

Gastroduodenal mucus bicarbonate barrier: protection against acid and pepsin

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Allen, Adrian, and Gunnar Flemström. Gastroduodenal mucus bicarbonate barrier: protection against acid and pepsin. *Am J Physiol Cell Physiol* 288: C1–C19, 2005; doi:10.1152/ajpcell.00102.2004.—Secretion of bicarbonate into the adherent layer of mucus gel creates a pH gradient with a near-neutral pH at the epithelial surfaces in stomach and duodenum, providing the first line of mucosal protection against luminal acid. The continuous adherent mucus layer is also a barrier to luminal pepsin, thereby protecting the underlying mucosa from proteolytic digestion. In this article we review the present state of the gastroduodenal mucus bicarbonate barrier two decades after the first supporting experimental evidence appeared. The primary function of the adherent mucus gel layer is a structural one to create a stable, unstirred layer to support surface neutralization of acid and act as a protective physical barrier against luminal pepsin. Therefore, the emphasis on mucus in this review is on the form and role of the adherent mucus gel layer. The primary function of the mucosal bicarbonate secretion is to neutralize acid diffusing into the mucus gel layer and to be quantitatively sufficient to maintain a near-neutral pH at the mucus-mucosal surface interface. The emphasis on mucosal bicarbonate in this review is on the mechanisms and control of its secretion and the establishment of a surface pH gradient. Evidence suggests that under normal physiological conditions, the mucus bicarbonate barrier is sufficient for protection of the gastric mucosa against acid and pepsin and is even more so for the duodenum.

acid-base transporters; cystic fibrosis transmembrane conductance regulator channel; surface pH gradient; mucus gels; trefoil peptides

MORE THAN A CENTURY AGO in 1892, Schierbeck (205) demonstrated high values of P_{CO_2} in dog stomach, and in 1898, Pavlov (180) postulated that an alkaline mucus layer protects the gastric mucosal surface. Hollander (109), some 50 years later, proposed a two-component protective gastric mucosal barrier of an alkaline mucus layer and a rapidly regenerating underlying epithelium. This two-component hypothesis emphasized an alkaline secretion of nonparietal origin in the intact stomach, but intraluminal neutralization could not at that time be experimentally distinguished from back diffusion of secreted acid into the epithelium. Metabolism-dependent secretion of bicarbonate (HCO_3^-) by intact gastric mucosa was first demonstrated in the mid-1970s by Flemström and coworkers (61, 66, 75) and was subsequently shown in the duodenum (64, 67, 214). A stable, unstirred layer, provided by surface mucus, was presumed to be a prerequisite for maintaining a pH higher at the epithelial surface than that in an acidic luminal solution (79, 244). The presence of a firm mucus gel layer adherent to gastric and duodenal mucosa was first directly demonstrated in the early 1980s by Allen and coworkers through visualization of unfixed mucosal sections and rheological studies (7, 15, 31, 131). At the same time, dynamic proof of the existence of a

gastroduodenal mucus bicarbonate barrier to luminal acid came from demonstrations of a pH gradient at the gastric mucosal surface by Turnberg and coworkers (25, 190, 260) and by Silen and coworkers (230) and at the duodenal mucosal surface by Flemström and Kivilaakso (72, 136).

Several agents, including un-ionized weak acids and ethanol, damage the gastric mucosa and cause a marked increase in the disappearance of acid from the gastric lumen. Davenport (52) and others (20) provided evidence for damage-induced transmucosal leakage of interstitial and plasma HCO_3^- neutralizing intraluminal acid. This leakage, which should be distinguished from metabolism-dependent transport of HCO_3^- from the undamaged mucosa, was later found to be important in mucosal repair by maintaining a neutral pH under the mucoid cap formed on top of the repairing epithelium (119, 146, 213). The protective mucoid cap was shown to be primarily a thick fibrin gel layer formed from leaking plasma fibrinogen along with interstitial HCO_3^- and distinct from the adherent mucus gel layer covering the undamaged mucosa (210).

In this article we review the current state of the gastroduodenal mucus bicarbonate barrier two decades after the first supporting experimental evidence appeared (see reviews, Refs. 12, 13, 66, and 78). Mucus is unique in the gastrointestinal tract in that the secretion, particularly in stomach, is a thick layer of gel adherent to the mucosal surface. The role of this gel layer in mucosal protection is a structural one: to create a stable, unstirred layer to support surface neutralization of acid and act

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as a protective physical barrier against luminal pepsin reaching the underlying epithelium. Therefore, the emphasis on mucus in this review is on the form and function of this adherent mucus gel layer. The role of mucosal HCO_3^- secretion is to neutralize acid diffusing into the mucus gel layer and to be quantitatively sufficient to maintain a near-neutral pH at the mucus-mucosal surface interface. The emphasis on mucosal HCO_3^- secretion in this review is on the mechanisms and neurohormonal control of its secretion, the establishment of a surface pH gradient, and, in particular, the stimulation of HCO_3^- secretion by acid in the lumen, which is of primary importance, particularly in duodenum.

ADHERENT MUCUS GEL LAYER IN SITU

A continuous layer of adherent mucus gel in stomach, duodenum, and more distal parts of the intestinal tract has been demonstrated using a number of methods *in vitro* (8, 36, 130, 131, 176) and *in vivo* (6, 24, 44, 111, 204, 238). In this context it should be emphasized that conventional histological methods using organic solvents and paraffin embedding result in shrinkage and dehydration of the mucus gel layer to give little or no extracellular mucus visible on mucosal sections (8). The original methods demonstrating an adherent gastric mucus gel circumvented these problems by either 1) indirectly measuring differences in refractive index with a slit lamp and pachymeter (36) or 2) directly observing unfixed mucosal sections on which the mucus gel appeared as a translucent layer of variable thickness (10–250 μm , in rats) between a clear bathing solution and the dense mucosa (131, 159). Subsequently, histological methods have been developed that preserve the surface adherent mucus layer with the use of cryostat sections, milder fixing conditions, and water-soluble mountants (18, 130). Mean mucus layer thickness values obtained with the use of these methods for antrum in humans and rats are 106 μm (minimum, 50 μm) and 166 μm (minimum, 90 μm), respectively (130, 168), and in rats approach values seen *in vivo* (24).

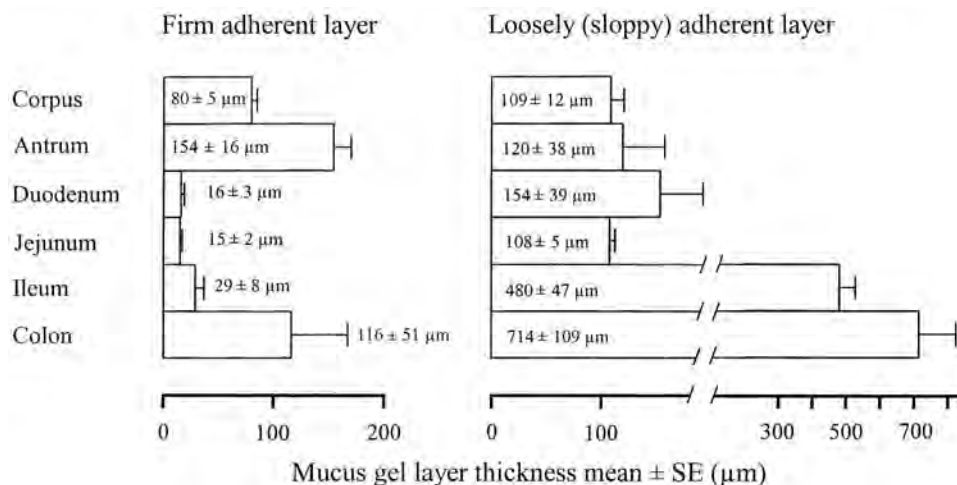
The paucity or absence of mucus on histological sections prepared using conventional procedures initially led to controversy as to whether a continuous layer of mucus existed over the gastric mucosal surface (166, 255). This was compounded by the misnaming, as mucus, of the mucoïd cap seen on histological sections of reepithelializing gastric mucosa follow-

ing acute damage (166). The mucoïd cap on top of the damaged and repairing mucosa is primarily a fibrin gel with necrotic cells and remains of the adherent mucus layer from the original, undamaged mucosa (8, 210). This mucoïd cap, in contrast to the adherent mucus gel, is three to four times thicker and can be preserved using standard histological fixation procedures.

An elegant method involving the use of intravital microscopy to observe adherent mucus gel layers on mucosal surfaces *in vivo*, initially developed for stomach, has now been applied throughout the gut of anesthetized rats (6, 24, 111, 197, 204, 238). With the use of this method, a continuous, translucent mucus gel layer can be seen on mucosal surfaces, with a mean thickness of 189 μm in corpus, 274 μm in antrum, and 170 μm in duodenum (24). What is particularly interesting is that two physical forms of mucus have been observed: a loosely adherent mucus layer that can be removed by suction and a firm adherent mucus layer that remains. This remaining firm adherent mucus is substantial and continuous in antrum, corpus, and colon, while it is very thin or absent in duodenum, jejunum, and ileum, where the primary function is absorption of nutrients (Fig. 1). In stomach *in vivo*, the firm adherent mucus layer presumably equates to the stable protective barrier, whereas the superficial loosely adherent layer, likely to be largely removed by the shear forces of the digestive process, would act more as a lubricant. The loosely adherent layer continually increases in thickness at a rate slowest in the stomach and greatest in the colon, where it is very thick (714 μm) and copious (24). Compatible with these observations *in vivo* is the recent isolation from pig stomach *in vitro* of two mucus secretions that differ distinctly in their gel rheology and, possibly, component mucin multimeric structure (242). There is a sloppy mucus gel that is readily broken down by low applied shear forces and an underlying firm adherent mucus gel that is resistant to applied shear and collapses only at shear forces two orders of magnitude higher.

An alternative approach, the application of confocal microscopy and fluorescent beads to observe the surface of the adherent mucus layer, has been used in anesthetized rat and mouse stomach *in vivo* (29, 44). Mucus layer thickness values (median, 50–75 μm) obtained for rat corpus by using this approach are ~30–40% of those obtained using intravital

Fig. 1. Thickness of mucus gel layers along the rat gastrointestinal tract. The gastrointestinal tissues of thiobarbiturate-anesthetized rats were mounted luminal side up for intravital microscopy, and mucus thickness was measured before and after partial removal by suction. A loosely (sloppy) adherent mucus layer was removed by suction to leave a firm adherent mucus layer attached to the mucosa. This loosely adherent mucus layer was continuous and did not follow the contours of the villi in the intestine. In stomach and colon, the firm adherent mucus layer was continuous, but in the small intestine, this firm mucus layer had a patchy distribution and was absent on individual villi. [Adapted from Atuma et al. (24).]



microscopy. This difference, in part, could be due to the use of a perfusion system in the confocal studies, likely to remove the loosely adherent mucus gel, whereas unstirred conditions pertain to intravital microscopy studies (24, 44). At the same time, mucus layer thickness values from confocal microscopy are still noticeably lower than those for the firm mucus layer (i.e., after suction) from intravital microscopy studies. A comparative study in vivo using both methodologies for observing mucus thickness and at varying rates of perfusion would be interesting.

STRUCTURE AND STABILITY OF MUCUS LAYER

Two factors that determine the efficacy of the adherent mucus layer as a barrier are, first, the gel structure, on which depend the stability and permeability of the adherent mucus layer, and second, the thickness of the adherent layer (see below). Compared with other gastrointestinal secretions, the adherent mucus gel form is physically unique, and an understanding of its structure and properties is essential to underpin physiological studies. Rheological studies have shown that adherent gastrointestinal mucus gels from stomach, duodenum, and colon are all well-defined viscoelastic gels that do not dissolve on dilution (8, 17, 30, 31, 211). Mucus secretions as gels are also unique in that they flow over a relatively long time (30–120 min), reannealing when sectioned. It is this property that distinguishes mucus gels from other noncovalent but rigid gels (e.g., agar). In functional terms, these flow properties are key to the adherent mucus gel layer forming a continuous cover over the mucosa in vivo. Mucus gels are stable, and exposure of isolated gastric mucus gel to pH 1–8, hypertonic salt (e.g., 2 M NaCl), or bile does not disperse the gel or affect its rheological properties (30, 31, 211). The mucus gel is dissolved by reduction with thiol agents or proteolysis, both of which destroy the multimeric structure of the component mucins (19, 31).

Mucus secretions from all regions of the gut have the same generic gel structure (8, 31, 211), and this is reflected by common structural patterns in the component gel-forming mucins. The central regions of all gel-forming mucin units have a protein core covered by a sheath of glycan chains that are flanked at both ends by nonglycosylated protein domains rich in cysteine. The cysteine-rich domains are the location of the interchain disulfide bridges between the mucin units that are polymerized end to end into large mucin multimers (7, 8, 19, 91, 155, 183). Gel-forming mucins are very large molecules (reported molecular masses of $5\text{--}45 \times 10^6$ Da) consisting of multimers of mucin units ($2\text{--}3 \times 10^6$ Da). The peptide core alone of each mucin unit contains $>5,000$ amino acids, around which are packed glycan chains that comprise the major portion by weight of the molecule (between 50 and 80%). Until recently, progress in this field was slow, primarily because of the structural complexity of the component mucins and the difficulties of performing meaningful rheological studies of mixtures of such molecules. The structure of each of the four gel-forming mucins, MUC2, MUC5AC, MUC5B, and MUC6 (published only in part), has now been elucidated with cloning and sequencing of the mucin genes. This has led, over the past decade, to an explosion of information on the complexities of mucin structure, biosynthesis, and secretion, and there are many reviews available (8, 38, 46, 47, 83, 88, 132, 164, 183,

252). Three of the four gel-forming mucins are well expressed in the normal gastrointestinal tract. In the stomach, MUC5AC is expressed by the surface mucous cells of cardia, fundus, and antrum, and MUC6 is expressed by the mucous neck cells of fundus and of antral glands in cardia and antrum (54, 103, 105). MUC2 is expressed by goblet cells from duodenum to colon, whereas MUC6 is expressed by Brunner's glands in duodenum (103, 105).

The general features of mucus gel structure are known, although there are many questions remaining. The multimeric structure (previously referred to as polymeric structure) of the mucins is essential for gel formation (7, 19, 31, 220). Gel strength and stability have been shown to relate directly to the percentage of multimeric mucin, relative to the monomeric form, in the gel (211). In gastric ulcer and *Helicobacter pylori* infection, the percentage of multimeric mucin in the adherent gastric mucus layer falls, indicative of a weaker and less stable mucus gel (10, 168, 264). The large, highly hydrated multimeric mucin molecules interact noncovalently to form the mucus gel network (8, 17), but the chemical nature of these interactions is unclear. Recent rheological studies point to a complexity of different, noncovalent interactions of a variety of types and strengths between the mucin molecules (241). More than 90% of these interactions are transient (i.e., make and break over a finite time), explaining the unique flow properties of mucus gels.

What has emerged from such studies is evidence that gel-forming mucins from different secretions or even the same secretion, while possessing a common structural pattern, show many specific structural differences in their protein core, glycan side chains, antigenic properties, and negative charge (8, 38, 46, 54, 83, 132). Furthermore, histological studies suggest that there maybe different structural patterns within the adherent mucus layer itself. Laminated layers of surface mucus and glandular mucus, which stain for different glycan structures, have been observed in the adherent gastric mucus layer in situ in humans (100, 176). Alternating layers of MUC5AC and MUC6, the two gastric mucin gene products, have been reported in the mucus layer in sections of human gastric mucosa (104). Moreover, recent studies using the novel technique of high-pressure freezing/freeze substitution, which preserves intact not only the mucus gel but also the fluid luminal phase, showed a triple lamination of different mucin structures within the secreted mucus gel layer at the mouth of the gastric pits (203). In the present context, the key question for the future is whether and to what extent these subtle structural differences, between and within the different mucin secretions, influence gel structure sufficiently to effect the stability and permeability of the adherent mucus gel layer.

TREFOIL PEPTIDES AND THE ADHERENT MUCUS LAYER

There is considerable interest in a putative structural role in mucus gel for the cosecreted, low-molecular-weight trefoil peptides. Three trefoil peptides have been identified in humans and have been shown to be key factors in stimulating cell migration and promoting epithelial repair after damage (59, 106, 185, 202, 240). TFF1 is cosecreted with MUC5AC mucin in stomach, TFF2 with MUC6 mucin in glands of stomach and duodenum, and TFF3 with MUC2 mucin from goblet cells. In TFF1-null mice, the antral and pyloric gastric mucosa exhibit

severe hyperplasia and are almost entirely devoid of mucus, showing that expression of the latter is dependent on the former (150). TFF1 is present in high levels in the adherent gastric mucus gel layer and is strongly, noncovalently bound to isolated mucin (167). In a yeast two-hybrid system, TFF1 interaction with the disulfide-bridging von Willebrand C domains of MUC5AC and MUC2 has been shown and interpreted as facilitating multimerization of the mucin. However, these interactions have yet to be confirmed by *in vitro* studies (248). In HT-29 cells, upregulation of trefoil factor secretion is stimulated by mucin secretagogues, and the former is bound to mucin in the adherent mucus layer (92). There are various studies suggesting that trefoil peptides influence mucus gel properties. Thus rat gastric or intestinal mucin acts cooperatively with TFF3 in the attenuation of damage to a human T84 colonic cancer cell line by a variety of agents (133). TFF2 addition dose-dependently decreased the rate of diffusion of H⁺ through a 5% solution of pig gastric mucus and slowed the initial acidification rate of gastric mucosal cells *in vivo* (237). These results were interpreted as interaction of TFF2 with the mucus gel, decreasing permeability of the latter to protons. Human TFF2 has been shown to increase the viscosity of a commercial preparation of pig gastric mucin, leading to formation of a gel, and this was interpreted as evidence of trefoil peptides interacting with the mucins to stabilize the gel network (246). However, it is difficult to relate these *in vitro* studies to the adherent gel secreted *in vivo*, because commercial pig gastric mucin preparations are substantially degraded and non-gel forming (8). Clearly, trefoil peptides are an integral part of the intracellular mucus secretory vesicles and the ensuing adherent mucus gel; furthermore, they interact strongly with the component mucins. However, it is still an open question as to whether trefoil factors have a structural role in the secreted mucus gel *in vivo* or the latter primarily provides a stable extracellular support for the former, similar to that for secretory IgA. Trefoil peptides may well play a role in the intracellular assembly and/or packaging of mucins.

