significant transepithelial leak [92]. In agreement with the results in Ref. [84] for LLC-PK1 renal epithelia, Fromm and Schulzke's groups observed that molecules >4000 Da did not exhibit increased transepithelial flux across an intestinal epithelium undergoing increased rates of apoptosis [93]. Also reflective of results seen with renal epithelia [7], recovery of the epithelium from conductance increases caused by multiple single-cell apoptoses was rapid (<10 mins) and dependent on cytoskeletal-based motility [6]. Caspase and metalloproteinase-related cleavage of certain TJ proteins, particularly occludin, were observed after induction of epithelial apoptosis with claudin-1 being a notable exception [94]. It is possible that these phenomena have a direct relationship to events in ulcerative colitis [95].

The state of chronic inflammation produces effects on epithelial barriers, whose net effect is to increase the leakiness of the barriers. With a few exceptions [83], there is decreased occludin expression as barrier function is diminished in a wide variety of tissues and chronic inflammatory conditions. Collagenous colitis occurs with decreased Rt and decreased expression of occludin and claudin-4 in the colon [96]. Occludin expression pattern in the epidermis is also altered in psoriasis [97]. Nematode infection of the intestines results in occludin degradation, which appears to be mediated by mast cells [98]. Systemic peripheral inflammation has been shown to cause leakiness in TJs across blood–brain barrier (BBB) with significant downregulation of occludin, a process that raises an interesting possibility for altered emotional states in chronic inflammatory conditions [99]. Decreased expression of claudin-3 has also been observed in TJs of blood vessels at inflammatory sites in a mouse model of encephalitis [100].

The best-investigated chronic inflammatory model with regard to epithelial barrier function is IBD, a condition that has long been associated with altered epithelial barrier function [101]. Both Rt and TJ strand number are decreased in biopsy samples of inflamed sigmoid colon of ulcerative colitis patients [95]. Although some have shown that inflamed IBD tissue is no different in its junctional protein expression when compared with those from normal control tissue [102], others have shown that occludin (but not ZO-1, claudin-1 or cell adhesion proteins) is downregulated, even in non-actively inflamed tissue in ulcerative colitis [103]. TNBS and ethanol-induced colitis in rats caused disruption of normal immunofluorescent-stained patterns of intestinal epithelial occludin (but not ZO-1 or cingulin), although freeze-fracture strand patterns seen in electron micrographs were normal [104]. However, in another experimental colitis model, an IL-2 knockout mouse, there was increased expression of occludin and increased barrier function in the colon [105].

In summary, cytokine-related disruption of epithelial barrier function and TJ proteins is evident in cell culture-based studies using both renal and GI cells. This transepithelial leak has both apoptotic and non-apoptotic causes. The leaks can be transient or long lasting, but are typically less severe than expected. The observed cell culture phenomena appear to have a direct bearing on at least one diagnosis of inflammatory disease, ulcerative colitis, and are probably involved in others. It is likely that the frequency of cytokine-induced apoptosis could have an important and as yet unrealized bearing on the leak phenomenon. Above a certain frequency, apoptotic cells will be bordered not by non-apoptotic cells, but by other apoptotic cells, thereby changing remodeling patterns and the severity of leak.

Diabetes: metabolic dysregulation spatially

In contrast to the microbial and inflammatory diseases, there is no teleological reason why diabetes might 'want' to generate leaky epithelial or endothelial TJs. There is little doubt that TJs do become leaky in diabetes, a case that is best documented in retinal endothelia (diabetic retinopathy).

It is tempting to speculate that hyperglycemia is a root cause of TJ leakiness in diabetes, but other alterations in signal transduction pathways might be at work. Increased glucose levels can bring about increased activity of PKC in cells [106,107]. This presumably occurs through increased diacylglycerol synthesis from (elevated) glycolytic intermediates such as dihydroxyacetone phosphate [108]. Increased activity of PKC probably yields increased TJ permeability in many different preparations [22,109–112]. Hyperglycemia-induced increase of vascular endothelial growth factor (VEGF) production is thought to be involved in TJ leakage of the blood–retinal barrier in diabetes [113], a phenomenon also seen in cancer.

Altered TJs in diabetic tissues have been known for some time [114,115]. Significantly reduced expression of occludin has been observed in endothelia of the blood–retinal barrier of diabetic rats, simultaneously with increased permeability to macromolecules [116,117]. The elevated level of VEGF is thought to be in part responsible for these changes, in addition to the altered phosphorylation state of occludin [118]. Occludin content was reduced in brain capillaries of diabetic rats, although ZO-1 content was unchanged [119]. A similar result was observed for occludin in endothelial junctions in placenta [120].

Cancer: the leak in the dike

Cancer cells are renowned for their tendency to spread and disperse metastatically, which is mirrored in the laboratory setting by their very high rate of cell motility and diminished sense of cell adhesion [124,125]. Although much is written on the loss or downregulation of cell–cell adhesion in metastasis, the TJ has also been abrogated in the process.

Elimination and/or reduction of TJ barriers in cancer are essential to allow metastatic cells to break in and out of blood vessels [127,128]. However, in the context of specific epithelial barriers (and epithelial tumors), it would also allow (to a growing epithelial tumor) an additional source of nutrients and other molecules to be obtained from luminal fluids (this is important because nutrient transporters and hormonal receptors are generally on the basal-lateral cell surfaces of epithelia). This can help to keep the leading edge of the tumor growing aggressively as tumor cells stratify. Eventually, the tumors undergo necrosis of their inner layers, cutting off vascular supply in certain regions and force-feeding from wherever it can be done, for example, from luminal fluids. TJ junction leakage might also be one of the final stages in the process of promoting a focus of transformed epithelial cells to 'cross the line' from dormancy into actual neoplasia. Molecules other than nutrients are able to cross leaky TJs, and luminal fluids can have high concentrations of growth-stimulatory peptides and proteins [129].

The evidence supporting an alteration of TJs in transformed and tumor epithelia goes back for >30 years [130]. Although the disorganization and/or disappearance of TJs do not occur in all tumors [131], it is a hallmark of many tumors. Attenuation of TJs is observed in urinary bladder carcinomas [132]. Circumferential discontinuity in TJs was observed in murine mammary adenocarcinomas [133]. TJ strand abnormalities have been seen in human thyroid carcinomas [134]. Poorly differentiated colon adenocarcinomas were observed to have the diminished structural organization, as seen in fetal colon TJs [135]. N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced gastric cancers and pre-cancerous gastric lesions in rats exhibit disappearance of apical TJs [136]. Decreased numbers of TJ strands in transitional carcinoma of urinary bladder have been observed [137]. TJ disorganization and diminishment was detected in freeze-fracture electron microscopy of hepatocellular carcinomas [138]. Pre-cancerous colon of mice treated with chemical carcinogens exhibit decreased transepithelial barrier function as measured by transepithelial electrical impedance [139]. A similar pattern of decreased impedance has been recorded in inflamed esophageal tissue, and an even lower impedance is seen in dysplastic esophageal tissue [140]. In Crohn's disease, the human colon tissue is at increased risk of cancer onset, which is probably associated with increased TJ permeability to paracellular probes [101]. This also applies to specific, noninflamed ileal tissue from Crohn's disease patients, in which there is an increased TJ permeability in response to sodium caprate (a fatty acid that could cause TJ leak by potentially activating PKC) compared with that by control noninflamed ileal tissue [141]. Whereas the epithelial to mesenchymal transition that typifies epithelial neoplasia occurs rather late in the process, decreases of TJ proteins such as ZO-1 occur earlier [142].

