

The Gastric Mucosal Barrier

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Abstract

Most gastroduodenal ulcer disease results from a weakness in the normal gastric mucous barrier against the penetration of acid secreted by the stomach. Based on meticulous and insightful research, the distinguished physiologist Franklin Hollander hypothesized that the stomach is protected against its own acid secretion by a dynamic two-component mucus-mucosal barrier. Hollander and his co-workers defined the physical and chemical characteristics of the mucus components of this barrier, as well as the defense provided by the surface epithelial cell layer, which he viewed as the second line of defense (the second component). Barrier investigators at Mount Sinai demonstrated the effects of impairment of barrier function with resultant increased back-diffusion of acid, and they defined the consequences of this acid penetration into the gastric epithelium. The contribution of these workers included important observations on the natural impermeability of the gastric corpus and fundus as well as the normally increased permeability of the antrum. They also presented evidence on the role of bile in duodenogastric reflux in gastric ulcer disease and the presence of impaired barrier function in patients with gastric ulcer and pernicious anemia. Further studies included demonstration that stress and carcinogens could disrupt the gastric mucosal barrier. Disruption of the barrier, in turn, was shown to allow carcinogenesis to occur by permitting the absorption of certain carcinogens which otherwise are warded off by the barrier. The Hollander two-component gastric mucosal barrier hypothesis has, in recent years, been increasingly validated by experimental data coming from other laboratories. **Key Words:** Gastric mucus, gastric mucosal barrier, gastric epithelium, gastric acid.

The Gastric Mucosal Barrier

IT IS NOW GENERALLY AGREED that almost all gastroduodenal ulcer disease results from an abnormality in the mucosal barrier. In recent years, attention has focused on *Helicobacter pylori* and ASA/NSAIDs as important causes of this failure of barrier function. Uncommonly, ulcer disease of the upper GI tract is attributable to excessive secretion of hydrochloric acid rather than to a primary failure of the barrier itself.

Background

An early hypothesis on the inherent resistance of the stomach to autodigestion was that of John Hunter in 1772 (1). On observing the rapidity of post-mortem gastric autolysis, he ascribed the

ability of the stomach not to digest itself during life to the presence of a "living principle." This "living principle" depended, in Hunter's view, on the continuing circulation of blood through the gastric tissue. In 1853, Virchow (2) refined this hypothesis, proposing that the acid in the gastric juice diffused back into the mucosa, where it was neutralized by circulating alkaline blood. Gastric ulcers were considered to be secondary to a restriction in local blood supply, with resultant ineffective neutralization of absorbed acid, leading to localized areas of autodigestion. Pavy agreed, publishing strong endorsements in 1863 (3) and 1869 (4).

Beaumont's epic observations provided a meticulous account of the dynamic nature of the gastric lining of his patient, Alexis St. Martin, a gunshot victim with an open gastric fistula (5). Beaumont described the inner lining of the stomach to be "constantly covered with a very thin transparent viscid mucus lining the whole interior of the organ." Beaumont documented this mucus layer to be a distinct alkaline entity of widely varying appearance and physical characteristics,

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often dependent on the general state of his patient.

An earlier reference to the protective quality of the gastric mucus layer itself was published by Glover in 1800 (6). Glover believed the function of the mucus to be "lubricating and that it must likewise defend the internal surface of the stomach and the intestines from the action of the gastric juice." Some 60 years later, Harley (7), who is usually credited with the initial hypothesis regarding the protective nature of the mucus layer, stated: "It is chiefly, if not solely, the mucus which protects the stomach from the chemical action of its own gastric juice." In 1855, Claude Bernard (8) recognized the apparent impermeability of the gastric lining to pepsin. Bernard also pointed out the importance of the dynamic quality of this gastric surface epithelium: "the epithelial layer is destroyed and renewed with great ease."

Bernard described the gastric lining: "The epithelium of the gastric mucosa, especially the glutinous mucus which covers the inner wall of this viscus, and which is seen very well when one opens the living animal, encloses the gastric juice as in a vase as impermeable as though it were made of porcelain." This view regarding the one-component nature of the protective barrier dominated physiologic thinking until the 1940s, when the importance of a dynamic two-component barrier was proposed by Hollander.

Hollander's Two-Component Barrier

During the first half of the 20th century, the prevalence of peptic ulcer disease increased alarmingly. The thinking of most clinicians and investigators turned to excessive secretion of HCl as the major causative factor in this disease. However, despite this consensus, Hollander proposed that the stomach's primary defense against peptic ulcers was the mucus-mucosal barrier. Hollander's insight into the functioning of the mucus-mucosa as a virtually impenetrable barrier to the acid which the stomach itself secretes grew out of his fundamental studies on the mechanism of gastric acid secretion. It became apparent to him, early on, that the parietal cell secretion consisted of nearly pure concentrated HCl; he was able to demonstrate that it remained almost unchanged within the gastric lumen over long periods of time. He also pointed out that peptic ulcer is a localized disease, rather than one diffusely affecting the gastric or duodenal lining, i.e., if hypersecretion of the acid were the sole cause of ulcer disease, then that disease should be more diffuse. He also drew attention to the fact

that most individuals with hypersecretion of acid did not suffer from peptic ulcer disease and that many patients with peptic ulcer disease did not demonstrate hypersecretion of acid. To Hollander, the conclusion was obvious: some localized defect or weakness in the protective mucus-mucosal barrier must be primary in the causation of peptic ulcer disease. By 1944, Hollander presented his early ideas of the "mucous barrier" as a two-component, self-renewing system.

Franklin Hollander

Franklin Hollander had come to The Mount Sinai Hospital as director of the Gastrointestinal Physiology Research Laboratory in 1936, and he continued there until his death in 1966 (see above, chapter 6). His studies on gastric physiology began a decade earlier in the laboratory of Lafayette Mendel at Yale. In all, he published a total of 272 scientific papers. His final days were spent painfully but quietly at home, continuing almost daily meetings with those of us whom he was guiding through ongoing studies on gastric pathophysiology. In accordance with his request, his name did not appear on any papers published after his death. Hollander devoted the bulk of his time and energies to basic science rather than to clinically oriented research, yet he was always looking for direct clinical applications of his work and gave generously of his time and support to clinicians and trainees. It was Hollander's conviction that a grounding in bench research and in basic gastrointestinal physiology was essential to the development of the complete gastroenterologist.

In his earliest publication (9), Hollander reported that gastric juice collected from isolated canine gastric pouches had a pH of less than 1, provided special care was taken to avoid irritation of the pouch during collection of juice, thereby avoiding any stimulus to the stomach to secrete diluting or neutralizing substance. Hollander's new technique involved the construction of a sphincter at the mouth of the pouch. Although he left it unstated at the time, Hollander later referred to this observation as a crucial one, indicating to him that there must be very little (or virtually no) loss of acid via back-diffusion into the mucosa; otherwise, such low pH values could not have been achieved. When he published this observation in its final form (10), Hollander was able to report the pouch juice to have a constant pH value of less than 1.

His early interest in the barrier was already apparent in 1929 (11). Commenting on the high

concentration of hydrochloric acid in the gastric secretion, "about 0.15 N.," Hollander stated: "Even more remarkable are the intracellular organizations which permit the existence of living normally functioning tissue in intimate contact with so corrosive a liquid. True, the fluid contents in the lumen of the stomach is much less acid, by reason of the neutralizing action exerted by food stuffs, saliva, and regurgitated intestinal juices. Nevertheless, even this less acid fluid will exert a marked digestive action on many tissues which may be immersed in it, even though these tissues are living organs transplanted into the stomach. The acid secretion, however, unaffected by admixture with the above-mentioned alkaline fluids exerts no such corrosive action on the cells which are immediately concerned in its elaboration. While the effective agent in the protection of these cells may be the mucus, no one has yet conclusively demonstrated that it is so, and thus, the stability of these tissues in the presence of digestive juices which they themselves manufacture remains as great a mystery as ever."