FACTORS INFLUENCING MUCUS LAYER THICKNESS

A primary factor in determining the protective efficacy of the mucus barrier *in vivo* is the thickness of the adherent firm mucus layer. Thickness of the mucus layer is the result of a dynamic balance between its secretion and its erosion mechanically by shear forces of the digestive process and by proteolytic degradation, particularly from luminal pepsin in stomach (8, 9). There is a wealth of work on secretion of the component mucins, the intracellular control of this process, and the response to neural, hormonal, and paracrine stimulation, as well as the effect of inflammatory mediators. A variety of *in vitro* and *in vivo* models, including cell culture, have been used in these studies, and there are many reviews (8, 46, 82, 83, 239, 252). It is difficult, however, to relate these studies on mucin secretion directly to the effective thickness of the adherent mucus gel *in vivo*, because of variables such as mucin concentration in the gel, rates of mucus erosion *in vivo*, and proportions of sloppy (loosely adherent) and firm mucus in the secreted product. Where gastroduodenal mucus thickness has been measured directly, it has been shown to be increased by hormonal, paracrine, and neural stimulation and, in duodenum, by topical acid.

In rat stomach *in vivo*, mucus layer thickness, measured on unfixed mucosal sections, is increased up to threefold by topical prostaglandins, carbachol (intraperitoneal), and secretin (intravenous) (8, 9, 131, 159). Rat gastric mucus layer thickness was unchanged after exposure to acid pH 1 or 2 over 3 h *in vivo* (16). Direct observation in rat stomach *in vivo* showed no change in adherent mucus layer thickness over time in the presence of acid pH 1.7, whereas pentagastrin (intravenous), cimetidine (intraperitoneal), and prostaglandin E₂ (PGE₂) all increased mucus layer thickness by up to one-third (170, 171). Further evidence that exposure to luminal acid does not change gastric mucus layer thickness was provided by reports that no significant increase in thickness occurred in pentagastrin-stimulated rats *in vivo*, with or without inhibition of acid secretion by omeprazole (204). Central vagal stimulation by injection of thyrotropin-releasing hormone analog RX 77368 increased gastric mucus layer thickness by up to 30%, and this was unaffected by acid pH 1, indomethacin, or omeprazole (238).

In rat duodenum, in contrast to rat stomach, exposure to luminal acid does increase mucus layer thickness, and this is mediated via extrinsic sensory nerve fibers that release CGRP followed by stimulation via nitric oxide (NO) and cyclooxygenase (COX). The rate of continuous replenishment of duodenal mucus (sloppy) in rat *in vivo*, following its removal by suction, was increased (44%) by exposure to HCl pH 2 for 10 min and inhibited by indomethacin or NO synthase inhibitor N^ω-nitro-L-arginine (197). More recently, rat duodenal mucus layer thickness *in vivo* was shown to be increased by >50% in response to luminal acid, primarily by stimulation of a capsaicin-sensitive pathway including NO, vanilloid receptors, and afferent nerves (3, 6). PGE₂ (luminal or intravenous) similarly increased duodenal mucus layer thickness, but, interestingly, indomethacin also abolished the acid/capsaicin-mediated response, suggesting that COX pathways may be the common mechanism for duodenal mucus secretion. Once the acid stimulus is withdrawn, the mucus layer thickness values returned to basal levels of the order of 100 μm (3), indicating that the increase may have reflected primarily changes in sloppy mucus gel, which is subsequently removed by the shear forces of the perfusion system used. The acid-induced stimulation of duodenal mucus layer thickness in the duodenum is part of a series of mucosal protective mechanisms in response to increased luminal acidity, including increased HCO₃⁻ secretion and blood distribution (69, 94, 114).

MUCUS AND PROTECTION AGAINST PEPSIN

Pepsin, in contrast to acid, has received relatively little attention as the other endogenous aggressor in gastric juice. Yet, in an anesthetized rat stomach model, mildly excess pepsin (2 mg/ml, maximal secretory levels in humans over 2 h) causes extensive mucosal damage under conditions in which acid pH 1 or 2 alone is ineffective (11, 16). Pepsin damage is characterized by focal areas of discontinuity in the adherent mucus gel layer, localized hemorrhagic punctuate ulcers with bleeding into the lumen, and no evidence of reepithelialization or mucoïd cap formation. Damage by pepsin is markedly different from that caused by ethanol (70%, 45 s) or 2 M NaCl (14, 16, 210, 213). These agents rapidly penetrate the mucus barrier, resulting in exfoliation of the epithelial layer with a dramatic increase in mucosal permeability, followed by reepi-

thelialization under a fibrin-based mucoid cap. Pepsin digestion of the adherent mucus layer on *Necturus* gastric mucosa, bathed in acid pH 2.5, resulted in a fall in pH at the epithelial surface from pH 5.22 to pH 2.35 over 2 h (137). In esophagus, where squamous epithelium is devoid of a significant adherent mucus gel layer (56), evidence from animal models (247) and clinically in humans (102) points to pepsin rather than acid as the critical factor in gastroesophageal reflux damage.

The adherent mucus gel layer is a physical barrier to luminal pepsin accessing the underlying mucosa. The rate of diffusion of solutes through mucus gel decreases progressively with increasing molecular size (14, 16, 27, 29, 55), and it takes several hours for pepsin (pH 7.0) to permeate a thin layer of mucus gel in vitro (11, 14). The physical explanation for this is that measured rates of diffusion of macromolecules in unstirred solution are very slow, of the order of 1×10^{-6} cm²/s, and calculations show that it would take hours for pepsin (32 kDa) to diffuse through the adherent gastric mucus layer in vivo (11). There also may be some steric hindrance to macromolecular permeability through a 5% mucus gel. In duodenum in vivo, controlled removal of the thin mucus layer by papain digestion markedly enhanced the HCO₃⁻ secretory response to cholera toxin (85 kDa), *H. pylori* Vac A toxin (89 kDa), and glucagon (3.5 kDa) (68). These studies show that even the thin layer of firm mucus gel in the duodenum in vivo limits access of larger-molecular-weight toxins to the mucosa. They also provide a note of caution in secretory studies involving luminal application of such agents. Interestingly, in these studies, removal of the mucus layer also enhanced the stimulation of HCO₃⁻ by PGE₂ (335 Da), but this effect is most likely explained by the binding of the relatively small amounts of secretagogue to mucin (68).

Because luminal pepsin cannot permeate the continuous adherent mucus layer within a physiologically meaningful time scale, it follows that the latter is an effective barrier, probably the major barrier in vivo, against proteolytic digestion of the underlying epithelium (14, 16). At the same time, luminal pepsin at acidic pH slowly hydrolyzes and erodes the adherent mucus layer. However, at the levels of luminal pepsin in vivo, this is normally balanced by new secretion (9, 14, 31). Lack of interest in pepsin as a mucosal damaging agent has been due primarily to the pharmaceutical success of acid inhibition in peptic ulcer treatment and the absence of a good selective inhibitor of pepsin secretion. The proteolytic activity of pepsin in gastric juice on an average protein substrate falls rapidly above pH 3 (102, 181), and it has been assumed that above this pH most, if not all, of the pepsin activity in vivo is lost. However, depending on the substrate and pepsin type, there can be significant pepsin activity well above pH 3. Thus pepsin 1, which rises fivefold in peptic ulcer disease to reach 20% of the total activity in gastric juice (243), has substantial mucolytic activity between pH 3 and 5 (14, 181). Levels of serum pepsinogen 1 and 2 have been shown to be increased in *H. pylori* infection and peptic ulcer disease, whereas low serum pepsinogen levels are a good indicator of atrophic gastritis (35, 201). However, the relationship between serum pepsinogen levels and luminal gastric pepsin activity has yet to be clarified experimentally. Pepsin-induced mucosal damage and activity of the secreted individual pepsin isoforms in peptic ulcer disease and gastroesophageal reflux disease, as well as how

luminal pepsin activity relates to serum pepsinogen levels, all merit further study.

pH GRADIENT AT THE GASTRIC MUCOSAL SURFACE

The conventional view is that the primary role of the adherent mucus layer in protection against acid is to form a stable, unstirred layer at the mucosal surface that prevents immediate mixing of the secreted HCO₃⁻ with the excess of acid in the lumen. The mucus gel, by acting as a mixing barrier, enables the stabilization of a pH gradient from acid in the lumen to near neutral at the mucosal surface (11, 13, 78). Direct evidence of the presence of a surface pH gradient on top of gastric mucosa comes from numerous demonstrations of a pH gradient across the mucus layer. This has been shown for human (25), rabbit (260), and amphibian gastric mucosa in vitro (137, 230) and for rat (184, 204, 227), canine (179), and human stomach in vivo (190, 212). Various other studies support such a role for the mucus layer; for example, in *Necturus* stomach, in vitro removal of the mucus layer by pepsin or *N*-acetyl-cysteine causes intracellular and juxtamucosal surface pH to fall on perfusion of luminal acid pH 2.5 (137). There is an inverse correlation between adherent mucus layer thickness and the initial acidification rate of mucosal cells in rats in vivo (57). In mouse stomach in vivo, mucus has been shown to strengthen the surface unstirred layer effect (29). Also, a collapse of the alkaline surface pH gradient, which would otherwise inhibit pepsin activity, must occur when luminal excess of this enzyme causes loss of the adherent mucus layer and digestion of the underlying mucosa in vivo (16).

Under conditions of acid secretion and HCl (pH 1) instilled into the lumen, a pH gradient across the gastric mucus layer is maintained with a pH of >7 at the mucosal surface (Fig. 2) (184). Other workers using a similar system in rat and mouse stomach, but with confocal imaging and the fluorescent dye Cl-*NERF*, demonstrated a surface pH gradient with superfusion at luminal pH 3.0 (28, 44, 49). Furthermore, in this system, surface and intraglandular alkalinity were increased by luminal administration of dimethyl PGE₂, a compound previously shown to stimulate both gastric and duodenal mucosal HCO₃⁻ secretion (23, 65, 235). Also, at luminal pH 3.0, the relatively alkaline surface pH 4.3 ± 0.1 was acidified by indomethacin (to pH 3.6 ± 0.2), and subsequently dimethyl-PGE₂ restored surface pH to 4.2 ± 0.2 (28). The authors concluded that the preepithelial alkaline layer in the mouse stomach is regulated by endogenous COX activity. Similar increases in surface alkalinity in response to E-type prostaglandins have been observed in rat stomach (195).

Results with the noninvasive confocal imaging technique at luminal pH 3.0 are thus broadly in line with those recorded on penetration of the mucus layer with pH-sensitive microelectrodes at higher luminal acidities of pH 1–2. However, with superfusion at luminal pH 5.0 in both rat and mouse stomach (28, 44, 49), the surface pH gradient was shown to be reversed with a more acidic juxtamucosal pH. This acidity was enhanced by pentagastrin and eliminated by omeprazole, indicating its dependence on acid secreted by the parietal cells. Moreover, it was associated with marked variations of surface pH with the rate of luminal perfusion. A higher juxtamucosal pH at luminal pH 3.0 compared with that at luminal pH 5.0 could reflect higher gastric HCO₃⁻ export stimulated by the

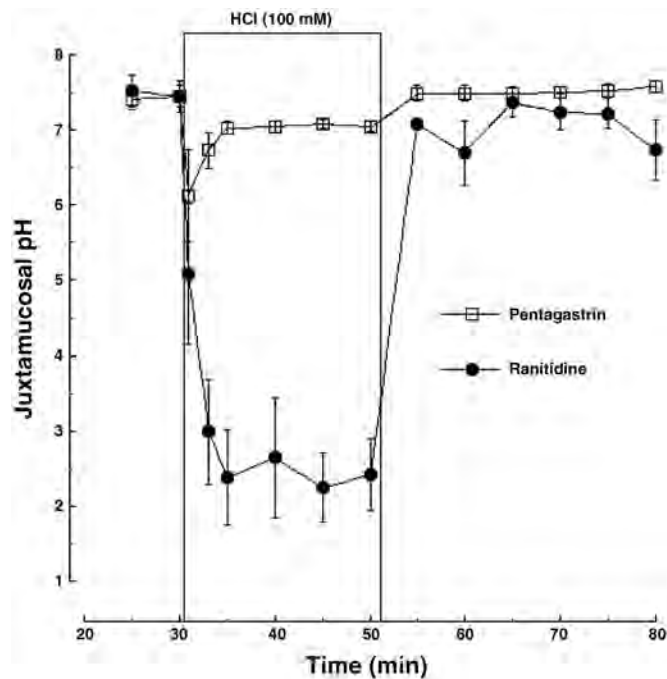


Fig. 2. Gastric juxtamucosal pH in pentagastrin- and ranitidine-treated rats in vivo. The mucus layer was left intact, and juxtamucosal (mucoepithelial interface) pH was measured with H^+ -sensitive microelectrodes. After a 15-min control period with isotonic saline, the gastric lumen was exposed to 100 mM HCl for 10 min, as indicated. This was followed by a second control period with isotonic saline. Note the only slight and transient decline in juxtamucosal pH in animals treated with pentagastrin ($40 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ iv, starting 30 min before acid exposure) to stimulate parietal cell acid secretion. In contrast, there was a marked decline in juxtamucosal pH in animals treated with the histamine H_2 antagonist ranitidine (1 mg/kg iv, 30 min before acid exposure) to inhibit parietal cell acid secretion. [Adapted from Phillipson et al. (184).]

higher luminal acidity (28). A possible explanation for the reversal of the pH gradient observed at pH 5 is that the zone of CO_2 released within the gastric mucus gel, during neutralization of H^+ by HCO_3^- , moves closer to the epithelial surface at lower (less stimulated) rates of HCO_3^- secretion (62, 87, 244). The dependence of gastric HCO_3^- export on interstitial/blood HCO_3^- supply (see below) also may influence the juxtamucosal pH. Further and comparative studies with pH-sensitive microelectrodes and confocal imaging, particularly above luminal pH 3 and under different rates of perfusion, are needed to clarify the dynamics of the pH gradient at the gastric surface.

The adherent mucus layer per se is not a diffusion barrier to acid that diffuses rapidly through the gel matrix both in vitro and in vivo. Numerous studies have measured diffusion rates of H^+ through fresh preparations of gastric mucus gel in vitro and have shown that after initial saturation of binding sites, such diffusion rates are retarded 4- to 10-fold compared with those in an equivalent "unstirred" layer of solution (57, 169, 236, 259). These results have been interpreted to show that mucus slows the mobility of H^+ by acting as a barrier to its free diffusion. Various possible mechanisms for this effect have been reviewed (57). However, calculations from these measured rates of diffusion of H^+ in vitro indicate that acid would still rapidly permeate a layer of adherent mucus gel 100–200 μm thick in a matter of minutes in vivo (11). Studies in vivo confirm that the adherent mucus gel layer is not a significant permeability barrier to H^+ (as opposed to a stable unstirred

layer and mixing barrier). Thus acidification of juxtamucosal surface pH, as monitored by electrodes, can occur very rapidly through the mucus layer when acid is applied to the luminal side (137, 179, 184) (Fig. 2). Recently, elegant experiments in mouse stomach in vivo showed that the rate of diffusion of the low-molecular-mass (400 Da) fluorescent dye CL-NERF through the mucus is the same as that through an unstirred layer of luminal solution (29). This provides clear evidence that, in vivo, the adherent mucus layer is freely permeable to ions and small-sized molecules.

There have been suggestions that mucus undergoes a conformational change at pH ~ 4 that affects its permeability properties with the result that it becomes more impermeable to H^+ at higher levels of luminal acidity. The evidence to support this comes from numerous different studies on mucin solutions involving viscosity (34), viscous fingering (33), and light scattering (41). The evidence from in vivo studies in rat and mouse stomach and *Necturus* stomach in vitro does not support such pH effects on H^+ permeability through mucus in vivo (29, 137, 179, 184). Furthermore, rheological results obtained with mucin solutions cannot necessarily be extrapolated to the native gel, where mucin molecules are restricted by interactions in a gel network and may not undergo the conformational changes seen in free solution, particularly where changes in ionic strength are involved. Thus the rheological characteristics of intact mucus gel are unaffected by exposure to low ionic strength or acid down to pH 1 (31, 211), yet both of these conditions result in a dramatic increase in mucin solution viscosity (17, 34, 41).

Considerable interest has been generated in the role of lipids within the adherent mucus layer providing a barrier to H^+ (101, 153, 154). Evidence for a lipid barrier comes primarily from high contact angle measurements over the gastric mucosa, indicating a hydrophobic surface and the presence of lipids, including surfactants, in adherent gastric mucus. Loss of mucosal hydrophobicity, measured as sharp decreases in contact angle, and decreased phospholipid occur after exposure to known mucosal barrier breakers (e.g., bile and aspirin) (90, 154, 156). The reverse is seen with EGF, which is reported to increase mucosal resistance to acid (157). How lipids in mucus could form a barrier to acid is unclear, although monolayers or multilayers of phospholipid molecules within the mucus layer have been proposed (101, 154). Furthermore, it is uncertain to what contact angle measurements refer. Full drying times of 40–50 min are necessary to obtain consistent results (223, 224), and during this process the adherent mucus gel is progressively dehydrated (11). Even if a lipid barrier does exist in the adherent mucus layer, the evidence of the free permeability to H^+ through mucus in vivo (discussed above) demonstrates that it is not effective against acid.