From aberrant crypts in colon mucosa to dysplasia in Barrett's esophagus, TJ integrity appears to be almost universally compromised in epithelial and endothelial cancers [140,143,144]. Processes and agents known to augment the promotional stage of cancer (e.g. tumor-promoting phorbol esters) induce TJ leakiness [112]. Transforming growth factor-α (TGF-α) is observed to override the stimulation of TJ formation by glucocorticoids in mammary tumor cells [145]. Oncogenes are known to induce TJ leakiness [146] (Mullin *et al.*, unpublished). SV-40 virus exposure induces TJ leakiness and occludin downregulation [147], as does hepatocyte growth factor (HGF; also known as scatter factor) [148].

Occludin expression decreases progressively with the increasing carcinoma grade in human endometrial cancer. The downregulation of TJ protein expression is common in many tumors. A similar phenomenon occurring with TJ proteins in colon cancer (e.g. loss of claudin-1 [149]) might have a bearing on the dispersal of carcinoembryonic antigen in colon tumor cells from its normally polarized distribution [150,151]. Occludin distribution and expression is similarly altered in increasing Gleason grades of prostate cancer [152]. Poorly differentiated adenocarcinomas of the GI tract exhibit reduced occludin expression [153]. Occludin expression is sharply decreased or otherwise altered in hydatiform moles of the placenta [154]. Microvessels of human brain tumors, the leaks in which TJs are responsible for cerebral edema in certain types of brain cancer, are excellent examples of downregulation of occludin in cancer, a phenomenon perhaps attributable to increased secretion of VEGF [155,156]. These effects on TJ proteins and leakage could arise from increased VEGF secretion by tumor cells in the vicinity of microvessels [157].

Claudin-1 is absent from many tumor microvessels of human glioblastoma multiforme [143] and is frequently absent or downregulated in breast tumors, implicating its potential as a tumor suppressor gene [158], which was also considered for ZO-1 [159]. However, claudin-1 expression has been observed to be upregulated in certain colorectal cancers when compared with expression in adjacent normal mucosae [160]. Claudin-1 is expressed in benign perineuriomas, but is absent in (the more serious) fibromyxoid sarcomas, dermatofibrosarcoma protuberans and desmoplastic fibroblastoma [161]. The expression of claudin-7 is reduced in invasive ductal carcinoma of the breast [162]. Claudins 3 and 4 are overexpressed in some ovarian cancers, and claudin-16 is uniquely expressed in certain ovarian tumors, not being found in normal ovarian tissue [163,164].

In an intriguing case of a well-known tumor marker [prostate-specific antigen (PSA)], two variant forms of claudin-7, themselves subject to androgen regulation, could possess the ability to regulate PSA expression [165].

In summary, TJs are typically deranged, downregulated or simply absent during neoplasia, the process starting very early in the progression, with interesting possibilities for diagnosis, prognosis and even mechanism of tumor progression. Breakdown of barrier function is nearly universal for developing or established epithelial tumors. However, this cannot be extrapolated as a generalization when dealing with specific TJ proteins that, in the process of neoplasia, can be upregulated, downregulated or unchanged depending on the TJ protein in question and the tissue in which the tumor originates. The two realities are not in conflict. The emerging picture of TJ structure at the molecular level is one of homotypic and heterotypic interactions among occludin and claudin molecules. Alterations in proportions of specific molecules, down or up, will disrupt the balance involved in the 'weave' of TJ proteins and TJ fibrils. The result should be aberrant pores (in size, charge and/or number) in the paracellular pathway.

Genetic diseases – inherited leakiness

TJs comprise a complex of multiple proteins, many of uncertain function [166,167]. Some of these proteins are cytoplasmic in location, some are associated with the cytoskeleton and some are integral membrane proteins associated with the TJ, including occludin [168], junction-associated membrane proteins 1 and 2 [169,170], Coxsackie- and adenovirus-associated receptor [171], IG8 antigen [172] and claudins [173]. Claudins are the most heterogeneous integral membrane proteins and they consist of a family of >20 isoforms [19,174]. Each isoform has a tissue-specific and segment-specific pattern of distribution in epithelia. Therefore, it is speculated that TJ ionic permeabilities are determined by varying combinations and expression levels of different claudins. Normal or pathological changes in claudins are expected to alter epithelial barrier properties.

PCLN-1 and claudin-16

The kidney has a major role in the regulation of extracellular magnesium concentration, a crucial cofactor in various biological activities. The renal reabsorption of Mg^{2+} occurs predominantly by paracellular flux in the thick ascending limb (TAL) of the nephron [175]. The conductance of this paracellular pathway is highly regulated, with urine Mg^{2+} excretion ranging from 1% to 80% of the filtered load [176]. A familial hypomagnesemia with hypercalciuria and nephrocalcinosis has been recently described as an autosomal recessive disease [177]. Affected persons are characterized by a significant loss of Mg^{2+} resulting in severe hypomagnesemia, which cannot be corrected by oral or intravenous Mg^{2+} supplementation. Other features of this disease are urinary tract infections, kidney stones, hyperuricemia and ocular findings. The massive renal Mg^{2+} loss implicated an important defect in transepithelial reabsorption. The identification of the human gene responsible for this defect was reported to be paracellin-1 (PCLN-1), which caused loss of renal Mg^{2+}. PCLN-1 is only expressed in the kidney and colocalizes with occludin, indicating that PCLN-1 is a component of the TJ. PCLN-1 was localized to HSA3q and is a member of the claudin family [178]. These results identify PCLN-1 as a renal TJ protein which, when mutated, causes massive renal Mg^{2+} wasting, resulting in hypomagnesemia and hypercalciuria.

More recently, a novel autosomal recessive renal disorder was described in Japanese black cattle, presenting as chronic interstitial nephritis with diffuse zonal fibrosis (CINF) [179]. CINF was diagnosed preliminarily by increased levels of blood urea and urinary protein. The symptoms suggested that CINF results from defects in selective filtration and absorption in the tubular renal epithelium. The CINF locus was assigned to the central region of bovine chromosome 1 (BTA1) close to a microsatellite marker BM9019. The human–cattle comparative map studies show that HSA3q, for PCLN-1, corresponds to BTA1 where claudin-16 is located. The nucleotide sequence of claudin-16 shares 90% homology with that of PCLN-1. The authors concluded that claudin-16 is a cattle orthologue of PCLN-1 and the product of PCLN-1/claudin-16 is probably required for selective paracellular conductance and forms an intercellular pore, allowing paracellular passage of Mg^{2+} and Ca^{2+} through TJs of the TAL.

One specific homozygous claudin-16 mutation results in inactivation of a PDZ-domain binding motif. This disrupts the association of claudin-16 with ZO-1, and claudin-16 then accumulates in the lysosomes rather than in the TJ regions [180].

Claudin-14

The cochlea of the inner ear has two fluid compartments with different ionic compositions. The perilymph, with low K^+ concentration but high Na^+ concentration, is similar to cerebrospinal fluid, whereas the endolymph is similar to an intracellular microenvironment, high in K^+ and low in Na^+ [181]. This large K^+ gradient contributes to an 80–100 mV endocochlear potential, which is crucial for the depolarization of sensory hair cells [182,183]. To maintain the high resting potential in the endolymph, cells bordering this fluid are 'sealed' with various types of intercellular TJs. In the cochlea, the expression of claudin-14 is restricted to the organ of Corti and is not found in other regions of the membranous labyrinth. Ultrastructural studies show that multiple types of TJ complexes are present in the cochlea. The importance of claudin-14 in the cochlea is demonstrated by observations of two different claudin-14 mutations co-segregating with recessive deafness in two large families with multiple consanguineous intermarriages. In addition, claudin-14 expression is specific to the sensory epithelium of the cochlea. The authors sug-

gest that other proteins with functions important for inner ear cell–cell junctions are excellent candidates for causes of deafness because of the compartmentalization of the cochlea. Claudin-14 is one of many genes located in the Down's syndrome crucial region [184]. The relationship between overexpression of claudin-14 (as a result of a third copy of this gene in Down's trisomy) and the etiology of age-related sensorineural hearing loss in Down's syndrome patients could be addressed using transgenic mice overexpressing claudin-14.

