Gastroduodenal HCO$_3^-$ transport: characteristics and proposed role in acidity regulation and mucosal protection

**Flemström, Gunnar, and Andrew Garner.** Gastroduodenal HCO$_3^-$ transport: characteristics and proposed role in acidity regulation and mucosal protection. Am. J. Physiol. 242 (Gastrointest. Liver Physiol. 5): G183-G193, 1982.—Gastric HCO$_3^-$ transport (basal) studied in isolated amphibian mucosa and mammalian stomach in vivo amounts to 2-10% of maximal H$^+$ secretion. Duodenal mucosa, devoid of Brunner's glands, transports HCO$_3^-$ at a greater rate (per unit surface area) than either stomach or jejunum in vitro and in vivo. Gastric (but not duodenal) HCO$_3^-$ transport is stimulated by dibutyryl cGMP, carbachol, and cholecystokinin and duodenal (but not gastric) transport by dibutryl cAMP and gastric inhibitory peptide. Glucagon and E- and F-type prostaglandins stimulate, whereas histamine, gastrin, and secretin are without effect in both stomach and duodenum. Gastric transport very probably occurs by Cl$^-$-HCO$_3^-$ exchange at the luminal membranes of the surface epithelial cells. In addition to this mechanism, the duodenum also transports HCO$_3^-$ electrogenically. Lowering the luminal pH increases transport in both the stomach and duodenum. This response, probably mediated via both local production of prostaglandins and tissue-specific humoral agents, may be important in mucosal protection against acid. Metabolism-dependent transport of HCO$_3^-$, stimulated by acid, seems quantitatively sufficient to account for all of the duodenal and most of the gastric mucosa's ability to remove luminal acid.

bicarbonate transport; duodenal mucosa; gastric mucosa; gastrointestinal hormones; prostaglandins

Evidence indicating that gastric mucosa secretes HCO$_3^-$ in addition to H$^+$ was presented as early as 1892 by the Danish physiologist Schierbeck (79). Furthermore, his results suggested that HCO$_3^-$ secretion by the stomach could be stimulated. Thus, in carefully controlled experiments in dogs, it was observed that a sham-feeding maneuver increased not only acid secretion but also the amount of CO$_2$ appearing in the gastric lumen. Levels of CO$_2$ tension attained (>100 mmHg) were considerably higher than in blood and indicative of gastric intraluminal neutralization of H$^+$ by HCO$_3^-$ Later, Hollander (52) postulated secretion of an alkaline fluid that was produced at a constant rate and originated from nonparietal sources.

In recent years, use of spontaneously secreting antral preparations and fundic preparations after potent inhibition of H$^+$ secretion has enabled direct measurement and characterization of some of the properties of gastric HCO$_3^-$ transport. Studies with in vitro amphibian antral (16, 17, 30) and fundic (16, 17, 21, 33) mucosa and rabbit (32) antral mucosa have shown that transport is dependent on tissue metabolism, sensitive to a variety of agents, and very probably originates from surface epithelial cells. Sensitivity of HCO$_3^-$ transport to several potential ulcerogens and the stimulatory effect of ulceroprotective agents such as prostaglandins and glucagon have led to the proposal that it may serve a protective function (3, 16, 17, 18, 26, 30, 37).

Techniques have also been developed for direct studies on gastric HCO$_3^-$ transport in the mammalian stomach in vivo. Transport of HCO$_3^-$ with properties similar to those occurring in amphibian mucosa in vitro has been demonstrated in the guinea pig (24, 35), dog (8, 38, 39, 57, 67), and cat (42), and work in humans (72) has been initiated.

The finding of HCO$_3^-$ transport by gastric surface epithelial cells and the possibility that it serves a protective function made it interesting to study whether duodenal epithelium possesses a similar ability to transport HCO$_3^-$. It was found that duodenal preparations, devoid of Brunner's glands, secrete HCO$_3^-$ at considerably higher rates than the stomach or lower parts of the small intestine both in amphibian mucosa in vitro (19, 26, 81, 82).
and in mammals in vivo (25, 29, 41). The stimulatory pathways as well as the mechanism of duodenal \( \text{HCO}_3^- \) transport do, however, show distinct differences from those in the stomach.

