editorial review

Barrier function of epithelia

POWELL, DON W. *Barrier function of epithelia.* Am. J. Physiol. 241 (Gastrointest. Liver Physiol 4): G275–G288, 1981.—The ability of an epithelium to prevent permeation of noxious agents has not been well studied except in the gastrointestinal tract where exclusion of H^+ has clinical significance. This article reviews the permeation routes across epithelia both as elucidated in the extensive electrophysiological work done in recent years and as demonstrated in morphological studies. We thus place concepts about gastrointestinal barrier function into the framework of transport physiology. Both the permeability and permselectivity of epithelial barriers are reviewed here. The effects of physical agents (pressure and electric current), polyvalent cations, organic compounds with both specific (channel blocking) and nonspecific (detergent) membrane properties, cyclic nucleotides, microfilament-active agents, and particularly H^+ on both the barrier function (permeability and permselectivity) and transport function of epithelia are considered. Based on the available data, an important role for active Na^+ transport in the maintenance of the epithelial barrier function can be postulated.

cell membranes; electrical resistance; paracellular shunt path; permeability; permselectivity; active and passive transport; electrolytes; cell junctions; gastric mucosal barrier

FOR THE LAST HALF CENTURY, epithelial transport has flourished as an investigative science. The mechanisms whereby nutrients, electrolytes, and water are transferred from the environment across the epithelia into the organism have been defined in varying detail for most epithelia and in a number of animal species. However, physiologists have paid less attention to the other major function of epithelia: their ability to serve as barriers between the outside world and the internal milieu of the organism. No doubt this is because the epithelial properties that allow the frog skin to absorb sodium from concentrations as low as 1 mM in pond water or the rabbit urinary bladder "to prevent loss of Na^+ from the body during Na^+ depletion" (94) are the same properties that allow these epithelia to keep the pond out of the frog and the urine out of the rabbit. Thus, studies directed toward understanding epithelial transport function can be assumed to explain barrier functions as well (36, 37, 168).

Another group of scientists, working from an entirely different perspective, has been more concerned with how epithelia act as barriers. Since the pioneering work of Davenport (31), who developed the concept of the "gastric mucosal barrier," clinically oriented researchers have studied how acid (H^+) affects the mucosa of the gastrointestinal tract. The barrier properties seem paramount to these investigators, as they are constantly reminded of the results of violated epithelia. Unfortunately, workers in the barrier field have rarely attempted to apply the concepts and the experimental techniques of the transport physiologists in their approach. This article reviews some of the literature, seeking a mutual understanding of both groups of researchers. Although I will consider diverse epithelia, I have concentrated on information available about the gastrointestinal tract. I have not addressed here the protein-antigen (macromolecule) uptake in the intestine (172), although this is another important barrier function in the gastrointestinal tract.

PERMEATION PATHWAYS IN EPITHELIA: ELECTROPHYSIOLOGICAL CONSIDERATIONS

Extensive investigations by transport physiologists over the past years have developed the paradigm of two transport routes across epithelia (37, 147, 150). The two routes of movement for solutes across single-layered epithelia are *1)* a transcellular route that consists of two barriers, an apical and a basolateral cell membrane; and *2)* a paracellular pathway (shunt path) that can be considered as either a single barrier, the tight junction, or as two barriers, the tight junction and the intercellular space. Because the major permeability barrier of multilayered epithelia is thought to be the first living cell layer (109), stratified squamous epithelia can be visualized as having a similar division of permeation routes. As a first approximation, epithelia can be depicted as a simple electrical circuit of batteries and resistances. Figure 1 shows only the four major resistances. There are two

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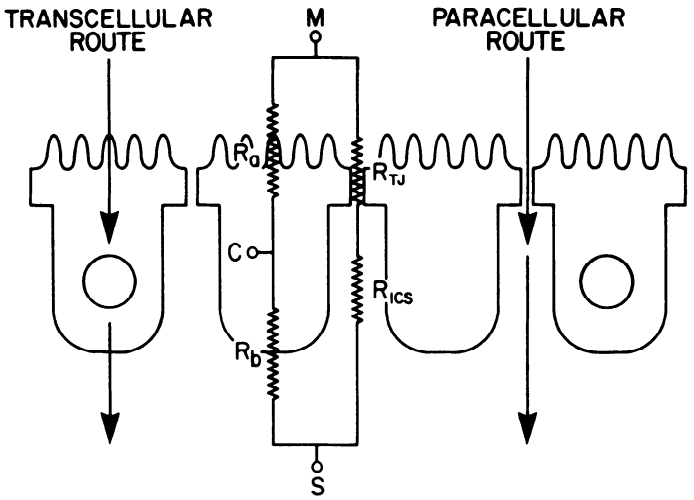

$$R_C = R_a + R_b$$

$$R_S = R_{TJ} + R_{ICS}$$

$$R_T = \frac{(R_C)(R_S)}{R_C + R_S}$$

$$G_T = G_C + G_S$$

$$\frac{G_S}{G_T} = \frac{G_S}{G_C + G_S}$$

FIG. 1. Two permeation routes across epithelia, the transcellular resistance (R_c) and resistance of paracellular shunt path (R_s), operate in parallel. R_c and R_s each contain 2 resistances in series: R_a and R_b are resistances of apical and basolateral cell membranes, respectively, while R_{tj} and R_{ics} are resistances of tight junction proper and intercellular space, respectively. Relations between resistances and conductances (G) are given by simple electrical circuit analysis.

resistances in parallel between the mucosal solution of the epithelium and the serosal solution: the cellular resistance (R_c) and the resistance of the paracellular shunt path (R_s). In turn, each of these parallel resistances is made up of two resistances in series: R_c is composed of the apical membrane (R_a) in series with the basolateral membrane (R_b), while R_s is composed of the tight junction (R_{tj}) and the intercellular spaces (R_{ics}). The mathematical relationships of these resistances, and conductances (G_t, G_c, and G_s), as determined from an analysis of simple electrical circuits (16, 149), are also shown in Fig. 1.

Each of these epithelial barriers has two properties that can be measured experimentally: the general *permeability* (magnitude) of the barrier, which may be simply quantified by the electrical resistance, and the *permselectivity* of the barrier, which is a qualitative measure of its ability to discriminate and show preference for either cations or anions and within a series of cations or anions.