OSP

Another example of a pathology resulting from an alteration in the expression of a specific TJ protein is seen in oligodendrocyte-specific protein (OSP; also known as claudin-11) null mice studies [185]. OSP was recently identified as a transmembrane protein strongly expressed in the CNS myelin and testis. OSP is localized to TJs in myelin sheaths and Sertoli cells, and is expressed at low levels elsewhere. This null-mouse study showed the function of the OSP protein by ablating its expression in embryonic stem cells. The data suggest that OSP is a component of specialized TJs because deletion of *Osp* gene results in disappearance of TJ strands in CNS myelin and Sertoli cells. Interestingly, such mice are also deaf [181,186].

WNK4

Mutations in the *Wnk4* gene are believed to cause an autosomal-dominant disorder of hyperkalemia and hypertension known as pseudohyperaldosteronism Type II (PHAII). WNK4 is thought to be a protein kinase and is localized to the region of the TJ *in vivo*. *In vitro*, WNK4 can bind to and phosphorylate claudins 1 to 4, and give rise to increased paracellular Cl^- conductance [187].

Miscellaneous diseases and conditions

Disruption of occluding-staining patterns of BBB endothelia is a hallmark of HIV-associated encephalitis [188]. TJ and barrier alterations in intestinal epithelia were observed as a result of cytokine production by white blood cells after HIV exposure [189]. Celiac sprue is also characterized by a decrease in TJ strand number in all regions of the jejunal surface-crypt axis [190]. The paracellular permeability of the upper GI tract is increased in sprue as evidenced by increased transmucosal permeability of sucrose, mannitol and lactulose [191]. Zonulin is also increased in expression during the acute phase of celiac disease [41]. Experimentally induced pancreatitis manifests delocalization of occludin, but not ZO-1, from TJs [192]. Menetrier's disease is characterized by abnormal widening of TJs in the gastric mucosa [193,194]. Serum from multiple sclerosis patients can reduce the expression of occludin in endothelial cell cultures [195]. This is consistent with BBB leakages in multiple sclerosis patients *in vivo*, in addition to discontinuities in immunofluorescent-staining patterns for occludin along cell borders [196]. Cancer chemotherapy, whether by transcellular or TJ mechanisms, induces a transient increase of intestinal permeability to carbohydrates [197]. Emotional stress has been observed to alter mammary gland TJ permeability to lactose presumably through change in cortisol levels [198]. Finally, aging itself has been linked with declining occludin content in BBB endothelia, although the ZO-1 content was unaltered [199]. Decreased barrier function to macromolecules is perhaps ongoing in human intestine with age [200,201].

Summary

In conclusion, there is little doubt about the importance of barrier function in tissue, organ and organism homeostasis. This survey of literature and consideration of the concepts presents a cogent case that diseases in general – of disparate etiology – make a point of attacking barrier function. The upshots for designed attempts at making barriers leaky for purposes of, for example, drug delivery are simple: (i) ideally, such attempts should be specifically targeted, occurring in a specific organ (or preferably segment of an organ) only; (ii) the attempts should be brief in duration, with the paracellular 'openings' spontaneously and reverting back to control, barrier-restored states quickly; and (iii) make the paracellular leaks specific for only the class of molecules one wishes to deliver. A consideration of different modalities of achieving increased TJ permeability (for the purposes of uptake of gene therapy agents) and their biomedical drawbacks was recently performed [202].

Future research should be focused on the mechanisms by which barriers can be made less leaky because, in so doing, we could encounter a common thread in many disease states. Myosin light chain kinase and the PKC family are likely places to begin for drug targets. Further research aimed at identifying signaling intermediates in regulating TJs is pivotal in this regard. Clues as to how we might tighten barriers are found in the transepithelial effects of agents as diverse (and surprising) as EGF, steroids and probiotics. EGF can decrease TJ permeability in several epithelial cell culture models [2,3,203,204]. In MDCK cells, the EGF-induced decrease in TJ permeability correlated with decreased claudin-2 expression [204]. Hydrocortisone resulted in increased occludin expression and decreased transepithelial permeability across retinal endothelia [205]. The synthetic glucocorticoid, dexamethasone, increased Rt and decreased transepithelial permeability of mannitol and insulin across mammary epithelial cell sheets [206]. Src kinase inhibition can enhance barrier function in keratinocytes, while MEK1 inhibition achieves similar results in renal epithelia [146]. Suppression of the β-catenin–T-cell factor–lymphoid-enhancer factor (TCF–LEF) complex in colorectal epithelial also enhances barrier function [207]. Inhibition of Rho-associated coiled-coil-forming protein serine–threonine kinase (ROCK) has likewise augmented barrier function [208]. Exposure of intestinal epithelial cell sheets to probiotics such as

Lactobacilli increased Rt, while altering the phosphorylation state of occludin [209]. *Lactobacilli* inhibit neutrophil migration across intestinal cell sheets exposed to the bacteria [210]. Probiotic *E. coli* have inhibited the TJ disruptive effects of *Salmonella* bacteria [211].

Although the guardians of modern research discourage studies that are not aimed precisely at molecular mechanism and structure, attaining the clinical means of tightening epithelial barriers will be spearheaded by phenomenological studies across a wide variety of epithelial cell culture and epithelial tissue models, using a host of different agents. Considering the morbidity that could be relieved by such studies, one can only hope that they will be undertaken. Aspirin was after all in use and improving life for many decades before cyclooxygenases were known to exist or be understood.