The purpose of the present survey is to discuss some of the methodology developed for studying gastroduodenal \( \text{HCO}_3^- \) transport, to summarize present knowledge about its properties including the stimulatory effect of luminal acid, and to discuss its possible role in acidity regulation and mucosal protection.

**Gastric Mucosa**

*Methods in vitro.* With antral mucosa mounted as a flat sheet in an in vitro chamber, luminal alkalinization can be titrated directly (17, 30). This preparation has no or very few \( \text{H}^+ \)-secreting cells. However, studies using gastric fundus must contend with the problem that \( \text{HCO}_3^- \) transport is usually masked by a simultaneous and higher rate of \( \text{H}^+ \) transport. It can be disclosed by inhibition of \( \text{H}^+ \) transport with specific inhibitors such as thiocyanate (SCN\(^-\)) or histamine \( \text{H}_2 \)-receptor antagonists or by use of species where spontaneous arrest of \( \text{H}^+ \) transport occurs (17). Neither SCN\(^-\) (30) nor histamine \( \text{H}_2 \)-antagonist (37) affects gastric transport of \( \text{HCO}_3^- \).

Use of luminal pH levels of 7.40 or higher excludes the possibility of alkalinization of the luminal side resulting from passive diffusion of \( \text{H}^+ \) ions into the mucosa. Luminal alkalinization may then reflect secretion of \( \text{HCO}_3^- \) (or \( \text{OH}^- \)) or active absorption of \( \text{H}^+ \). If secretion of \( \text{HCO}_3^- \) is the case or if the net effect of another transport process is appearance of \( \text{HCO}_3^- \) on the luminal side (cf. Ref. 9), it should be pointed out that continuous gassing of the luminal bathing solution with 100\% \( \text{O}_2 \) as usually used, by removal of \( \text{CO}_2 \) from \( \text{HCO}_3^- \) (and thus formation of \( \text{OH}^- \)), will increase its pH. The pH level used for titration may thus differ from that of the original alkaline secretion, but titration will nevertheless, under steady-state conditions, be a quantitative estimate of the amount of \( \text{HCO}_3^- \) transported. Removal of \( \text{CO}_2 \) requires rapid gassing of the luminal fluid in contact with the mucosa. Chambers appropriate for this purpose have been described by Rehm (73).

Transport of \( \text{HCO}_3^- \) by amphibian mucosa is abolished by anoxia (gassing with \( \text{N}_2 \)) and antimetabolites [cyanide (CN\(^-\)) or 2,4-dinitrophenol] and stimulated by the cyclic nucleotide dibutyryl cGMP (DBcGMP), prostaglandins, and low concentrations of some hormones, strongly suggesting it to be an active process. It is also independent of \( \text{HCO}_3^- \) (and \( \text{CO}_2 \)) on the nutrient side (17, 26, 37). In most experiments in vitro, however, the nutrient bathing solution is buffered with this ion (and \( \text{CO}_2 \)), comparable with gastric mucosa in vivo that is exposed to tissue and blood \( \text{HCO}_3^- \). This raises the problem that some agents, by increasing paracellular or other pathways of the normally tight (84) gastric fundic epithelium, may induce leakage of \( \text{HCO}_3^- \) into the luminal solution. It is important to distinguish between this phenomenon and metabolism-dependent \( \text{HCO}_3^- \) transport. Experiments illustrating this point with respect to the effects of ethanol on frog fundic mucosa are shown in Fig. 1. At a nutrient-side concentration of 14\% (vol/vol), ethanol after a transient decline increases the amount of alkali titrated (at pH 7.40) on the luminal side. Simultaneously, there are decreases in mucosal electrical potential difference (PD) and resistance, indicating increased mucosal ionic permeability. Use of N-2-hydroxyethylpiperazine-\( \text{N}'\)-2-ethanesulfonic acid (HEPES\(^-\)) buffer (gassed with 100\% \( \text{O}_2 \)) instead of \( \text{HCO}_3^- \) on the nutrient side can be used to show that ethanol is a potent inhibitor of metabolism-dependent transport (Fig. 1). Both nutrient \( \text{HCO}_3^- \) and HEPES\(^-\) very probably permeate the ethanol-treated low-resistance mucosa, but only \( \text{HCO}_3^- \) being a volatile buffer is titrated. Lower concentrations of ethanol inhibit metabolism-dependent \( \text{HCO}_3^- \) transport without inducing passive leakage of this ion (21). It is also probable that, if very high concentrations of ethanol or other permeability-increasing agents are used, only the increased passive leakage of \( \text{HCO}_3^- \) may be recorded. The gastric antrum is a more "leaky" epithelium than the fundus (83), and