Barrier Resistance

Table 1 lists the resistances of various epithelia and, when known, the individual components that make up the total resistance. Epithelia can be classified as leaky or tight by these values. Leaky epithelia are those with a tissue resistance (R_t) less than 1,000 $\Omega \cdot cm^2$, those with an R_c greater than R_s ($R_c/R_s > 1$), or those in which the shunt conductance (G_s) is greater than 50% of the total tissue conductance (G_t). When considering the gastrointestinal tract, it is useful to classify epithelia in three categories: leaky (gallbladder and small intestine), moderately leaky or moderately tight (colon and gastric antrum), and tight (gastric fundus and esophagus). The esophagus has not been well characterized so it is not shown in Table 1. However, given its total tissue resistance of 1,000–2,500 $\Omega \cdot cm^2$, esophageal epithelia would fall into the tight group (19, 129). When considering the resistances of the membranes among species, no distinct patterns of classification emerge, although in general the resistances of amphibian membranes appear to be greater than those of warm-blooded animals.

Barrier Permselectivity

If one considers only the rates and not the mechanisms whereby ionic species cross epithelia, tissue permselectivity combines contributions from both cell membrane and shunt activities. The permselectivities of the apical and basolateral cell membranes are a reflection of the carriers and pumps as well as the passive permeation channels in those individual membranes. The Na^+-transporting epithelia usually have a $P_K > P_{Cl} > P_{Na}$ in the basolateral membrane (97, 175). According to the Koefoed-Johnson-Ussing model of Na^+-transporting epithelia, one might expect an opposite sequence of permeabilities in the apical membrane. Indeed, some Na^+-transporting epithelia do have large apical membrane Na^+ permeabilities (96). Similarly, Cl^--transporting epithelia may have a large Cl^- conductance in the apical membrane (3, 155). However, actual measurements in *Necturus* gallbladder (137), which also transports Na^+, have shown the same apical membrane permeabilities ($P_K > P_{Cl} > P_{Na}$) as seen in the basolateral membranes.

More is known about the permselectivity of the shunt path. Electrokinetic potentials created by osmotic flow or by placing unequal ion concentrations on either side of the epithelium reflect the permselectivity of the shunt path in leaky tissues. The relative permeabilities of the ions in question can be determined from these potentials. Measurements of shunt path permselectivity in tight epithelia are more difficult. These tissues do not produce large electrokinetic potentials, and determination of the relative permeabilities depends on prior perturbations such as hypertonic mucosal solutions or the passage of electrical current to open shunt paths (51, 107). In addition, hyperpolarization or depolarization of the tissue has often been used to create the movement of the ions in order to determine their relative permeabilities (58). Such maneuvers may in themselves alter the shunt path permselectivity (see below).

Table 2 shows the calculated relative permeabilities of the shunt paths of several epithelia. When possible, permeabilities are given with P_{Na} normalized to 1.00. With a few exceptions, the shunt path of both leaky and tight epithelia are cation selective (P_K and $P_{Na} > P_{Cl}$), and P_K is always greater than P_{Na}. This has been explained by the nature of the paracellular pathway, which is lined with negative charges (carboxyl, phosphoric acid, or sulfuric acid radicals) that discriminate against the

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TABLE 1. *Electrical resistances of various epithelia*

Tissue	Species	Resistances, Ω·cm²						100 G_s/G_t, %
		R_a	R_b	R_c	R_s	R_t	R_c/R_s	
Proximal renal tubule	Dog, rabbit (17, 99)				6–7	6–7		99
Gallbladder	*Necturus* (65)	4,470	2,280	6,750	324	310	21	96
	Necturus (136)	3,350	2,750	6,100	480	450	31	94
	Rabbit (75)	156	143	299	21	20	14	93
	Goose (75)	625	378	1,003	30	29	33	97
	Tortoise (75)	1,607	861	2,468	99	94	25	95
	Trout (75)	528	212	740	29	28	26	96
Small intestine								
Duodenum	Rat (124)					98		95
Jejunum	Rat (120)				67	51		76
	Rat (124)					66		94
Ileum	Rat (124)					88		94
	Rabbit (60)				115	100		85
	Rabbit (145)*				28	21		75
Colon	Rabbit (151)	1,570	100	1,670	345	286	5	83
	Rabbit (175)*	705	95	800	730	385	1	53
	Turtle (34)*			1,351	781	495	2	63
Gastric mucosa								
Antrum	*Necturus* (159)*	4,343	3,354	7,697	2,241	1,730	3	78
Fundus	*Necturus* (160)*	1,779	1,047	2,826	10,573	2,230	0.3	21
Amphibian urinary bladder	Toad (139)	4,040	3,215	7,255	13,245	3,755	0.6	28
	Frog (93)		<2,500	5,000†	>35,000	4,500†	0.1	13
Amphibian skin	Toad (21)			8,092	13,513	763	0.6	6
	Frog (93)		<5000	10,000†	>65,000	8,700†	0.2	13
Mammalian urinary bladder	Rabbit (96)	4,000–150,000	5,000–10,000	9,000–160,000	6,000–300,000	5,000–10,000	<0.1	<3

Numbers in parentheses are reference numbers. R_a, apical cell membrane resistance; R_b, basolateral cell membrane resistance; R_c, total cellular resistance; R_s, shunt path resistance; R_t, total resistance; 100 G_s/G_t, % of total tissue conductance due to shunt path. * Muscle and serosal layers removed. † Calculated value, assuming $R_a = R_b$.

TABLE 2. *Shunt path permselectivities of various epithelia*

Tissue	Species	P_{K^+} :	P_{Na^+} :	P_{Cl^-}
Kidney				
Proximal renal tubules	Dog (17)	1.10	1.00	0.72
Cultured kidney cells	Dog (132)	1.41	1.00	0.23
Gallbladder	Rabbit (38)	2.30	1.00	0.33
	Necturus (169)	1.81	1.00	0.32
Small intestine				
Jejunum	Rat (176)	1.20	1.00	0.10
	Rat (120)	1.60	1.00	0.20
Ileum	Rabbit (60)	1.14	1.00	0.55
	Rabbit (128)	1.14	1.00	0.40
Colon	Rabbit (175)	1.00	1.00	
	Rabbit (58)*	14.30	1.00	1.57
Gastric mucosa				
Antrum	*Necturus* (159)	1.00		0.86
	Necturus (5)	1.00		0.95
	Bullfrog (5)	1.00		0.71
Urinary bladder	Toad (51)	1.41	1.00	0.72
	Toad (51)†	1.41	1.00	1.39
	Toad (148)†	1.39	1.00	1.02
Amphibian skin	Toad (21)		1.00	0.53
	Frog (107)‡	1.33	1.00	4.46
Free solution		1.47	1.00	1.52

Numbers in parentheses are reference numbers. * Hyperpolarized and depolarized tissue. † Hyperpolarized tissue. ‡ Depolarized tissue.

passive movement of anions through the hydrated channel. Anion-selective shunt paths have been found in the rabbit colon and frog skin (Table 2). An important study of Finn and Bright (51) may explain these discrepancies. These investigators found a cation-selective shunt path ($P_K > P_{Na} > P_{Cl}$) in the toad urinary bladder by measuring ionic fluxes into a hypertonic mucosal solution. When they repeated these same experiments in bladders that were voltage clamped at +25 mV, the permselectivity sequence was altered such that now $P_K = P_{Cl} > P_{Na}$. Thus, the passage of current itself altered the charge on the shunt path, making it more anion (less cation) selective. Both the studies of Frizzell et al. (58) in rabbit colon and those of Mandel and Curren (107) in frog skin were performed in tissues that had been either hyperpolarized or depolarized.