References

1 Jinguji, Y. and Ishikawa, H. (1992) Electron microscopic observations on the maintenance of the tight junction during cell division in the epithelium of the mouse small intestine. *Cell Struct. Funct.* 17, 27–37
2 Soler, A.P. *et al.* (1993) Effects of epidermal growth factor versus phorbol ester on kidney epithelial (LLC-PK1) tight junction permeability and cell division. *Exp. Cell Res.* 207, 398–406
3 Saladik, D.T. *et al.* (1995) Cell division does not increase transepithelial permeability of LLC-PK1 cell sheets. *Exp. Cell Res.* 220, 446–455
4 Kojima, T. *et al.* (2001) Occludin and claudin-1 concentrate in the midbody of immortalized mouse hepatocytes during cell division. *J. Histochem. Cytochem.* 49, 333–340
5 Madara, J. (1990) Maintenance of the macromolecular barrier at cell extrusion sites in intestinal epithelium: physiological rearrangement of tight junctions. *J. Membr. Biol.* 116, 177–184
6 Florian, P. *et al.* (2002) Single-cell epithelial defects close rapidly by an actinomyosin purse string mechanism with functional tight junctions. *J. Physiol.* 545, 485–499
7 Peralta Soler, A. *et al.* (1996) Tissue remodeling during tumor necrosis factor-induced apoptosis in LLC-PK1 renal epithelial cells. *Am. J. Physiol.* 270, F869–F879
8 Schulzke, J.D. *et al.* (1986) Epithelial and subepithelial resistance of rat large intestine: segmental differences, effect of stripping, time course, and action of aldosterone. *Pflugers Arch.* 407, 632–637
9 Fromm, M. *et al.* (1985) Epithelial and subepithelial contributions to transmural electrical resistance of intact rat jejunum, *in vitro*. *Pflugers Arch.* 405, 400–402
10 Groot, J. *et al.* (1981) Analysis of the ouabain-induced increase in transepithelial electrical resistance in the goldfish intestinal mucosa. *Pflugers Arch.* 392, 67–71
11 Nagel, W. *et al.* (1983) Cellular and paracellular conductance patterns in voltage-clamped frog skin. *Prog. Clin. Biol. Res.* 126, 221–231
12 Finn, A. and Reuss, L. (1975) Effects of changes in the composition of the serosal solution on the electrical properties of the toad urinary bladder epithelium. *J. Physiol.* 250, 541–558
13 Dvorak, A. and Dickersin, G. (1980) Crohn's disease: transmission electron microscopic studies. I. Barrier function. Possible changes related to alterations of cell coat, mucous coat, epithelial cells, and Paneth cells. *Hum. Pathol.* 11(Suppl. 5), 561–571
14 Farhadi, A. *et al.* (2003) Intestinal barrier: an interface between health and disease. *J. Gastroenterol. Hepatol.* 18, 479–497
15 Podolsky, D.K. (1999) Mucosal immunity and inflammation. V. Innate mechanisms of mucosal defense and repair: the best offense is a good defense. *Am. J. Physiol.* 277, G495–G499
16 Baumgart, D. and Dignass, A. (2002) Intestinal barrier function. *Curr. Opin. Clin. Nutr. Metab. Care* 5, 685–694
17 Schneeberger, E.E. and Lynch, R.D. (1992) Structure, function, and regulation of cellular tight junctions. *Am. J. Physiol.* 262, L647–L661
18 Anderson, J. and Van Itallie, C.M. (1999) Tight Junctions:closing in on the seal. *Curr. Biol.* 9, R922–R924
19 Tsukita, S. and Furuse, M. (2000) The structure and function of claudins, cell adhesion molecules at tight junctions. *Ann. N. Y. Acad. Sci.* 915, 129–135
20 Gonzalez-Mariscal, L. *et al.* (2003) Tight junction proteins. *Prog. Biophys. Mol. Biol.* 81, 1–44
21 Hopkins, A.M. *et al.* (2000) Modulation of tight junction function by G protein-coupled events. *Adv. Drug Deliv. Rev.* 41, 329–340
22 Lacaz-Vieira, F. (2000) Tight junction dynamics: oscillations and the role of protein kinase C. *J. Membr. Biol.* 178, 151–161
23 Clarke, H. *et al.* (2000) Modification of tight junction function by protein kinase C isoforms. *Adv. Drug Deliv. Rev.* 41, 283–301
24 Kottra, G. and Vank, C. (2003) The forskolin-induced opening of tight junctions in *Xenopus* gallbladder epithelium is mediated by protein kinase C. *Cell Mol. Bio.l (Noisy-le-grand).* 49, 33–43
25 Duffey, M. *et al.* (1981) Regulation of epithelial tight junction permeability by cyclic AMP. *Nature* 294, 451–453
26 Ma, T.Y. *et al.* (2000) Mechanism of extracellular calcium regulation of intestinal epithelial tight junction permeability: role of cytoskeletal involvement. *Microsc. Res. Tech.* 51, 156–168
27 Hecht, G. *et al.* (1996) Expression of the catalytic domain of myosin light chain kinase increases paracellular permeability. *Am. J. Physiol.* 271, C1678–C1684
28 Turner, J.R. *et al.* (1997) Physiological regulation of epithelial tight junctions is associated with myosin light-chain phosphorylation. *Am. J. Physiol.* 273, C1378–C1385
29 Stuart, R.O. *et al.* (1994) Critical role for intracellular calcium in tight junction biogenesis. *J. Cell. Physiol.* 159, 423–433
30 Gonzalez-Mariscal, L. *et al.* (1990) Role of calcium in tight junction formation between epithelial cells. *Am. J. Physiol.* 259, C978–C986
31 Siliciano, J.D. and Goodenough, D.A. (1988) Localization of the tight junction protein, ZO-1, is modulated by extracellular calcium and cell-cell contact in Madin-Darby canine kidney epithelial cells. *J. Cell Biol.* 107, 2389–2399
32 Denker, B.M. *et al.* (1996) Involvement of a heterotrimeric G protein alpha subunit in tight junction biogenesis. *J. Biol. Chem.* 271, 25750–25753
33 Hirase, T. *et al.* (2001) Regulation of tight junction permeability and occludin phosphorylation by Rhoa-p160ROCK-dependent and -independent mechanisms. *J. Biol. Chem.* 276, 10423–10431
34 Jou, T. *et al.* (1998) Structural and functional regulation of tight junctions by RhoA and Rac1 small GTPases. *J. Cell Biol.* 142, 101–115
35 Meyer, T. *et al.* (2002) Zonula occludens-1 is a scaffolding protein for signaling molecules. Galpha(12) directly binds to the Src homology 3 domain and regulates paracellular permeability in epithelial cells. *J. Biol. Chem.* 277, 24855–24858
36 Rubenstein, N.M. *et al.* (2003) Glucocorticoid down-regulation of RhoA is required for the steroid-induced organization of the junctional complex and tight junction formation in rat mammary epithelial tumor cells. *J. Biol. Chem.* 278, 10353–10360
37 Sawada, N. *et al.* (2003) Tight junctions and human diseases. *Med. Electron Microsc.* 36, 147–156
38 McCarthy, K.M. *et al.* (1996) Occludin is a functional component of the tight junction. *J. Cell Sci.* 109, 2287–2298
39 Holman, G. and Naftalin, R. (1979) Fluid movements across rabbit ileum coupled to passive paracellular ion movements. *J. Physiol.* 290, 351–366
40 Fasano, A. *et al.* (1991) *Vibrio cholerae* produces a second enterotoxin, which affects intestinal tight junctions. *Proc. Natl. Acad. Sci. U. S. A.* 88, 5242–5246
41 Fasano, A. (2000) Regulation of intercellular tight junctions by zonula occludens toxin and its eukaryotic analogue zonulin. *Ann. N. Y. Acad. Sci.* 915, 214–222
42 Balkovetz, D. and Katz, J. (2003) Bacterial invasion by a paracellular route: divide and conquer. *Microbes Infect.* 5, 613–619
43 Berkes, J. *et al.* (2003) Intestinal epithelial responses to enteric pathogens: effects on the tight junction barrier, ion transport, and inflammation. *Gut* 52, 439–451
44 Clayburgh, D. *et al.* (2004) A porous defense: the leaky epithelial barrier in intestinal disease. *Lab. Invest.* 84, 282–291
45 Hofman, P. (2003) Pathological interactions of bacteria and toxins with the gastrointestinal epithelial tight junctions and/or the zonula adherens: an update. *Cell Mol. Biol. (Noisy-le-grand).* 49, 65–75
46 Sears, C.L. (2000) Molecular physiology and pathophysiology of tight junctions V. assault of the tight junction by enteric pathogens. *Am. J. Physiol. Gastrointest. Liver Physiol.* 279, G1129–G1134
47 Philpott, D.J. *et al.* (1998) Signal transduction pathways involved in enterohemmorrhagic *Escherichia coli*-induced alterations in T84 epithelial permeability. *Infect. Immun.* 66, 1680–1687

48 Li, Z. *et al.* (1999) Shiga toxin-producing *Escherichia coli* can impair T84 cell structure and function without inducing attaching/effacing lesions. *Infect. Immun.* 67, 5938–5945

49 Obiso, R.J., Jr *et al.* (1997) The *Bacteroides fragilis* toxin fragilysin disrupts the paracellular barrier of epithelial cells. *Infect. Immun.* 65, 1431–1439

50 Gogarten, W. *et al.* (1994) Effect of acute *Yersinia enterocolitica* infection on intestinal barrier function in the mouse. *Scand. J. Gastroenterol.* 29, 814–819

51 Singh, U. *et al.* (2000) CaCo-2 cells treated with *Clostridium perfringens* enterotoxin form multiple large complex species, one of which contains the tight junction protein occludin. *J. Biol. Chem.* 275, 18407–18417

