Gastroduodenal mucus bicarbonate barrier: protection against acid and pepsin

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MORE THAN A CENTURY AGO in 1892, Schierbeck (205) demonstrated high values of PCO₂ 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 (HCO₃⁻) 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 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 HCO₃⁻ neutralizing intraluminal acid. This leakage, which should be distinguished from metabolism-dependent transport of HCO₃⁻ 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 HCO₃⁻ 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 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.

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.

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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 \( \text{HCO}_3^- \) 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 \( \text{HCO}_3^- \) secretion in this review is on the mechanisms and neurohormonal control of its secretion, the establishment of a 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 \( \mu \text{m} \), 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 fixation 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 \( \mu \text{m} \) (minimum, 50 \( \mu \text{m} \)) and 166 \( \mu \text{m} \) (minimum, 90 \( \mu \text{m} \)), 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 mucoid cap seen on histological sections of reepithelializing gastric mucosa following acute damage (166). The mucoid 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 mucoid 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 intravitral 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 \( \mu \text{m} \) in corpus, 274 \( \mu \text{m} \) in antrum, and 170 \( \mu \text{m} \) 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 \( \mu \text{m} \)) 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 \( \mu \text{m} \)) obtained for rat corpus by using this approach are ~30–40% of those obtained using intravitral...
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 mucous gel, whereas unstirred conditions pertain to intravitral microscopy studies (24, 44). At the same time, mucous layer thickness values from confocal microscopy are still noticeably lower than those for the firm mucous layer (i.e., after suction) from intravitral microscopy studies. A comparative study in vivo using both methodologies for observing mucous 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 mucous layer as a barrier are, first, the gel structure, on which depend the stability and permeability of the adherent mucous layer, and second, the thickness of the adherent layer (see below). Compared with other gastrointestinal secretions, the adherent mucous 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 mucous 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 mucous gels from other noncovalent but rigid gels (e.g., agar). In functional terms, these flow properties are key to the adherent mucous gel layer forming a continuous cover over the mucosa in vivo. Mucus gels are stable, and exposure of isolated gastric mucous 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 mucous gel is dissolved by reduction with thiol agents or proteolysis, both of which destroy the multicimeric 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 mucins contain a protein core covered by a sheath of glycan chains that are 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, 91, 155, 183). Gel-forming mucins are very large molecules (reported molecular masses of $5-45 \times 10^6$ Da) consisting of multimers of mucin units (2–3 $\times 10^6$ 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 by 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, 54, 83, 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 mucous gel structure are known, although there are many questions remaining. The multicimeric 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 mucous layer falls, indicative of a weaker and less stable mucous gel (10, 168, 264). The large, highly hydrated multicimeric mucin molecules interact noncovalently to form the mucous 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 mucous 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 may be different structural patterns within the adherent mucous layer itself. Laminated layers of surface mucus and glandular mucus, which stain for different glycan structures, have been observed in the adherent gastric mucous layer in situ in humans (100, 176). Alternating layers of MUC5AC and MUC6, the two gastric mucin gene products, have been reported in the mucous 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 mucous gel but also the fluid luminal phase, showed a triple lamination of different mucous structures within the secreted mucous 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 mucous gel layer.

TREFOIL PEPTIDES AND THE ADHERENT MUCUS LAYER

There is considerable interest in a putative structural role in mucous 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 mucous 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 influenced 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 Nω-nitro-L-arginine (197). More recently, rat duodenal mucous layer thickness in vivo was shown to be increased by >50% in response to luminal acid, primarily by stimulation of a capsacin-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 mucous 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 mucoid 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-
the thickening 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 penetrate a thin layer of mucus gel in vivo (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 $1 \times 10^{-6}$ cm$^2$/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 pepsin digestion markedly enhanced the HCO$_3^-$ 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 HCO$_3^-$ by PGE$_2$ (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 HCO$_3^-$ 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, mucin 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 CI-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 PGE$_2$, a compound previously shown to stimulate both gastric and duodenal mucosal HCO$_3^-$ 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-PGE$_2$ 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 prostanoids 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 HCO$_3^-$ export stimulated by the...
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, 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 micro-electrodes 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 4–10-fold compared with those in an equivalent “unstirred” layer of solution. 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 $\sim$ 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 mcosa, 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 mucus gel 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 H$_2$-receptor agonist imipramine. In contrast, there is an acidification of the surface mucus gel during inhibition of mucosal HCl secretion by the histamine H$_2$-receptor antagonist ranitidine. The maintenance of the surface pH gradient in acid-exposed gastric surface. Furthermore, secretion by the histamine H$_2$-receptor antagonist ranitidine, i.e., the “alkaline tide,” carried to the surface parietal cells, i.e., the “alkaline tide,” carried to the surface mucosa by the gastric mucosal vasculature (65, 86).