The important point for the investigator interested in gastrointestinal barrier function and in H^+ permeation is that the shunt path permselectivities of the stomach and small intestine appear to be cation selective. Thus, the permeation system does not naturally resist H^+ diffusion across the epithelium.

Agents Altering Epithelial Resistance

Several physical and chemical agents can alter epithelial resistance. It is not always clear whether the change in R_t comes about through an effect on the cellular or the paracellular pathway. A general perspective can be gained by considering epithelial conductance ($G_t = G_c + G_s$) and the relative effect on G_t that would result from changes in G_c and G_s. In leaky tissues, agents that decrease G_c would probably have little relative effect on G_t, inasmuch as over 75% of the current flow (or ionic flux) takes place across the shunt pathway. Similarly, agents

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that increase G_s have more significant effects on G_t than do agents that increase G_c. Conversely, in tight epithelia, anything that would decrease G_s would have less relative effect on G_t, because G_s accounts for less than 25% of G_t, whereas increases in G_c (but not G_s) would cause nearly the same percentage increase in G_t. Thus, significant increases or decreases in epithelial resistance come about predominantly by changes in R_s in leaky tissues and by changes in R_c in tight tissues.

Physical agents. Two physical agents that affect epithelial resistance are the passage of electrical current and pressure (osmotic and/or hydrostatic). Both leaky (gallbladder) and tight (frog skin and stomach) epithelia respond to strong electrical current by a decrease in R_t (13, 107, 123). This effect is presumably on the shunt path, inasmuch as it is accompanied by increased permeation of water-soluble solutes such as urea or mannitol, which are thought to transverse the paracellular pathway primarily.

Luminal hypertonicity also alters permeability. In tight epithelia such as amphibian skin (48, 105), urinary bladder (40, 171), and colon (34), the change in osmotic pressure decreases total R_t. Both electrophysiological and morphological techniques indicate that the effect in these tissues is on the shunt path. In contrast, in leaky epithelia such as gallbladder, luminal hypertonicity increases both R_t and R_s (138). Serosal hypertonicity has the opposite effect in the gallbladder, decreasing resistance (13, 174). Because these changes in resistance are accompanied by closing or dilation of the intercellular spaces, Wiedner and Wright (174) believe this indicates a role for the intercellular space in the shunt path permeability of leaky tissues. They conclude that the major barrier to paracellular permeation of leaky epithelia is probably the tight junction proper (R_{tj}) when the lateral spaces are dilated and in the intracellular space (R_{ics}) when the spaces are collapsed. These various and differential effects of mucosal solution hypertonicity should be borne in mind by investigators studying the effect of ethanol on barrier integrity of the gastrointestinal tract, because ethanol is usually added to the mucosal solution in concentrations that result in significant hypertonicity.

Another example of the effects of altered hydrostatic and/or oncotic pressure is the increase in paracellular permeability of both the renal tubule (15, 79) and the intestine (76, 80) in response to intravenous saline loading (volume expansion). However, because systemic volume expansion might produce hormonal changes, a permeation relationship explained solely by pressure change cannot be established.

Electrolytes and organic compounds. In tight tissues, changes in permeability of the apical cell membrane can have drastic effects on the total R_t. For instance, variations in Cl^- concentration in the lumen of rabbit submaxillary duct (a tissue that transports Cl^- from lumen to blood) can cause a 3- to 10-fold change in R_t (155). In fact, this tissue is considered by some to be a tight epithelium (if defined by $R_c/R_s < 1$), which has an R_t in the range of very leaky tissues (10 $\Omega \cdot cm^2$) because of the high apical membrane Cl^- permeability (3, 4). Similarly, the apical membrane of rabbit colon (175) and rabbit urinary bladder (94, 96) are Na^+ selective, and decreases in the Na^+ concentration of mucosal solutions can change R_a. For instance, in rabbit urinary bladder, R_a can increase from 4,000 to 150,000 $\Omega \cdot cm^2$ by removing Na^+ from the mucosal solution. This change in R_a is accompanied by large changes in R_t.

Polyvalent cations, both heavy metals such as Ca^{2+} and organic compounds such as 2,4,6-triaminopyrimidinium (TAP), block the Na^+ permeation pathway in the apical cell membrane of Na^+-transporting tight epithelia (presumably by binding to negative charges in the Na^+ channel) with a coincidental increase in both R_a and R_t (6, 50, 94, 96, 177). Specific Na^+ channel blockers such as amiloride have a similar effect (6, 94, 96, 151, 167), whereas aldosterone is thought to decrease resistance (and increase Na^+ transport) by creating new Na^+ channels (59). There also seems to be an important internal control of Na^+ permeability in these tissues. Agents that inhibit Na^+ exit from the basolateral membrane (e.g., Na-K-ATPase inhibitors or metabolic inhibitors such as cyanide or anoxia) also reduce Na^+ entry across the apical cell membrane (94, 96). Agents that increase Na^+ exit (e.g., serosal HCO_3) increase apical membrane Na^+ conductance. Thus, there seems to be an important negative feedback mechanism that allows the cell to protect and maintain its volume (94, 96, 166, 167).

The major ionic conductance pathway in leaky tissues is paracellular. However, polyvalent cations will also increase R_t in these epithelia by increasing R_s (100, 101, 116, 117, 177). This does not necessarily imply that the molecular nature of the groups conferring cation selectivity to the apical membranes of tight epithelia or to the shunt paths of leaky epithelia are the same, only that both channels are lined with negatively charged ligands. Changes in mucosal solution Ca^{2+} concentrations cause the tissue resistance to vary in both gallbladder and intestine (23, 177), primarily by blocking Na^+ permeability in the shunt pathway. Similar changes in tissue resistance have been observed with moderately tight epithelia such as mammalian stomach (28, 152) and colon (57). However, because these tissues also transport Na^+, it is not entirely clear whether the Ca^{2+} is blocking Na^+ conductance across the cells or across the shunt.