52 Sonoda, N. *et al.* (1999) *Clostridium perfringens* enterotoxin fragment removes specific claudins from tight junction strands: Evidence for direct involvement of claudins in tight junction barrier. *J. Cell Biol.* 147, 195–204

53 Wu, Z. *et al.* (2000) Distinct effects of *Vibrio cholerae* haemagglutinin/protease on the structure and localization of the tight junction-associated proteins occludin and ZO-1. *Cell. Microbiol.* 2, 11–17

54 Chen, M. *et al.* (2002) Protein kinase C signaling regulates ZO-1 translocation and increased paracellular flux of T84 coloncytes exposed to *Clostridium difficile* toxin A. *J. Biol. Chem.* 277, 4247–4254

55 Nusrat, A. *et al.* (2001) *Clostridium difficile* toxins disrupt epithelial barrier function by altering membrane microdomain localization of tight junction proteins. *Infect. Immun.* 69, 1329–1336

56 MacIntyre, A. *et al.* (2002) *Chlamydia pneumoniae* infection alters the junctional complex proteins of human brain microvascular endothelial cells. *FEMS Microbiol. Lett.* 217, 167–172

57 Chen, S. *et al.* (2003) *Cryptococcus neoformans* induces alterations in the cytoskeleton of human brain microvascular endothelial cells. *J. Med. Microbiol.* 52, 961–970

58 Terres, A.M. *et al.* (1998) *Helicobacter pylori* disrupts epithelial barrier function in a process inhibited by protein kinase C activators. *Infect. Immun.* 66, 2943–2950

59 Suzuki, K. *et al.* (2002) SS1 *Helicobacter pylori* disrupts the paracellular barrier of the gastric mucosa and leads to neutrophilic gastritis in mice. *Virchows Arch.* 440, 318–324

60 Papini, E. *et al.* (1998) Selective increase of the permeability of polarized epithelial cell monolayers by *Helicobacter pylori* vacuolating toxin. *J. Clin. Invest.* 102, 813–820

61 Dickman, K.G. *et al.* (2000) Rotavirus alters paracellular permeability and energy metabolism in caco-2 cells. *Am. J. Physiol. Gastrointest. Liver Physiol.* 279, G757–G766

62 Tafazoli, F. *et al.* (2001) NSP4 enterotoxin of rotavirus induces paracellular leakage in polarized epithelial cells. *J. Virol.* 75, 1540–1546

63 Nava, P. *et al.* (2004) The rotavirus surface protein VP8 modulates the gate and fence function of epithelial cells. *J. Cell Sci.* 117, 5509–5519

64 Madara, J.L. and Pappenheimer, J.R. (1987) Structural basis for physiological regulation of paracellular pathways in intestinal epithelia. *J. Membr. Biol.* 100, 149–164

65 Adler, J. *et al.* (1973) Chemotaxis toward sugars in *Escherichia coli*. *J. Bacteriol.* 115, 824–847

66 van der Drift, C. *et al.* (1975) Chemotaxis of a motile *Streptococcus* toward sugars and amino acids. *J. Bacteriol.* 124, 1142–1147

67 Meggs, W. *et al.* (1996) Nasal pathology and ultrastructure in patients with chronic airway inflammation (RADS and RUDS) following an irritant exposure. *J. Toxicol. Clin. Toxicol.* 34, 383–396

68 Wan, H. *et al.* (2001) The transmembrane protein occludin of epithelial tight junctions is a functional target for serine peptidases from faecal pellets of *Dermatophagoides pteronyssinus*. *Clin. Exp. Allergy* 31, 279–294

69 Wan, H. *et al.* (1999) Der p 1 facilitates transepithelial allergen delivery by disruption of tight junctions. *J Clin. Invest.* 104, 123–133

70 Baker, S. *et al.* (2003) Peptidase allergen Der p 1 initiates apoptosis of epithelial cells independently of tight junction proteolysis. *Mol. Membr. Biol.* 20, 71–81

71 Bates, P. *et al.* (1995) Effect of cotton, hemp, and flax dust extracts on lung permeability in the guinea pig. *Exp. Lung Res.* 21, 643–665

72 Robinson, C. *et al.* (2001) Peptidase allergens, occludin and claudins. Do their interactions facilitate the development of hypersensitivity reactions at mucosal surfaces? *Clin. Exp. Allergy* 31, 186–192

73 Parkos, C.A. *et al.* (1992) Neutrophil migration across a cultured epithelial monolayer elicits a biphasic resistance response representing sequential effects on transcellular and paracellular pathways. *J. Cell Biol.* 117, 757–764

74 Colgan, S. *et al.* (1993) Neutrophil migration across cultured intestinal epithelial monolayers is modulated by epithelial exposure to IFN-gamma in a highly polarized fashion. *J. Cell Biol.* 120, 785–798

75 Huber, D. *et al.* (2000) Occludin modulates transepithelial migration of neutrophils. *J. Biol. Chem.* 275, 5773–5778

76 Nash, S. *et al.* (1988) The selective and superoxide-independent disruption of intestinal epithelial tight junctions during leukocyte transmigration. *Lab. Invest.* 59, 531–537

77 Burns, A. *et al.* (2000) Analysis of tight junctions during neutrophil transendothelial migration. *J. Cell Sci.* 113, 45–57

78 Michail, S.K. *et al.* (2003) Enteropathogenic *Escherichia coli*: stimulating neutrophil migration across a cultured intestinal epithelium without altering transepithelial conductance. *J. Pediatr. Gastroenterol. Nutr.* 36, 253–260

79 Edens, H. *et al.* (2002) Neutrophil transepithelial migration: evidence for sequential, contact-dependent signaling events and enhanced paracellular permeability independent of transjunctional migration. *J. Immunol.* 169, 476–486

80 Mrsny, R.J. *et al.* (2004) Identification of hepoxilin A3 in inflammatory events: a required role in neutrophil migration across intestinal epithelia. *Proc. Natl. Acad. Sci. U. S. A.* 101, 7421–7426

81 Burns, A. *et al.* (1997) Neutrophil transendothelial migration is independent of tight junctions and occurs preferentially at tricellular corners. *J. Immunol.* 159, 2893–2903

82 Poritz, L.S. *et al.* (2004) Tumor necrosis factor alpha disrupts tight junction assembly. *J. Surg. Res.* 116, 14–18

83 Lechner, J. *et al.* (1999) Effects of interferon alpha-2b on barrier function and junctional complexes of renal proximal tubular LLC-PK1 cells. *Kidney Int.* 55, 2178–2191

84 Mullin, J.M. *et al.* (1997) Different size limitations for increased transepithelial paracellular solute flux across phorbol ester and tumor necrosis factor-treated epithelial cell sheets. *J. Cell. Physiol.* 171, 226–233

85 Madara, J.L. and Stafford, J. (1989) Interferon-gamma directly affects barrier function of cultured intestinal epithelial monolayers. *J. Clin. Invest.* 83, 724–727

86 Ma, T. *et al.* (2004) TNF-alpha-induced increase in intestinal epithelial tight junction permeability requires NF-kappa B activation. *Am. J. Physiol. Gastrointest. Liver Physiol.* 286, G367–G376

87 Adams, R. *et al.* (1993) IFN-gamma modulation of epithelial barrier function. Time course, reversibilty, and site of cytokine binding. *J. Immunol.* 150, 2356–2363

88 Rodriguez, P. *et al.* (1995) Tumour necrosis factor-alpha induces morphological and functional alterations of intestinal HT29 cl.19A cell monolayers. *Cytokine* 7, 441–448

89 Madsen, K. *et al.* (1997) Interleukin 10 prevents cytokine-induced disruption of T84 monolayer barrier integrity and limits chloride secretion. *Gastroenterology* 113, 151–159