ROLE OF THE ALKALINE TIDE IN GASTRIC MUCOSAL PROTECTION

Luminal acidities below pH 2.0–3.0 have been reported to dissipate the gastric mucosal surface pH gradient with consequent exposure of the epithelial cell surface to the acidity of the bulk luminal solution. However, this reduction of surface pH occurred in stomachs in which gastric mucosal secretion of HCl was low or absent. As illustrated in Fig. 2, recent work (184, 227) has shown that the pH gradient in the surface mucus

gel is markedly resistant to luminal acid pH 1.0 after stimulation of gastric acid secretion by pentagastrin or the histamine H₂-receptor agonist impromidine. In contrast, there is an acidification of the surface mucus gel during inhibition of mucosal HCl secretion by the histamine H₂-receptor antagonist ranitidine. The maintenance of the surface pH gradient in acid-secreting stomachs can be explained by the increased supply of HCO₃⁻ released to the interstitium from the HCl-secreting parietal cells, i.e., the “alkaline tide,” carried to the surface mucosa by the gastric mucosal vasculature (65, 86).

The importance of plasma/interstitial HCO₃⁻ in protecting the gastric mucosa against acid-induced damage was first demonstrated in dogs (51) and rats (135) *in vivo*, where parenteral administration of exogenous HCO₃⁻ prevented ulceration of the acid-exposed gastric surface. Furthermore, serosal (basolateral)-side administration of HCO₃⁻ has a similar acid-protective action in amphibian (137, 138) and rat (191) mucosa *in vitro*. Studied in the amphibian preparation, serosal-side administration of other buffers, e.g., HEPES, did not protect the mucosa, and pretreatment with DIDS, or removal of serosal-side Na⁺, prevented the protective action of serosal-side HCO₃⁻. The combined results provide strong evidence that uptake of HCO₃⁻ by basolateral Na⁺-HCO₃⁻ cotransport enhances alkaline secretion by the surface epithelial cells (65), resulting in an increased ability of the gastric epithelial surface to resist luminal acid. The presence of HCO₃⁻ in the serosal-side solution furthermore enhances the rapid reconstitution of epithelial integrity in damaged gastric mucosa *in vitro* (213).

SECRETION OF ACID AND PEPSIN FROM GASTRIC GLANDS ACROSS THE MUCUS LAYER

Acid and pepsin, secreted very rapidly from the gastric glands upon stimulation, must gain access to the stomach lumen across a continuous adherent mucus layer. The best explanation is that gastric juice transverses the mucus layer through channels under pressure from the glands (8, 111). Compatible with such an explanation for acid is that pH measurements *in vivo* under secretory conditions do not show lateral diffusion of H⁺ away from the gastric glands at the mucosa-mucus interface (111, 184, 204). Also, it is difficult to explain how pepsin could transverse the mucus layer if it were not through channels. Channels containing acid in mucus layers impregnated with the acid-sensitive dye Congo red have been observed via direct microscopy in secreting rat stomach *in vivo* (111, 127). These Congo red-stained, 5- to 7- μ m-wide channels of acid arise from crypt openings, appear to be discrete structures in that they remain after transient inhibition of acid secretion, and are formed during the secretion process (127). Congo red precipitates at acid pH, and it is not yet clear whether these channels naturally have a defined structure within the mucus gel or, alternatively, are formed in some way by interaction of the mucus gel with Congo red. In the same system, high intraglandular pressures (5–20 mmHg) in the gastric glands during acid secretion have been demonstrated, and this would provide a driving force for pushing secretions through the mucus layer into the lumen (110, 225, 226, 228). An *in vitro* analogy has been made with the phenomenon of viscous fingering, where a discrete boundary is observed between a high-viscosity mucus solution and low-viscosity HCl injected under pressure into it (27, 33).

Other studies have failed to show acid channels in the adherent mucus gel during acid secretion. With the use of confocal microscopy and the fluorescent acid-sensitive dye CL-NERF, no channels were observed in the mucus layer in secreting rat stomach *in vivo* (44), despite the spatial resolution of the technique and its demonstrated ability to measure differences in pH between glandular pits and the surrounding epithelium. Why channels are not seen with confocal imaging but are seen with direct microscopy is not yet clear, and comparative experiments using both methodologies in the same preparation would help resolve this. In another study *in vitro*, with the use of sensitive, double-barreled microelectrodes, no evidence was found for acid channels in the mucus layer of acid-secreting guinea pig mucosa (206). From this study, a model has been proposed in which protons transverse the mucus gel layer buffered by the mucin and are released from the latter by conversion of pepsinogen to pepsin at the lower pH values nearer the lumen (206, 207). There are difficulties with this model in that it would require rapid turnover of the firm mucus gel layer at a substantially much greater rate than that seen *in vivo* (24). Also, pepsinogen is converted to pepsin autocatalytically at acid pH, and it is likely that it will be, almost entirely, the activated enzyme that emerges from the gastric gland. Furthermore, the observed stability of the gastric mucus gel to acid *in vitro* and *in vivo* is not compatible with the postulate that it is widely impregnated with pepsin or pepsinogen, which under conditions of low pH would result in mucolysis (16, 31).

Finally, secreted mucosal HCO₃⁻ presumably diffuses freely from the surface of the gastric epithelium through the mucus gel layer. Because the alkali secretion (HCO₃⁻ export) by the gastric mucosa in the main reflects a Cl⁻/HCO₃⁻ exchange process in the apical membrane of the surface epithelial cells (see below), an association between gastric crypt secretion, volume flow, and alkali secretion would appear unlikely.

GASTRIC BICARBONATE SECRETION

In frog gastric mucosa mounted as sheets in Ussing chambers (61, 75), characteristics of the HCO₃⁻ secretion by antral mucosa, devoid of parietal and chief cells, and fundic mucosa point to the surface epithelium, common to both preparations, as the origin of the secretion. Similar characteristics have been seen for fundic and antral alkaline secretion in conscious dogs with pouches (142, 143). Studies of frog (66, 231) and *Necturus* mucosa (137, 138) show Na⁺-HCO₃⁻ cotransport at the basolateral membrane as the major mechanism for import of HCO₃⁻. Recently, it was demonstrated that expression of the Na⁺-HCO₃⁻ cotransporters NBC1 and NBC2 in rabbit stomach is at a higher level (5.5- and 2.5-fold higher, respectively) in the mucus containing surface cells than in the parietal cells (196). The inhibition of alkaline secretion *in vitro* and *in vivo* by carbonic anhydrase inhibitors, although at relatively high doses (61, 75, 193), does suggest that some HCO₃⁻ is also formed intracellularly from CO₂ and H₂O. Secretion of HCO₃⁻ by frog fundic mucosa *in vitro* is dependent on luminal but not serosal Cl⁻ (63) and is, in contrast to that of H⁺ transport, not associated with changes in the transmucosal electrical potential difference (61). Recent immunological staining of rat and rabbit gastric mucosa demonstrated expression of a Cl⁻/HCO₃⁻ exchanger of the SLC4 family, termed anion exchanger iso-

form 4 (AE4), in the apical membranes of gastric surface epithelial cells (262). These results provide strong evidence that $\text{Cl}^-/\text{HCO}_3^-$ exchange is indeed a HCO_3^- export process in the gastric mucosa. Interestingly, recent work (48) also suggested an association between nonacidic Cl^- secretion and HCO_3^- secretion.

Recordings of pH within the lumen of the gastric glands have provided further information on the secretion of HCO_3^- by the gastric mucosa. Intraglandular pH in acid-inhibited, cimetidine-pretreated sheets of frog (*Rana esculenta*) fundic mucosa was measured with double-barreled pH glass microelectrodes inserted into the glandular lumen (53). The fluid was found to be slightly more alkaline than the bathing solution, and, interestingly, both carbachol and gastrin increased transport of alkali into the intraglandular lumen. The authors proposed that oxyntopeptic cells contribute to the gastric alkaline secretion in this species. In another study using double-barreled pH-sensitive microelectrodes with guinea pig fundic mucosa (206, 207), there was a gradient of increasing pH along the crypt lumen from 3.0 in the parietal cell region to 4.6 in crypt outlets. However, it should be stressed that the rate of acid secretion by mammalian gastric mucosa in vitro usually is much lower ($\leq 5\%$) than that in vivo. This difference in rates may reflect insufficient oxygenation of the circulation deprived mammalian tissues in vitro. Furthermore, oxygen-deficient mucosal cells may swell and release mucus. The pH-sensitive indicator Lysosensor yellow-blue was used for measurement of net acid transport within isolated gastric glands in a recent elegant study (182). From the transient nature of the response to carbachol, it was proposed that this secretagogue may induce intraglandular secretion of alkali and/or proteins, such as pepsinogens and mucins, from chief and other nonparietal cells, that may buffer acid in the intraglandular lumen. An only slightly acidic intraglandular pH (pH ~ 5.3) was found upon confocal microscopy of rat fundic mucosa in vivo, and treatment with dimethyl-PGE₂ increased intraglandular pH (49). E-type prostaglandins, which stimulate both gastric and duodenal alkaline secretion (65, 235), are also well-known inhibitors of gastric H^+ secretion (256). These combined studies of intraglandular pH provide evidence that some HCO_3^- and/or acid-buffering substance originates from cells within the gastric glands, but this is unlikely to be quantitatively significant in net gastric mucosal HCO_3^- secretion in vivo.

DUODENAL ALKALINE SECRETION

In all amphibian and mammalian species tested, the duodenal mucosal surface enterocytes secrete HCO_3^- , and they do so at higher rates (per unit surface area) than does the stomach or more distal small intestine (43, 61, 64, 99, 143, 173, 214). Secretion of HCO_3^- by the duodenum was originally observed in proximal duodenum in dogs, and the submucosal glands (i.e., Brunner's glands) were then assumed to be the origin (65, 78). However, secretion by Brunner's glands could not be separated from that of the mucosa itself in early experiments. Brunner's glands are unique to mammalian species and are confined mainly to the submucosa. Secretory units consist primarily of a mucin-producing cell type, and in addition to mucus, these cells are known to secrete epidermal growth factor and trefoil peptides (144). Video microscopy of the acinar lumen of Brunner's glands in guinea pig duodenum in

vitro demonstrated secretory responses to cholinergic vagal fibers but not to capsaicin-sensitive or enteric intrinsic nerves (165). Secretion of a limited amount of HCO_3^- by Brunner's glands cannot be excluded in some species, but this has not been experimentally confirmed, and micropuncture studies of the gland or duct lumen need to be done.

Knowledge about the HCO_3^- transport processes in the duodenal epithelium per se originates from numerous studies (65, 69) involving animals and humans in vivo, amphibian and mammalian mucosa in vitro, isolated mammalian duodenal enterocytes, isolated cell membranes, and, more recently, genetically modified mice. Microfluorospectrophotometric studies of mixed villous and crypt enterocytes isolated from proximal duodenum and loaded with the pH-sensitive fluoroprobe BCECF (117) confirmed previous hypotheses based on studies of amphibian mucosa in vitro (66), namely, that duodenal enterocytes possess at least three mechanisms for acid-base transport (Fig. 3). These are 1) amiloride-sensitive Na^+/H^+ exchange, which extrudes acid; 2) duodenal enterocytes, which import HCO_3^- at the basolateral membrane by $\text{Na}^+(\text{n})\text{-HCO}_3^-$ cotransport and export HCO_3^- via an apical anion conductive pathway and $\text{Cl}^-/\text{HCO}_3^-$ exchange; and in addition, 3) paracellular migration of HCO_3^- , which is dependent on transmucosal hydrostatic pressure in vitro (64) and intestinal motility in

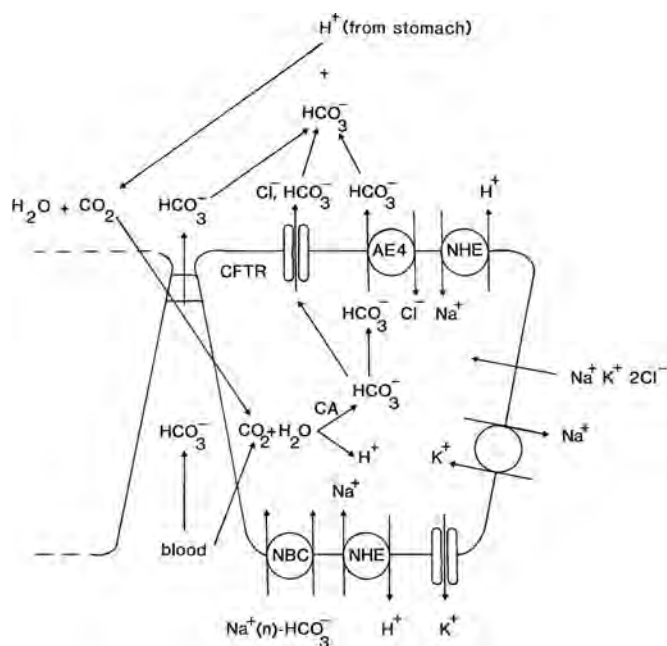


Fig. 3. Duodenal acid-base transporters. The model is based on studies of isolated duodenal enterocytes, membrane vesicles, and duodenal mucosa in vitro and in vivo in several species and, more recently, from studies of genetically modified mice. Species differences, if any, seem small, but the mucosal location of the transport processes (villus and/or crypt) has not yet been fully determined. HCO_3^- reaches the epithelium via blood as well as secondary to the intracellular carbonic anhydrase (CA) conversion of $\text{CO}_2 + \text{H}_2\text{O}$. HCO_3^- is imported via $\text{Na}^+\text{-HCO}_3^-$ transport and exported via an apical conductance (CFTR) pathway and $\text{Cl}^-/\text{HCO}_3^-$ exchange. In addition, there is paracellular migration of HCO_3^- , dependent on intestinal transmucosal hydrostatic pressure and motility. Na^+/H^+ exchanger isoforms NHE1, NHE2, and NHE3 are all reported to be expressed and contribute to intracellular pH regulation in duodenal enterocytes. Evidence for electrogenic as well as electroneutral $\text{Na}^+\text{-HCO}_3^-$ cotransporters (NBC) has been presented. Any factors that diminish HCO_3^- export may increase the vulnerability of the mucosa to acid injury. AE, ion exchanger.

vivo (172, 174). Studies of duodenum from knockout mice deficient in the cystic fibrosis transmembrane conductance regulator (CFTR) (45, 93, 107, 129, 209) provide strong evidence that CFTR is the membrane-spanning conductance transporting HCO_3^- as well as Cl^- (Fig. 3). CFTR is presently considered a principal anion channel pathway in Cl^- and HCO_3^- export in airway and intestinal epithelia, but it also exerts several cellular actions independent of channel activity (32). Endoscopic specimens mounted in Ussing chambers have been used in a study of CFTR activity in human duodenum (186). In line with findings from mouse duodenum, basal HCO_3^- secretion and short-circuit current were significantly lower in biopsies from patients with cystic fibrosis than in those from normal subjects. Present evidence thus strongly suggests that CFTR is the important apical conductive pathway in agonist-stimulated duodenal mucosal HCO_3^- secretion mediated by cAMP as well as by cGMP and intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) (22, 45, 107, 129, 209, 222). Basal HCO_3^- secretion, in contrast, seems mainly dependent on apical $\text{Cl}^-/\text{HCO}_3^-$ exchange (222, 254). Recently, PAT1 (SLC26A6) was identified as a major $\text{Cl}^-/\text{HCO}_3^-$ exchanger in the apical membrane of the duodenum (257), along with the DRA (SLC26A3) $\text{Cl}^-/\text{HCO}_3^-$ exchanger (121, 161).

Parallel operation of CFTR Cl^- channels and apical $\text{Cl}^-/\text{HCO}_3^-$ exchangers is a possible mechanism for cellular export of HCO_3^- (251). The CFTR channel would provide Cl^- to the luminal surface and act as a Cl^- leak pathway, preventing intracellular accumulation of Cl^- . However, it was found in rabbit and rat duodenum in vitro that $\text{Cl}^-/\text{HCO}_3^-$ exchange is neither necessary nor increased upon cAMP-induced stimulation of mucosal HCO_3^- secretion (222). In contrast, CFTR-dependent $\text{Cl}^-/\text{HCO}_3^-$ exchange does occur in cAMP-stimulated mouse duodenum (45). The difference between species may reflect relatively greater rates of secretion in the mouse duodenum, a thinner preparation that would seem easier to maintain well oxygenated in vitro. It should be noted that uroguanylin, inducing cGMP-mediated HCO_3^- secretion, also causes some increase in transmucosal short-circuit current in duodenum from CFTR knockout mice (129). This may suggest cGMP-induced activation of a minor electrogenic pathway, obscured in wild-type animals by the larger CFTR conductance.

It has been believed that intestinal secretions are of crypt origin, whereas absorptive functions reside in villi. Interestingly, recent work (21, 22) suggested involvement of the CFTR channel in secretion by duodenal villus enterocytes. Expression of CFTR in human and rat duodenum was characterized by immunofluorescence and immunoelectron microscopy by using anti-CFTR and enzyme marker antibodies. A subpopulation (2.5%) of villus enterocytes expressed very high levels of CFTR throughout the cells, with the greatest concentration apically. Thus CFTR-dependent export of HCO_3^- may be a property of villus as well as crypt cells. Finally, it was recently observed in clusters of rat and human duodenal enterocytes (218) that melatonin-induced $[\text{Ca}^{2+}]_i$ signaling spread throughout the clusters, indicating that duodenal enterocytes interact and that the mucosa functions as a syncytium. This would enhance an integrated action of duodenal secretagogues.