Certain compounds appear to have a more general effect on epithelial permeability. For instance, polyene antibiotics such as amphotericin B and nystatin markedly decrease tissue resistance and increase Na^+ transport in Na^+-absorbing epithelia (7, 11, 61, 95, 112, 135, 143, 144, 175). These agents are thought to work by increasing Na^+ permeability at the apical cell membrane, although others (143, 144) have suggested that they may also affect R_s. Dihydroxy bile salts (11, 12, 67), hydroxylated fatty acids (11, 66), and other detergents such as dioctyl sodium sulfosuccinate (42) markedly increase G_t in the colon and reversibly increase epithelial permeability to large molecules such as inulin, implicating R_s as the major locus of action. However, these lipid-soluble agents have important effects on cellular activities (such as active electrolyte transport) that are due to their effects on cell membranes. Indeed, Duane and Wiegand (43) have demonstrated that bile salts will leach significant quantities of cholesterol and phospholipids from gastric mucosal membranes by micellar solubilization.

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Cyclic nucleotides and microfilament-active agents. Recent studies indicate that shunt path permeability may be under cellular control. Cholera toxin- or theophylline-induced increases in cellular cyclic AMP content are related to significant increases in epithelial resistance in both leaky and moderately tight tissue such as rabbit ileum (128) and colon (67). Interestingly, theophylline has the opposite effect in tight epithelia such as frog skin [i.e., the drug significantly decreases resistance (105, 106)]. The swelling of the cells, an event that occurs during cyclic AMP-induced secretion in small intestine, may close off the intercellular space in a manner similar to that exerted by hypertonic mucosal solutions in leaky tissues (39). However, cell swelling is not so evident in the colon or in frog skin. In recent studies of *Necturus* gallbladder, Bentzel et al. (8, 9) have presented evidence that the tight junction proper is under cellular control. Plant cytokinins such as kinetin and zeatin (N-6-substituted purine derivatives that induce division of plant cells in tissue culture) and two other microfilament-active drugs, cytochalasin B and phalloidine, were shown to increase R_t reversibly. Microelectrode studies showed no significant change in the ratio of R_a to R_b, indicating that the effect of these drugs was primarily on R_s. Anatomic changes in the tight junctions (see below) further implicate a change in R_{tj}. Because these plant kinins increase cyclic AMP content in lymphocytes, the effect of these agents may be mediated through cyclic nucleotides. The interactions between cyclic nucleotides and Ca^{2+}, the effect of Ca^{2+} (and calmodulin) on microfilaments, and the known effects of Ca^{2+} on tissue permeability suggest several possible mechanisms, as yet not investigated, whereby the cells might control the junctions.

Hydrogen ion and barrier breakers. Backdiffusion of H^+ is considered to be the primary cause of acid-peptic disease of the esophagus, stomach, and duodenum. Therefore, the ability of these epithelia to resist H^+ permeation represents the major barrier function of the gastrointestinal tract. As pointed out by Sachs (146), proton diffusion in free solution is the most rapid of any ion because the proton can hop from water molecule to water molecule. This is probably its primary method of movement through a hydrated epithelial shunt path. However, other mechanisms may promote the permeation. Protons can rapidly move across cell membranes by following the peptide backbone of membrane proteins, by diffusing through lipid, by exchanging through specific carriers (e.g., Na-H exchange mechanisms), or possibly by moving via endogenous membrane ionophores.

Recent studies of H^+ or HCl movement through lipid are especially pertinent. Although there is disagreement concerning the exact rates of permeation and the mechanism, the transport rate of H^+ across liposomes (121, 122) or molecular HCl across lipid bilayers (70) is several orders of magnitude higher than that of monovalent electrolytes. Furthermore, agents that belong to the "barrier breaker" category such as alcohol (69), detergents (74), and weak acid uncouplers of oxidative phosphorylation (such as aspirin) (110) can markedly increase lipid permeability. In fact, the weak acid uncouplers are so good at facilitating H^+ diffusion that they have been named "protonophores."

In the gastrointestinal tract, investigators must be concerned with the effect of H^+ on both the stratified squamous epithelium of the esophagus and on the simple columnar epithelia of the gastric and duodenal mucosa. Such studies are complicated by the inhomogeneity of the mucosae. Most esophagi (the rabbit being the exception) have submucosal glands as well as the stratified squamous epithelium of the mucosa proper. The duodenum has submucosal glands as well as its absorptive (villus) and secretory (crypt) epithelia. The gastric mucosa is made up of surface cells that absorb Na^+ and secrete mucus (and possibly HCO_3), parietal cells that secrete HCl, and the chief cells whose electrolyte secretions are unknown. In addition, electrolyte transport in the gastric antrum may be different from that in the fundus. Therefore, the effects of H^+ on transport in such tissues are difficult to interpret because one cannot be sure which of the elements of the mucosa are being perturbed. Therefore, studies of the effect of low pH on simple epithelia such as frog skin and mammalian urinary bladder can give insight into transport in the esophagus because of the structural similarity. Likewise, studies of the gallbladder add to our understanding of the stomach and duodenum.

Lowering the pH of the mucosal solution bathing the frog skin, mammalian urinary bladder, and esophagus results in changes in permeability of both the cells and shunt path. The effect on the shunt path is evident by changes in R_t that occur concomitantly with loss of H^+ from the mucosal solution and increased permeation of water-soluble solutes such as mannitol or sucrose (27, 52, 68, 126). The evidence for an effect of lower pH on cell function comes from the observation that short-circuit current in the frog skin, mammalian urinary bladder, and esophagus increases initially (52, 94, 96, 126). Because all of these epithelia transport Na^+ from lumen to blood, the increased short-circuit current may be due to increased Na^+ entry across the apical cell membrane, resulting in an overall increase in transepithelial transport. However, stimulated anion secretion is also a possible explanation that must be ruled out by ion flux measurements. With continued exposure to high H^+, Na^+ transport falls and then ceases (106, 126).

"Barrier breakers" are a diverse group of agents that act synergistically to enhance the noxious effects of H^+. They markedly accelerate the action of H^+ on stratified squamous epithelia. Bile salts, ethanol, pepsin, trypsin, and lysolecithin all increase the rate of H^+ loss from the lumen (20, 26, 71, 85, 126). These agents are more active in the presence of a low pH, with the exception of trypsin and deconjugated bile salts. Trypsin has a known neutral pH optimum, and the deconjugated bile salts have poor solubility at low pH. In addition, there is evidence that ethanol has an effect on active electrolyte transport in such epithelia that is separate from but additive to the effect due to acid damage on permeability (20, 26).