In the 1930s, Hollander continued his studies on the nature and mechanisms of gastric acid secretion (11–19). His observations refined and extended the views of Pavlov, and he formally proposed a two-component acid secretory process: (1) the parietal cell secretion, containing isosmotic HCl (160 mM) and no other ions except for "perhaps a little potassium," and (2) an alkaline component comprised of mucus, enzymes and a transudate of interstitial fluid. These were conceived to be relatively invariant physiologic entities with all alterations in the concentrated (isosmotic) primary HCl secretion resulting from dilution and neutralization by the non-parietal cell alkaline component. Absorption or back-diffusion of H^+ was, in Hollander's view, not a factor.

The major evidence supporting an opposing viewpoint was presented by the renowned physiologist Torsten Teorell (20, 21). Based on experiments in pylorus-ligated cats, as well as in an *in vitro* membrane preparation, Teorell concluded that the hydrogen ions of strong acids diffuse out of the stomach quite easily, and that this hydrogen ion loss occurs via an exchange diffusion for Na ions across the surface mucosa. He viewed the gastric mucosa as an ion exchange membrane whose function it was to reduce the intraluminal gastric acidity. Teorell's back-diffusion model received some support (22). One of the more interesting aspects of Teorell's 1939 report (21) was his observation that weak acids disappear from the stomach even more rapidly than do

strong acids, a phenomenon ascribed to entry of un-ionized acid molecules via paths (solution in the cell membrane) other than those open to ions. The inflammatory effects upon the mucosa of the entry of these weak acids were noted, but better appreciation of their significance awaited the work of later investigators (23).

Hollander (24) objected to Teorell's hypothesis: "The notion that the concentration of HCl within the stomach may be reduced by a physical process of absorption is one which as yet has not acquired many adherents." Hollander pointed out that Teorell had not demonstrated directly the existence of an ionic exchange in the mammalian stomach, but rather had utilized *in vitro* non-vital membranes, as an "analogous" model. Hollander held Teorell's views to be "worthy of consideration and even of experimental examination," but they received little of either by that time.

Hollander's own early observation that highly acidic gastric secretions can be retained within canine gastric pouches up to 9 hours without any significant diminution in acidity cast considerable doubt on the likelihood of any such process of absorption or ionic exchange (12).

Hollander's studies on the physical and chemical properties of gastric mucus began with publication of an abstract written with Robert Felberg in 1941 (25). In it, they stated that "previously published data on mucus are too scant and divergent to yield criteria of purity of the secretion or quantitative correlations among its chemical characteristics," and then described their earliest efforts to obtain mucus secretion from canine fundic pouches. The abstract ends with: "these studies will be extended"; and, so they were.

In 1943, Hollander and Stein published their first joint paper on some of the characteristics of gastric mucus (26). They reported that a major part of the mucus secreted after pilocarpine administration is squeezed out of the surface epithelial cells by muscular activity. In particular, they saw oozing of blood from the mucosa after pilocarpine, a finding later featured in a study by Davenport (23). Seven years later, Janowitz, Hollander and Jackson (27) found that topical application of acetylcholine resulted in secretion of alkaline, cell-free mucus, confirming Hollander's previous conclusion that the exfoliation of gastric-surface epithelial cells is not an essential part of the process of mucus secretion.

Hollander first mentioned a "two-component barrier" in 1941 (28), when he suggested that such a barrier provided laboratory animals with their normal resistance to the induction of gastric

cancer by topical application of carcinogens. He presented his concept of the barrier more formally in an editorial in *Gastroenterology* in 1944 (29) "Mucous secretion (including desquamation of affected surface mucosal cells) constitutes the most effective protective agent for the healthy mucosa — against tumor formation, as well as peptic ulceration. Some such modification in (mucus) secretory activity may obtain in the human precancerous stomach."

Hollander amplified this view in 1945 (30): "The one aspect of gastric physiology that might prove to be of fundamental importance in relation to gastric carcinogenesis is mucus secretion. This importance derives from its function as a protective agent against all forms of irritation, but particularly the chemical ones. Hence, any deficiency in output of mucus or in its essential physical properties might well serve to predispose the gastric mucosa to attack by an exogenous carcinogenic agent. This barrier involves not only the viscous mucus secretion, but also the mucous cells themselves. Mildly irritating solutions result in a flow of thick, jelly-like mucus. Histologic examination smears reveal considerable quantities of desquamated columnar epithelium. Spontaneous secretion, to the contrary, is frequently free of such desquamated columnar cells. . . . Obviously, if any cancerous change is induced, when these columnar cells make contact with a carcinogen, it will immediately elicit a shedding of these cells so affected making the development of an adequate tumor impossible."

Seeking confirmation of this view, Hollander and co-workers studied the effects of mild irritants and noted that the occurrence and volume of mucus and of desquamated surface epithelium increased with the irritating power of the agent applied (31). Hollander credits Pavlov (32) with stating that the copious flow of mucus after application of irritants to the gastric mucosa does not reflect serious pathologic condition but only a normal physiologic reaction to an irritant which "wards off danger which threatens the more important elements of the mucus membrane beneath."

Hollander and Lauber, studying multiple stimuli, found that clove oil water emulsion is the most effective stimulus of gastric mucus secretion (33). However, clove oil is a mixture of several compounds, including 80% eugenol (4-allyl-2-methoxyphenol). Therefore, they investigated pure eugenol as a topical stimulus and concluded that it is as effective a stimulus as clove oil and "has the advantage of being a single chemical individual." After exhaustive studies on the effects of many

irritants and other stimuli, Hollander proposed that eugenol be adopted as a standard stimulus for study of the gastric mucus (34).

Hollander and co-workers explored the effects of repeated eugenol instillation in isolated canine gastric pouches (35). In these "fatigue experiments," striking changes occurred, with an initial flow of opaque jelly-like mucus decreasing, to be replaced by large volumes of secretion of clear alkaline fluid. These studies formed the basis for the later works of Code (36) and Davenport (37). In a companion paper (38) with Sonnenblick and Sober, Hollander documented cytologically the progressive desquamation of layers of epithelium after eugenol.

Hollander and Goldfisher (39) then described (by histology) a remarkably rapid 3-stage regenerative process after repeated eugenol applications: (1) the preliminary resurfacing of the denuded mucosa with flat cells, evident 30–60 minutes after removal of eugenol from the pouch, (2) the transformation of these new cells into low and tall columnar cells, and (3) the reformation of crypts in these areas of smoothly resurfaced mucosa. This entire process occurred within 36 hours following deep shedding of the mucosa as far down as the bottoms of the foveolae.

The definitive paper by Sober, Hollander, and Sonnenblick on the effects of topical eugenol application (40) established a new experimental approach to some stomach diseases — notably gastritis, peptic ulcer and carcinoma of the stomach. Hollander and co-workers looked at the possible beneficial effect of topical application of eugenol to the gastric lining of patients with peptic ulcer via increased mucus secretion (41). Ten out of 14 patients had complete or partial relief of symptoms, suggesting that prolonged stimulation of mucus secretion might be beneficial.