A role for the CFTR channel in duodenal HCO_3^- export has been questioned on the basis of a study comparing transport of acid-base equivalents across the plasma membrane of proximal

duodenal enterocytes in CFTR-deficient mice with that in normal littermates (188). The acid extrusion and the alkaline extrusion rates were unaffected by CFTR deficiency, and acid-base transport seemed mediated almost exclusively by Na^+/H^+ exchange, $\text{Cl}^-/\text{HCO}_3^-$ exchange, and $\text{Na}^+-\text{HCO}_3^-$ cotransport. Absence of CFTR conductance in these studies might reflect the use of enterocytes rather than intact mucosa. The possibility cannot be excluded that cell isolation procedures induce cellular changes preventing migration of cytoplasmic CFTR (22, 251) to the duodenal enterocyte apical surface. The molecular expression and localization of $\text{Na}^+-\text{HCO}_3^-$ cotransporters (NBC) in mouse duodenum were recently studied using RT-PCR, sequence analysis, and immunohistochemistry (189). Enterocytes expressed mRNA encoding two electrogenic NBC1 isoforms and the electroneutral NBCn1. Both NBC1 and NBCn1 were localized to the basolateral membrane of duodenal villus enterocytes, whereas crypt enterocytes did not label with the anti-NBC antibodies. In membrane vesicles from rabbit duodenal enterocytes (120), pH gradient-driven Na^+ uptake was partly HCO_3^- and DIDS sensitive and partly dependent on the Na^+/H^+ exchange isoform NHE1.

Na^+/H^+ exchange would supply intracellular HCO_3^- by export of H^+ formed on hydration of CO_2 to H^+ and HCO_3^- . The Na^+/H^+ exchanger isoforms NHE1 as well as NHE2 and NHE3 are expressed in mouse duodenal enterocytes, and all three isoforms were reported to contribute to regulation of intracellular pH (pH_i) in these cells (187). In contrast, specific inhibition of the apical isoform NHE3 by the compound S3226 did not affect pH_i in rat villus enterocytes in situ (85). However, the latter study (85) also showed that this compound causes a slowly developing rise in HCO_3^- secretion by duodenum in anesthetized rats. A rise in net alkaline (HCO_3^- minus H^+) secretion could reflect inhibition of a smaller, and therefore concealed, H^+ export. Luminal administration of the anion channel inhibitor 5-nitro-2-(3-phenylpropylamino)benzoic acid prevented the rise in net alkaline secretion, indicating an action on HCO_3^- export per se. The authors suggested interaction between NHE3 exchanger activity and the CFTR channel. Finally, entry of Cl^- for secretion across epithelial apical membranes predominantly depends on import of Cl^- by basolateral $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ cotransport (NKCC). Basal and cAMP-stimulated HCO_3^- secretion in duodenum from NKCC1 knockout mice was very similar to that in wild-type animals, indicating that apical export of HCO_3^- does not depend on basolateral $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ cotransport activity (254).

STIMULATION OF GASTRODUODENAL BICARBONATE SECRETION BY LUMINAL ACID

Presence of acid in the lumen is a powerful stimulant of gastric and duodenal HCO_3^- secretion. This has been demonstrated in several species, including humans (50, 116, 118), dogs (141), cats (67, 74), rats (72, 112, 216, 233), pigs (2), and frogs (99). The presence of a pH gradient within the mucus gel adherent to both gastric and duodenal mucosae raises the interesting question of how acid present in the lumen is sensed by the HCO_3^- -secreting epithelium if the mucus pH gradient does indeed prevent a lowering of pH at the mucosal surface. An explanation for this is that the secreting mucosa senses PCO_2 rather than pH. In support of this, CO_2 generated at the

surface during reaction between luminal H^+ and secreted HCO_3^- has been shown to be the stimulus of alkaline secretion in rat *in vivo* (112). Acid and/or PCO_2 -sensitive cell filaments (221) or neural receptors protruding into the surface mucus gel could be an additional mechanism for sensing bulk luminal acidity.

The impaired alkaline response to acid in patients with duodenal ulcer disease (40, 108, 118, 163) has focused interest on mediation of this duodenal response. A low pH in the duodenal lumen (\sim pH 5 in rat and \sim pH 3 in human) caused a marked, up to fivefold (60, 72) rise in the secretion. The response is mediated by neural reflexes and mucosal production of prostaglandins (23, 65, 235) and also, very likely, by locally produced uroguanylin (129). Several transmitters, including VIP and acetylcholine, are mediators of the efferent limb of the neural response. Chemical deafferentiation by capsaicin inhibits the rise in HCO_3^- secretion in response to luminal acid (114). The response to exogenous PGE_2 , in contrast, is not affected by the destruction of the afferent neurons. Thus this suggests that luminal acidification involves the enteric nervous system, whereas PGE_2 acts directly on the HCO_3^- -producing cells (23, 235). Recent findings suggest that prostaglandins stimulate duodenal HCO_3^- secretion by acting on duodenal EP_3 receptors as well as EP_4 receptors. Furthermore, it has been proposed (23) that stimulation via EP_4 receptors is mediated by cAMP, whereas that via EP_3 receptors is mediated by cAMP production as well as $[Ca^{2+}]_i$ signaling. In addition, there is an interaction between these stimulatory pathways. In the stomach, prostaglandins stimulate HCO_3^- by affecting the gastric EP_1 receptors (235). Affirming a role for prostaglandins and epithelial HCO_3^- in protection against mucosal injury, the duodenal mucosa in EP_3 knockout mice has a markedly decreased ability to resist luminal acid damage (234). NO is another interesting mediator of the rise in duodenal alkaline secretion in response to luminal acid. Luminal acid stimulates the expression of inducible NO synthase in the duodenal villi, and it is proposed that this induces synthesis of NO (113).

It would seem physiologically rational that the presence of acid in the gastric lumen would result in an anticipatory rise in alkaline secretion by duodenal mucosa about to receive an acid load. However, instillation of acid into the ligated stomach or, conversely, decreasing gastric acidity by inhibition of acid secretion, does not affect duodenal HCO_3^- secretion (65, 70, 99).

NEUROHUMORAL CONTROL OF BICARBONATE SECRETION

The stimulatory action of sham feeding demonstrates that gastric (80, 81, 141) and duodenal (26, 141) alkaline secretions are under central nervous system influence. Vagally mediated inhibition of duodenal HCO_3^- absorption also has been reported and explained by inhibition of villus Na^+/H^+ exchanger activity by an atropine-sensitive cholinergic mechanism (160). Studies in fasting rats demonstrated circadian rhythms in the gastric secretion of H^+ , HCO_3^- , and mucus (147, 148). Interestingly, the secretion of HCO_3^- and that of H^+ followed circadian rhythms with different peak times. This could, in theory, result in circadian rhythmicity of mucosal vulnerability to acid injury. Intracerebroventricular infusion of neurotransmitters and drugs has been used to further elucidate the central nervous system influence on gastroduodenal alkaline secretion. Centrally elicited stimulation of the duodenal secretion has

been observed with some neuropeptides, including thyrotropin-releasing hormone (TRH), corticotropin-releasing factor (CRF), and bombesin (70, 151, 152), and with some benzodiazepines (199). Furthermore, the α_1 -receptor agonist phenylephrine (Fig. 4) caused a marked, up to fivefold, centrally elicited duodenal HCO_3^- secretion (149, 216, 217). Intrahypothalamic injection of CRF increased HCO_3^- secretion and also enhanced mucosal protection in rat stomach (95).

A variety of peripherally acting agents have been found to influence HCO_3^- secretion in the duodenum and are summarized in Table 1. Primary sites of action include the enteric nervous systems, enterocyte membrane receptors, and local mucosal production of eicosanoids. Some of the more recently described secretagogues, namely, melatonin, uroguanylin, orexin A, and stress-related inhibitors of the duodenal secre-

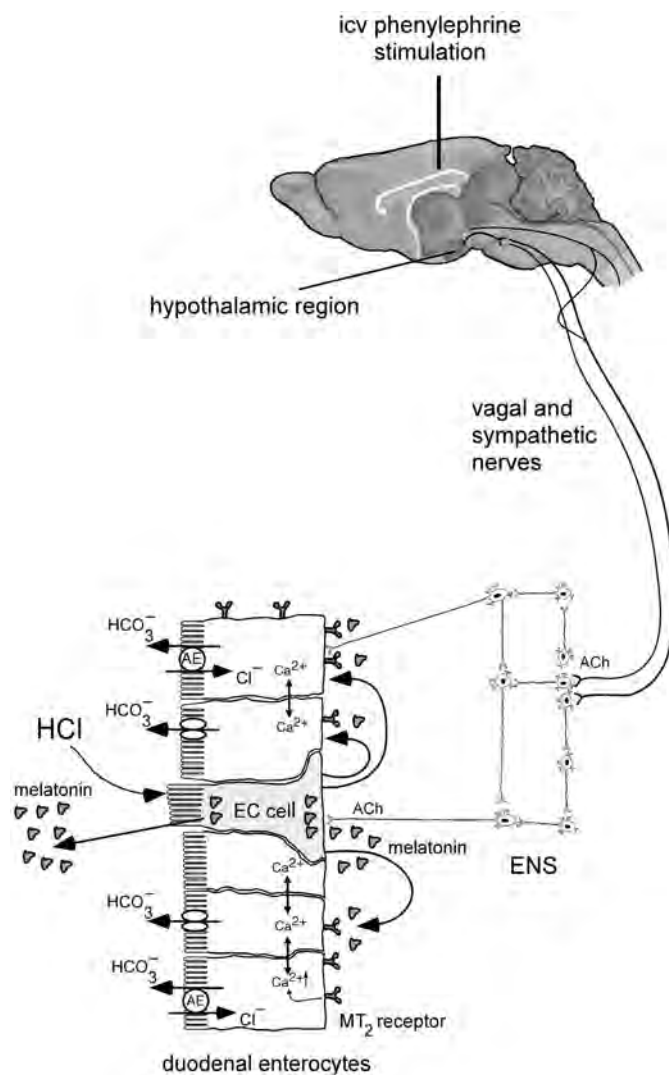


Fig. 4. Proposed role of melatonin in control of duodenal mucosal HCO_3^- secretion. Melatonin released from intestinal enterochromaffin cells stimulates the protective secretion via an action at enterocyte MT_2 receptors. Release is initiated by central nervous system (intracerebroventricular) administration of adrenoceptor agonist phenylephrine and by a low pH in the duodenal lumen. Neural pathways involve sympathetic as well as vagal components and the enteric nervous system (ENS). The action of melatonin at enterocyte membrane receptors also has been demonstrated in clusters of human duodenal enterocytes. [Adapted from Sjöblom et al. (216).]

Table 1. *Peripherally acting transmitters and physiological conditions that affect duodenal mucosal bicarbonate secretion*

Stimulation	
cAMP mediated	Dopamine D ₁ (76, 198), PACAP (89, 232), prostaglandin EP ₃ and EP ₄ (23, 64, 74, 116, 234, 235), VIP (43, 73, 175, 194, 198, 261), 5-HT ₄ (249, 250)
cGMP mediated	Guanylin (93, 192), uroguanylin (129), heat-stable enterotoxin (93, 129)
[Ca ²⁺] _i mediated	CCK ₁ (42, 218), melatonin MT ₂ (217, 218), muscarinic M ₃ (42, 218), CCK ₁ (42, 218), prostaglandin EP ₄ (23)
Mediator not determined	Angiotensin II AT ₂ (126), β-endorphin (71, 151), cholera toxin (68, 124), glucagon (1, 67, 93, 258), orexin A (77), neurokinin A (96, 97)
Physiological conditions	Luminal acid (2, 4, 5, 74, 99, 112, 118, 233), sham feeding (26, 141)
Inhibition	
Neurotransmitters	α ₂ -adrenoceptor (58, 69, 125, 128, 139), neuropeptide Y (69)
Enzyme inhibitors	Acetazolamide (45, 64, 140, 233), COX inhibitors (64, 72, 162, 235)
Other	<i>Helicobacter pylori</i> infection (108)

Representative types of agents that alter duodenal mucosal HCO₃⁻ secretion in animals and humans by a peripheral action. Effects of NO (not listed) are complex in that both inhibitory and stimulatory actions as well as species differences are reported (113, 172). In addition to these agents with a peripheral action, some hormones, neurotransmitters, and drugs influence the central nervous system's control of HCO₃⁻ secretion. COX, cyclooxygenase; [Ca²⁺]_i, intracellular Ca²⁺ concentration.

tion, are discussed below. Melatonin is released from the pineal gland in the central nervous system and from enterochromaffin cells in the intestinal epithelium. The amount produced in the intestine is ~400 times greater than that in the central nervous system, but the role of melatonin in gastrointestinal function has not been elucidated (39). Recent studies (215, 217) have demonstrated that close intraarterial infusion of melatonin and agonists into the duodenum in rats increased HCO₃⁻ secretion, with a low dose of melatonin (20 nmol·kg⁻¹·h⁻¹) causing maximal stimulation. Furthermore, melatonin induces enterocyte [Ca²⁺]_i signaling in clusters of human and rat duodenal enterocytes (218). The main pattern of response was similar to that observed in response to carbachol and cholecystokinin octapeptide (42) in duodenal enterocytes in primary culture. The responses to melatonin in vivo as well as on enterocyte signaling were inhibited by MT₂-selective melatonin antagonists. Furthermore, the antagonist luzindole almost abolished the marked rise in secretion induced by intracerebroventricular infusion of the adrenoceptor agonist phenylephrine but did not affect the rise in release of melatonin to the duodenal lumen (216) induced by the central nervous system stimulation. This rise in secretion in response to central nervous system phenylephrine was also abolished by sublingual ligation of all nerves around the carotid arteries but was unaffected by removal of either the pineal gland or pituitary gland. Melatonin thus stimulates duodenal HCO₃⁻ secretion via action at enterocyte MT₂ receptors and mediates neural stimulation of the secretion. Interestingly, there is a strong disturbance of melatonin secretion in the exacerbation as well as remission stages of duodenal ulcer disease in patients (158).

Uroguanylin and guanylin are endogenous ligands for the transmembrane guanylate C receptor and increase enterocyte cGMP production. Both peptides are present throughout the length of the (rat) intestine. However, uroguanylin mRNA is most abundant in proximal and guanylin mRNA in distal segments of the small intestine, and uroguanylin, unlike guanylin, is resistant to digestion by the pancreatic enzyme chymotrypsin (84). Luminally applied uroguanylin (129) and guanylin (93, 192), like *Escherichia coli* heat-stable enterotoxin (St_a), stimulate HCO₃⁻ secretion by rat and mouse duodenal mucosa. The ratio between the HCO₃⁻ and total anion (Cl⁻ plus HCO₃⁻) secretory rates is considerably higher after stimulation with guanylin than after stimulation with a cAMP-dependent agonist such as PGE₂ or VIP (93). Interestingly, acidification (pH 5.0–5.5) of the lumen of isolated mouse duodenum enhanced the stimulatory action of uroguanylin on mucosal HCO₃⁻ transport and short-circuit current (129). An important role of cGMP-dependent agonists in mucosal acid protection is further supported by the recent observation (192) that the rise in duodenal HCO₃⁻ secretion in response to luminal acid is markedly smaller in guanylate C receptor knockout mice than in wild-type animals.

Orexins are involved in the central nervous system's control of appetite and behavior and appear to be involved in short-term regulation of feeding rather than long-term regulation of body weight (134, 145, 200). These peptides are present in endocrine cells and/or neurons in the intestine, but their effects on mucosal function and protection are largely unknown. Recent studies (77) have shown that close intraarterial infusion of low doses of orexin A (Fig. 5) caused a marked and dose-dependent stimulation of the duodenal HCO₃⁻ secretion in

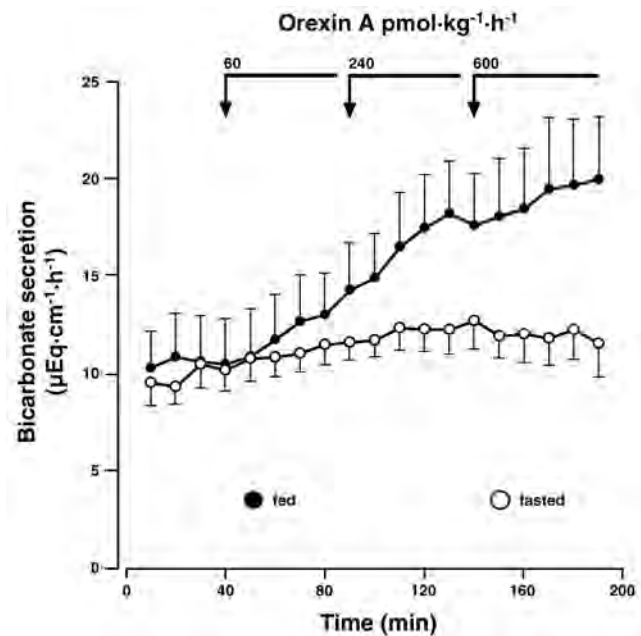


Fig. 5. Short (overnight) food deprivation markedly decreases the sensibility of the HCO₃⁻ secreting rat duodenum to the appetite-regulating peptide orexin A. Similarly, short fasting caused a 100-fold increase in the amount of the muscarinic agonist bethanechol required for stimulation of the secretion. In contrast, the secretory responses to VIP and melatonin were not affected. Compounds were administered by close intraarterial infusion and HCO₃⁻ secretion continuously titrated. [Reprinted from Flemström et al. (77).]

rats. However, stimulation occurred only in animals that had continuous access to their regular food. Short (overnight) deprivation of food abolished the stimulation of the duodenal HCO_3^- secretion. Similarly, short fasting caused a 100-fold increase in the amount of the muscarinic agonist bethanechol required for stimulation of the secretion. In contrast, the HCO_3^- secretory responses to VIP and melatonin were not affected (Fig. 5). An attractive explanation for the changes in sensitivity to orexin A and the muscarinic agonist would be that food constituents, either directly or indirectly by central or peripheral mechanisms, stimulate the activity or expression of signal pathways or receptors in the intestinal mucosa. It should also be noted that overnight fasting is a standard experimental procedure in studies of gastrointestinal function and pathophysiology in humans and animals. In view of these differences in response between the fed and fasted states, studies made on neuroendocrine control of mucosal protection and intestinal secretion may require reevaluation with respect to feeding status.