90 Schmitz, H. *et al.* (1999) Tumor necrosis factor-alpha (TNFalpha) regulates the epithelial barrier in the human intestinal cell line HT-29/B6. *J. Cell Sci.* 112, 137–146

91 Gitter, A. *et al.* (2000) Leaks in the epithelial barrier caused by spontaneous and TNF-alpha-induced single-cell apoptosis. *FASEB J.* 14, 1749–1753

92 Bojarski, C. *et al.* (2000) Apoptosis and intestinal barrier function. *Ann. N. Y. Acad. Sci.* 915, 270–274

93 Bojarski, C. *et al.* (2001) Permeability of human HT-29/B6 colonic epithelium as a function of apoptosis. *J. Physiol.* 535, 541–552

94 Bojarski, C. *et al.* (2004) The specific fates of tight junction proteins in apoptotic epithelial cells. *J. Cell Sci.* 117, 2097–2107

95 Gitter, A. *et al.* (2001) Epithelial barrier defects in ulcerative colitis: characterization and quantification by electrophysiological imaging. *Gastroenterology* 121, 1320–1328

96 Burgel, N. *et al.* (2002) Mechanisms of diarrhea in collagenous colitis. *Gastroenterology* 123, 433–443

97 Yoshida, Y. *et al.* (2001) Altered expression of occludin and tight junction formation in psoriasis. *Arch. Dermatol. Res.* 293, 239–244

98 McDermott, J. *et al.* (2003) Mast cells disrupt epithelial barrier function during enteric nematode infection. *Proc. Natl. Acad. Sci. U. S. A.* 100, 7761–7766

99 Huber, J. *et al.* (2001) Inflammatory pain alters blood-brain barrier permeability and tight junctional protein expression. *Am. J. Physiol. Heart Circ. Physiol.* 280, H1241–H1248

100 Wolburg, H. *et al.* (2003) Localization of claudin-3 in tight junctions of the blood-brain barrier is selectively lost during experimental autoimmune encephalomyelitis and human glioblastoma multiforme. *Acta Neuropathol. (Berl.)* 105, 586–592

101 Hollander, D. (1988) Crohn's disease–A permeability disorder of the tight junction. *Gut* 29, 1621–1624

102 Gassler, N. *et al.* (2001) Inflammatory bowel disease is associated with changes of enterocytic

junctions. *Am. J. Physiol. Gastrointest. Liver Physiol.* 281, G216–G228
103 Kucharzik, T. *et al.* (2001) Neutrophil transmigration in inflammatory bowel disease is associated with differential expression of epithelial intercellular junction proteins. *Am. J. Pathol.* 159, 2001–2009
104 Fries, W. *et al.* (1999) Experimental colitis increases small intestine permeability in the rat. *Lab. Invest.* 79, 49–57
105 Barmeyer, C. *et al.* (2004) Mechanisms of diarrhea in the interleukin-2-deficient mouse model of colonic inflammation. *Am. J. Physiol. Gastrointest. Liver Physiol.* 286, G244–G252
106 Lee, C. *et al.* (1989) Demonstration of the role of prostate-specific antigen in semen liquefaction by two-dimensional electrophoresis. *J. Androl.* 10, 432–438
107 Derubertis, F. and Craven, P. (1994) Activation of protein kinase C in glomerular cells in diabetes. Mechanisms and potential links to the pathogenesis of diabetic glomerulopathy. *Diabetes* 43, 1–8
108 Rossi, F. *et al.* (1991) *De novo* synthesis of diacylglycerol from glucose. A new pathway of signal transduction in human neutrophils stimulated during phagocytosis of beta-glucan particles. *J. Biol. Chem.* 266, 8034–8038
109 Mullin, J.M. and O'Brien, T.G. (1986) Effects of tumor promoters on LLC-PK1 renal epithelial tight junctions and transepithelial fluxes. *Am. J. Physiol.* 251, C597–C602
110 Balda, M.S. *et al.* (1991) Assembly and sealing of tight junctions: Possible participation of G-proteins, phospholipase C, protein kinase C and calmodulin. *J. Membr. Biol.* 122, 193–202
111 Tai, Y.H. *et al.* (1996) Regulation of tight junction resistance in T84 monolayers by elevation in intracellular Ca^{2+}: A protein kinase C effect. *J. Membr. Biol.* 149, 71–79
112 Mullin, J.M. *et al.* (1997) Transepithelial paracellular leakiness induced by chronic phorbol ester exposure correlates with polyp-like foci and redistribution of protein kinase C-alpha. *Carcinogenesis* 18, 2339–2345
113 Antcliff, R. and Marshall, J. (1999) The pathogenesis of edema in diabetic maculopathy. *Semin. Ophthalmol.* 14, 223–232
114 Caldwell, R. and Slapnick, S. (1992) Freeze-fracture and lanthanum studies of the retinal microvasculature in diabetic rats. *Invest. Ophthalmol. Vis. Sci.* 33, 1610–1619
115 Gardner, T. (1995) Histamine, ZO-1 and increased blood-retinal barrier permeability in diabetic retinopathy. *Trans. Am. Ophthalmol. Soc.* 93, 583–621
116 Antonetti, D. *et al.* (1998) Vascular permeability in experimental diabetes is associated with reduced endothelial occludin content: vascular endothelial growth factor decrease occludin in retinal endothelial cells. Penn State Retina Research Group. *Diabetes* 47, 19953–19959
117 Barber, A. and Antonetti, D. (2003) Mapping the blood vessels with paracellular permeability in the retinas of diabetic rats. *Invest. Ophthalmol. Vis. Sci.* 44, 5410–5416
118 Antonetti, D. *et al.* (1999) Vascular endothelial growth factor induces rapid phosphorylation of tight junction proteins occludin and zonula occluden 1. A potential mechanism for vascular permeability in diabetic retinopathy and tumors. *J. Biol. Chem.* 274, 23463–23467
119 Chehade, J. *et al.* (2002) Diabetes-related changes in rat cerebral occludin and zonula occludens-1 (ZO-1) expression. *Neurochem. Res.* 27, 249–252
120 Babawale, M. *et al.* (2000) Effects of gestational diabetes on junctional adhesion molecules in human term placental vasculature. *Diabetologia* 43, 1185–1196
121 Racker, E. (1972) Bioenergetics and the problem of tumor growth. *Am. Sci.* 60, 56–63
122 Wallach, D.F. (1969) Generalized membrane defects in cancer. *N. Engl. J. Med.* 280, 761–767
123 Hume, D. and Weidemann, M. (1979) Role and regulation of glucose metabolism in proliferating cells. *J. Natl. Cancer Inst.* 62, 3–8
124 Strauli, P. and Haemmerli, G. (1984) The role of cancer cell motility in invasion. *Cancer Metastasis Rev.* 3, 127–141
125 Carter, S. (1965) Principles of cell motility: the direction of cell movement and cancer invasion. *Nature* 208, 1183–1187
126 Fraumeni, J.F. *et al.* (1989) Epidemiology of Cancer. In *Cancer: Principles and Practice of Oncology* (3rd edn) (de Vita, V.T. *et al,*. eds), pp. 196–209, Lippincott
127 Martin, T. *et al.* (2004) Hepatocyte growth factor disrupts tight junctions in human breast cancer cells. *Cell Biol. Int.* 28, 361–371
128 Tan, X. *et al.* (2004) Analysis of invasion-metastasis mechanism in pancreatic cancer: involvement of tight junction transmembrane protein occludin and MEK/ERK signal transduction pathway in cancer cell dissociation. *Oncol. Rep.* 11, 993–998
129 Mullin, J.M. (2004) Epithelial barriers, compartmentation, and cancer. *Science STKE 2004,* p. 2
130 Martinez-Palomo, A. (1970) Ultrastructural modifactions of intercellular junctions. *In Vitro* 6, 15–20
131 Posalaky, Z. and McGinley, D. (1984) Occluding junctions in an epithelioid mesothelioma: a freeze-fracture study. *Ultrastruct. Pathol.* 6, 177–183
132 Alroy, J. (1979) Ultrastructure of canine urinary bladder carcinoma. *Vet. Pathol.* 16, 693–701
133 Robenek, H. *et al.* (1981) Structure and function of the junctional complement of spontaneous and transplanted muring mammary carcinomas. *J. Submicrosc. Cytol.* 13, 347–363
134 Kerjaschki, D. *et al.* (1979) The structure of tight junctions in human thyroid tumors. A systematic freeze-fracture study. *Am. J. Pathol.* 96, 207–225
135 Polak-Charcon, S. *et al.* (1980) Tight junctions of epithelial cells of human fetal hindgut, normal colon and colon adenocarcinoma. *J. Natl. Cancer Inst.* 65, 53–57
136 Aoyagi, K. *et al.* (2000) Morphological change in the MNNG-treated rat gastric mucosa. *Kurume Med. J.* 47, 31–36
137 Saito, T. (1984) Ultrastructural changes on the junctional complexes in the human urinary bladder carcinoma by thin sectioning and freeze fracture. *J Clin Electron Micros* 17, 201–209
138 Swift, J.G. *et al.* (1983) Intercellular junctions in hepatocellular carcinoma. *J. Submicrosc. Cytol.* 15, 799–810
139 Davies, R. *et al.* (1989) Detection of the cancer-prone colon, using transepithelial impedance analysis. *Arch. Surg.* 124, 480–484
140 Gonzalez-Correa, C. *et al.* (2003) Low frequency electrical bioimpedance for the detection of inflammation and dysplasia in Barrett's. *Physiol. Meas.* 24, 291–296
141 Soderholm, J.D. *et al.* (2002) Augmented increase in tight junction permeability by luminal stimuli in the non-inflamed ileum of Crohn's disease. *Gut* 50, 307–313
142 Blanco, D. *et al.* (2004) Altered expression of adhesion molecules and epithelial-mesenchymal transition in silica-induced rat lung carcinogenesis. *Lab. Invest.* 84, 999–1012
143 Liebner, S. *et al.* (2000) Correlation of tight junction morphology with the expression of tight junction proteins in blood-brain barrier endothelial cells. *Eur. J. Cell Biol.* 79, 707–717
144 Soler, A.P. *et al.* (1999) Increased tight junctional permeability is associated with the development of colon cancer. *Carcinogenesis* 20, 1425–1431
145 Guan, Y. *et al.* (2002) Transforming growth factor-alpha abrogates the glucocorticoid stimulation of tight junction formation and reverses the steroid-induced down-regulation of fascin in rat mammary epithelial tumor cells by a Ras-dependent pathway. *Exp. Cell Res.* 273, 1–11
146 Chen, Y. *et al.* (2000) Restoration of tight junction structure and barrier function by down-regulation of the mitogen-activated protein kinase pathway in ras-transformed Madin-Darby canine kidney cells. *Mol. Biol. Cell* 11, 849–862
147 Nunbhakdi-Craig, V. *et al.* (2003) Simian virus 40 small tumor antigen induces deregulation of the actin cytoskeleton and tight junctions in kidney epithelial cells. *J. Virol.* 77, 2807–2818
148 Jiang, W. *et al.* (1999) Hepatocyte growth factor/scatter factor decreases the expression of occluding and transendothelial resistance (TER) and increases paracellular permeability in human vascular endothelial cells. *J. Cell. Physiol.* 181, 319–329
149 Resnick, M.B. *et al.* Claudin-1 is a strong prognostic indicator in stage II colonic cancer: a tissue microarray study. *Mod. Pathol.* (in press)
150 Tobioka, H. *et al.* (2004) Sawada, N. Occludin expression decreases with the progression of human endometrial carcinoma. *Hum. Pathol.* 35, 159–164
151 Tobioka, H. *et al.* (2002) Polarized distribution of carcinoembryonic antigen is associated with a tight junction molecule in human colorectal adenocarcinoma. *J. Pathol.* 198, 207–212
152 Busch, C. *et al.* (2002) Down-regulation of CEACAM1 in human prostate cancer: correlation with loss of cell polarity, increased proliferation rate, and Gleason grade 3 to 4 transition. *Hum. Pathol.* 33, 290–298
153 Kimura, Y. *et al.* (1997) Expression of occludin, tight-junction-associated protein, in human digestive tract. *Am. J. Pathol.* 151, 45–54
154 Marzioni, D. *et al.* (2001) Expression of ZO-1 and occludin in normal human placenta and in hydatidiform moles. *Mol. Hum. Reprod.* 7, 279–285
155 Davies, R. (2002) Blood-brain barrier breakdown in septic encephalopathy and brain tumours. *J. Anat.* 200, 639–646
156 Papadopoulos, M.C. *et al.* (2001) Occludin expression in microvessels of neoplastic and non-neoplastic human brain. *Neuropathol. Appl. Neurobiol.* 27, 384–395
157 Wang, W. *et al.* (2001) VEGF increases BMEC monolayer permeability by affecting occludin expression and tight junction assembly. *Am. J. Physiol. Heart Circ. Physiol.* 280, H434–H440
158 Kramer, F. *et al.* (2000) Genomic organization of claudin-1 and its assessment in hereditary and sporadic breast cancer. *Hum. Genet.* 107, 249–256