The importance of plasma/interstitial HCO$_3^-$ 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 HCO$_3^-$ prevented ulceration of the acid-exposed gastric surface. Furthermore, serosal (basolateral)-side administration of HCO$_3^-$ 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 HCO$_3^-$ (65). The combined results provide strong evidence that uptake of HCO$_3^-$ by basolateral Na$^+$.HCO$_3^-$ 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 HCO$_3^-$ in the serosal-side solution furthermore enhances the rapid reconstitution of epithelial integrity in damaged gastric mucosa in vitro (213).

SECRETION OF ACID AND PEP SIN 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 though 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 mucous 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-μm-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 mm Hg) 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 mucous 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 HCO$_3^-$ presumably diffuses freely from the surface of the gastric epithelium through the mucus gel layer. Because the alkali secretion (HCO$_3^-$ export) by the gastric mucosa in the main reflects a Cl$^-$/HCO$_3^-$ 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 HCO$_3^-$ 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$^+$.HCO$_3^-$ cotransport at the basolateral membrane as the major mechanism for import of HCO$_3^-$. Recently, it was demonstrated that expression of the Na$^+$.HCO$_3^-$ 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 HCO$_3^-$ is also formed intracellularly from CO$_2$ and H$_2$O. Secretion of HCO$_3^-$/H$^+$ 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$^-$/HCO$_3^-$ 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 Cl⁻/HCO₃⁻ exchange is indeed a HCO₃⁻ export process in the gastric mucosa. Interestingly, recent work (48) also suggested an association between nonacidic Cl⁻ secretion and HCO₃⁻ secretion.

Recordings of pH within the lumen of the gastric glands have provided further information on the secretion of HCO₃⁻ by the gastric mucosa. Intraluminal pH in acid-inhibited, cimetidine-pre-treated 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 intraluminal lumen. The authors proposed that oxytropic 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 (≤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 intraluminal secretion of alkali and/or proteins, such as pepsinogens and mucins, from chief and other nonparietal cells, that may buffer acid in the intraluminal lumen. An only elegant study (182). From the transient nature of the response to carbachol, it was proposed that this secretagogue may induce intraluminal secretion of alkali and/or proteins, such as pepsinogens and mucins, from chief and other nonparietal cells, that may buffer acid in the intraluminal lumen. There are several 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 HCO₃⁻ at the basolateral membrane by Na⁺(n)-HCO₃⁻ cotransport and export HCO₃⁻ via an apical anion conductive pathway and CI⁻/HCO₃⁻ exchange; and in addition, 3) paracellular migration of HCO₃⁻, which is dependent on transmucosal hydrostatic pressure in vitro (64) and intestinal motility in vivo.

DUODENAL ALKALINE SECRETION

In all amphibian and mammalian species tested, the duodenal mucosal surface enterocytes secrete HCO₃⁻, 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 HCO₃⁻ 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 epithelial 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 HCO₃⁻ 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 HCO₃⁻ 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 HCO₃⁻ at the basolateral membrane by Na⁺(n)-HCO₃⁻ cotransport and export HCO₃⁻ via an apical anion conductive pathway and Cl⁻/HCO₃⁻ exchange; and in addition, 3) paracellular migration of HCO₃⁻, which is dependent on transmucosal hydrostatic pressure in vitro (64) and intestinal motility in vivo.
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 HCO₃⁻ as well as Cl⁻ (Fig. 3). CFTR is presently considered a principal anion channel pathway in Cl⁻/HCO₃⁻ 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 HCO₃⁻ 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 HCO₃⁻ secretion mediated by cAMP as well as by cGMP and intracellular Ca²⁺ concentration ([Ca²⁺]), (22, 45, 107, 129, 209, 222). Basal HCO₃⁻ secretion, in contrast, seems mainly dependent on apical Cl⁻/HCO₃⁻ exchange (222, 254). Recently, PAT1 (SLC26A6) was identified as a major Cl⁻/HCO₃⁻ exchanger in the apical membrane of the duodenum (257), along with the DRA (SLC26A3) Cl⁻/HCO₃⁻ exchanger (121, 161).