![FIG. 1. Ethanol was added as indicated to nutrient (serosal) side of alkaline-secreting (metiamide-treated) frog (Rana temporaria) fundic mucosa. Luminal side was always bathed with an unbuffered solution (gassed with 100\% \( \text{O}_2 \)), and pH on this side was kept constant at 7.40 by infusion of HCl. This nutrient side was buffered with either 17.8 m\( \text{M} \) \( \text{HCO}_3^- \) (gassed with 95\% \( \text{O}_2 \)-5\% \( \text{CO}_2 \)) or 5 m\( \text{M} \) N-2-hydroxyethylpiperazine-\( \text{N}'\)-2-ethanesulfonic acid (HEPES\(^-\)) (gassed with 100\% \( \text{O}_2 \)). Note that metabolism-dependent transport of \( \text{HCO}_3^- \) alone is titrated with HEPES\(^-\)\( \text{O}_2 \) on nutrient side. With nutrient \( \text{HCO}_3^-\text{CO}_2 \), both this transport and \( \text{HCO}_3^- \) permeating passively across low-resistance mucosa contribute to luminal alkalinization (Ref. 21). PD, potential difference.](http://ajpgi.physiology.org/)}
(untreated) antrum possesses pathways for passive migration of $\text{HCO}_3^-$ in addition to metabolism-dependent transport of this ion (17, 32).

Control of preoperative handling and feeding of animals is important in studies with stimulants and inhibitors of gastric (and duodenal) $\text{HCO}_3^-$ transport both in vitro and in vivo. Intraluminal acid increases transport in vitro (47, 48) and in vivo (38, 39, 41), and part of this response is most probably mediated via an increase in tissue prostaglandin levels. Variations in the level of endogenous prostaglandin (or other mediator controlling $\text{HCO}_3^-$ transport) may thus affect the sensitivity to their exogenous administration. Such variations may be present in control preparations (e.g., seasonal or species variation), induced by preoperative conditions (e.g., storage or feeding), or result from experimental procedures (e.g., operative trauma or exposure to acid). Hence exposure of cat duodenum to acid rendered this tissue insensitive to $E$-type prostaglandins (41), whereas frog gastric (37, 70) and guinea pig duodenal (25, 29) mucosa did not respond to prostaglandin $E_2$ (PG$E_2$) unless pretreated with inhibitors of prostaglandin synthesis. Also, stimulation of gastric $\text{HCO}_3^-$ transport by the synthetic analogue 16,16-dimethyl PG$E_2$ has been observed in frog (Rana temporaria) preparations in vitro (37) and in other species in vivo (8, 39, 42, 57, 67), whereas Schiessel et al. (80) reported this agent to be without effect on $\text{HCO}_3^-$ transport by bullfrog fundic mucosa. It seems thus preferable to use control animals pretreated with indomethacin or another inhibitor of tissue prostaglandin synthesis for work on gastroduodenal $\text{HCO}_3^-$ transport.

Methods in vivo. The occurrence of titratable $\text{HCO}_3^-$ in gastric secretion in vivo was first demonstrated in canine antral pouches (43) and in fundic pouches where $H^+$ transport had been decreased by vagotomy and antrectomy (52). It has also been demonstrated in humans during spontaneous $H^+$ secretory arrest (64). More recently, use of histamine $H_2$-antagonists has permitted quantitation of gastric $\text{HCO}_3^-$ secretion in the perfused fundic pouch (38, 39, 57, 67). With this technique, adequate removal of $\text{CO}_2$ formed during reaction between secreted $\text{HCO}_3^-$ and titrant (acid) is necessary. Accumulated $\text{CO}_2$ may otherwise oxidize the perfusate and thus apparently inhibit alkalinization.