There are no detailed studies of permeability properties of the duodenal epithelium after H^+ exposure to a low pH. However, it is known that, in the rabbit ileum, serosal exposure to pH 7.0 increases conductance and also stimulates Na^+ absorption (154). In the gallbladder, high H^+ concentrations decrease Na^+ conductance

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through the shunt, but R_t remains the same because of a simultaneous increase in Cl^- conductance (118).

Although the gastric mucosal barrier has been the subject of investigations for the past 25 yr (31–33, 81,140), investigators are just beginning to dissect apart the separate effects of H^+ (and the barrier breakers) on the various transport pathways. The gastric mucosa is resistant to damage by its own secretions. In fact, an initial effect of increasing H^+ is to increase epithelial resistance by blocking Na^+ entry across an amiloride-sensitive channel in the apical membrane of the gastric surface epithelial cells (102). However, the gastric mucosa does have a finite, measurable permeability to H^+ (1, 10, 164). With prolonged exposure to a high concentration of H^+ or with addition of one of the synergistic barrier-breaking agents, the epithelial resistance and potential difference decrease, H^+ loss from the lumen is accelerated, Na^+ enters the gastric lumen, Cl^- may initially be lost from the lumen and then gained, and eventually plasma proteins and even red blood cells enter the stomach (1, 10, 14, 31–33, 35, 53, 62–64, 81, 82, 86, 88, 90–92, 102, 134, 140, 153, 157, 158, 164).

In the early stages of the process in vivo, there ia a one-for-one stoichiometry between H^+ loss and Na^+ gain to the lumen and an apparent Cl-HCO_3^- exchange (32, 33, 81, 84). However, there is no proof that these phenomena result from some cell membrane-exchange carriers activated by the H^+ or by the barrier-breaking agents. The stoichiometry may be due to changes in shunt path permeability (with or without altered permselectivity) along with changes in both active and passive transport across the cells. There is an increase in the gastric mucosal permeability to water-soluble probes that indicates a functional change in the shunt path, although anatomic changes in the tight junctions have been difficult to discern (see below). It is clear that high H^+ concentration and the barrier-breaking agents affect both the active and passive cellular transport of H^+, Cl^-, Na^+, and K^+ (14, 35, 53, 62–64, 82, 86, 88, 90–92, 134, 153, 157, 158) and that these changes occur either before or simultaneously with the changes in passive permeability. Net transport of these ions can be either stimulated or inhibited, and one reponse can follow the other.

Cell-to-cell communication is another area where low pH may alter epithelia. Cell-to-cell coupling decreases (see below) as cellular Ca^{2+} rises (142). Lowering intracellular pH also induces uncoupling, and perhaps this may be due to H^+ mobilization of Ca^{2+} from intracellular stores (141) or perhaps to the low pH itself. The role that cell-to-cell coupling may play in maintaining barrier integrity clearly needs further investigation.

Agents Altering Shunt Path Permselectivity

Electrical current is one agent affecting the permselectivity of the paracellular pathway (see above). In fact, many agents can alter the relative permeability of shunt paths. One of the best studied is H^+. Most if not all shunt paths are cation selective, and, at a neutral physiological pH, these pathways are relatively permeable to H^+. However, as the pH of the mucosal solution is decreased, these epithelia may suddenly switch from cation to anion selectivity. The pH at which this dramatic alteration in permselectivity occurs has been called the isoelectric point (Table 3), which in most tissue is between 2.7 and 5.1. Thus, the negatively charged groups that line the shunt path and govern the permselectivity of this aqueous channel appear to have a pK_a in the range of 4–5. These tissues, therefore, have mechanisms to exclude H^+ permeation when there are drastic decreases in luminal pH. Table 3 lists an isoelectric point for canine gastric mucosa in the range of 9–11 (83). This was derived not from formal measurements of cation-anion selectivity but rather from measurements of Na^+ permeation at different pH levels. If the isoelectric point in the mammalian stomach is 9–11, this would protect the stomach against H^+ permeation, inasmuch as the shunt path under these circumstances would be anion selective until an alkaline pH is reached.

Polyvalent cations might be expected to titrate the negative changes of the shunt path. Indeed, Th^{4+}, La^{3+}, Ba^{2+}, Ca^{2+}, Cu^{2+}, and (least of all) Mg^{2+} all decrease cation permeation in the gallbladder (38, 100, 101, 177). Al^{3+} does also, but, because it is an amphoteric substance that undergoes reactions in neutral aqueous solutions that give off H^+, it is difficult to be sure whether the effect is due to the Al^{3+} itself or to the released hydrogen ion (177). This may have therapeutic implications, inasmuch as hydroxides of these cations are used as antacids. Ca^{2+}, Mg^{2+}, and Al^{3+} may also decrease hydrogen ion permeation across the cellular pathway by reacting with lipid membranes to "stabilize" them by decreasing membrane fluidity (73, 170). Thus, it is possible that these heavy metal hydroxides might decrease both shunt path and cellular H^+ permeation across gastrointestinal epithelia in addition to neutralizing HCl.

Large organic cations such as TAP also block Na^+ permeation of the shunt paths of gallbladder and small intestine, presumably by H^+ bonding to the anionic ligands in the channel (116, 117, 119). Other agents such as cyclic nucleotides and microfilament-active agents such as kinetin and phalloidine that alter the resistance of the intestinal shunt path also alter the permselectivity. Cholera toxin and theophylline change the relative permeability of the small intestine from a P_K:P_{Na}:P_{Cl} of 1.18:1.00:0.44 to 1.17:1.00:0.60 (128), while kinetin reduces dilution potentials in the gallbladder (9). Thus, these agents decrease the cation and/or increase anion permeability of the shunt. Because cyclic AMP may alter Ca^{2+} homeostasis in epithelia, it is possible that Ca^{2+} is reponsible

TABLE 3. *Isoelectric points of various epithelia*

Tissue	Species	Isoelectric Point
Gallbladder	Rabbit (177)	3.0
	Frog (118)	3.1
Jejunum-ileum	Rat (156)	2.7
Gastric mucosa		
Antrum	*Necturus* (5)	3.0
	Frog (5)	4.5
Fundus	Dog (83)	10.0
Urinary bladder	Toad (98)	3.5
Amphibian skin	Frog (2)	5.1

Numbers in parentheses are reference numbers.