Formulation of the Two-Component Barrier Theory

After a decade of study on the nature of the gastric mucus and the underlying epithelium, Hollander formally proposed the concept of a two-component, self-regenerating barrier (42). According to his hypothesis, the gastric mucous barrier is a composite of two integrated structural units. The layer of viscous mucus constituted the first line of defense and the second was the layer of columnar and cuboidal cells of the surface of crypt epithelium. "The mucus layer constitutes a first line of defense in the gastric wall by reason of its tenacious adherence to the underlying tissue, and its ability to maintain considerable thick-

ness and its impermeability to destructive chemical agents.” Hollander looked upon the cellular layer as providing defense by immediately shedding the injured columnar cells and concomitantly reinforcing the secreted layer (mucus) with material of extra-high viscosity. He documented the rapidity with which this cellular layer regenerates itself and pointed it out as a crucial element in this hypothesis. Hollander commented that “it is curious that although Claude Bernard anticipated the importance of this dynamic quality of the surface layer in protecting the stomach against autodigestion, its role in this regard has almost completely been neglected during the intervening century.” The facts suggested to him that ulcer etiology was more closely dependent upon predisposition to autodigestion than on secretion of excessive gastric juice, and he stressed the need for a shift of emphasis in research, holding this position long before any understanding of *H. pylori* or the role of NSAIDs in altering the mucosal barrier and in the causation of peptic ulcer disease.

Hollander proposed the gastric “mucus-mucosa” to be a virtually impenetrable barrier to the diffusion or absorption of HCl or to the exchange of H⁺ for other ions across it. Therefore, all alterations in the concentration and composition of HCl collected from the gastric lumen resulted from neutralization and dilution. Support for Hollander’s hypothesis came from Cope and co-workers (43, 44), who in 1943 demonstrated in dogs a strong gastric “epithelial barrier” to the diffusion of H⁺ and other ions, as did Reitmeier et al. in humans, in 1957 (45). In 1963, Code and co-workers reported that the presence of acid within the gastric lumen led to a striking, almost complete, restriction of the movement of Na⁺ ions across the membrane (46).

It remained unclear, however, whether this barrier to diffusion of ions (most notably H⁺ and Na⁺) was at the level of the surface epithelium or at the mucus layer, or both. Nevertheless, most authors began to refer to the epithelium as the site of restriction of ionic diffusion. This view arose in part from the apparent ease with which HCl secreted by the parietal cells found its way across the mucus layer and into the gastric lumen, as well as experiments which demonstrated the free movement of H⁺ in both directions across refined films of gastric mucus *in vitro*. This objection to the mucus layer as being the protective barrier against acid in the lumen was met by Hollander with an educated speculation (42): “This seeming inconsistency in behavior might result from the formation of short-lived channels in the mucus

layer. Immediately thereafter, the uncoagulated mucus surrounding the minute hole, by reason of its high surface tension, will flow over the opening and seal it.”

Breaking the Barrier

This next stage in barrier research began in 1955 with a brief abstract (47) in which Hollander drew attention to Davenport’s report that acid secretion by mouse stomachs *in vitro* was inhibited by the enzyme inhibitors iodoacetamide and N-ethyl maleimide (48). Hollander stated that “at Davenport’s suggestion, we have attempted to confirm this effect *in vivo*.” Hollander found that topical application of these substances to the mucosa, followed by parenteral histamine stimulation, resulted in a marked increase in volume rate of secretion without a drop in pH (49). Surprisingly, neither Hollander in this study (49), nor Davenport in his (48), considered the possibility that this finding might have resulted from a disruption of the mucous barrier, and that hydrochloric acid continued to be secreted by the parietal cells only to back-diffuse across this impaired barrier and be lost before it could be recovered for titration. This possible explanation may have been obscured by the fact that, in the absence of histamine, these enzyme inhibitors led to the output of significant volumes of viscous alkaline mucus secretion. Similarly, in 1952, when Janowitz, Colcher and Hollander concluded that secretion of HCl by canine gastric pouches in response to repeated injections of histamine had been markedly inhibited by acetazolamide (a new and powerful carbonic anhydrase inhibitor), they too ignored the possibility that acetazolamide might be causing a disruption of the barrier, with loss of secreted hydrogen ions by back-diffusion rather than by true inhibition at the parietal cell level (50). In support of their conclusion, Janowitz, Colcher and Hollander referred to a paper by Davies and Edelman (51), who studied tied tubes of frog mucosa and found “acid already secreted began to leak back through the mucosa” after application of carbonic anhydrase inhibitors. They ascribed this result to damaged parietal cells (51). Janowitz and Hollander also failed to make the connection and did not mention the possibility that the gastric mucosal barrier was being impaired by acetazolamide.

This oversight was corrected, quite by accident, in the fall of 1960. Mario Altamirano, a brilliant young physiologist from Santiago, Chile, who had established an international reputation with his studies on the permeability of the gastric

mucosa, came to work with Hollander. He brought with him his ingenious lucite chamber device, in which a segment of gastric fundus with intact blood supply was mounted between two layers of lucite so that the mucosa formed the bottom of a cup. This so-called *vivo-vitro* preparation allowed for nearly quantitative instillation and collections, as well as continuous direct observation of the gastric mucosa itself (52). I borrowed Altamirano's chamber and in the course of a pilot experiment on the effects of acetazolamide on potassium in gastric secretion, I noted the sudden appearance of striking gross mucosal damage after intravenous acetazolamide when HCl was in the chamber in contact with the mucosa. This unexpected observation prompted a series of experiments designed to evaluate the degree to which mucosal damage might have contributed to the apparent inhibition of acid secretion following administration of this compound. Test solutions were placed in the chamber to control the acidity of the fluid bathing the mucosa, and acid secretion was stimulated by histamine. Administration of acetazolamide to preparations with high concentrations of HCl in contact with the mucosa resulted in prompt gross damage, with hemorrhage and edema. When the acidity of the chamber fluid was reduced by the addition of a buffer (glycine), the degree of damage and the degree of acid inhibition diminished proportionately to the reduction in acidity. The resulting paper (53) was the first to describe the consequences of impairment of the gastric mucosal barrier function leading to back-diffusion of H^+ . It was also the first to point out that what appeared to be inhibition of stimulated acid secretion was in fact a consequence of H^+ loss by back-diffusion. When this paper was presented, Charles Code questioned the results, because the experiments had been done using a chambered gastric segment preparation, which in his view might have led to damage of the mucosa even though the blood supply was thought to be intact. He proposed that the experiment needed to be repeated in isolated canine pouches and emphasized that Janowitz, Colcher, and Hollander had not found any such damage or impairment of the barrier (50).

In 1962, Horace Davenport, chairman of the Department of Physiology at the University of Michigan, spent a sabbatical year at the Mayo Clinic with Dr. Code. At the Mayo Clinic, he held the title of visiting professor, but he asked Code (36) to "treat him as a post-doctoral fellow and assign a research project to him." Early in the fall of 1962, Hollander received a telephone

call from Code and Davenport, asking his advice. Hollander beamed as he recounted to me the details of the long conversation: Code would assign Davenport a study on acid and sodium movement across the mucosa in canine gastric pouches damaged by eugenol, in accordance with Hollander's eugenol experiments. By Hollander's account, information was freely shared, and he was delighted at the prospect of these highly regarded physiologists starting a project so close to Hollander's heart, and so much derived from his ideas and previous work.

In 1981, at the time of his presentation of the Friedenwald Medal to Davenport, Code spoke (36) of the arrival of Davenport at Code's laboratory, and of the barrier research project: "The investigation, I thought, was one for which he was uniquely qualified. I had found a year or two earlier that when I washed out canine gastric pouches with an emulsion of eugenol, the active ingredient of the oil of cloves, the secretion of the pouch in response to histamine changed from hydrochloric acid to sodium chloride. Was the eugenol altering the product of the parietal cells, or was the hydrogen ion they secreted escaping after its formation?" The rest is history. To quote Code, "Davenport took off like a jackrabbit. . . . He had two papers published before I had ours written!"