An alternative approach enables gastric secretion of both $\text{HCO}_3^-$ and $H^+$ to be measured simultaneously from determination of gastric intraluminal pH and $\text{CO}_2$ tension (23, 35). A prerequisite for this technique is that gastric mucosal permeability to $\text{CO}_2$ is low so that intraluminal $\text{CO}_2$ originates mainly from reaction secreted $\text{HCO}_3^-$ and $H^+$. Differential effects of $\text{CO}_2$ applied to secretory (mucosal) and nutrient (serosal) sides of in vitro frog preparations (54) suggest low $\text{CO}_2$ permeability. Experimental data in vivo have demonstrated low mucosal permeability to $\text{CO}_2$ in both the guinea pig (35) and dog (58) stomach, and methodology based on intragastric pH and $\text{CO}_2$ tension measurement has recently been adapted for studies of $\text{HCO}_3^-$ transport in humans (72).

Direct titrations at low values of gastric intraluminal pH cannot distinguish between $H^+$ removal due to passive diffusion and secretion of $\text{HCO}_3^-$. In addition, measurement of $\text{HCO}_3^-$ from intraluminal $\text{CO}_2$ under such conditions (58) is likely to give an underestimate of $\text{HCO}_3^-$ transport; reaction between $\text{HCO}_3^-$ and $H^+$ with release of $\text{CO}_2$ occurring in closer vicinity to the luminal cell membranes (see below) may thus facilitate increased absorption of $\text{CO}_2$.

Increases in mucosal permeability with ensuing passive leakage of $\text{HCO}_3^-$ constitute a similar problem when evaluating the nature of increases in $\text{HCO}_3^-$ appearance in gastric lumen in vivo as in vitro. Davenport (11) demonstrated that high concentrations of acetylsaliclycic acid not only increase apparent removal of $H^+$ from the gastric lumen but also induce leakage of plasma proteins and glucose into the lumen, indicating creation of pathways for their passive transmucosal migration. Increases in mucosal passive permeability in vivo have also been estimated from gastric absorption of saccharide macro-molecules (27, 28) and mucosal permeability to horseradish peroxidase (68). It has been observed that some procedures, including gastric intra-arterial injection of acetylcholine (4), increase ultrafiltration of large amounts of protein and ions, including $\text{HCO}_3^-$, into the gastric lumen.

Gastric output of $Na^+$ has been used frequently as an index of nonparietal alkaline secretion (52, 66). However, $\text{HCO}_3^-$ transport probably occurs by $Cl^--\text{HCO}_3^-$ exchange (21), and passive components of $Na^+$ flux across gastric mucosa (51, 56) have been demonstrated. Interestingly, pathways for $Na^+$ transport depend on luminal pH in both antral (5) and fundic (59) mucosa. Thus, $Na^+$ in gastric juice may not be a precise quantitative estimate of $\text{HCO}_3^-$ transport.

Properties and origin of metabolism dependent gastric $\text{HCO}_3^-$ transport. Properties of gastric $\text{HCO}_3^-$ transport examined in amphibian fundic mucosa are summarized in Table 1. Basal transport amounts to 0.2-0.5 $\mu$eq cm$^{-2}$ h$^{-1}$, and DBcGMP, carbachol (17), cholecystokinin, glucagon (26), and prostaglandins (37) increase transport. Stimulation by calcium ions very probably reflects mucosal release of acetylcholine, inasmuch as it is inhibited by atropine or magnesium ions (23). Inhibition by acetazolamide is asymmetric because $10^{-4}$ M is sufficient to produce an effect on the luminal side, whereas $10^{-2}$ M is required on the nutrient side (17). Atropine abolishes stimulation by carbachol but does not affect basal $\text{HCO}_3^-$ transport (23, 35). Well-known stimulants of gastric $H^+$ transport such as histamine, gastrin, or DBcAMP are without effect on gastric $\text{HCO}_3^-$ transport (17, 18), indicating that stimulatory pathways for $\text{HCO}_3^-$ are different from those for $H^+$ transport.