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for the changes in permselectivity. Bajaj et al. (5) have investigated the possibility that alterations in H^+ permeation of shunt paths could account for the therapeutic action of carbenoxolone, an effective antiulcer agent with an unknown mechanism of action. Paradoxically, this agent was found to lower further the isoelectric point in *Necturus* and bullfrog gastric mucosa from 3.0–4.5 to 2.5. Because this is the opposite of the desired effect, it must be apparent that carbenoxolone has its salutary therapeutic effects through some other mechanism, perhaps by altering cell membrane permeability.

PERMEATION PATHWAYS IN EPITHELIA: ANATOMIC CONSIDERATIONS

Epithelia are made up of cells joined together by cell junctions (Fig. 2). The ability of the epithelium to act as a barrier between the outside and inside world depends on the integrity of these junctions and of the cell membranes proper. The study of various types of cellular junctions and their anatomic, biochemical, and physiological characteristics is an exciting field of cell biology described well recently in several excellent reviews (49, 111, 127, 162, 163). Certain definitions must be established here to develop points in this review.

Types of Junctions

Gap junctions do not impede the permeation of materials from the lumen to the blood. Rather, they present a hexagonal array of cylindrical tubules that connect adjacent cells and allow the passage of small molecules for cell-to-cell communication.

Desmosomes are of two types: spot desmosomes and belt desmosomes. Spot desmosomes (macula adherens) are analogous to spot welds that join cells together at specific punctate locations. They are very prominent in tissues such as stratified squamous epithelia that are subjected to great mechanical stress. Belt desmosomes (intermediate junctions or zonula adherens) are bandlike

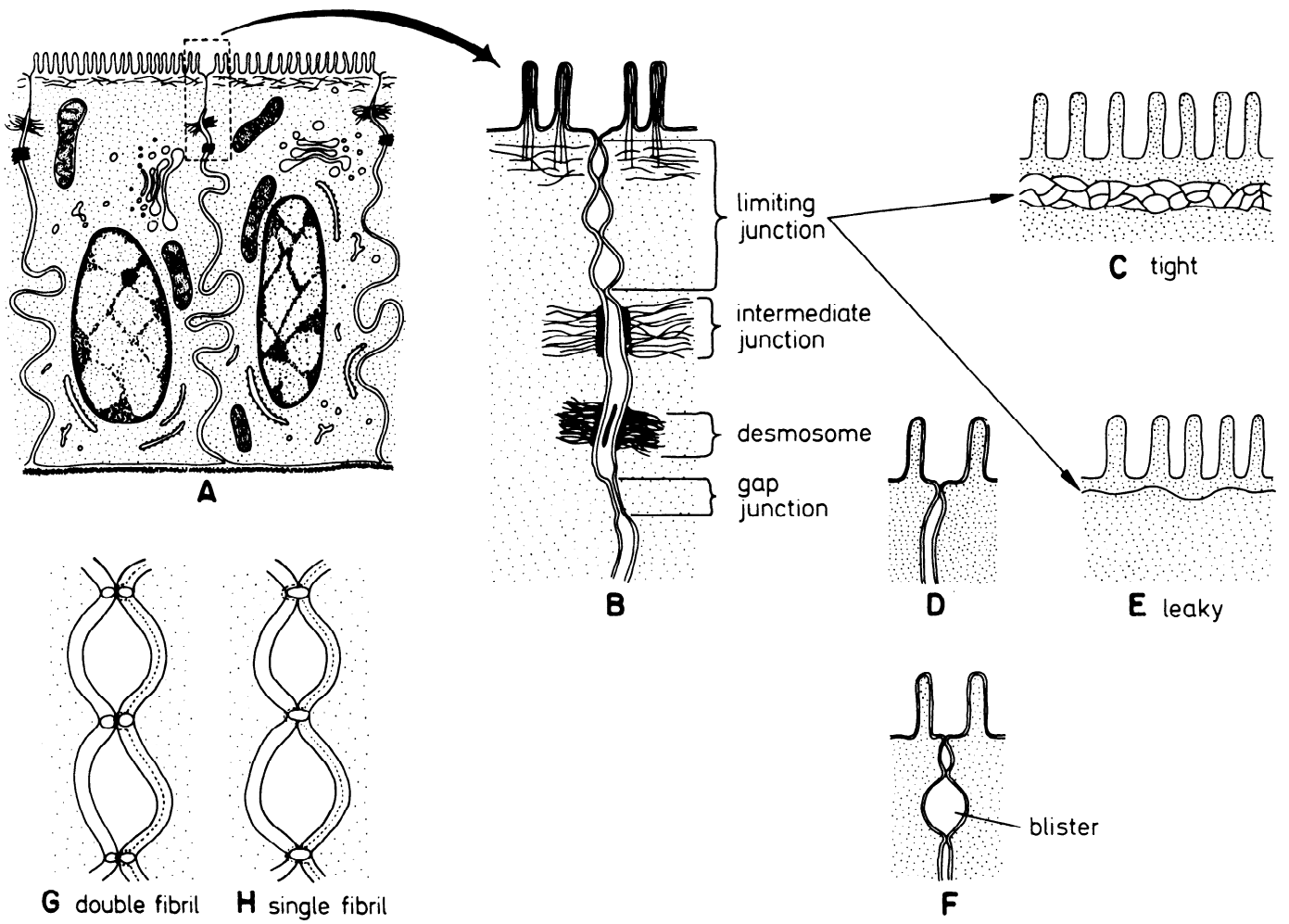

FIG. 2. Intercellular junction of epithelia (*A* and *B*) are gap junctions, which allow cell-to-cell communication; spot desmosomes (macula adherens), which bind cells together at several locations to protect against mechanical stress; intermediate junctions (zonula adherens), which attach to microfilaments and give cell membranes active movement; and limiting or tight junctions (zonula occludens), which seal off intercellular space from lumen. Tight junctions (*C–H*) are composed of rows of integral membrane protein that form fibrils or strands that meet at intercellular space to hold cell membranes together. In general, junctions of tight epithelia (*B* and *C*) are composed of several strands, whereas junctions of leaky epithelia (*D* and *E*) have 1 or 2 strands. Hypertonic mucosal solutions may cause blisters or blebs in tight junction (*F*). *Dashed line* through membrane and around junctional fibrils in *G* and *H* represents possible freeze-fracture planes with either double- or single-fibril model. (Reprinted with permission from Ref. 127.)

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areas of cell-to-cell attachment that completely encircle the cells. Actin-containing filaments attach to these desmosomes and, by contracting in reponse to ATP, Ca^{2+}, and Mg^{2+}, give the cell membrane active movement.