It is likely that stress-induced reactions contribute to gastroduodenal damage. Splanchnicotomy or adrenergic blockade ameliorates stress-induced gastroduodenal ulceration in animals (65), whereas increased plasma levels of noradrenalin have been reported in patients with duodenal ulcer disease (123). The effects on mucosal HCO_3^- secretion resulting from elicitation of sympathetic reflexes and administration of α -adrenoceptor ligands and some neuropeptides have been studied in animals and humans (58, 128, 219). α_2 -Adrenoceptors (58, 62, 128, 139) as well as neuropeptide Y1 receptors (69) mediate sympathetic inhibition of mucosal HCO_3^- secretion and defense.

DUODENAL PROTECTION AGAINST ACID

In the first part of the duodenum, mucosal HCO_3^- is secreted in immediate proximity to the epithelial cell surface and also proximal to the entry of HCO_3^- from pancreaticobiliary duct at the tubercle (papilla) of Vater. Despite this entry of pancreatic HCO_3^- distal to the duodenal bulb, luminal pH of the latter falls below pH 2.0 only sporadically and in short (5–10 s) spikes, and this is so even in humans with exocrine pancreatic secretory deficiency (177). Median pH in the duodenal bulb during the first hour after a meal was 3.99 in healthy controls and 3.79 in patients with pancreatic deficiency. In a recent study in fasting humans (122), gastric acid discharge and duodenal alkaline secretion were found to be dynamically coordinated with very few values of pH within the duodenal bulb being below pH 5.0. The juxtamucosal pH in rat duodenum *in situ* (72, 136) remained at neutrality during 15-min exposure to 10 mM HCl (pH 2.0) and decreased only slightly on exposure to 20 mM HCl. In healthy humans, the juxtamucosal pH in the duodenal bulb has been reported to remain at neutrality when luminal pH was as low as pH 1.5 (190). These combined data provide good evidence that in the healthy duodenum, the mucosa exports HCO_3^- at rates sufficient for maintaining its surface neutrality. In proximal duodenum, the adherent mucus layer is thinner than that in stomach, considerably so if one considers the firm mucus layer not removed by suction (Fig. 1). However, it would appear that, when necessary, it is of sufficient thickness to maintain a stable surface pH gradient under conditions of motility and low luminal pH *in vivo*.

Studies in several species with a variety of modulators of secretion demonstrate the key importance of epithelial HCO_3^- secretion in duodenal mucosal protection against luminal acid. Stimulation and inhibition of alkaline secretion respectively increase and decrease the juxtamucosal pH at the duodenal mucosal cell surface (72, 136). Stimulation of mucosal HCO_3^- secretion by VIP, at doses not affecting mucosal blood flow, protected the duodenal mucosa in rat (175) and pig (1) against morphological damage induced by 10 mM luminal HCl and 30 mM luminal HCl, respectively. Stimulation of mucosal HCO_3^- secretion by glucagon significantly reduced the duodenal mucosal damage induced by 50 mM HCl in the rabbit (258). Inhibition of mucosal HCO_3^- secretion by parenteral administration of NH_4Cl , vasopressin, or furosemide, in contrast, increased duodenal mucosal damage (258). Modest, acute hypovolemia markedly inhibited mucosal alkaline secretion in rat duodenum (125, 128). This procedure also significantly increased the morphological changes induced by 15-min exposure of the duodenal lumen to 100 mM HCl (125), although it had no effect at the lower concentration (10 mM) of acid. Furthermore, E-type prostaglandins, which increase duodenal mucosal alkaline secretion in all species tested, *in vivo* as well as *in vitro*, also increase mucosal resistance to luminal acid (64, 116, 233, 234, 235, 263). Inhibition of mucosal endogenous prostaglandin production by COX inhibitors reduces the alkaline secretion in humans (162) and in several (2, 64, 72, 233, 235) but not all (172) experimental models, and these compounds are well-known inducers of duodenal ulceration in experimental animals as well as in humans (229, 256).

An acid-protective role for the duodenal mucosal alkaline secretion is further supported by the finding that HCO_3^- secretion in proximal duodenum (40, 108, 118, 163), and, in particular, the rise in secretion in response to luminal acid is depressed in patients with duodenal ulcer disease. It has also been reported the pH at the surface of this epithelium in duodenal ulcer patients is lower than that in healthy subjects (190). The HCO_3^- secretion is reported to normalize after eradication of the bacterium *Helicobacter pylori* in patients with such infection (108). Interestingly, evidence has been presented that *H. pylori* infection inhibits antral mucosal production of NO (253), a transmitter thought to be important in mediation of the duodenal alkaline response (113). Perhaps surprisingly, E-type prostaglandin release from duodenal mucosa in patients with duodenal ulcer disease was greater than that in healthy controls (40). This could indicate that decreased sensitivity to E-type prostaglandins also may be part of the depression of the alkaline secretion in duodenal ulcer disease.

One additional mechanism of duodenal defense, namely, intracellular neutralization of acid, has been proposed from studies of pH_i in apical villus cells in rat duodenum *in situ*. pH_i was measured using pH-sensitive microelectrodes (178) or fluorescence microscopy of the duodenal surface after loading with the pH-sensitive compound BCECF-AM (4, 5). During acid perfusion, pH_i decreased, whereas mucus gel thickness and blood flow increased. At the highest luminal acidity tested (pH 2.2), the recorded decrease in pH_i after 5 min was from ~ 7.10 to ~ 6.30 with the fluorescence microscopy technique. The changes in pH_i recorded with intracellular microelectrodes, and in response to a somewhat higher acidity (10 mM HC), were smaller (from pH 7.46 to pH 7.21) than those recorded with fluorescence microscopy. With both techniques,

this decrease in pH_i was smaller on a second brief exposure to acid. This interesting adaptation to rapid shifts in duodenal luminal acidity was sensitive to DIDS and was explained by acid-induced augmented cellular uptake of HCO_3^- by the enterocyte basolateral $\text{Na}^+\text{-HCO}_3^-$ cotransporter. Subsequent perfusion with neutral luminal solution increased pH_i above baseline values (4), and, in line with the higher rates of transcellular HCO_3^- transport in proximal duodenum (115, 116, 214), acid-induced decreases in pH_i were smaller in proximal than in distal duodenum (178). It thus would seem likely that intracellular neutralization of acid reflects the basolateral part of the processes for transcellular transport of HCO_3^- into the duodenal lumen (Fig. 3). It should be noted that, mainly, pH_i in apical enterocytes in the duodenal villi was examined in these studies and that decreases in pH_i may reflect acidification by high levels of CO_2 (98, 112, 122) formed within the duodenal mucus gel during reaction between acid from the lumen and secreted HCO_3^- .

CONCLUSIONS

Since the first experimental evidence for the mucus bicarbonate barrier was reported nearly three decades ago, it has become firmly established as a key component of the gastroduodenal mucosal protective mechanisms against acid and pepsin. The secretion of HCO_3^- into a stable, adherent mucus gel layer creates a pH gradient at the epithelial surface in stomach and duodenum and provides the first line of mucosal defense against luminal acid. The mucus gel layer is an effective barrier to luminal pepsin, and evidence points to it as the major protective mechanism against proteolytic digestion of the underlying epithelium by this enzyme.

The numerous pH gradient studies are good experimental evidence for the existence of the mucus bicarbonate barrier in vivo and the establishment of a nearly neutral pH at the epithelial surface. The gastric epithelial cells may be slightly and transiently acidified during acute exposure to high concentrations of luminal acid, but mainly, the surface epithelium in acid-secreting gastric mucosa exports HCO_3^- at rates sufficient to maintain a neutral pH at the apical cell surfaces. During acid secretion, parietal cells release HCO_3^- into the mucosal interstitium and vasculature. This increased interstitial HCO_3^- , the alkaline tide, is imported by the surface epithelial cells, significantly enhancing mucosal alkaline secretion and surface alkalinity. Uncertainties have been raised as to whether a more alkaline or acidic juxtamucosal pH exists at the mucosal surface at higher luminal pH values (pH 5), and more experimentation is needed to resolve this question. The two approaches to studying pH gradients are not directly comparable either, because pH-sensitive microelectrode studies, at luminal pH 1–3, have been conducted primarily in unstirred conditions, whereas the confocal imaging studies at pH 3–5 were performed in a perfused system.

In duodenum, the mucosal HCO_3^- secretion is currently accepted as the primary defense mechanism against acid discharge from the stomach. Thus the duodenal epithelium secretes HCO_3^- at higher rates (per unit surface area) than does the stomach or, for that matter, the more distal small intestine. In the first part of the duodenum, mucosal HCO_3^- , secreted proximal to the entry of HCO_3^- from the pancreaticobiliary duct, maintains a pH gradient with a neutral pH at the mucus

epithelial interface under all luminal acidities encountered in the healthy organ. Presence of acid in the lumen is a powerful stimulant in duodenum and causes up to a fivefold increase in duodenal HCO_3^- secretion as well as an increased mucus gel secretion. Furthermore, there is an impaired HCO_3^- secretory response to acid in patients with duodenal ulcer disease. The alkaline response to acid is mediated by neural reflexes and mucosal production of prostaglandins and, as shown recently, by locally produced uroguanylin. Melatonin released from enterochromaffin cells in the duodenal mucosa, furthermore, may be involved in control of HCO_3^- secretion, and a role also has been proposed for local mucosal dopamine.

Finally, this review is confined to aspects of the mucus bicarbonate barrier and this barrier's role in protection against the natural endogenous aggressors acid and pepsin. Although there is an array of other aggressive factors that have been shown to be integral to the pathology of peptic ulceration, the principle as proposed by Schwartz in 1910 (208) still holds: no peptic activity, no ulcer. Furthermore, it should be stressed that the mucus bicarbonate barrier is one of many integrated components that form the complete protective mucosal barrier. It is the initial, and only, preepithelial barrier between the lumen and the gastric surface epithelium, and as discussed in this review, evidence suggests that it is sufficient for protection against luminal acid and pepsin during normal digestive processes. Mucosal protection for parietal and chief cells within the gastric crypts is different, with their cell apical membranes apparently resistant to their intracryptal secretions (37, 245). When the mucus bicarbonate barrier is overwhelmed or when it breaks down in disease, then there are a whole series of protective mechanisms that come into play, including intracellular neutralization of acid, rapid epithelial repair and maintenance, and distribution of mucosal blood flow.

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REFERENCES

1. Ainsworth MA, Fenger C, Svendsen P, and Schaffalitzky de Muckadell OB. Effect of stimulation of mucosal HCO_3^- secretion on acid-induced injury to porcine duodenal mucosa. *Scand J Gastroenterol* 28: 1091–1097, 1993.
2. Ainsworth MA, Svendsen P, Glad H, Andersen NJ, Olsen O, and Schaffalitzky de Muckadell OB. Duodenal mucosal bicarbonate secretion in pigs is accompanied by compensatory changes in pancreatic and biliary HCO_3^- secretion. *Scand J Gastroenterol* 29: 889–896, 1994.
3. Akiba Y, Furukawa O, Guth PH, Engel E, Nastaskin I, and Kaunitz JD. Sensory pathways and cyclooxygenase regulate mucus gel thickness in rat duodenum. *Am J Physiol Gastrointest Liver Physiol* 280: G470–G474, 2001.
4. Akiba Y, Furukawa O, Guth PH, Engel E, Nastaskin I, and Kaunitz JD. Acute adaptive cellular base uptake in rat duodenal epithelium. *Am J Physiol Gastrointest Liver Physiol* 280: G1083–G1092, 2001.
5. Akiba Y, Furukawa O, Guth PH, Engel E, Nastaskin I, Sassani P, Dukkkipatis R, Pushkin A, Kurtz I, and Kaunitz JD. Cellular bicarbonate protects rat duodenal mucosa from acid-induced injury. *J Clin Invest* 108: 1807–1816, 2001.
6. Akiba Y, Guth PH, Engel E, Nastaskin I, and Kaunitz JD. Dynamic regulation of mucus thickness in the rat duodenum. *Am J Physiol Gastrointest Liver Physiol* 279: G437–G447, 2000.

7. **Allen A.** Structure of gastrointestinal mucus and the viscous and gel-forming properties of mucus. *Br Med Bull* 34: 28–33, 1978.
8. **Allen A.** Gastrointestinal mucus. In: *Handbook of Physiology. Gastrointestinal Physiology. Salivary, Gastric, Pancreatic, and Hepatobiliary Secretion*. Bethesda, MD: Am. Physiol. Soc. 1989, sect. 6, vol. III, chapt. 19, p. 359–382.
9. **Allen A and Carroll NJH.** Adherent and soluble mucus in the stomach and duodenum. *Dig Dis Sci* 30: 55S–62S, 1985.
10. **Allen A, Cunliffe WF, Pearson JP, and Venerables CW.** The adherent gastric mucus gel barrier in man and changes in peptic ulceration. *J Intern Med* 228, Suppl 732: 83–90, 1990.
11. **Allen A, Flemström L, Garner A, and Kivilaakso E.** Gastroduodenal mucosal protection. *Physiol Rev* 73: 823–857, 1993.
12. **Allen A, Flemström G, Garner A, Silen W, and Turnberg L.** *Mechanisms of Mucosal Protection in the Upper Gastrointestinal Tract*. New York: Raven, 1984.
13. **Allen A and Garner A.** Gastric mucus and bicarbonate secretion and their possible role in mucosal protection. *Gut* 21: 249–262, 1980.
14. **Allen A, Hutton DA, Leonard AJ, Pearson JP, and Sellers LA.** The role of mucus in the protection of the gastroduodenal mucosa. *Scand J Gastroenterol* 21, Suppl 125: 71–77, 1986.
15. **Allen A, Hutton D, McQueen S, Garner A.** Dimensions of the gastroduodenal surface pH gradients exceed those of the adherent mucus gel layer. *Gastroenterology* 85: 463–476, 1983.
16. **Allen A, Leonard AJ, Pearson JP, Sellers LAA, and Bennet MA.** Pepsin induced mucosal damage compared with that with ethanol or hypertonic saline: the role of the adherent mucus barrier. In: *Mechanisms of Injury and Mucosal Repair in the Upper Gastrointestinal Tract*, edited by Garner A and O'Brien, P. New York: Wiley, 1991, p. 7–18.
17. **Allen A, Pain RH, and Robson T.** Model for the structure of gastric mucus gel. *Nature* 264: 88–89, 1976.
18. **Allen A and Pearson JP.** The gastrointestinal adherent mucous gel barrier. In: *Glycoprotein Methods and Protocols: The Mucins*, edited by Corfield AP. Totowa, NJ: Humana, 2000, p. 57–64.
19. **Allen A and Snary D.** The structure and function of gastric mucus. *Gut* 13: 666–672, 1972.
20. **Altamirano M, Requena M, and Perez TZ.** Interstitial fluid pressure and gastric alkaline secretion. *Am J Physiol* 229: 1421–1426, 1975.
21. **Ameen NA, Ardito T, Kashgarian M, and Marino CR.** A unique subset of rat and human intestinal villus cells express the cystic fibrosis transmembrane conductance regulator. *Gastroenterology* 108: 1016–1023, 1995.
22. **Ameen NA, Mårtensson B, Bourguignon L, Marino C, Isenberg J, and McLaughlin GE.** CFTR channel insertion to the apical surface in rat duodenal villus epithelial cells is upregulated by VIP in vivo. *J Cell Sci* 112: 887–894, 1999.
23. **Aoi M, Aihara E, Nakashima M, and Takeuchi K.** Participation of prostaglandin E receptor EP₄ subtype in duodenal bicarbonate secretion in rats. *Am J Physiol Gastrointest Liver Physiol* 287: G96–G103, 2004.
24. **Atuma C, Strugala V, Allen A, and Holm L.** The adherent gastric mucus gel layer: thickness and physical state in vivo. *Am J Physiol Gastrointest Liver Physiol* 280: G922–G929, 2001.
25. **Bahari H, Ross IN, and Turnberg LA.** Demonstration of a pH gradient across the mucus layer on the surface of human gastric mucosa in vitro. *Gut* 23: 513–516, 1982.
26. **Ballesteros MA, Wolosin JD, Hogan DL, Koss MA, and Isenberg JJ.** Cholinergic regulation of human proximal duodenal mucosal bicarbonate secretion. *Am J Physiol Gastrointest Liver Physiol* 261: G327–G331, 1991.
27. **Bansil R, Stanley E, and Lamont JT.** Mucin biophysics. *Annu Rev Physiol* 57: 635–657, 1995.
28. **Baumgartner HK, Kirbiyik U, Coskun T, Chu S, and Montrose MH.** Endogenous cyclooxygenase activity regulates mouse gastric surface pH. *J Physiol* 544: 871–882, 2002.
29. **Baumgartner HK and Montrose MH.** Regulated alkali secretion acts in tandem with unstirred layers to regulate mouse gastric surface pH. *Gastroenterology* 126: 774–783, 2004.
30. **Bell A, Allen A, Morris ER, and Ross-Murphy SB.** Functional interactions of gastric mucus glycoprotein. *Int J Biol Macromol* 6: 309–315, 1984.
31. **Bell A, Sellers LA, Allen A, Cunliffe WJ, Morris ER, and Ross-Murphy SB.** Properties of gastric and duodenal mucus: effect of proteolysis, disulfide reduction, bile, acid, ethanol and hypertonicity on mucus gel structure. *Gastroenterology* 88: 269–280, 1985.
32. **Bertrand CA and Frizzell RA.** The role of regulated CFTR trafficking in epithelial secretion. *Am J Physiol Cell Physiol* 285: C1–C18, 2003.
33. **Bhaskar K, Garik P, Turner BS, Bradley JD, Bansil R, Stanley HE, and Lamont JT.** Viscous fingering of HCl through gastric mucin. *Nature* 360: 458–461, 1992.
34. **Bhaskar K, Gong D, Bansil R, Pajevic S, Hamilton JA, Turner BS, and Lamont JT.** Profound increase in viscosity and aggregation of pig gastric mucin at low pH. *Am J Physiol Gastrointest Liver Physiol* 261: G827–G833, 1991.
35. **Biasco G, Paganelli GM, Vaira D, Holton J, Di Febo G, Brillanti S, Miglioli L, Barbara L, and Samloff MI.** Serum pepsinogen I and II concentrations and IgG antibody to *Helicobacter pylori* in dyspeptic patients. *J Clin Pathol* 46: 826–828, 1993.
36. **Bickel M and Kauffman GL.** Gastric gel mucus thickness—effect of distention, 16,16-dimethyl prostaglandin-E₂, and carbenoxolone. *Gastroenterology* 80: 770–775, 1981.
37. **Boron WF, Waisbren SJ, Modlin IM, and Geibel JP.** Unique permeability barrier of the apical surface of parietal and chief cells in isolated perfused gastric glands. *J Exp Biol* 196: 347–360, 1994.
38. **Brockhausen I and Schacter H.** Glycosyltransferases involved in N- and O-glycan biosynthesis. *Glycosciences, Status and Perspectives*, edited by Gabius H-J and Gabius S. London: Chapman, 1997, p. 1–14.
39. **Bubenik GA.** Gastrointestinal melatonin: localization, function, and clinical relevance. *Dig Dis Sci* 47: 2336–2348, 2002.
40. **Bukhave K, Rask-Madsen J, Hogan DL, Koss MA, and Isenberg JJ.** Proximal duodenal prostaglandin E₂ release and mucosal bicarbonate secretion are altered in patients with duodenal ulcer. *Gastroenterology* 99: 951–955, 1990.
41. **Cao X, Bansil R, Bhaskar KR, Turner BS, LaMont JT, Niu N, and Afdhal NH.** pH-dependent conformational change of gastric mucin leads to sol-gel transition. *Biophys J* 76: 1250–1258, 1999.
42. **Chew CS, Säfsen B, and Flemström G.** Calcium signaling in cultured human and rat duodenal enterocytes. *Am J Physiol Gastrointest Liver Physiol* 275: G296–G304, 1998.
43. **Chikhlsa AR, Gharzouli A, Charpin G, Descroix-Vagne M, and Pansu D.** Comparison of VIP-induced electrolyte secretion in rat small intestine. *Reprod Nutr Dev* 32: 37–45, 1992.
44. **Chu S, Tanaka S, Kaunitz J, and Montrose MH.** Dynamic regulation of gastric surface pH by luminal pH. *J Clin Invest* 103: 605–612, 1999.
45. **Clarke LL and Harline MC.** Dual role of CFTR in cAMP-stimulated HCO₃⁻ secretion across murine duodenum. *Am J Physiol Gastrointest Liver Physiol* 274: G718–G726, 1998.
46. **Corfield A, Carroll D, Myerscough N, and Probert SJ.** Mucus in the gastrointestinal tract in health and disease. *Front Biosci* 6: D1321–D1357, 2001.
47. **Corfield A, Myerscough N, Myerscough N, Longman R, Sylvester P, Arul S, and Pignatelli M.** Mucins and mucosal protection in the gastrointestinal tract: new prospects or mucins in the pathology of gastrointestinal disease. *Gut* 47: 589–594, 2000.
48. **Coskun T, Baumgartner HK, Chu S, and Montrose MH.** Coordinated regulation of gastric chloride secretion with both acid and alkali secretion. *Am J Physiol Gastrointest Liver Physiol* 283: G1147–G1155, 2002.
49. **Coskun T, Chu S, and Montrose MH.** Intragastric pH regulates conversion from net acid to net alkaline secretion by the rat stomach. *Am J Physiol Gastrointest Liver Physiol* 281: G870–G877, 2001.
50. **Crampton JR, Gibbons LC, and Rees WDW.** Effect of luminal pH on the output of bicarbonate and PGE₂ by normal human stomach. *Gut* 28: 1291–1295, 1987.
51. **Cummings GM, Grossman MI, and Ivy AC.** An experimental study of the acid factor in ulceration of the gastrointestinal tract in dogs. *Gastroenterology* 10: 714–726, 1948.
52. **Davenport HW.** Fluid produced by the gastric mucosa during damage by acetic and salicylic acids. *Gastroenterology* 50: 487–499, 1966.
53. **Debellis L, Caroppo R, Frömter E, and Curci S.** Alkaline secretion by frog gastric glands measured with pH microelectrodes in the gland lumen. *J Physiol* 513: 235–241, 1998.
54. **De Bolos C, Francisco XR, and Lopez-Ferrer A.** Regulation of mucin and glycoconjugate expression: from normal epithelium to gastric tumors. *Front Biosci* 6: D1256–D1263, 2001.
55. **Desai M, Mutlu M, and Vadgama P.** A study of macromolecular diffusion through native porcine mucus. *Experientia* 48: 22–26, 1992.
56. **Dixon J, Strugala V, Griffin SM, Welfare MR, Dettmar PW, Allen A, and Pearson JP.** Eosophageal mucin: an adherent mucus gel barrier is