Gastric mucosal surface epithelial cells contain most of the mucosal activity of cGMP diesterase (87) and carbonic anhydrase (46), and the concentrations of DBcGMP or acetazolamide affecting $\text{HCO}_3^-$ transport are considerably lower than those necessary to exert an effect on $H^+$ secretion (17). Antral mucosa is composed mainly of surface epithelial cells, and there is close morphological similarity between surface epithelial cells in fundus and antrum. In addition, fundic and antral metabolism-dependent $\text{HCO}_3^-$ transports display almost identical properties. These findings strongly suggest that transport originates from the surface epithelial cells (17). It is interesting that cholinergic stimuli elevate mucosal con-
TABLE 1. Properties of HCO₃⁻ transport in amphibian isolated gastric mucosa

<table>
<thead>
<tr>
<th>Stimulants</th>
<th>DBCGMP (10⁻⁶ M), calcium, carbachol (10⁻⁴ M), cholecystokinin (10⁻⁸ to 10⁻⁷ M), pancreatic glucagon (10⁻⁵ M), PGE₁ (10⁻⁵ M), PGE₂ (10⁻⁷ M), CGMP, CN⁻, acetazolamide (10⁻⁴ M),* anoxia, CN⁻ (10⁻² M), atropine (10⁻⁴ M),* 2,4-dinitrophenol (10⁻⁴ M), ethanol (3%, vol/vol), histamine (10⁻⁴ M), hydrocortisone (500 mg/ml), isoprenaline (10⁻⁴ M), motilin (10⁻⁴ M), secretin (10⁻⁴ M),* serotonin (10⁻⁴ M), somatostatin (10⁻⁷ M), substance P (10⁻⁶ M), uracil (10⁻⁷ M), vasoactive intestinal peptide (10⁻⁸ M)*</th>
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<tr>
<td>No effect</td>
<td>DBCAMP (10⁻³ M), ACTH (0.3 IU/ml), amiloride (10⁻⁴ M), carbenoxolone (10⁻⁴ M), gastrins (10⁻⁸ to 10⁻⁷ M), histamine (10⁻⁶ M), hydrocortisone (500 mg/ml), isoprenaline (10⁻⁴ M), motilin (10⁻⁴ M), secretin (10⁻⁴ M),* serotonin (10⁻⁴ M), somatostatin (10⁻⁷ M), substance P (10⁻⁷ M), uracil (10⁻⁷ M), vasoactive intestinal peptide (10⁻⁸ M)*</td>
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With the exception of acetazolamide and taurocholate (applied luminaly), agents were administered on the nutrient side of fundic mucosa after inhibition of H⁺ transport by histamine H₂-antagonists. Stimulants of gastric H⁺ transport were also tested in spontaneously alkalinizing antral mucosa. Concentrations of stimulants and inhibitors in parentheses are the minimal concentration tested that produced a significant (P < 0.05) effect on HCO₃⁻ transport. In the case of agents without effect, maximal concentrations tested are given. Atropine had no effect on "basal" transport but abolished stimulation by carbachol. Ouabain was tested in mucosae stimulated by prostaglandin F₂α (PGE₂), and PGE₁, in mucosae inhibited by thupropfen. (Refs. 16–21, 23, 24, 26, 30, 33, 36, 37, 70, 71, and unpublished data.) DBcGMP, dibutyryl cGMP; CN⁻, cyanide. * Agents have been tested and found to exert a similar response in mammals in vivo.

centrations of cGMP in both fundus and antrum (10) together with increasing HCO₃⁻ transport. This suggests that nucleotide increases are located in the surface cells, and cGMP may serve the role of an intracellular messenger in cholinergic stimulation of HCO₃⁻ transport. In this connection, histamine H₂-receptor antagonists attenuate the rise in cAMP (but not cGMP) in response to acetylcholine in fundic mucosa (15).

Removal of luminal (but not nutrient) Cl⁻ abolishes HCO₃⁻ transport by frog fundic mucosa, and stimulation of transport, in contrast to H⁺ transport, is not associated with changes in mucosal PD or electrical resistance. These findings together with its greater sensitivity to luminal acetazolamide suggest that alkalization occurs by electroneutral Cl⁻-HCO₃⁻ exchange at the luminal cell membrane (20, 21). Most of the carbonic anhydrase activity in surface epithelial cells is localized to the apical cytoplasmic matrix and the microvillar cores (86).