Tight junctions (limiting junctions or zonula occludens) are near the apices of cells, encircling them completely and attaching each cell to its neighbor. However, this junction seals off the space between the cells completely and thus acts as a passive diffusion barrier. Along with the cell membranes, the tight junction allows the development of osmotic gradients between the lumen and the interstitial space of the organ. When injured, they can be repaired within 30 min (78). Tight junctions are the most important intercellular barrier in all epithelia, with the possible exception of mammalian stratified squamous epithelia. Although tight junctions are the intercellular barrier in amphibian skin (109), Elias et al. (46, 47) have suggested that the tight junctions in mammalian skin do not completely encircle the cells. In these tissues, the tight junctions may be of the macula occludens type that, like desmosomes, serve only to spot weld the cells together and not to provide effective permeability barriers. In mammalian stratified squamous epithelia, the intercellular permeability barrier may be a protein-lipid lamellar substance (intercellular cement) that is secreted in the form of membrane-coating granules by the middle layers of the epithelium (46, 47, 72, 161). Although this concept seems firmly established in the case of mammalian skin, more investigation is necessary in other stratified squamous epithelia such as mammalian esophagus.

Morphological Correlates of Permeability

There are three morphological hallmarks of altered shunt path permeability: *1*) there may be anatomic alterations in the junction, such as blister or bleb formation (Fig. 2*F*) or collapse and dilation of intercellular spaces (13, 171); *2*) there may be evidence of movement of electron-opaque molecules such as lanthanum or horseradish peroxidase through the junctions; and *3*) there may be a relationship between the number of sealing strands in the junction as studied by freeze-fracture technology and permeability (Fig. 2, *B–E*).

Transmission electron microscopic studies of leaky epithelia such as renal tubules (165, 173), intestine (101), and gallbladder (101) indicate that these tight junctions are permeable to lanthanum. Conversely, similar studies of tight epithelia such as amphibian urinary bladder (171) and amphibian skin (109) show truly tight junctions through which lanthanum cannot pass. Perturbations that increase the permeability of tight epithelia increase lanthanum permeation through the junction (171). Similarly, the protein-lipid lamellar substance (intercellular cement) of mammalian stratified squamous epithelia can be shown to arrest the permeation of lanthanum (46, 47, 72, 161).

Freeze-fracture studies of tight junctions reveal a honeycomb network of interconnecting sealing strands or fibrils (Fig. 2, *C*,*E*,*G*, and *H*). When the freeze-fracture plane extends through the protoplasmic face of the plasma membrane, the strands appear as ridges; when the fracture plane shows the half of the plasma membrane facing the extracellular space, the strands appear as grooves or furrows. The tight junctional strands seem to be composed of two rows of integral membrane proteins drawn from the plasma membranes of two adjacent cells. If the fracture plane jumps from the intermembrane region of one cellular membrane to the intermembrane region of the adjacent cell, the ridges can be seen to span the width of both membranes. Thus, the two rows of protein seem to fit like a zipper at the intercellular space to hold the two plasma membranes together and to seal off the space.

Several investigators (30, 56, 162) have shown that there is a rough correlation between the number of tight junctional strands (or the total junctional depth because of the number of strands) and the permeability of the epithelium. Claude (29) has suggested that there is an exponential relation between the number of strands and the electrical resistance. Thus, the proximal renal tubule and gallbladder (leaky epithelia) may average two to four strands, while the stomach and urinary bladder (tight epithelia) may average eight strands. In the intestine, cells in the ileum appear to have more strands than jejunal cells, again seeming to reflect the differences in permeability of these two segments of intestine (104). In addition, villus cell tight junctions are more complex than those of crypt cells, suggesting that there may be differences in permeability between the two regions of the epithelium. Additional evidence comes from the studies of Bentzel et al. (9). The increase in epithelial resistance and changes in permselectivity with the microfilament-active agent were accompanied by an increase in the disorder of the meshwork of strands such that the depth of the junction was increased. There was no evidence, however, that these agents caused the development of entirely new strands.

Other investigators have challenged the correlation between the number of strands and resistance. For instance, changes in permeability during embryological development of the choroid plexus of sheep brain failed to correlate with differences in the number of strands (115). In addition, rabbit ileum seems to have just as many strands as toad bladder epithelium in spite of the differences in resistance of these two tissues (108). Finally, perturbations that are known to increase epithelial permeability do not seem to have corresponding changes in freeze-fracture morphology (108). This is further complicated by the possibility that there may not be a uniform permeability or uniform number of strands at all points in the circumferential junctions. In cultured kidney epithelia, the same cell may have two strands at one point and eight strands at another (24).

Morphological Changes With H^+ and Barrier Breakers

Numerous investigators have used morphological studies to determine at what point along the gastric epithelium the barrier-breaking agents were acting. The tight junctional blebs seen in the toad bladder treated with hypertonic mucosal solutions (171) are also seen in the gastric mucosa with a luminal instillation of hypertonic

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urea or alcohol (45). Therefore, by increasing hypertonicity, these agents primarily affect the shunt path. It is less clear that lower concentrations of alcohol, Ca^{2+}-free solutions, bile salts, or salicylates affect the junction. These agents do not appear to cause significant microscopic changes in the esophageal, intestinal, or gastric tight junctions (23, 44, 54, 77). At most, they cause incompletely broken junctions (55) or only slight separation of the membranes of the junction (152). Lanthanum permeation can be used as the hallmark of loss of integrity of tight junctions. Therefore, one cannot rule out the possibility that a significant increase in junctional permeability has occurred unless such electron-opaque markers are used. There is evidence of anatomic changes in the intercellular space in the early phase of H^+ damage. Studies in the esophagus (22, 126) and the stomach (84) show reversible dilatation of the intercellular spaces early in the time sequence of exposure to high concentrations of H^+. Dilated intercellular spaces are generally considered to be anatomic evidence of increased water transport by epithelia. This could be a result of H^+ initially stimulating Na^+ absorption by the cells, perhaps due to increases in apical cell membrane permeability to Na^+ after exposure to high H^+ concentrations. Alternatively, it could be due to increased movement of H_2O across a more permeable epithelium in response to the same amount of active Na^+ transport. Both processes occur in frog skin after exposure to low pH (52).