In his early publication on the subject in 1964, Davenport and colleagues followed Hollander's lead, using eugenol to alter the barrier, and showed that eugenol damage did not alter acid secretion but rather that secreted hydrogen ions back-diffused across a damaged barrier more rapidly (37). Davenport's important and highly regarded body of work from 1962 through 1982 enlarged and enhanced our understanding of the gastric mucosal barrier. As part of his quest to locate the site of the barrier, he went on to publish many papers documenting the effects of a variety of barrier breakers. Davenport clearly can be credited with the reawakening of interest in the gastric mucosal barrier, for it was these many critical and concise reports, published in popular clinical journals, which caught the attention of the medical world. At the same time, the Mount Sinai group continued its own studies on the barrier. Multiple publications by Hollander, Altamirano, Werther, Chapman, Dycke, Rudick, Janowitz, Himal, Lindner, and Berkowitz (*vide infra*) followed.

Interest in the gastric mucosal barrier was not only reawakened: it flourished. In fact, it became an exciting subject for research and filled many a program at national meetings. By this time, how-

ever, Hollander was terminally ill. Although treated with respect, Hollander and his work were generally met with a polite indifference and at times went unrecognized. Even the eloquent article by Johnson (54) stated that “all of those papers, written by numerous physiologists, pharmacologists, gastroenterologists, surgeons, anatomists, and probably others as well, on acetic acid, bile acids, aspirin, and ethanol damage to the gastric mucosa were prompted by Davenport’s original studies.” When Davenport’s paper (37) was published in *Gastroenterology*, its editor, Dr. Morton I. Grossman, wrote that it would revolutionize the physiology of the stomach and added, without references: “Substances which break the gastric mucosal barrier cause desquamation of cells which are quickly replaced.”

The final paper on the gastric mucous barrier co-authored by Hollander and published in his lifetime was the definitive report of the effects of barrier disruption by acetazolamide (55). The apparent inhibition of acid secretion after acetazolamide was in fact shown to result from increased loss of secreted H^+ from the luminal fluid by back-diffusion. Even very prolonged contact (5 hours) of the mucosa with strong HCl (160 mEq per liter) never resulted in damage. This remarkable ability of the mucosa to maintain solutions of high acidity within the gastric lumen was abruptly lost after acetazolamide administration. H^+ losses accelerated over time, and the increase in H^+ penetration caused more mucosal damage. Furthermore, mucosal damage did not depend upon active HCl secretion *per se* and therefore was not due to the accumulation of alkali within oxyntic cells, as had previously been thought (51). Microscopic examination of the tissues did not show early damage to the oxyntic cells, but instead found it to be in the surface mucosal cells.

Davenport initially suggested that the increase in Na^+ gain and H^+ loss associated with impairment of the mucosal barrier resulted from an acceleration of the normally minimal equilibration of these ions across the membrane. Opposing this view, the Mount Sinai group showed that a large part of the increased Na^+ output from damaged mucosae was associated with net water movement into the lumen (55). In later publications, Davenport accepted these findings. The second Mount Sinai group of barrier investigators (working apart from Hollander), Lindner, Cohen, Dreiling and Janowitz, studied the effects of acetazolamide in humans and also agreed (56). Altamirano (57) confirmed the absence of a transport mechanism involving Na^+ for H^+ exchange across the gastric mucosa and concluded that both

ions diffuse independently, for which he suggested the term “interdiffusion.”

Hollander died in 1966. At this point, Davenport (58) still disagreed strongly with Hollander’s two-component theory: “It should be noted that Hollander included the epithelial cells as half the barrier. . . although the layer of mucus is equivalent to a thin sheet of unstirred fluid, it provides little chemical protection for the mucosa; its chief function is lubrication. . . . Acid quickly diffuses through it to reach the apical border of the cells.”

Thus, Davenport dismissed the mucus layer as nothing more than a lubricant. He gave no direct evidence to support this view, but referred to the work of others using refined mucus films. Davenport proposed that most of the barrier function of the mucosa was at the level of the apical membrane of the surface epithelial cells and at the tight intercellular junctions of the gastric mucosa. The luminal surface of the epithelial cell has no carrier for the ionic transport (as do the basolateral cell membranes) and the tight junctions appear to be much tighter than those found elsewhere in the GI tract. However, the mucus layer was later shown to possess a notable pH gradient from acidic at its luminal surface to neutral at the mucosal surface (59). This near-neutrality was maintained at the epithelial membrane surface by bicarbonate secretion into the mucus layer from the mucosa itself.

It was not long before Davenport began to retract his opposition to Hollander’s views. In February 1968 (60), two years after Hollander’s death, he published a subscript dedication: “This paper is dedicated to the memory of Franklin Hollander in gratitude for early encouragement and in appreciation of his contributions to the physiology of the gastric mucosal barrier.” In this paper, Davenport wrote, “Although the exact locus and nature of the barrier are unknown, one component must be the plasma membrane of the mucosal cells, and another component may be the mucus layer at the tips of the epithelial cells.” In addition, he credited Hollander’s 1954 paper (43) with defining the two-component barrier. Davenport speculated that detergents might loosen the tight junctions and/or disrupt the plasma membrane of cells, and he cited evidence (61) that urea is capable of dissolving gastric mucus.

After Hollander

In the post-Hollander period, the Mount Sinai barrier investigators continued their studies.

Some are presented here as representative of that group.

Previous studies showing a "tight" barrier to H^+ and Na^+ had utilized *vivo-vitro* preparations (55) or isolated canine pouches, which consisted of oxyntic gland area only (62). Studies on the intact human stomach indicated a more rapid movement of hydrogen and sodium ions across the membrane than would have been anticipated from the canine studies, which employed oxyntic gland area only (63). Citing this disparity, as well as the known physiological relationship between antral acidification and the inhibition of gastric release, Walter Dyck, then a fellow in Gastroenterology at Mount Sinai, working with Werther, Rudick and Janowitz, studied separated canine pouches constructed from antrum or corpus (64). The antral mucosa absorbed more H^+ and put out greater amounts of Na^+ and K^+ than did the oxyntic mucosa. Dyck continued his work on the barrier and on other aspects of gastric pathophysiology during his fellowship and later as chief of the Gastroenterology Division and director of Research and Education at the Scott and White Clinic and Texas A and M University Health Science Center.

At this point, it had been established that the administration of acetazolamide, as well as topical chemical injury to the gastric mucosa, resulted in a loss of resistance of the mucosal barrier to acid absorption. Practically nothing was known about the nature and importance of this process in the diseased human stomach. Perhaps the hypoacidity as well as the ulcers in patients with gastric ulcer disease resulted from an altered barrier. Therefore, Chapman, Werther, and Janowitz studied the responses of the normal and abnormal human gastric mucosa to an instilled acid load (65). The patients with larger and more proximal ulcers were found to have a greater gastric permeability to H^+ and Na^+ , as did patients with pernicious anemia.

Increased bile regurgitation into the stomach occurs in gastric ulcer disease (66–68) and the concentration of bile salts within the stomach returns to normal after the ulcers have healed. Davenport had shown that dog bile and sodium taurocholate caused an increased permeability of the gastric mucosa to H^+ in oxyntic-gland-area canine pouches (60); however, peptic ulcers of the stomach occur in pyloric-gland-area mucosa, not oxyntic. Werther and colleagues therefore studied the effects of human hepatic bile on electrolyte movements across the pyloric gland area in canine pouches and compared them with oxyntic gland area in the same dog (69). Their findings

indicated that brief exposure to human bile in relatively low concentration markedly increased the net flux of Na^+ , K^+ , and H^+ across the pyloric-gland-area gastric mucosa. These results supported the hypothesis that at least part of the mucosal abnormality in gastric ulcer disease may reflect damage from increased back-diffusion of H^+ through a functional barrier which has been diminished by contact with bile. In the same issue of *Gastroenterology*, in 1970, Ivey and colleagues reported similar effects of bile salts on the gastric mucosa of human subjects (70).