Parallel operation of CFTR Cl⁻ and apical Cl⁻/HCO₃⁻ exchangers is a possible mechanism for cellular export of HCO₃⁻ (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 Cl⁻/HCO₃⁻ exchange is neither necessary nor increased upon cAMP-induced stimulation of mucosal HCO₃⁻ secretion (222). In contrast, CFTR-dependent Cl⁻/HCO₃⁻ 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 HCO₃⁻ 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 HCO₃⁻ 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 [Ca²⁺]²⁺ 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 HCO₃⁻ 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, Cl⁻/HCO₃⁻ exchange, and Na⁺-HCO₃⁻ 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 Na⁺-HCO₃⁻ cotransporters (NBC) in mouse duodenum were recently studied using RT-PCR, sequence analysis, and immunochemistry (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 HCO₃⁻ and DIDS sensitive and partly dependent on the Na⁺/H⁺ exchange isoform NHE1.

Na⁺/H⁺ exchange would supply intracellular HCO₃⁻ by export of H⁺ formed on hydration of CO₂ to H⁺ and H₂O. 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 (pHi) in these cells (187). In contrast, specific inhibition of the apical isoform NHE3 by the compound S3226 did not affect pHi in rat villus enterocytes in situ (85). However, the latter study (85) also showed that this compound causes a slowly developing rise in HCO₃⁻ secretion by duodenum in anesthetized rats. A rise in net alkaline (HCO₃⁻ 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 HCO₃⁻ 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 Na⁺-K⁺-2Cl⁻ cotransport (NKCC). Basal and cAMP-stimulated HCO₃⁻ secretion in duodenum from NKCC1 knockout mice was very similar to that in wild-type animals, indicating that apical export of HCO₃⁻ does not depend on basolateral Na⁺-K⁺-2Cl⁻ 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 HCO₃⁻ 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 HCO₃⁻-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 PCO₂ rather than pH. In support of this, CO₂ generated at the
surface during reaction between luminal H⁺ and secreted HCO₃⁻ has been shown to be the stimulus of alkaline secretion in rat in vitro (112). Acid and/or Pco₂-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 (~pH 5 in rat and ~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 response. Therefore, the role of these transmitters and drugs has been used to further elucidate the central nervous system influence on gastroduodenal alkaline secretion. 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 HCO₃⁻ 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. Vagal mediated inhibition of duodenal HCO₃⁻ 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⁺, HCO₃⁻, and inosine triphosphate (147, 148). Interestingly, the secretion of HCO₃⁻ 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 α₁-receptor agonist phenylphrine (Fig. 4) caused a marked, up to fivefold, centrally elicited duodenal HCO₃⁻ secretion (149, 216, 217). Intrahypothalamic injection of CRF increased HCO₃⁻ secretion and also enhanced mucosal protection in rat stomach (95).

A variety of peripherally acting agents have been found to influence HCO₃⁻ 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-
agonists into the duodenum in rats increased $\text{HCO}_3^-$ has not been elucidated (39). Recent studies (215, 217) have shown that close intraarterial infusion of low doses of orexin A (Fig. 5) caused a marked and dose-dependent stimulation of the duodenal $\text{HCO}_3^-$ secretion in vivo as well as on enterocyte 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 $\text{MT}_2$-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 sublaryngeal 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 $\text{HCO}_3^-$ secretion via action at enterocyte $\text{MT}_2$ 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 (Sta), stimulate $\text{HCO}_3^-$ secretion by rat and mouse duodenal mucosa. The ratio between the $\text{HCO}_3^-$ and total anion (Cl$^-$ plus $\text{HCO}_3^-$) secretory rates is considerably higher after stimulation with guanylin than after stimulation with a cAMP-dependent agonist such as PGE$_2$ 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 $\text{HCO}_3^-$ 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 $\text{HCO}_3^-$ 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 $\text{HCO}_3^-$ secretion in