In contrast to the uncertainty of anatomic changes in junctions and in the intercellular spaces with these agents, there seems to be an almost uniform morphological response of the epithelial cells after exposure to high H^+ concentration alone or in combination with the barrier-active agents. Studies with low-dose ethanol (41, 45), bile salts (44, 54), and salicylates (55, 77, 133) in the stomach and prolonged acid perfusion (with and without pepsin) in the esophagus (22) show profound changes in cells. These changes are very similar whether gastric surface epithelial cells or epithelial cells of the stratum spinosum of the esophagus are studied. The cells of both epithelia show clumping and margination of the nuclear chromatin (karyolysis) and a decrease in cytoplasmic density that progresses on to the production of frank vacuoles in the cytoplasm (cellular edema). The apical cell membrane of gastric epithelial cells becomes distorted and eventually ruptures with discharge of the cytoplasmic contents (54). The esophageal cells in the midzone of the epithelium also burst, leaving remnants of the cell membranes in the space (22). With continued acid exposure, there is desquamation of clumps of gastric mucosal cells and extension of the damage in the esophagus to deeper layers, resulting in transmural ulceration in both epithelia.

IMPLICATIONS FOR GASTROINTESTINAL BARRIER FUNCTION

Several changes in our approach to gastrointestinal barrier function and its response to H^+ are suggested by the studies reviewed here. First, the "mucosal barrier," as described above, should be conceived as a system made up of both cell membranes and the junction-intercellular space complex. Second, H^+ and the barrier breakers affect both cell membranes and the shunt path. It is also clear that H^+ and the barrier-breaking agents may have a generalized effect that first decreases and, with long exposure, increases the permeabilities of the barriers. Certain pH levels and agents can have more specific effects by altering the permselectivity of the barriers. Thus, H^+ and the barrier breakers may have time- and concentration-dependent effects, and a true understanding of their actions can be obtained only if these variables are considered and controlled. Third, interpretation of the damage in epithelial function brought about by high concentrations of H^+ and barrier agents is extremely difficult because of the complex nature of the epithelia. For example, the decrease in potential difference with barrier damage can be the result of a decreased resistance of the epithelium or inhibition of active transport. Resistance changes can occur in the shunt path, the transcellular path, or both, and a change in one may be followed by a change in the other. What appears to be ionic exchange based on stoichiometry could be complex changes in passive diffusion along with simultaneous changes in active transport. The more complicated the epithelium, the more difficult it is to sort out the various results of H^+ damage.

It is evident that H^+ and the barrier agents are capable of inhibiting the active transport of ions by epithelia. This property needs further investigation, particularly as it relates to Na^+ transport, which is an important function of esophageal, gastric, and duodenal epithelia. Studies of the rabbit esophagus, a simple stratified squamous epithelium devoid of submucosal glands, have suggested that Na^+ transport plays an important role in the barrier function. H^+ damage to the epithelial cells of the esophagus occurs concomitantly with inhibition of cellular Na-K-ATPase (126) and with abolition of Na^+ transport (125). Studies in the stomach, a more complex mucosa, also implicate an important role for sodium. Menguy and Masters (113) have shown inhibition of Na-K-ATPase in the gastric surface cells by H^+ and bile salts. Studies of Kuo and Shanbour on gastric mucosa showed that Na^+ transport is inhibited when exposed to H^+ in conjunction with aspirin (91) or bile salts (90). In addition, Jacobson's laboratory (18, 25) has reported that treatment with cyclic AMP (or agents such as prostaglandins and theophylline, which increase cyclic AMP content in the gastric epithelial cells) will reverse the permeability changes and inhibition of Na^+ transport brought about by indomethacin in the gastric mucosa. The rather uniform histological changes of the esophagus and gastric mucosa with H^+ and the barrier agents (see above) is what would be expected if the cells were losing their ability to regulate osmolality and were thus swelling and bursting. Na^+ transport may be the major determinant of cell volume regulation in epithelia, although direct conclusive proof of this generally held concept is still lacking (103). It is possible that, regardless of any initial effects on membrane permeability, H^+ and the barrier-breaking agents eventually increase cell membrane and junctional permeability to ions, including Na^+ and H^+. As the pH in the environment around the Na-K-ATPase

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decreases, either as a result of a lowered intracellular pH or a lowered pH in the intercellular space, Na^+ transport may be inhibited, volume regulation disturbed, and the cells may die. Desquamation of surface epithelial cells in the stomach and extension of the damage through all layers of the esophagus then result in ulceration.

Kivilaakso and Silen (87) have recently reviewed the role of mucosal permeability, blood flow, H^+ secretion (and the resulting "alkaline tide" that accompanies H^+ secretion), pepsin, carbonic anhydrase, and prostaglandins in gastrointestinal barrier function. The relationships among findings of many studies suggest that the salutory and adverse effects of these different events may be explained within a framework of altered ion transport and disturbed cell volume regulation. As pointed out by Miller and Jacobson (114), cytoprotection can also be explained by considering the transport properties of epithelia. Agents that reduce H^+ permeability of cell membranes or tight junctions and agents that might improve the cells' ability to extrude Na^+ would be cytoprotective. Cyclic AMP seems to have such effects in the stomach (25, 89), gallbladder (8, 9), and intestine (67, 128) but not in the esophagus (19). Taken from this perspective, the seemingly conflicting differences in prostaglandin cytoprotection in these different segments of the gastrointestinal tract become understandable.

SUMMARY

This review emphasizes the fundamental interaction that exists between the transport and barrier functions of epithelia. The concept is an obvious one theoretically, but its practical implications have not been explored. For example, the problem of acid damage in the gastrointestinal tract could benefit from a broader approach that considers the sequence of deterioration of transport function to irreversible damage (impaired barrier function). Few studies in transport physiology, however, attempt to model the diseased epithelia, although the method is potentially available through the noxious agents often used in the basic preparations. The transport physiologists usually strive to maintain an experimental preparation that approximates normal function. The clinically oriented investigator, whether studying experimental animals or humans, begins with a damaged barrier function. These studies cannot assess the prior events that lead to irreversible damage. Such considerations could, however, suggest means of influencing early and reversible changes and preventing permanent damage. I propose that an integration of these two orientations toward viewing epithelia will lead to promising new avenues of research in the function of epithelia.

ADDENDUM

Since the submission of this review, Dr. Davenport has reminded me of Dr. Code's early involvement in the study of mucosal barriers. An early report by Dr. Code appeared in 1963 (*J. Physiol. London* 166: 110–119, 1963) and an abstract in 1955 (*Am. J. Physiol.* 183: 604, 1955). Like so many areas of gastrointestinal physiology, Dr. Code's influence was felt here as well. However, although Code, Teorell, and others were the "explorers" in this field, Dr. Code remains to be the "pioneer."

The author thanks Drs. Arthur Finn and Roy Orlando for helpful discussions of this paper; Suzy Cashwell for manuscript preparation; and Lou Ann Brower for editorial assistance.

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