Jack Rudick was appointed assistant professor of surgery at the Mount Sinai School of Medicine in 1966. Before he arrived at Mount Sinai, he had been a research associate at the University of Washington School of Medicine in Seattle. In 1968, he joined Werther, Dyck, Chapman and Janowitz in their studies on the barrier. Rudick's surgical skills, intellect and high energy added importantly to the work of this group, which found that atropine administration in both fundic and antral pouches in dogs resulted in an increased H^+ diffusion into the mucosa (71). They concluded that some of the apparent reduction in secretory rate after atropine was, in fact, the result of these H^+ losses. Other investigators did not agree (72), finding to the contrary that atropine did not affect the permeability of the gastric mucosa.

From the earliest observations on the gastric resistance to autodigestion, investigators had suggested that impairment of blood flow (especially localized) might be responsible for decreased resistance. The Mount Sinai group then validated aminopyrine clearance as a measure of mucosal blood flow, but aminopyrine itself was shown in this study to alter the permeability of both antral and fundic mucosa for H^+ and Na^+ (73, 74).

The Mount Sinai barrier group also reported that pentagastrin infusion tightened the barrier (75) and others agreed (76). Overholt, a student of Davenport, had previously attempted to estimate H^+ back-diffusion rates by utilizing glycine to trap secreted H^+ in the gastric lumen in human subjects (77). However, glycine had been shown to stimulate acid secretion (78), negating Overholt's results. Chapman, Werther, Rudick and Janowitz applied this glycine technique to patients with gastric ulcer, duodenal ulcer and gastric cancer and found increased permeability of the stomach to endogenous and exogenous H^+ ions in gastric ulcer disease and diminished permeability in duodenal ulcer disease (79). They corrected the problem of glycine augmentation of acid secretion by utilizing the glycine trap against

a background of maximum acid output during full dose pentagastric infusion. Chapman was then a fellow in gastroenterology at Mount Sinai, later rising to associate clinical professor. He made important contributions to the work of the Mount Sinai gastric mucosal barrier study group and was lead author or co-author on seven papers relating to the gastric mucosal barrier.

Utilizing *vivo-vitro* chamber mounts of distal canine duodenum (80), the group then showed that the rate of movement of H^+ , Na^+ and K^+ across duodenal mucosa was twice the rate for the antrum and 30–100 times that of the fundus of the stomach.

Werther and Horowitz clarified the controversial role of the gastric mucosal barrier to back-diffusion of H^+ in the pathogenesis of gastric ulcerations produced by restraint-stress in rats (81). Acid secretion did not decrease during restraint-stress, but there was a marked increase in hydrogen ion back-diffusion and loss across the gastric mucosa, which was clearly correlated with gross mucosal damage. They concluded that abnormal gastric mucosal permeability to hydrogen ions played an important role in restraint-stress-induced gastric ulceration.

Certain carcinogens, such as methylcholanthrene, are not absorbed by the normal stomach (82) and cause cancer experimentally only when they are surgically implanted in the gastric wall. Bile salts, which may reflux back into the stomach from the duodenum, promote absorption of such carcinogens by micelle formation. Horowitz and Werther showed that radiolabelled micelles of 3-methylcholanthrene, prepared *in vitro*, were absorbed by the gastric mucosa of the rat (83).

Some nitrosamines (N-methyl-N-nitro-N-nitrosoguanidine [MNNG]) are potent carcinogens site-specific for the stomach. Early inflammatory changes in the gastric mucosa occurred after administration of MNNG, followed by the occurrence of gastric cancer. Horowitz, Toth and Werther showed that the gastric mucosal barrier to hydrogen ions was markedly impaired after the administration of MNNG (84). Increased hydrogen back-diffusion was observed three hours after small doses of MNNG, both with carcinogenic, as well as with subcarcinogenic concentrations, so that barrier disruption by MNNG may play a role in gastric carcinogenesis by this agent.

The Mount Sinai group then reported (85) the effects of stress, aspirin and sodium taurocholate on the activity of MNNG in the stomach of the Buffalo rat, which is uniquely resistant to gastric carcinogenesis from any source, including MNNG. Adenocarcinomas and even greater

numbers of gastric leiomyosarcomas were produced in these animals after barrier disruption by these agents. However, Buffalo rats receiving MNNG in the absence of restraint-stress, aspirin or taurocholate did not develop either gastric adenocarcinoma or leiomyosarcoma, so that at least part of the resistance of this unique strain to gastric cancer may be related to its mucosal barrier.

Mucus Revisited

The intense focus on gastric epithelium as a barrier gradually waned and was supplanted (in 1979) by a wave of interest in a phenomenon referred to as gastric "cytoprotection." This term related to the remarkable ability of gastric mucosa to resist gross injury by some extremely aggressive substances, such as boiling water, absolute alcohol, and concentrated corrosive solutions. Cytoprotection could be induced by prior exposure of the mucosa to a substance which would be irritating minimally injurious to the gastric lining. This gastric adaptation enabled the stomach to remain intact after exposure to these strongly aggressive agents. Since adaptive cytoprotection was blocked by inhibition of prostaglandin, it was suggested that exposure to a conditioning agent increased production of prostaglandin in the gastric mucosa, which in turn, in some mysterious way, was cytoprotective. However, there soon followed studies, utilizing both light and electron microscopy, which found that, in fact, the integrity of the surface epithelium was not maintained by this process. The term "cytoprotection" came into disfavor; however, the concept of mucosal protection by the effects of prostaglandins was documented and clarified. Increased thickness of the mucus layer provides most of the prostaglandin-induced cytoprotection (86–93). Recently, the mucus layer itself has been proposed as the site of the primary barrier (94).

If we accept the evidence that the mucus layer acts as a powerful diffusion barrier to hydrogen ions in the gastric lumen, how then does HCl secreted at the base of the gastric glands by the parietal cell traverse this mucus layer? Until 1990, the answer was obscure. Hollander had speculated on the possibility of short-lived channels but did not pursue this and presented no evidence. Fabry, working independently at Mount Sinai, provided the answer (95). Fabry described a process of viscous fingering, which is a hydrodynamic phenomenon by which fluids of low viscosity pass through fluids of high viscosity without mixing. In Fabry's model, pulses of hydrated HCl wind their way back through the mucus layer

by means of short-lived channels or fingers. He proposed that surface tension makes the flat surface which the mucus layer presents to the lumen almost impermeable to the entry of hydrochloric acid. Driven by the secretory pressure of oxyntic cells, pulses of hydrochloric acid find their way through these channels in the mucus layer and into the lumen. After the secretory pressure drops, at the end of the pulse of acid secretion, the finger closes and disappears.

Lamont's group (96) confirmed Fabry's hypothesis. They documented viscous fingering patterns and showed the process to be dependent upon pH, so that "HCl secreted by the gastric gland can penetrate the mucus gel layer at the epithelial surface (pH 5–7) through these narrow fingers. Whereas, HCl in the lumen (pH 2) is prevented from diffusing back to the epithelium by the high viscosity of the gastric mucus gel on the luminal side."

In the same year (1990), it was shown (97) that the mucus gel contained numerous phospholipids, and that its luminal surface was coated with a film of phospholipid. This surfactant layer accounts for the remarkably strong hydrophobic nature of the gastric luminal surface, which, in turn, provides protection against damaging agents. This phospholipid surfactant is a secretory component of the gastric surface mucosal cell.