159 Willott, E. *et al.* (1993) The tight junction protein ZO-1 is homologous to the Drosophila discs-large tumor suppressor protein of septate junctions. *Proc. Natl. Acad. Sci. U. S. A.* 90, 7834–7838

160 Miwa, N. *et al.* (2000) Involvement of claudin-1 in the beta-catenin/Tcf signaling pathway and its frequent upregulation in human colorectal cancers. *Oncol. Res.* 12, 469–476

161 Folpe, A. *et al.* (2002) Expression of claudin-1, a recently described tight junction-associated protein, distinguishes soft tissue perineurioma from potential mimics. *Am. J. Surg. Pathol.* 26, 1620–1626

162 Kominsky, S.L. *et al.* (2003) Loss of the tight junction protein claudin-7 correlates with histological grade in both ductal carcinoma *in situ* and invasive ductal carcinoma of the breast. *Oncogene* 22, 2021–2033

163 Rangel, L.B. *et al.* (2003) Tight junction proteins claudin-3 and claudin-4 are frequently overexpressed in ovarian cancer but not in ovarian cystadenomas. *Clin. Cancer Res.* 9, 2567–2575

164 Rangel, L.B. *et al.* (2003) Characterization of novel human ovarian cancer-specific transcripts (HOSTs) identified by serial analysis of gene expression. *Oncogene* 22, 7225–7232

165 Zheng, J.Y. *et al.* (2003) Regulation of the expression of the prostate-specific antigen by claudin-7. *J. Membr. Biol.* 194, 187–197

166 Fanning, A. *et al.* (1999) Transmembrane proteins in the tight junction barrier. *J. Am. Soc. Nephrol.* 10, 1337–1345

167 Tsukita, S. and Furuse, M. (1999) Occludin and claudins in tight-junction strands:leading or supporting players? *Cell Biology* 9, 268–273

168 Furuse, M. *et al.* (1993) Occludin: A novel integral membrane protein localizing at tight junctions. *J. Cell Biol.* 123, 1777–1788

169 Martin-Padura, I. *et al.* (1998) Junctional adhesion molecule, a novel member of the immunoglobulin superfamily that contributes at intercellular junctions and modulates monocyte transmigration. *J. Cell Biol.* 142, 117–127