- absent in the normal esophagus but present in columnar-lined Barrett's esophagus. *Am J Gastroenterol* 96: 2575–2583, 2001.
57. Engel E, Guth PH, Nishizaki Y, and Kaunitz JD. Barrier function of the gastric mucus gel. *Am J Physiol Gastrointest Liver Physiol* 269: G994–G999, 1995.
 58. Fändriks L, Jönson C, and Nylander O. Effects of splanchnic nerve stimulation and of clonidine on gastric and duodenal HCO_3^- secretion in the anaesthetized cat. *Acta Physiol Scand* 130: 251–258, 1987.
 59. Farrell J, Taupin D, Koh TJ, Chen D, Zhao CM, Podolsky DK, and Wang TC. TFF2/SP-deficient mice show decreased gastric proliferation, increased acid secretion, and increased susceptibility to NSAID injury. *J Clin Invest* 109: 193–204, 2002.
 60. Feitelberg SP, Hogan DL, Koss MA, and Isenberg JI. pH threshold for human duodenal bicarbonate secretion and diffusion of CO_2 . *Gastroenterology* 102: 1252–1258, 1992.
 61. Flemström G. Active alkalization by amphibian gastric fundic mucosa. *Am J Physiol Endocrinol Metab Gastrointest Physiol* 233: E1–E12, 1977.
 62. Flemström G. Effect of catecholamines, Ca^{++} and gastrin on gastric HCO_3^- secretion. In: *Gastric Ion Transport: Proceedings from the Symposium on Gastric Ion Transport in Uppsala, Sweden, July 24–28, 1977*, edited by Öbrink KJ and Flemström G. Uppsala, Sweden: Acta Physiologica Scandinavica, 1978, p. 81–90.
 63. Flemström G. Cl^- dependence of HCO_3^- transport in frog gastric mucosa. *Ups J Med Sci* 85: 303–309, 1980.
 64. Flemström G. Stimulation of bicarbonate transport in isolated bullfrog duodenum by prostaglandins. *Am J Physiol Gastrointest Liver Physiol* 239: G198–G203, 1980.
 65. Flemström G. Gastric and duodenal mucosal bicarbonate secretion. In: *Physiology of the Gastrointestinal Tract* (3rd ed.), edited by Johnson LR, Jacobson ED, Christensen J, Alpers D, and Walsh JH. New York: Raven, 1994, p. 1285–1309.
 66. Flemström G and Garner A. Gastroduodenal bicarbonate transport: characteristics and proposed role in acidity regulation and mucosal protection. *Am J Physiol Gastrointest Liver Physiol* 242: G183–G193, 1982.
 67. Flemström G, Garner A, Nylander O, Hurst BC, and Heylings JR. Surface epithelial bicarbonate transport by mammalian duodenum in vivo. *Am J Physiol Gastrointest Liver Physiol* 243: G348–G358, 1982.
 68. Flemström G, Hällgren A, Nylander O, Engstrand L, Wilander E, and Allen A. Adherent surface mucus gel restricts diffusion of macromolecules in rat duodenum in vivo. *Am J Physiol Gastrointest Liver Physiol* 277: G375–G382, 1999.
 69. Flemström G and Isenberg JI. Gastroduodenal mucosal alkaline secretion and mucosal protection. *News Physiol Sci* 16: 23–28, 2001.
 70. Flemström G and Jedstedt G. Stimulation of duodenal mucosal bicarbonate secretion in the rat by brain peptides. *Gastroenterology* 97: 412–420, 1989.
 71. Flemström G, Jedstedt G, and Nylander O. β -Endorphin and enkephalins stimulate duodenal mucosal alkaline secretion in the rat in vivo. *Gastroenterology* 90: 368–372, 1986.
 72. Flemström G and Kivilaakso E. Demonstration of a pH gradient at the luminal surface of the rat duodenum and its dependence on mucosal alkaline secretion. *Gastroenterology* 84: 787–794, 1983.
 73. Flemström G, Kivilaakso E, Bridén S, Nylander O, and Jedstedt G. Gastroduodenal bicarbonate secretion in mucosal protection. Possible role of vasoactive intestinal peptide and opiates. *Dig Dis Sci* 30: 63S–68S, 1985.
 74. Flemström G and Nylander O. Stimulation of duodenal epithelial HCO_3^- transport in the guinea pig and cat by luminal prostaglandin E_2 . *Prostaglandins* 21, Suppl: 47–52, 1981.
 75. Flemström G and Sachs G. Properties of isolated antral mucosa. I. General characteristics. *Am J Physiol* 228: 1188–1198, 1975.
 76. Flemström G, Säfsten B, and Jedstedt G. Stimulation of mucosal alkaline secretion in rat duodenum by dopamine and dopaminergic compounds. *Gastroenterology* 104: 825–833, 1993.
 77. Flemström G, Sjöblom M, Jedstedt G, and Åkerman KEO. Short fasting dramatically decreases rat duodenal secretory responsiveness to orexin-A but not to VIP or melatonin. *Am J Physiol Gastrointest Liver Physiol* 285: G1091–G1096, 2003.
 78. Flemström G and Turnberg LA. Gastroduodenal defense mechanisms. *Clin Gastroenterol* 13: 327–354, 1984.
 79. Florey H. Mucin and protection of the body. *Proc R Soc Lond B Biol Sci* 143: 144–148, 1955.
 80. Forssell H, Lind T, and Olbe L. Comparative potency of carbachol, sham feeding, fundic distension and 16,16-dimethyl prostaglandin E_2 in stimulating human gastric bicarbonate secretion. *Acta Physiol Scand* 134: 75–78, 1988.
 81. Forssell H, Stenquist B, and Olbe L. Vagal stimulation of human gastric bicarbonate secretion. *Gastroenterology* 89: 581–586, 1985.
 82. Forstner G. Signal transduction packaging and secretion of mucins. *Annu Rev Physiol* 57: 585–605, 1995.
 83. Forstner JF and Forstner G. Gastrointestinal mucus. In: *Physiology of the Gastrointestinal Tract* (3rd ed.), edited by Johnson LR, Jacobson ED, Christensen J, Alpers D, and Walsh JH. New York: Raven, 1994, p. 1255–1283.
 84. Forte LR. Guanylin regulatory peptides: structures, biological activities mediated by cyclic GMP and pathobiology. *Regul Pept* 81: 25–39, 1999.
 85. Furukawa O, Bi LC, Guth PH, Engel E, Hirokawa M, and Kaunitz JD. NHE3 inhibition activates duodenal bicarbonate secretion in the rat. *Am J Physiol Gastrointest Liver Physiol* 286: G102–G109, 2004.
 86. Gannon B, Browning J, O'Brien P, and Rogers P. Mucosal microvascular architecture of the fundus and body of the human stomach. *Gastroenterology* 86: 866–875, 1984.
 87. Garner A and Flemström G. Gastric bicarbonate secretion in the guinea pig. *Am J Physiol Endocrinol Metab Gastrointest Physiol* 234: E535–E542, 1978.
 88. Gendler S and Spicer AP. Epithelial mucin genes. *Annu Rev Physiol* 57: 607–634, 1995.
 89. Glad H, Ainsworth MA, Svendsen P, Fahrenkrug J, and Schaffalitzky de Muckadell OB. Effect of vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide on pancreatic, hepatic and duodenal mucosal bicarbonate secretion in the pig. *Digestion* 67: 56–66, 2003.
 90. Goddard P, Kao YCJ, and Lichtenburger L. Luminal surface hydrophobicity of canine gastric mucosa is dependent on surface mucous gel. *Gastroenterology* 98: 361–370, 1990.
 91. Godt K, Johansson MEV, Lidell ME, Morgelin M, Karlsson H, Olson FJ, Gum JR, Kim YS, and Hansson GC. The N terminus of the MUC2 mucin forms trimers that are held together within a trypsin-resistant core fragment. *J Biol Chem* 277: 47248–47256, 2002.
 92. Gouyer V, Wiede A, Buisine MP, Dekeyser S, Moreau O, Lesuffleur T, Hoffman W, and Huet G. Specific secretion of gel-forming mucins and TFF peptides in HT-29 cells of mucin-secreting phenotype. *Biochim Biophys Acta* 1539: 71–84, 2001.
 93. Guba M, Kuhn M, Forssmann WG, Classen M, Gregor M, and Seidler U. Guanylin strongly stimulates rat duodenal HCO_3^- secretion: proposed mechanism and comparison with other secretagogues. *Gastroenterology* 111: 1760–1763, 1996.
 94. Guha S and Kaunitz JD. Gastroduodenal mucosal defense: an integrated protective response. *Curr Opin Gastroenterol* 18: 650–657, 2002.
 95. Gunion MW, Kauffman GI, and Taché Y. Intrahypothalamic corticotropin-releasing factor elevates gastric bicarbonate secretion and inhibits stress ulcers in rats. *Am J Physiol Gastrointest Liver Physiol* 258: G152–G157, 1990.
 96. Hällgren A, Flemström G, Hellström PM, Lördal MS, Hellgren S, and Nylander O. Neurokinin A increases duodenal mucosal permeability, bicarbonate secretion, and fluid output in the rat. *Am J Physiol Gastrointest Liver Physiol* 273: G1077–G1086, 1997.
 97. Hällgren A, Flemström G, and Nylander O. Interaction between Neurokinin A, VIP, prostanoids and enteric nerves in regulation of duodenal function. *Am J Physiol Gastrointest Liver Physiol* 275: G95–G103, 1998.
 98. Harmon JW, Woods M, and Gurll NJ. Different mechanisms of hydrogen ion removal in stomach and duodenum. *Am J Physiol Endocrinol Metab Gastrointest Physiol* 235: E692–E698, 1978.
 99. Heylings JR, Garner A, and Flemström G. Stimulation of gastroduodenal HCO_3^- transport by luminal acid in the frog in vitro. *Am J Physiol Gastrointest Liver Physiol* 246: G235–G242, 1984.
 100. Hidaka E, Ota H, Hidaka H, Hayama M, Matsuzawa K, Akamatsu T, Nakayama J, and Katsuyama T. *Helicobacter pylori* and two ultrastructurally distinct layers of gastric mucous cell mucins in the surface mucous gel layer. *Gut* 49: 474–480, 2001.
 101. Hills BA. Gastric mucosal barrier stabilisation of the hydrophobic lining to the stomach by mucus. *Am J Physiol Gastrointest Liver Physiol* 249: G342–G349, 1985.
 102. Hirschowitz B. Pepsin and the esophagus. *Yale J Biol Med* 72: 133–143, 1999.