Prostaglandin induces maintenance of the stomach's nonwettable surface properties protecting the underlying epithelium from aqueous acidic/peptolytic damaging agents in the lumen (98). This hydrophobic acid-resistant property of the gastric surface active phospholipid layer (SAPL) is rapidly attenuated by NSAIDs. As in humans, *Helicobacter* infection in mice is associated with a significant reduction in both gastric surface hydrophobicity and the phospholipid concentration of the oxyntic mucosa (99).

In a recent leading article in *Gut*, Hills emphasized that the stomach wall is strikingly hydrophobic (100). A droplet of water placed upon it "beads up as if on polyethylene." This hydrophobicity is largely eliminated by major "barrier breakers" such as bile salts and aspirin. A layer of hydrophobic surfactant transforms the hydrophilic mucoid layer into a surface so hydrophobic that the contact angle in many normal stomachs can exceed 90 degrees — that is, approaching that of polyethylene (93 degrees) or Teflon (108 degrees). The SAPL resides on the outermost layer of the gastric mucus (101). The source of this SAPL seems to be lamellar bodies found in parietal cells and mucus neck cells with some reported in chief cells.

The surfactant phospholipid is more permeable to lipids than to aqueous solutes. This finding fits well with the studies of discussed above in this paper. The Slomianys also found that stress ulcers are associated with a change in lipid profile of the gastric mucosa and that each of the known barrier-breakers displays some affinity for SAPL (102, 103). Bile salts form a chemical complex with SAPL, ethanol is a solvent for SAPL, while NSAIDs inhibit the production of prostaglandins controlling SAPL. Lichtenberger (99) has also shown that NSAIDs disrupt the surfactant phospholipid layer directly.

Recently, Hills (104), in a reply to a letter by Bernhard and Postle (105), agreed "on the major issue that surface active phospholipid (SAPL) protects the stomach wall from autodigestion by providing the gastric mucosal barrier to hydrated protons $H(H_2O)_3^+$." However (104), "protons are far too polarizing to exist alone in an aqueous environment, the water of hydration being repelled by any hydrophobic domain."

Conclusion

Controversy over the locus of the gastric barrier has gradually faded and been replaced by disagreement. The importance of the mucus layer continues in its re-ascendancy; however, some physiologists continue to consider its major function to be lubrication (106). The importance of cytoprotection by prostaglandin- E_2 now receives considerable attention because of increasing concerns regarding NSAID-induced ulcers. However, these prostaglandins reduce gastric mucosal blood flow, which theoretically should be deleterious rather than helpful. Most would agree that enhanced mucus production, with thickening of the mucus layer, is the key to prostaglandin-induced cytoprotection. The apical cell membrane and the very tight gastric surface mucosal tight junctions are generally resistant to acid back-diffusion and may serve further to retard hydrogen ion back-diffusion. Some regard this membrane as the primary barrier. The layer of surfactant residing on the surface of the mucus layer is probably of great importance, but this is still being disputed. Most would agree that the pH gradient across the mucus layer exists and is important in the protective process. The problem with this latter hypothesis is that *H. pylori* may dwell close to the mucosal surface. *H. pylori* has very high levels of urease activity to make it an acid-tolerant neutrophil. This observation might lead one to the conclusion that the pH in the

deeper mucus layer is, in fact, more acidic than microelectrode problems would indicate.

So what is the view from Mount Sinai at this time? We interpret the published evidence to show that the key protective mucosal barrier resides in the mucus layer itself, and that this derives from the nonwetable surfactant layer on its surface. The physico-chemical effect of the very high viscosity of the mucus layer and its ability to retain bicarbonate secreted by the epithelium with maintenance of a pH gradient is real and an important factor in protection. Our view of the barrier includes the surface epithelial cell layer for: (1) its easy desquamation, (2) its remarkably rapid regeneration, and (3) production of its bicarbonate and phospholipid surfactant. Last but not necessarily least is the resistance of the apical membrane of the surface epithelial cells to back-diffusion from ions.

All the work of a multitude of investigators, including the Mount Sinai group and Davenport, on the “barrier breakers” was important in understanding the nature of the barrier and the consequences of its disruption. However, since all these barrier breakers may function to disrupt the SAPL and mucus layer, as well as the surface epithelial cell at the apical membrane, the data do not give us critical information regarding the locus of the barrier. Evidence from the predilection of *H. pylori* to colonize the mucus layer close to the epithelium is circumstantial and indirect and cannot yet be considered definitive negation of the importance of the surface mucosal resistance to acid penetration. From the Mount Sinai point of view, the preferred area for *Helicobacter* colonization is the gastric antrum (pyloric gland area), because of the increased permeability of the antral mucus layer to acid as opposed to that of the nearly impenetrable surface of the oxyntic gland area mucus. From this viewpoint, sufficient acid passes through the antral mucus layer to satisfy *Helicobacter* and to reach the acid-sensitive neuroendocrine cells residing in the antral surface epithelium. We speculate that factors which increase the permeability of mucus overlying oxyntic gland areas lead to back-diffusion of enough acid to encourage cephalad extension of *Helicobacter* colonization into the gastric corpus and fundus.

References

- Hunter J. Digestion of the stomach after death. *Phil Trans R Soc Lond* 1772; 62:447–454.
- Virchow R. Historisches, Kritisches und Positives zur Lehre der Unterleibsaffektionen. *Arch Pathol Anat* 1853; 5:281–375.
- Pavy FW. On the immunity enjoyed by the stomach from being digested by its own secretion during life. *Phil Trans R Soc Lond* 1863; 153:161–171.
- Pavy FW. A treatise on the function of digestion, its disorders and their treatment. 2nd ed. London: J. Churchill; 1869. pp. 70–86.
- Beaumont W. Experiments and observations on the gastric juice and the physiology of digestion. Plattsburgh (NY): F.P. Allen; 1833.
- Glover J. An attempt to prove that digestion in man depends on the united causes of solution and fermentation. (Dissertation, University of Pennsylvania). Philadelphia: Way and Groff; 1800. p. 39.
- Harley G. Contribution to our knowledge of digestion. *Br Foreign Med Chir Rev* 1860; 25:206–214.
- Bernard C. Leçons de physiologie expérimentale appliquée à la médecine, Vol. II. Paris: JB Ballière; 1856. p. 406.
- Hollander F. The mechanism of gastric secretion: Preliminary report on the technique for collecting gastric juice of constant and reproducible pH. *J Biol Chem* 1927; 74:23–24.
- Hollander F. The mechanism of gastric secretion: The nature of gastric juice of constant maximum acidity. *Proc Soc Exp Biol Med* 1928; 25:486–487.
- Hollander F. Theories of hydrochloric acid formation in the stomach. *J Am Inst Homeopathy* 1929; 22:311–321.
- Hollander F, Cowgill GR. Studies in gastric secretion. I. Gastric juice of constant acidity. *J Biol Chem* 1931; 91:151–182.
- Hollander F. What is the acidity of pure gastric juice? A review of the experimental literature. *J Am Inst Homeopathy* 1931; 24:491–500.
- Hollander F. Studies in gastric secretion. III. Evidence in reputation of the Rosemann theory of hydrochloric acid formation. *Am J Physiol* 1931; 98:551–555.
- Hollander F. A quantitative relation between the chloride and acid concentrations in gastric juice. *Proc Soc Exp Biol Med* 1932; 29:640–641.
- Hollander F. Composition of gastric juice as a function of its acidity. Some properties of parietal secretion. *J Biol Chem* 1932; 97:41–43.
- Hollander F. Studies in gastric secretion. IV. Variations in the chlorine content of gastric juice and their significance. *J Biol Chem* 1932; 97:585–604.
- Hollander F. Studies in gastric secretion. V. The composition of gastric juice as a function of its acidity. *J Biol Chem* 1934; 104:33–42.
- Hollander F. The composition of pure gastric juice. *Am J Dig Dis* 1934; 1:319–329.
- Teorell T. Untersuchungen über die magensaftsekretion. *Skand Arch Physiol* 1933; 66:225–317.
- Teorell T. On the permeability of the stomach mucosa for acids and some other substances. *J Gen Physiol* 1939; 23:263–268.
- Elliot A, Rosholm L, Obrink AJ. The exchange diffusion of hydrochloric acid through the gastric mucosa in man. *Acta Med Scand* 1942; 110:267–271.
- Davenport HW. Gastric mucosal injury by fatty and acetylsalicylic acids. *Gastroenterology* 1964; 46:245–53.
- Hollander F. Factors which reduce gastric acidity: A survey of the problem. *Am J Dig Dis* 1938; 5:364–372.
- Hollander F, Felberg RS. Gastric mucus secretion [abstract]. *J Biol Chem* 1941; 140:62–63.
- Hollander F, Stein J. Secretion in the stomach following the injection of pilocarpine. *Amer J Physiol* 1943; 140:136–147.
- Janowitz HD, Hollander F, Jackson C. Stimulation of cell free gastric mucus by the topical application of acetylcholine. *Proc Exp Biol* 1950; 76:578–580.