170 Cunningham, S. *et al.* (2000) A novel protein with homology to the junctional adhesion molecule. Characterization of leukocyte interactions. *J. Biol. Chem.* 275, 34750–34756

171 Cohen, C. *et al.* (2001) The coxsackievirus and adenovirus receptor is a transmembrane component of the tight junction. *Proc. Natl. Acad. Sci. U. S. A.* 98, 15191–15196

172 Nasdala, I. *et al.* (2002) A transmembrane tight junction protein selectively expressed on endothelial cells and platelets. *J. Biol. Chem.* 277, 16294–16303

173 Furuse, M. *et al.* (1998) Claudin-1 and -2: Novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. *J. Cell Biol.* 141, 1539–1550

174 Tsukita, S. and Furuse, M. (2000) Pores in the wall: claudins constitute tight junction strands containing aqueous pores. *J. Cell Biol.* 149, 13–16

175 Shareghi, G.R. and Agus, Z.S. (1982) Magnesium transport in the cortical thick ascending limb of Henle´s loop of the rabbit. *J. Clin. Invest.* 69, 759–769

176 Quamme, G.A. and Dirks, J.H. (1980) Magnesium transport in the nephron. *Am. J. Physiol.* 239, F393–F401

177 Praga, M. *et al.* (1995) Familial hypomagnesemia with hypercalcinuria and nephrocalcinosis. *Kidney Int.* 47, 1419–1425

178 Simon, D.B. *et al.* (1999) Paracellin-1, a renal tight junction protein required for paracellular Mg2+ resorption. *Science* 285, 103–106

179 Hirano, T. *et al.* (2000) Null mutation of PCLN-1/Claudin-16 results in bovine interstitial nephritis. *Genome Res.* 10, 659–663

180 Muller, D. *et al.* (2003) A novel claudin 16 mutation associated with childhood hypercalciuria abolishes binding to ZO-1 and results in lysosomal mistargeting. *Am. J. Hum. Genet.* 73, 1293–1301

181 Wilcox, E.R. *et al.* (2001) Mutations in the gene encoding tight junction claudin-14 cause autosomal recessive deafness DFNB29. *Cell* 104, 165–172

182 Johnstone, B. and Sellick, P. (1972) The peripheral auditory apparatus. *Q. Rev. Biophys.* 5, 1–75

183 Gratton, M. *et al.* (1997) Decline in the endocochlear potential corresponds to decreased Na, K-ATPase activity in the lateral wall of quiet-aged gerbils. *Hear. Res.* 108, 9–16

184 Shapiro, B.L. (1999) The Down syndrome critical region. *J. Neural Transm. Suppl.* 57, 41–60

185 Gow, A. *et al.* (1999) CNS Myelin and sertoli cell tight junction strands are absent in Osp/Claudin-11 null mice. *Cell* 99, 649–659

186 Gow, A. *et al.* (2004) Deafness in Claudin 11-null mice reveals the critical contribution of basal cell tight junctions to stria vascularis function. *J. Neurosci.* 24, 7051–7062

187 Yamauchi, K. *et al.* (2004) Disease-causing mutant WNK4 increases paracellular chloride permeability and phosphorylates claudins. *Proc. Natl. Acad. Sci. U. S. A.* 101, 4690–4694

188 Dallasta, L. *et al.* (1999) Blood-brain barrier tight junction disruption in human immunodeficiency virus-1 encephalitis. *Am. J. Pathol.* 155, 1915–1927

189 Schmitz, H. *et al.* (2002) Supernatants of HIV-infected immune cells affect the barrier function of human HT-29/B6 intestinal epithelial cells. *AIDS* 16, 983–991

190 Schulzke, J.D. *et al.* (1998) Epithelial tight junction structure in the jejunum of children with acute and treated celiac sprue. *Pediatr. Res.* 43, 435–441

191 Vogelsang, H. *et al.* (1998) Changes in gastrointestinal permeability in celiac disease. *Dig. Dis.* 16, 333–336

192 Schmitt, M. *et al.* (2004) Disruption of paracellular sealing is an early event in acute caerulein-pancreatitis. *Pancreas* 28, 181–190

193 Takaya, J. *et al.* (1997) Menetrier's disease evaluated serially by abdominal ultrasonography. *Pediatr. Radiol.* 27, 178–180

194 Oderda, G. *et al.* (1990) Increased tight junction width in two children with Menetrier's disease. *J. Pediatr. Gastroenterol. Nutr.* 11, 123–127

195 Minagar, A. *et al.* (2003) Serum from patients with multiple sclerosis downregulates occludin and VE-cadherin expression in cultured endothelial cells. *Mult. Scler.* 9, 235–238

196 Plumb, J. *et al.* (2002) Abnormal endothelial tight junctions in active lesions and normal-appearing white matter in multiple sclerosis. *Brain Pathol.* 12, 154–169

197 Keefe, D.M. *et al.* (1997) Effect of high-dose chemotherapy on intestinal permeability in humans. *Clin. Sci. (Lond.)* 92, 385–389

198 Stelwagen, K. *et al.* (2000) Short communication: effects of isolation stress on mammary tight junctions in lactating dairy cows. *J. Dairy Sci.* 83, 48–51

199 Mooradian, A.D. *et al.* (2003) Age-related changes in rat cerebral occludin and zonula occludens-1 (ZO-1). *Mech. Ageing Dev.* 124, 143–146

200 Hollander, D. and Tarnawski, H. (1985) Aging-associated increase in intestinal absorption of macromolecules. *Gerontology* 31, 133–137

201 Katz, D. *et al.* (1987) Aging-associated increase in intestinal permeability to polyethylene glycol 900. *Dig. Dis. Sci.* 32, 285–288

202 Johnson, L. *et al.* (2003) Safety and efficiency of modulating paracellular permeability to enhance airway epithelial gene transfer *in vivo*. *Hum. Gene Ther.* 14, 729–747

203 Chen, M. *et al.* (2001) Apical and basolateral EGF receptors regulate gastric mucosal paracellular permeability. *Am. J. Physiol. Gastrointest. Liver Physiol.* 280, G264–G272

204 Singh, A.B. and Harris, R.C. (2004) Epidermal growth factor receptor activation differentially regulates claudin expression and enhances transepithelial resistance in Madin-Darby canine kidney cells. *J. Biol. Chem.* 279, 3543–3552

205 Antonetti, D. *et al.* (2002) Hydrocortisone decreases retinal endothelial cell water and solute flux coincident with increased content and decreased phosphorylation of occludin. *J. Neurochem.* 80, 667–677

206 Zettl, K.S. *et al.* (1992) Glucocorticoid-induced formation of tight junctions in mouse mammary epithelial cells *in vitro*. *Proc. Natl. Acad. Sci. U. S. A.* 89, 9069–9073

207 Naishiro, Y. *et al.* (2001) Restoration of epithelial cell polarity in a colorectal cancer cell line by suppression of beta-catenin/T-cell factor 4-mediated gene transactivation. *Cancer Res.* 61, 2751–2758

208 Nakajima, M. *et al.* (2003) WF-536 inhibits metastatic invasion by enhancing the host cell barrier and inhibiting tumour cell motility. *Clin. Exp. Pharmacol. Physiol.* 30, 457–463

209 Resta-Lenert, S. and Barrett, K.E. (2003) Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive *Escherichia coli* (EIEC). *Gut* 52, 988–997

210 Michail, S. and Abernathy, F. (2003) Lactobacillus plantarum inhibits the intestinal epithelial migration of neutrophils induced by enteropathogenic *Escherichia coli*. *J. Pediatr. Gastroenterol. Nutr.* 36, 385–391

211 Otte, J.M. and Podolsky, D.K. (2004) Functional modulation of enterocytes by gram-positive and gram-negative microorganisms. *Am. J. Physiol. Gastrointest. Liver Physiol.* 286, G613–G626