103. **Ho S, Anway RE, and Khalil ZM.** Mucin gene expression in normal and neoplastic tissue and use of mucin mRNAs as markers for micro-metastatic disease. *J Clin Ligand Assay* 22: 358–363, 1999.
104. **Ho S, Anway RE, and Khalil ZM.** Spatial organization of MUC5AC and MUC6 mucins within the surface mucous layer of the stomach (Abstract). *Gastroenterology* 118: 1396, 2000.
105. **Ho S, Robertson AM, Shekels LL, Lyftogt CT, Niehans GA, and Toribara NW.** Expression cloning of gastric mucin complementary-DNA and localization of mucin gene-expression. *Gastroenterology* 109: 735–747, 1995.
106. **Hoffmann JW and Wiede A.** Molecular medicine of TFF-peptides: from gut to brain. *Histol Histopathol* 16: 319–334, 2001.
107. **Hogan DL, Crombie DL, Isenberg JI, Svendsen P, Schaffalitzky de Muckadell OB, and Ainsworth MA.** CFTR mediates cAMP- and Ca²⁺-activated duodenal epithelial HCO₃⁻ secretion. *Am J Physiol Gastrointest Liver Physiol* 272: G872–G878, 1997.
108. **Hogan Rapier RC DL, Dreilinger A, Koss MA, Basuk PM, Weinstein WM, Nyberg LM, and Isenberg JI.** Duodenal bicarbonate secretion: eradication of *Helicobacter pylori* and duodenal structure and function in humans. *Gastroenterology* 110: 705–716, 1996.
109. **Hollander F.** The two-component mucus barrier; its activity in protecting the gastroduodenal mucosa against peptic ulceration. *AMA Arch Intern Med* 93: 107–120, 1954.
110. **Holm L, Ågren J, and Persson AEG.** Stimulation of acid secretion increases the gastric luminal pressure in the rat. *Gastroenterology* 103: 1797–1803, 1992.
111. **Holm L and Flemström G.** Microscopy of acid transport at the gastric surface in vivo. *J Intern Med* 228, Suppl 732: 91–95, 1990.
112. **Holm M, Johansson B, Pettersson A, and Fändriks L.** Carbon dioxide mediates duodenal mucosal alkaline secretion in response to luminal acidity in the anesthetized rat. *Gastroenterology* 115: 680–685, 1998.
113. **Holm M, Powell T, Casselbrant A, Johansson B, Fändriks L.** Dynamic involvement of the inducible type of nitric oxide synthase in acid-induced duodenal mucosal alkaline secretion in the rat. *Dig Dis Sci* 46: 1765–1771, 2001.
114. **Holzer P.** Gastroduodenal mucosal defense: coordination by a network of messengers and mediators. *Curr Opin Gastroenterol* 17: 489–496, 2001.
115. **Isenberg JI, Flemström G, and Johansson C.** Mucosal bicarbonate secretion is significantly greater in the proximal versus distal duodenum in the in vivo rat. In: *Mechanisms of Mucosal Protection in the Upper Gastrointestinal Tract*, edited by Allen A, Flemström G, Garner A, Silen W, and Turnberg LA. New York: Raven, 1984, p. 175–180.
116. **Isenberg JI, Hogan DL, Koss MA, and Selling JA.** Human duodenal mucosal bicarbonate secretion. Evidence for basal secretion and stimulation by hydrochloric acid and a synthetic prostaglandin E₁ analogue. *Gastroenterology* 91: 370–378, 1986.
117. **Isenberg JI, Ljungström M, Säfsten B, and Flemström G.** Proximal duodenal enterocyte transport: evidence for Na⁺-H⁺ and Cl⁻-HCO₃⁻ exchange and NaHCO₃ cotransport. *Am J Physiol Gastrointest Liver Physiol* 265: G677–G685, 1993.
118. **Isenberg JI, Selling JA, Hogan DL, and Koss MA.** Impaired proximal duodenal mucosal bicarbonate secretion in duodenal ulcer patients. *N Engl J Med* 316: 374–379, 1987.
119. **Ito S, Lacy ER, Rutten MJ, Critchlow J, and Silen W.** Rapid repair of injured gastric mucosa. *Scand J Gastroenterol Suppl* 101: 87–95, 1984.
120. **Jacob P, Christiani S, Rossmann H, Lamprecht G, Vieillard-Baron D, Müller R, Gregor M, Seidler U.** Role of Na⁺HCO₃⁻ cotransporter NBC1, Na⁺/H⁺ exchanger NHE1, and carbonic anhydrase in rabbit duodenal bicarbonate secretion. *Gastroenterology* 119:406–419, 2000.
121. **Jacob P, Rossmann H, Lamprecht G, Kretz A, Neff C, Lin-Wu E, Gregor M, Groneberg DA, Kere J, and Seidler U.** Down-regulated in adenoma mediates apical Cl⁻/HCO₃⁻ exchange in rabbit, rat, and human duodenum. *Gastroenterology* 122: 709–724, 2002.
122. **Järbur K, Dalenbäck J, and Sjövall H.** Quantitative assessment of motility-associated changes in gastric and duodenal luminal pH in humans. *Scand J Gastroenterol* 38: 392–398, 2003.
123. **Järhult J, Angerås T, Farnebo LO, Graffner H, and Hamberger B.** Elevated plasma levels of noradrenaline in duodenal ulcer. *World J Surg* 7: 385–389, 1983.
124. **Jaup EA, Timar Peregrin A, Jodal M, and Lundgren O.** Nervous control of alkaline secretion in the duodenum as studied by the use of cholera toxin in the anaesthetized rat. *Acta Physiol Scand* 162: 165–174, 1998.
125. **Johansson B, Casselbrant A, Holm M, von Bothmer C, Johansson BR, and Fändriks L.** Effects of hypovolaemia on acid-induced duodenal mucosal damage in the rat. *Acta Physiol Scand* 171: 43–50, 2001.
126. **Johansson B, Holm M, Ewert S, Casselbrant A, Pettersson A, and Fändriks L.** Angiotensin II type 2 receptor-mediated duodenal mucosal alkaline secretion in the rat. *Am J Physiol Gastrointest Liver Physiol* 280: G1254–G1260, 2001.
127. **Johansson M, Synnerstad I, and Holm L.** Acid transport through channels in the mucous layer of rat stomach. *Gastroenterology* 119: 1297–1304, 2000.
128. **Jönson C, Tunbäck-Hanson P, and Fändriks L.** Splanchnic nerve activation inhibits the increase in duodenal HCO₃⁻ secretion induced by luminal acidification in the rat. *Gastroenterology* 96: 45–49, 1989.
129. **Joo NS, London RM, Kim HD, Forte LR, and Clarke LL.** Regulation of intestinal Cl⁻ and HCO₃⁻ secretion by uroguanylin. *Am J Physiol Gastrointest Liver Physiol* 274: G633–G644, 1998.
130. **Jordan N, Newton J, Pearson J, and Allen A.** A novel method for the visualization of the in situ mucus layer in rat and man. *Clin Sci (Lond)* 95: 97–106, 1998.
131. **Kerss S, Allen A, and Garner A.** A simple method for measuring thickness of the mucosal gel layer adherent to rat, frog and human gastric mucosa: influence of feeding prostaglandin, N-acetyl and other agents. *Clin Sci (Lond)* 63: 187–195, 1982.
132. **Kim Y, Gum J, and Brockhausen I.** Mucin glycoproteins in neoplasia. *Glycoconj J* 13: 693–707, 1996.
133. **Kindon H, Pothoulakis C, Thim L, Lynch-Devaney K, and Podolsky DK.** Trefoil protection of intestinal epithelial barrier function: cooperative interaction with mucin glycoprotein. *Gastroenterology* 109: 516–523, 1995.
134. **Kirchgessner AL.** Orexins in the brain-gut axis. *Endocr Rev* 23: 1–15, 2002.
135. **Kivilaakso E.** High plasma HCO₃⁻ protects gastric mucosa against acute ulceration in the rat. *Gastroenterology* 81: 921–927, 1981.
136. **Kivilaakso E and Flemström G.** Surface pH gradient in gastroduodenal mucosa. *Scand J Gastroenterol* 19, Suppl 105: 50–52, 1984.
137. **Kiviluoto T, Ahonen M, Bäck N, Häppölä O, Mustonen H, Paimela H, and Kivilaakso E.** Pre-epithelial mucus-bicarbonate layer protects against intracellular acidosis in acid-exposed gastric mucosa. *Am J Physiol Gastrointest Liver Physiol* 264: G57–G63, 1993.
138. **Kiviluoto T, Paimela H, Mustonen H, and Kivilaakso E.** Intracellular pH in isolated Necturus antral mucosa exposed to luminal acid. *Gastroenterology* 98: 901–908, 1990.
139. **Knutson L and Flemström G.** Duodenal mucosal bicarbonate secretion in man. Stimulation by acid and inhibition by the α₂-adrenoceptor agonist clonidine. *Gut* 30: 1708–1715, 1989.
140. **Knutson TW, Koss MA, Hogan DL, Isenberg JI, and Knutson L.** Acetazolamide inhibits basal and stimulated HCO₃⁻ secretion in the human proximal duodenum. *Gastroenterology* 108: 102–107, 1995.
141. **Konturek SJ, Bilski J, Tasler J, and Laskiewicz J.** Gastroduodenal alkaline response to acid and taurocholate in conscious dogs. *Am J Physiol Gastrointest Liver Physiol* 247: G149–G154, 1984.
142. **Konturek SJ, Bilski J, Tasler J, and Laskiewicz J.** Gut hormones in stimulation of gastroduodenal alkaline secretion in conscious dogs. *Am J Physiol Gastrointest Liver Physiol* 248: G687–G691, 1985.
143. **Konturek SJ, Tasler J, Bilski J, and Kania J.** Prostaglandins and alkaline secretion from oxyntic, antral and duodenal mucosa in the dog. *Am J Physiol Gastrointest Liver Physiol* 245: G539–G546, 1983.
144. **Krause WJ.** Brunner's glands: a structural, histochemical and pathological profile. *Prog Histochem Cytochem* 35: 259–367, 2000.
145. **Kukkonen JP, Holmqvist T, Ammoun S, and Åkerman KE.** Functions of the orexinergic/hypocretinergic system. *Am J Physiol Cell Physiol* 283: C1567–C1591, 2002.
146. **Lacy E and Ito S.** Rapid epithelial restitution of the rat gastric mucosa after ethanol injury. *Lab Invest* 51: 573–585, 1984.
147. **Larsen KR, Moore JG, and Dayton MT.** Circadian rhythms of acid and bicarbonate efflux in fasting rat stomach. *Am J Physiol Gastrointest Liver Physiol* 260: G610–G614, 1991.
148. **Larsen KR, Moore JG, and Dayton MT.** Circadian rhythms of gastric mucus efflux and residual mucus gel in the fasting rat stomach. *Dig Dis Sci* 36: 1550–1555, 1991.
149. **Larson GM, Jedstedt G, Nylander O, and Flemström G.** Intracerebral adrenoceptor agonists influence rat duodenal mucosal bicarbonate secretion. *Am J Physiol Gastrointest Liver Physiol* 271: G831–G840, 1996.

150. Lefebvre O, Chenard MP, Masson R, Linares J, Dierich A, LeMeur M, Wendling C, Tomasetto C, Chambon P, and Rio MC. Gastric mucosa abnormalities and tumorigenesis in mice lacking the pS2 trefoil protein. *Science* 274: 259–262, 1996.
151. Lenz HJ. Regulation of duodenal bicarbonate secretion during stress by corticotropin-releasing factor and β -endorphin. *Proc Natl Acad Sci USA* 86: 1417–1420, 1989.
152. Lenz HJ, Vale WW, and Rivier JE. TRH-induced vagal stimulation of duodenal HCO_3^- mediated by VIP and muscarinic pathways. *Am J Physiol Gastrointest Liver Physiol* 257: G677–G682, 1989.
153. Lichtenberger L. The hydrophobic barrier properties of gastrointestinal mucus. *Annu Rev Physiol* 57: 565–583, 1995.
154. Lichtenberger L, Graziani LA, Dial EJ, Butler BD, and Hills BA. Role of surface active phospholipids in cytoprotection. *Science* 219: 1327–1329, 1983.
155. Lidell M, Johansson MEV, Morgelin M, Asker N, Gum JR, Kim YS, and Hansson GC. The recombinant C terminus of the human MUC2 mucin forms dimers in Chinese hamster ovary cells and heterodimers with full length Muc 2 in LS 174T cells. *Biochem J* 372: 335–345, 2003.
156. Lugea A, Antolin M, Mourelle M, Guraner F, and Malagelada J. Deranged hydrophobic barrier of gastroduodenal mucosa after parenteral nonsteroidal anti-inflammatory drugs. *Gastroenterology* 112: 1931–1939, 1997.
157. Lugea A, Mourelle M, Domingo A, Salas A, Guraner F, and Malagelada JR. Epidermal growth factor increases surface hydrophobicity and resistance to acid in the rat duodenum. *Am J Physiol Gastrointest Liver Physiol* 280: G774–G779, 2001.
158. Malinovskaya NK, Komarov FI, Rapoport SI, Voznesenskaya LA, and Wetterberg L. Melatonin production in patients with duodenal ulcer. *Neuroendocrinol Lett* 22: 109–117, 2001.
159. McQueen S, Hutton D, Allen A, and Garner A. Gastric and duodenal surface mucus gel thickness in rat: effects of prostaglandins and damaging agents. *Am J Physiol Gastrointest Liver Physiol* 245: G388–G393, 1983.
160. Mellander A and Sjövall H. Indirect evidence for cholinergic inhibition of intestinal bicarbonate absorption in humans. *Gut* 44: 353–360, 1999.
161. Melvin JE, Park K, Richardson L, Schultheis PJ, and Shull GE. Mouse down-regulated in adenoma (DRA) is an intestinal $\text{Cl}^-/\text{HCO}_3^-$ exchanger and is up-regulated in colon of mice lacking the $\text{NHE3 Na}^+/\text{H}^+$ exchanger. *J Biol Chem* 274: 22855–22861, 1999.
162. Mertz-Nielsen A, Hillingsø J, Bukhave K, and Rask-Madsen J. Indomethacin decreases gastroduodenal mucosal bicarbonate secretion in humans. *Scand J Gastroenterol* 30: 1160–1165, 1995.
163. Mertz-Nielsen A, Hillingsø J, Frøkiær H, Bukhave K, and Rask-Madsen J. Gastric bicarbonate secretion and release of prostaglandin E_2 are increased in duodenal ulcer patients but not in *Helicobacter pylori*-positive healthy subjects. *Scand J Gastroenterol* 31: 38–43, 1996.
164. Moniaux N, Escande F, Porchet N, Aubert JP, and Batra SK. Structural organization and classification of the human mucin genes. *Front Biosci* 6: D1192–D1206, 2001.
165. Moore BA, Kim D, and Vanner S. Neural pathways regulating Brunner's gland secretion in guinea pig duodenum in vitro. *Am J Physiol Gastrointest Liver Physiol* 279: G910–G917, 2000.
166. Morris G, Harding RK, and Wallace JL. A functional-model for extracellular gastric mucus in the rat. *Virchows Arch* 46: 239–251, 1984.
167. Newton J, Allen A, Westley BM, and May FEB. The human trefoil peptide, TFF1, is present in different molecular forms that are intimately associated with the adherent mucus gel in normal stomach. *Gut* 46: 312–320, 2000.
168. Newton J, Jordan N, Oliver L, Strugala V, Pearson JP, James OJ, and Allen A. *Helicobacter pylori* in vivo causes structural changes in the adherent gastric mucus layer but barrier function is not compromised. *Gut* 43: 470–475, 1998.
169. Nicholas C, Desai M, Vadgama P, McDonnell MB, and Lucas S. pH dependence of hydrochloric acid diffusion through gastric mucus: correlation with diffusion through a water layer using a membrane-mounted glass pH electrode. *Analyst* 116: 463–467, 1991.
170. Nishizaki Y, Guth PH, Kim G, Wayland H, and Kaunitz JD. Penta-gastrin enhances gastric mucosal defenses in vivo: luminal acid-dependent and independent effects. *Am J Physiol Gastrointest Liver Physiol* 267: G94–G104, 1994.
171. Nishizaki Y, Guth PH, Quintero E, Bover J, Del Rivero M, and Kaunitz JD. Prostaglandin E_2 enhances gastric defense-mechanisms against acid injury in uremic rats. *Gastroenterology* 107: 1382–1389, 1994.
172. Nylander O, Hällgren A, and Sababi M. COX inhibition excites enteric nerves that affect motility, alkaline secretion, and permeability in rat duodenum. *Am J Physiol Gastrointest Liver Physiol* 281: G1169–G1178, 2001.
173. Nylander O, Kvietyts P, and Granger DN. Effects of hydrochloric acid on duodenal and jejunal mucosal permeability in the rat. *Am J Physiol Gastrointest Liver Physiol* 257: G653–G660, 1989.
174. Nylander O, Pihl L, and Perry M. Hypotonicity-induced increases in duodenal mucosal permeability facilitates adjustment of luminal osmolality. *Am J Physiol Gastrointest Liver Physiol* 285: G360–G370, 2003.
175. Nylander O, Wilander E, Larson GM, and Holm L. Vasoactive intestinal polypeptide reduces hydrochloric acid-induced duodenal mucosal permeability. *Am J Physiol Gastrointest Liver Physiol* 264: G272–G279, 1993.
176. Ota H and Katsuyama T. Alternating laminated array of two types of mucin in the human gastric surface mucous layer. *Histochem J* 24: 86–92, 1992.
177. Ovesen L, Bendtsen F, Tage-Jensen U, Pedersen NT, Gram BR, and Rune SJ. Intraluminal pH in the stomach, duodenum, and proximal jejunum in normal subjects and patients with exocrine pancreatic insufficiency. *Gastroenterology* 90: 958–962, 1986.
178. Paimela H, Kiviluoto T, Mustonen H, Sipponen P, and Kivilaakso E. Tolerance of rat duodenum to luminal acid. *Dig Dis Sci* 35: 1244–1248, 1990.
179. Patronella C, Vanek I, and Bowen JC. In vivo measurement of gastric mucus pH in canines: effect of high luminal acidity and prostaglandin E_2 . *Gastroenterology* 95: 612–618, 1988.
180. Pavlov J. Die Arbeit der Verdauungsdrüsen. Wiesbaden, Germany: Bergman, 1898.
181. Pearson J, Ward RA, Allen A, Roberts NG, and Taylor W. Mucus degradation by pepsin: comparison of mucolytic activity of human pepsin 1 and pepsin 3: implications for peptic ulceration. *Gut* 27: 243–248, 1986.
182. Pérez JF, Ruiz MC, and Michelangeli F. Simultaneous measurement and imaging of intracellular Ca^{2+} and H^+ transport in isolated rabbit gastric glands. *J Physiol* 537: 735–745, 2001.
183. Perez-Vilar J and Hill R. The structure and assembly of secreted mucins. *J Biol Chem* 274: 31751–31754, 1999.
184. Phillipson M, Atuma C, Henriksen J, and Holm L. The importance of mucus and bicarbonate transport in preservation of gastric juxtamucosal pH. *Am J Physiol Gastrointest Liver Physiol* 282: G211–G219, 2002.
185. Poulson R and Wright NA. Trefoil peptides: a newly recognized family of epithelial mucin associated molecules. *Am J Physiol Gastrointest Liver Physiol* 265: G205–G213, 1996.
186. Pratha VS, Hogan DL, Mårtensson BA, Bernard J, Zhou R, and Isenberg JI. Identification of transport abnormalities in duodenal mucosa and duodenal enterocytes from patients with cystic fibrosis. *Gastroenterology* 118: 1051–1060, 2000.
187. Prætorius J, Andreassen D, Jensen BL, Ainsworth MA, Friis UG, and Johansen T. NHE1, NHE2, and NHE3 contribute to regulation of intracellular pH in murine duodenal epithelial cells. *Am J Physiol Gastrointest Liver Physiol* 278: G197–G206, 2000.
188. Prætorius J, Friis UG, Ainsworth MA, Schaffalitzky de Muckadell OB, and Johansen T. The cystic fibrosis transmembrane conductance regulator is not a base transporter in isolated duodenal epithelial cells. *Acta Physiol Scand* 174: 327–336, 2002.
189. Prætorius J, Hager H, Nielsen S, Aalkjær C, Friis UG, Ainsworth MA, and Johansen T. Molecular and functional evidence for electrogenic and electroneutral $\text{Na}^+/\text{HCO}_3^-$ cotransporters in murine duodenum. *Am J Physiol Gastrointest Liver Physiol* 280: G332–G343, 2001.
190. Quigley EMM and Turnberg LA. pH of the microclimate lining human gastric and duodenal mucosa in vivo. Studies in control subjects and in duodenal ulcer patients. *Gastroenterology* 92: 1876–1884, 1987.
191. Ranta-Knuutila T, Mustonen H, and Kivilaakso E. Topical prostaglandin E_2 protects isolated gastric mucosa against acidified taurocholate-, but not ethanol- or aspirin-induced injury. *Dig Dis Sci* 45: 99–104, 2000.
192. Rao SP, Sellers Z, Crombie DL, Hogan DL, Mann EA, Childs D, Keely S, Sheil-Puopolo M, Giannella RA, Barrett KE, Isenberg JI, and Pratha VS. A role for guanylate cyclase C in acid stimulated