28. Hollander F. Discussion of paper by Stewart HL: Hyperplastic and neoplastic lesions of the stomach in mice. *J Natl Cancer Inst* 1941; 1:507–508.
29. Hollander F. Experimental gastric carcinoma and mucus secretion [editorial]. *Gastroenterology* 1944; 2:286–288.
30. Hollander F. Discussion of paper by Ivy AC: "Gastric physiology in relation to gastric carcinoma." *J Natl Cancer Inst* 1945; 5:330–331.
31. Hollander F, Stein J, Lauber FU. The consistency, opacity and columnar cell content of the gastric mucus secreted under the influence of several mild irritants. *Gastroenterology* 1946; 6:576–595.
32. Pavlov IP. *Work of the digestive glands*. Ed. 2. London: Charles Griffen & Co.; 1910. p. 237.
33. Hollander F, Lauber FU. Comparison with eugenol with other stimuli for gastric mucus secretion. *Communications 17th International Physiological Congress*; 1947; Oxford. Oxford: University Press; 1948. p. 155.
34. Hollander F, Lauber FU. Eugenol as a stimulant for gastric mucus secretion. *Proc Soc Exp Biol Med* 1948; 67:34–37.
35. Sober HA, Sonnenblick BP, Hollander F. Response of the Heidenhain pouch to repeated application of eugenol [abstract]. *Fed Proc* 1947; 11:292.
36. Code CF. On the occasion of presentation of the Friedenwald Medal of the American Gastroenterological Association to Horace W. Davenport. *Gastroenterology* 1981; 80:1–3.
37. Davenport HW, Warner HA, Code CF. Functional significance of the gastric mucosal barrier to sodium. *Gastroenterology* 1964; 47:142–151.
38. Sonnenblick BP, Sober HA, Hollander F. Influence of the repeated eugenol stimulation on the gastric mucosa as studied in mucus smears [abstract]. *Fed Proc* 1947; 6:292–293.
39. Hollander F, Goldfisher RL. Histologic study of the destruction and regeneration of the gastric mucous barrier following application of eugenol. Preliminary report. *J Nat Cancer Inst* 1949; 10:339–349.
40. Sober HA, Hollander F, Sonnenblick BP. Response of gastric mucous barrier in pouch dogs to repeated topical application of eugenol. *Am J Physiol* 1950; 162:120–130.
41. Bandes J, Samuels NA, Hollander F, et al. The clinical response of patients with peptic ulcer to a topical mucigogue (eugenol). *Gastroenterology* 1951; 18:391–399.
42. Hollander F. The two-component mucous barrier: Its activity in protecting the gastroduodenal mucosa against peptic ulceration. *Arch Intern Med* 1954; 93:107–120.
43. Cope OP, Cone WE, Brenizer AG, Jr. Gastric secretion. II. Absorption of radioactive sodium from pouches of the body and antrum of stomach of dog. *J Clin Invest* 1943; 22:103–110.
44. Cope OP, Blatt HAW, Ball MR. Gastric secretion. III. The absorption of heavy water from the pouches of the body and antrum of the dog. *J Clin Invest* 1943; 22:111–114.
45. Reitmeier RJ, Code CF, Orvis AL. Barrier offered by gastric mucosa of healthy persons to absorption of sodium. *J Appl Physiol* 1957; 10:261–266.
46. Code CF, Higgins JA, Moll JC, et. al. The influence of acid on the gastric absorption of water, sodium and potassium. *J Physiol* 1963; 166:110–119.
47. Hollander F. Reversibility of the inhibition of gastric acid secretion by iodoacetate amine (IAA) and N-ethyl maleimide (NEM) [abstract]. *Fed Proc* 1955; 14:75.
48. Davenport HW, Chavré VJ, Davenport VD. Inhibition of gastric acid secretion by iodoacetate amide and N-ethyl maleimide. *Am J Physiol* 1955; 182:221.
49. Hollander F. Inhibition of gastric secretion in vivo by iodoacetamide and N-ethyl maleimide. *Am J Physiol* 1956; 187:231–236.
50. Janowitz HD, Colcher H, Hollander F. Inhibition of gastric acid secretion in dogs by acetazolamide. *Am J Physiol*, 1952; 171:325–330.
51. Davies RW, Edelman J. Studying tied tubes of in vitro frog gastric mucosa. *Biochem J* 1951; 50:190–194.
52. Altamirano M., Chiang L, Bravo I. Affect of sympathetic stimulation on gastric secretion of pepsin. *Am J Physiol* 1960; 199:131–135.
53. Werther JL, Altamirano M., Hollander F. Affect of variation in pH of gastric contents upon inhibition by diamox of gastric acid secretion. *Gastroenterology* 1961; 40:806–807.
54. Johnson LR. Footnote to modern gastroenterology [special article]. *Gastroenterology* 1982; 82:1293–1294.
55. Werther JL, Hollander F, Altamirano M. The effect of acetazolamide on gastric mucus on canine vivo-vitro preparations. *Am J Physiol* 1965; 209:127–133.
56. Lindner AE, Cohen N, Dreiling DA, Janowitz HD. Effect of acetazolamide on secretion of sodium and potassium by the human stomach. *J Appl Physiol* 1962; 17:514–518.
57. Altamirano M.. Back-diffusion of H⁺ during gastric secretion. *Am J Physiol* 1970; 218:1–6.
58. Davenport HW. Salicylate damage to the gastric mucous barrier. *N Engl J Med* 1967; 1307–1312.
59. Williams SE, Turnberg LA. Demonstration of a pH gradient across mucus adherent to the rabbit gastric mucosa: Evidence for a "mucus bicarbonate" barrier. *Gut* 1981; 22:94–96.
60. Davenport HW. Destruction of the gastric mucous barrier by detergents and urea. *Gastroenterology* 1968; 54:175–181.
61. Edward DW, Skoryna SC. Properties of gel mucin of human gastric juice. *Proc Soc Exp Biol Med* 1964; 116:794–799.
62. Berkowitz JN, Janowitz HD. Alteration in the composition of hydrochloric acid solutions by the gastric mucosa. *Am J Physiol* 1966; 210:216–220.
63. Lindner AE, Cohn N, Dreiling DA, Janowitz HD. Electrolyte changes in the stomach following instillation of acid solutions. *Clin Sci* 1963; 25:195–203.
64. Dyck WP, Werther JL, Rudick J, Janowitz HD. Electrolyte movement across canine antral and fundal gastric mucosa. *Gastroenterology* 1969; 56:488–495.
65. Chapman MA, Werther JL, Janowitz HD. Response of the normal and pathological human gastric mucosa to an instilled acid load. *Gastroenterology* 1968; 55:344–353.
66. DuPlessis DJ. Pathogenesis of gastric ulceration. *Lancet* 1965; 1:974–978.
67. Rhodes J, Barnado BE, Phillips SF, et al. Increased reflux of bile into the stomachs of patients with gastric ulcer. *Gastroenterology* 1969; 57:241–252.
68. Capper WM. Factors in the pathogenesis of gastric ulcer. *Ann R Coll Surg England* 1967; 40:21–35.
69. Werther JL, Janowitz HD, Dyck WP, et al. The effect of bile on electrolyte movement across canine gastric antral and fundic mucosa. *Gastroenterology* 1970; 55:692–697.
70. Ivey KJ, DenBestin L, Clifton JA. Effect of bile salts on ionic movements across the human gastric mucosa. *Gastroenterology* 1970; 59:683–690.
71. Rudick J, Werther JL, Chapman ML, Janowitz HD. Ionic flux across the gastric mucosa effects of atropine on the permeability of the fundus and antrum. *Proc Soc Exp Bio Med* 1970; 135:605–608.
72. Overholt BF, Brody DA, Chase BJ. Effect of vagus nerve stimulation and salicylate administration on the permeability characteristics of the rat gastric mucosal barrier. *Gastroenterology* 1969; 56:651–658.