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

<table>
<thead>
<tr>
<th>Stimulation</th>
<th>Neuropeptide</th>
<th>Enzyme inhibitors</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>cAMP mediated</td>
<td>Dopamine D$_1$ (76, 198), PACAP (89, 232), prostaglandin EP$_1$ and EP$_2$ (23, 64, 74, 116, 234, 235), VIP (43, 73, 175, 194, 198, 261), 5-HT$_3$ (249, 250)</td>
<td>Acetazolamide (45, 64, 140, 233), COX, cyclooxygenase; [Ca$^{2+}$]$^i$-mediated</td>
<td>Helicobacter pylori infection (108)</td>
</tr>
<tr>
<td>cGMP mediated</td>
<td>Guanylin (93, 192), uroguanylin (129), heat-stable enterotoxin (93, 129)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Ca$^{2+}$]$^i$ mediated</td>
<td>CCK$_i$ (42, 218), melatonin MT$_2$ (217, 218), muscarinic M$_i$ (42, 218), CCK$_i$ (42, 218), prostaglandin EP$_2$ (23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mediator not determined</td>
<td>Angiotensin II AT$_2$ (126), 1,9-endorphin (71, 151), cholera toxin (68, 124), glucagon (1, 67, 93, 258), orexin A (77), neuropeptide Y (69)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physiological conditions</td>
<td>Luminal acid (2, 4, 5, 74, 99, 112, 118, 233), sham feeding (26, 141)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Inhibition

| Neurotransmitters | $\alpha_2$-adrenoreceptor (58, 69, 125, 128, 139), neuropeptide Y (69) |
| Enzyme inhibitors | Acetazolamide (45, 64, 140, 233), COX inhibitors (64, 72, 162, 235) |
| Other | Helicobacter pylori infection (108) |

Representative types of agents that alter duodenal mucosal $\text{HCO}_3^-$ 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 $\text{HCO}_3^-$ secretion. COX, cyclooxygenase; [Ca$^{2+}$]$^i$[mediated CCK$_1$ (42, 218), melatonin MT$_2$ (217, 192), prostaglandin EP$_2$ (23)] 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 MT$_2$-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 sublaryngeal 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 $\text{HCO}_3^-$ secretion via action at enterocyte MT$_2$ 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).

Fig. 5. Short (overnight) food deprivation markedly decreases the sensibility of the $\text{HCO}_3^-$ 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 $\text{HCO}_3^-$ 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 HCO₃⁻ 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 HCO₃⁻ 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 HCO₃⁻ 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). α₂-Adrenoceptors (58, 62, 128, 139) as well as neuropeptide Y1 receptors (69) mediate sympathetic inhibition of mucosal HCO₃⁻ secretion and defense.

DUODENAL PROTECTION AGAINST ACID

In the first part of the duodenum, mucosal HCO₃⁻ is secreted in immediate proximity to the epithelial cell surface and also proximal to the entry of HCO₃⁻ from pancreaticobiliary duct at the duodenal bulb (pH 2.2), the recorded decrease in pHi after 5 min was from pH 7.46 to pH 7.21) with the fluorescence microscopy technique. With both techniques, the juxtamucosal pH in rat duodenum during acid perfusion, pHi decreased, whereas mucus gel thickness decreased 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.

An acid-protective role for the duodenal mucosal alkaline secretion is further supported by the finding that HCO₃⁻ 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 pHi at the surface of this epithelium in duodenal ulcer patients is lower than that in healthy subjects (180). The HCO₃⁻ 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 prostaglandins are well-known inducers of duodenal ulceration in experimental animals as well as in humans (229, 256).

One additional mechanism of duodenal defense, namely, intracellular neutralization of acid, has been proposed from studies of pHi in apical villus cells in rat duodenum in situ. pHi 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, pHi decreased, whereas mucus gel thickness and blood flow increased. At the highest luminal acidity tested (pH 2.2), the recorded decrease in pHi after 5 min was from 7.10 to 6.30 with the fluorescence microscopy technique. The changes in pHi recorded with intracellular microelectrodes, and in response to a somewhat higher acidity (10 mM HCl), were smaller (from pH 7.46 to pH 7.21) than those recorded with fluorescence microscopy. With both techniques,
this decrease in pH, 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 Na$^+$/H$^+$-cotransporter. Subsequent perfusion with neutral luminal solution increased pH$_i$ above baseline values (4), and, in line with the higher rates of transepithelial 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 transepithelial 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 gastrointestinal mucus protective mechanisms against acid and peptic activity. 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 mucous 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 mucous 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 mucous 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 mucous bicarbonate barrier is overwhelmed or when it breaks down in disease, 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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