- duodenal mucosal bicarbonate secretion. *Am J Physiol Gastrointest Liver Physiol* 286: G95–G101, 2004.
193. **Reichstein BJ and Cohen MM.** Effect of acetazolamide on rat gastric mucosal protection and stimulated bicarbonate secretion with 16,16-dimethyl prostaglandin E₂. *J Lab Clin Med* 104: 797–804, 1984.
 194. **Reimer R, Odes HS, Muallem R, Schwenk M, Beil W, and Sewing KF.** Cyclic adenosine monophosphate is the second messenger of prostaglandin E₂- and vasoactive intestinal polypeptide-stimulated active bicarbonate secretion by guinea-pig duodenum. *Scand J Gastroenterol* 29: 153–159, 1994.
 195. **Ross I, Bahari HMM, and Turnberg LA.** The pH gradient across mucus adherent to rat fundic mucosa in vivo and effect of potential damaging agents. *Gastroenterology* 81: 713–718, 1981.
 196. **Rossmann H, Bachmann O, Vieillard-Baron D, Gregor M, and Seidler U.** Na⁺/HCO₃⁻ cotransport and expression of NBC1 and NBC2 in rabbit gastric parietal and mucous cells. *Gastroenterology* 116: 1389–1398, 1999.
 197. **Sababi M, Nilsson E, and Holm L.** Mucus and alkali secretion in the rat duodenum: effects of indomethacin, N^o-nitro-L-arginine, and luminal acid. *Gastroenterology* 109: 1526–1534, 1995.
 198. **Säfssten B and Flemström G.** Dopamine and vasoactive intestinal peptide stimulate cyclic adenosine-3',5'-monophosphate formation in isolated rat villus and crypt duodenocytes. *Acta Physiol Scand* 149: 67–75, 1993.
 199. **Säfssten B, Jedstedt G, and Flemström G.** Effects of diazepam and Ro 15-1788 on duodenal bicarbonate secretion in the rat. *Gastroenterology* 101: 1031–1038, 1991.
 200. **Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ, and Yanagisawa M.** Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 92: 573–585, 1998.
 201. **Samloff MI.** Peptic ulcer: the many proteinases of aggression. *Gastroenterology* 96: 586–595, 1989.
 202. **Sands B and Podolsky DK.** The trefoil peptide family. *Annu Rev Physiol* 58: 253–273, 1996.
 203. **Sawaguchi A, Ishihara K, Kawano J, Oinuma T, Hotta K, and Suganuma T.** Fluid dynamics of the excretory flow of zymogenic and mucin contents in rat gastric gland processed by high-pressure freezing/freezing substitution. *J Histochem Cytochem* 50: 223–234, 2002.
 204. **Schade C, Flemström G, and Holm L.** Hydrogen ion concentration in the mucus layer on top of acid-stimulated and acid-inhibited rat gastric mucosa. *Gastroenterology* 107: 180–188, 1994.
 205. **Schierbeck N.** Ueber Kohlensäure im Ventrikel. *Skand Arch Physiol* 3: 437–474, 1892.
 206. **Schreiber S, Nguyen TH, Stuben M, and Scheid P.** Demonstration of a pH gradient in the gastric gland of the acid-secreting guinea pig mucosa. *Am J Physiol Gastrointest Liver Physiol* 279: G597–G604, 2000.
 207. **Schreiber S and Scheid P.** Gastric mucus of the guinea pig: proton carrier and diffusion barrier. *Am J Physiol Gastrointest Liver Physiol* 272: G63–G70, 1997.
 208. **Schwarz K.** Ueber penetrierende Magen und Jejunalgeschwüre. *Beitr Klin Chir* 67: 96–128, 1910.
 209. **Seidler U, Blumenstein I, Kretz A, Vieillard-Baron D, Rossmann H, Colledge WH, Evans M, Ratcliff R, and Gregor M.** A functional CFTR protein is required for mouse intestinal cAMP-, cGMP- and Ca²⁺-dependent HCO₃⁻ secretion. *J Physiol* 505: 411–423, 1997.
 210. **Sellers LA, Allen A, and Bennett MK.** Formation of fibrin based gelatinous coat over repairing rat gastric epithelium after acute ethanol damage: interaction with adherent mucus. *Gut* 28: 835–843, 1987.
 211. **Sellers LA, Allen A, Morris E, and Ross-Murphy SB.** Mucus glycoprotein gels. Role of glycoprotein polymeric structure and carbohydrate side-chains in gel-formation. *Carbohydr Res* 178: 93–110, 1988.
 212. **Shorrock CJ, Prescott JR, and Rees WDW.** The effects of indomethacin on gastroduodenal morphology and mucosal pH gradient in the healthy human stomach. *Gastroenterology* 199: 334–339, 1990.
 213. **Silen W and Ito S.** Mechanisms for rapid re-epithelialisation of the gastric mucosal surface. *Annu Rev Physiol* 47: 217–229, 1985.
 214. **Simpson J, Merhav A, and Silen W.** Alkaline secretion by amphibian duodenum I. General characteristics. *Am J Physiol Gastrointest Liver Physiol* 240: G401–G408, 1981.
 215. **Sjöblom M and Flemström G.** Melatonin in duodenal lumen is a potent stimulant of mucosal bicarbonate secretion. *J Pineal Res* 34: 288–293, 2003.
 216. **Sjöblom M and Flemström G.** Central nervous α₁-adrenoceptor stimulation induces duodenal luminal release of melatonin. *J Pineal Res* 36: 1–6, 2004.
 217. **Sjöblom M, Jedstedt G, and Flemström G.** Peripheral melatonin mediates neural stimulation of duodenal mucosal bicarbonate secretion. *J Clin Invest* 108: 625–633, 2001.
 218. **Sjöblom M, Säfssten B, and Flemström G.** Melatonin-induced calcium signaling in clusters of human and rat duodenal enterocytes. *Am J Physiol Gastrointest Liver Physiol* 284: G1034–G1044, 2003.
 219. **Sjövall H, Forssell H, Häggendal J, and Olbe L.** Reflex sympathetic activation in humans is accompanied by inhibition of gastric HCO₃⁻ secretion. *Am J Physiol Gastrointest Liver Physiol* 255: G752–G758, 1988.
 220. **Snary D, Allen A, and Pain RH.** Structural studies on gastric mucoproteins lowering of molecular weight after reduction with 2-mercaptoethanol. *Biochem Biophys Res Commun* 40: 844–851, 1970.
 221. **Solcia E, Vassallo G, and Sampietro R.** Endocrine cells in the antropyloric mucosa of the stomach. *Z Zellforsch* 81: 474–486, 1967.
 222. **Spiegel S, Phillipper M, Rossmann H, Riederer B, Gregor M, and Seidler U.** Independence of apical Cl⁻/HCO₃⁻ exchange and anion conductance in duodenal HCO₃⁻ secretion. *Am J Physiol Gastrointest Liver Physiol* 285: G887–G897, 2003.
 223. **Spychal R, Goggin PM, Marrero JM, Saverymuttu SH, Yu CW, Corbishley CM, Maxwell JD, and Norfield TC.** Surface hydrophobicity of the gastric mucosa in peptic ulcer disease. Relationship to gastritis and *Campylobacter pylori* infection. *Gastroenterology* 98: 1250–1254, 1990.
 224. **Spychal R, Marrero JM, Saverymuttu SH, and Norfield TC.** Measurement of hydrophobicity of human gastrointestinal mucosa. *Gastroenterology* 97: 104–111, 1989.
 225. **Synerstad I, Ekblad E, Sundler F, and Holm L.** Gastric mucosal smooth muscles may explain oscillations in glandular pressure: role of vasoactive intestinal peptide. *Gastroenterology* 114: 284–294, 1998.
 226. **Synerstad I and Holm L.** Prostaglandin E₂ and aggressive factors increase the gland luminal pressure in the rat gastric mucosa in vivo. *Gastroenterology* 114: 1276–1286, 1998.
 227. **Synerstad I, Johansson M, Nylander O, and Holm L.** Intraluminal acid and gastric mucosal integrity: the importance of blood-borne bicarbonate. *Am J Physiol Gastrointest Liver Physiol* 280: G121–G129, 2001.
 228. **Synerstad I, Persson AEG, and Holm L.** Effect of inhibition of pentagastrin-stimulated acid secretion on gastric mucosal gland luminal pressure. *Acta Physiol Scand* 160: 103–111, 1997.
 229. **Takeuchi K, Furukawa O, Tanaka H, and Okabe S.** A new model of duodenal ulcers induced in rats by indomethacin plus histamine. *Gastroenterology* 90: 636–645, 1986.
 230. **Takeuchi K, Magee D, Critchlow J, Matthews J, and Silen W.** Studies of the pH gradient and thickness of frog gastric mucus gel. *Gastroenterology* 84: 331–340, 1983.
 231. **Takeuchi K, Merhav A, and Silen W.** Mechanism of luminal alkalization by bullfrog fundic mucosa. *Am J Physiol Gastrointest Liver Physiol* 243: G377–G388, 1982.
 232. **Takeuchi K, Takehara K, Kato S, and Yagi K.** PACAPs stimulate duodenal bicarbonate secretion at PACAP receptors in the rat. *Am J Physiol Gastrointest Liver Physiol* 272: G646–G653, 1997.
 233. **Takeuchi K, Tanaka H, Furukawa O, and Okabe S.** Gastroduodenal HCO₃⁻ secretion in anesthetized rats: effects of 16,16-dimethyl PGE₂, topical acid and acetazolamide. *Jpn J Pharmacol* 41: 87–99, 1986.
 234. **Takeuchi K, Ukawa H, Kato S, Furukawa O, Arachi H, Sugimoto Y, Ichikawa A, Ushikubo E, and Narumiya S.** Impaired duodenal bicarbonate secretion and mucosal integrity in mice lacking prostaglandin E-receptor subtype EP₃. *Gastroenterology* 117: 1128–1135, 1999.
 235. **Takeuchi K, Yagi K, Kato S, and Ukawa H.** Roles of prostaglandin E-receptor subtypes in gastric and duodenal bicarbonate secretion in rats. *Gastroenterology* 113: 1553–1559, 1997.
 236. **Tanaka S, Meiselman HHJ, Engel E, Guth PH, Furukawa O, Wenby RB, Lee J, and Kaunitz JD.** Regional differences of H⁺, HCO₃⁻, and CO₂ diffusion through native porcine gastroduodenal mucus. *Dig Dis Sci* 47: 967–973, 2002.
 237. **Tanaka S, Podolsky D, Engel E, Guth PH, and Kaunitz JD.** Human spasmolytic polypeptide decreases proton permeation through gastric

- mucus. *Am J Physiol Gastrointest Liver Physiol* 272: G1473–G1480, 1997.
238. **Tanaka S, Taché Y, Keneko H, Guth PH, and Kaunitz JD.** Central vagal activation increases mucus gel thickness and surface intracellular pH in the rat stomach. *Gastroenterology* 112: 409–417, 1997.
 239. **Tani S, Suzuki T, Kano S, Tanaka T, Sunaga K, Morishige R, and Tsuda T.** Mechanisms of gastric mucus secretion from cultured rat gastric epithelial cells induced by carbachol, cholecystokinin octapeptide, secretin, and prostaglandin E₂. *Biol Pharm Bull* 25: 14–18, 2002.
 240. **Taupin D and Podolsky DK.** Trefoil factors initiators of mucosal healing. *Nat Rev Mol Cell Biol* 4: 721, 2003.
 241. **Taylor C, Allen A, Dettmar PW, and Pearson JP.** The gel matrix of gastric mucus is maintained by a complex interplay of transient and nontransient associations. *Biomacromolecules* 4: 922–927, 2003.
 242. **Taylor C, Allen A, Dettmar PW, and Pearson JP.** Two rheologically different gastric mucus secretions with different putative functions. *Biochim Biophys Acta* 1674: 131–138, 2004.
 243. **Taylor W.** Pepsins of patients with peptic ulcer. *Nature* 227: 76–77, 1970.
 244. **Teorell T.** A method of studying conditions within diffusion layers. *J Biol Chem* 113: 735–748, 1936.
 245. **Thangarajah H, Wong A, Chow DC, Crothers JM, and Forte JG.** Gastric H-K-ATPase and acid-resistant surface proteins. *Am J Physiol Gastrointest Liver Physiol* 282: G953–G961, 2002.
 246. **Thim L, Madsen F, and Poulsen SS.** Effect of trefoil factors on the viscoelastic properties of mucus gels. *Eur J Clin Invest* 32: 519–527, 2002.
 247. **Tobey N, Hosseini SS, Caymaz-Bor C, Wyatt HR, Orlando GS, and Orlando RC.** The role of pepsin in acid injury to esophageal epithelium. *Am J Gastroenterol* 96: 3062–3070, 2001.
 248. **Tomasetto C, Masson R, Linares JL, Wendling C, Lefebvre O, Chenard MP, and Rio MC.** pS2/TFF1 interacts directly with the VWFC cysteine-rich domains of mucins. *Gastroenterology* 118: 70–80, 2000.
 249. **Tuo BG and Isenberg JI.** Effect of 5-hydroxytryptamine on duodenal mucosal bicarbonate secretion in mice. *Gastroenterology* 125: 805–814, 2003.
 250. **Tuo BG, Sellers Z, Paulus P, Barrett KE, and Isenberg JI.** 5-HT induces duodenal mucosal bicarbonate secretion via cAMP- and Ca²⁺-dependent signaling pathways and 5-HT₄ receptors in mice. *Am J Physiol Gastrointest Liver Physiol* 286: G444–G451, 2004.
 251. **Ulrich CD.** Bicarbonate secretion and CFTR: continuing the paradigm shift. *Gastroenterology* 118: 1258–1261, 2000.
 252. **Van Seuning I, Pigny P, Perrais M, Porchet N, and Aubert JP.** Transcriptional regulation of the 11p15 mucin genes. Towards new biological tools in human therapy, in inflammatory diseases and cancer? *Front Biosci* 6: D1216–D1234, 2001.
 253. **Von Bothmer C, Edebo A, Lönnroth H, Olbe L, Pettersson A, and Fändriks L.** *Helicobacter pylori* infection inhibits antral mucosal nitric oxide production in humans. *Scand J Gastroenterol* 37: 404–408, 2002.
 254. **Walker NM, Flagella M, Gawenis LR, Shull GE, and Clarke LL.** An alternate pathway of cAMP-stimulated Cl⁻ secretion across the NKCC1-null murine duodenum. *Gastroenterology* 123: 531–541, 2002.
 255. **Wallace JL.** Gastric resistance to acid: is the “mucus-bicarbonate barrier” functionally redundant? *Am J Physiol Gastrointest Liver Physiol* 256: G31–G38, 1989.
 256. **Wallace JL and Ma L.** Inflammatory mediators in gastrointestinal defense and injury. *Exp Biol Med* 226:1003–1015, 2001.
 257. **Wang Z, Petrovic S, Mann E, and Soleimani M.** Identification of an apical Cl⁻/HCO₃⁻ exchanger in the small intestine. *Am J Physiol Gastrointest Liver Physiol* 282: G573–G579, 2002.
 258. **Wenzl E, Feil W, Starlinger M, and Schiessel R.** Alkaline secretion. A protective mechanism against acid injury in rabbit duodenum. *Gastroenterology* 92: 709–715, 1987.
 259. **Williams S and Turnberg LA.** Retardation of acid diffusion by pig gastric mucus: a potential role in mucosal protection. *Gastroenterology* 79: 299–304, 1980.
 260. **Williams S and Turnberg LA.** Demonstration of a pH gradient across mucus adherent to rabbit gastric mucosa: evidence for mucus bicarbonate barrier. *Gut* 22: 94–96, 1981.
 261. **Wolosin JD, Thomas FJ, Hogan DL, Koss MA, O’Dorisio TM, and Isenberg JI.** The effect of vasoactive intestinal peptide, secretin, and glucagon on human duodenal bicarbonate secretion. *Scand J Gastroenterol* 24: 151–157, 1989.
 262. **Xu J, Barone S, Petrovic S, Wang Z, Seidler U, Riederer B, Ramaswamy Pradeep K, Dudeja PK, Shull GE, and Soleimani M.** Identification of an apical Cl⁻/HCO₃⁻ exchanger in gastric surface mucous and duodenal villus cells. *Am J Physiol Gastrointest Liver Physiol* 285: G1225–G1234, 2003.
 263. **Yao B, Hogan DL, Bukhave K, Koss MA, and Isenberg JI.** Bicarbonate transport by rabbit duodenum in vitro: effect of vasoactive intestinal polypeptide, prostaglandin E₂, and cyclic adenosine monophosphate. *Gastroenterology* 105: 957–959, 1993.
 264. **Younan F, Pearson JP, Allen A, and Venables CW.** Changes in the structure of the mucus gel on the mucosal surface of the stomach in association with peptic ulcer disease. *Gastroenterology* 82: 827–831, 1982.