73. Rudick J, Werther JL, Chapman ML, et al. Mucosal blood flow in canine antral and fundic pouches [abstract]. *Fed Proc* 1969; 28:787.
74. Rudick J, Werther JL, Chapman ML, et al. Mucosal blood flow in canine antral and fundic pouches. *Gastroenterology* 1971; 60:263–271.
75. Rudick J, Chapman ML, Werther JL, Janowitz HD. Ionic fluxes across canine antral mucosa: Effects of pentagastrin and histamine [abstract]. *Clin Res* 1970; 18:388.
76. Adair RK, Wlodek GK. Ionic changes in Pavlov pouches after insulin hypoglycemia gastrin and pentagastrin. *Arch Surg* 1968; 97:423–429.
77. Overholt B. Acid diffusion into the human gastric mucosa. *Gastroenterology* 1968; 54:182–189.
78. Moody FG, Durbin RP. Effects of glycine and other instillates concentration of gastric acid. *Am J Physiol* 1965; 209:122–126.
79. Chapman ML, Werther JL, Rudick J, Janowitz HD. Pentagastric infusion-glycine instillation as a measure of acid absorption in the human stomach: Comparison to an instilled acid load. *Gastroenterology* 1972; 63:962–972.
80. Himel H, Werther JL, Chapman ML, et al. Acid absorption in the canine duodenum. *Ann Surg* 1977; 184:481–487.
81. Werther JL, Horowitz I. The effects of stress on the gastric mucosal barrier in rats. *Exper Biol Med* 1977; 154:4015–4017.
82. Stewart HL, Hare WV. Variation in susceptibility of the fundic and pyloric portions of the glandular stomach with the rate to induction of neoplasia by 20-methylcholanthrene. *Acta Unio Internat Contra Cancrum* 1950; 7:176–177.
83. Horowitz I, Werther JL. Absorption of 3-methylcholanthrene, sodium taurocholate micelles by gastric mucosa [abstract]. *Gastroenterology* 1977; 72:1160.
84. Horowitz I, Toth LS, Werther JL. Effect of N-methyl-N1-nitro-N-nitrosoguanidine on the gastric mucosal barrier in rats. *Am J Dig Dis* 1978; 23:510–512.
85. Cohen A, Geller SA, Horowitz I, et al. Experimental models for gastric leiomyosarcoma. *Cancer* 1984; 53:1088–1092.
86. Robert A, Mezanimas JE, Lancaster C, Hanchar AJ. Cytoprotection by prostaglandin in rats. Prevention of gastric necrosis produced by alcohol, NaOH, hypertonic NaCl and thermal injury. *Gastroenterology* 1979; 77:433–443.
87. Lacey ER, Ito S. Microscopic analysis of ethanol damage to rat gastric mucosa after treatment with a prostaglandin. *Gastroenterology* 1982; 83:619–625.
88. Marrone G, Ito S, Silen W. Prostaglandins fail to prevent acetylsalicylic acid (ASA) induced injury of the isolated amphibian gastric mucosa [abstract]. *Gastroenterology* 1983; 84:1241.
89. Guth PH, Paulsen G, Negota H. Histologic and microcirculatory changes in acid induced gastric lesions in the rat: Effect of prostaglandin cytoprotection. *Gastroenterology* 1984; 84:1083–1090.
90. Terano A, Mach T, Stachura J, et al. Effect of 16, 16 dimethyl prostaglandin E2 on aspirin induced damage to the rat gastric epithelial cells in tissue culture. *Gut* 1984; 25:19–25.
91. Ito S, Lacey ER. Morphology of rat gastric mucosal damage defense and restitution in the presence of a luminal ethanol. *Gastroenterology* 1985; 88:250–260.
92. Ohno T, Ohtsuki H, Okabe S. Effect of 16, 16 dimethyl prostaglandin ethanol induced, aspirin induced gastric damage in the rat scanning electron microscopic study. *Gastroenterology* 1984; 88:351–361.
93. Schmidt KL, Penagan JN, Smith GS, et al. Prostaglandin cytoprotection against ethanol induced gastric injury in the rat: A histologic and cytologic study. *Gastroenterology* 1985; 88:649–659.
94. Kao Y-C, Lichtenberger L. Effect of 16, 16 dimethyl prostaglandin E2 on the lipidic organelles of rat gastric surface mucous cells. *Gastroenterology* 1993; 104:103–113.
95. Fabry TL. How the parietal secretion crosses the gastric mucus without being neutralized [abstract]. *Gastroenterology* 1990; 98:A42.
96. Bhaskar KR, Garik P, Turner BS, et al. Viscous fingering of HCl through gastric mucin. *Nature* 1992; 360:458–461.
97. Goddard PJ, Kao Y-C, Lichtenberger LM. Luminal surface hydrophobicity of canine gastric mucosa is dependent on a surface mucus gel. *Gastroenterology* 1990; 98:361–370.
98. Giraud MN, Motta C, Lichtenberger LM. Effect of aspirin (ASA) on the dynamic properties of gastric surface-active phospholipids (SAPL) [abstract]. *Gastroenterology* 1997; 112:A127.
99. Lichtenberger LM, Romero JJ, Fox JG. Alterations in gastric surface hydrophobicity and phospholipids in the *Helicobacter felis* infected mouse [abstract]. *Gastroenterology* 1997; 112:A199.
100. Hills BA. Gastric surfactant and the hydrophobic mucosal barrier. *Gut* 1996; 39:621–624.
101. Hills BA. A physical identity of the gastric mucosal barrier. *Med Australia* 1990; 153:76–81.
102. Slomiany BL, Piasek A, Sarosiek J, Slomiany A. The role of surface and intracellular mucus in gastric mucosal protection against hydrogen ion. *Scand J Gastroenterol* 1985; 20:1191–1196.
103. Slomiany BL, Sarosiek J, Liau YH, Slomiany A. Lysolecithin affects the viscosity permeability and the peptic susceptibility of gastric mucosa. *Scand J Gastroenterology* 1986; 21:1073–1079.
104. Hills BA. Reply [letter]. *Gut* 1997; 41:724.
105. Bernhard W, Postle AD. Is gastric surfactant related to lung surfactant? [letter]. *Gut* 1997; 41:723–724.
106. Sachs G, Modlin IN. Acid related diseases. Heidelberg (Germany): Springer Verlag; 1998. pp. 156–157.