Properties of the surface epithelial cells have been studied by micropuncture techniques in Necturus antrum (30). The luminal membrane displays an amiloride-sensitive Na⁺ conductance, whereas changes in luminal HCO₃⁻ concentration result in only small and slowly developing changes in the PD across this membrane. Ouabain has been demonstrated to bind to the basal membrane of gastric surface epithelial cells in the frog (14). This agent (10⁻⁶ M, nutrient side) was without effect on basal HCO₃⁻ transport by Necturus antrum (24) but inhibited alkalinization in frog (R. temporaria) fundus

stimulated by PGF₂α, (unpublished data). This might indicate a role for cell membrane Na⁺-K⁺-ATPase activity in maintenance of the intracellular electrolyte pattern rather than in the gastric HCO₃⁻-transport process.

**Gastric HCO₃⁻ in vivo.** Transport studied in experiments where mucosal permeability was not artificially increased exhibits sensitivity to stimulants and inhibitors very similar to that observed for metabolism-dependent transport in vitro. Thus, E-type prostaglandins increase gastric HCO₃⁻ transport in dogs (8, 39, 57, 67), cats (42), and probably humans (56). Carbachol and calcium ions stimulated transport of HCO₃⁻ (and H⁺) in the guinea pig (23), and low doses of acetylsalicylate (34) are inhibitory in this species. Secretin and vasoactive intestinal peptide are without effect (39), and acetazolamide (58) inhibits HCO₃⁻ transport in the dog. A difference has been observed with respect to effects of parathyroid hormone (24). This hormone inhibits transport by amphibian gastric mucosa in vitro but not in the guinea pig in vivo. It seemed likely, however, that stimulatory effects of released calcium masked any direct inhibitory effect of the hormone in vivo.

In the guinea pig, stimulation of HCO₃⁻ transport by carbachol (35) or inhibition by acetylsalicylate (34) were accompanied by changes in Na⁺ output of similar magnitude and in the same direction. The nature of this association is not known, but raising luminal pH increases mucosal permeability to cations both in antrum (5) and fundus (59). An increase in Na⁺ output might thus be secondary to alkalinization of the luminal surface by secreted HCO₃⁻.

**Duodenal Mucosa**

Duodenal secretion was studied in several mammalian species long ago by Florey and his collaborators (31). They demonstrated that feeding or intraluminal administration of acid increased the volume of secretion in transplanted proximal duodenal pouches and proposed humoral stimulation of an alkaline secretion originating from the Brunner's glands. Later, other investigators demonstrated that proximal and distal duodenum in dogs (12, 44, 49) and humans (94) disposes of instilled acid at about equal rates, although in both species Brunner's glands are confined mainly to the proximal portion of the duodenum. This suggests that duodenal surface epithelium rather than Brunner's glands accounts for removal of acid. It was also found that CO₂ tension in distal duodenal segments attained levels in excess of 100 mmHg when acid was instilled, indicating an intraluminal reaction between H⁺ and HCO₃⁻ (44). These findings and the demonstration of metabolism-dependent HCO₃⁻ transport by gastric mucosa initiated characterization of the nature of duodenal alkalization. Studies have been performed in vitro (19, 26, 81, 82) in isolated duodenum from the bullfrog, a species devoid of Brunner's glands, and in vivo (25, 29, 41) in duodenal segments from an area just distal to the Brunner's gland in cats, guinea pigs, rats, and rabbits.

**Methods of study.** Duodenal mucosa, isolated from the bullfrog and stripped of external muscle layers, was first mounted as a tube in an vitro chamber (19), and
luminal alkalinization was titrated continuously with HCl under automatic control from pH-stat equipment. Although this preparation probably minimizes edge damage, mucosae mounted as a flat sheet (81) have given similar values in regard to the rate of alkalinization (∼1 μeq·cm⁻²·h⁻¹) and PD (3–10 mV, lumen negative). In vivo, segments of duodenum in situ have been studied during rapid perfusion with unbuffered solutions (25, 29).

The in vitro preparation shows marked sensitivity to variations in transmucosal hydrostatic pressure. Changes of only 2 cmH₂O affect a component of passive migration (but not metabolism-dependent transport) of HCO₃⁻ and necessitate use of carefully controlled conditions (19). Duodenal HCO₃⁻ transport in vivo (25, 29) exhibits dependence on the depth of anesthesia and on body temperature because too deep (or superficial) an anesthesia and a body temperature below 37°C are inhibitory. Stretching of the mesentery during operation or rapid intravenous injection of large (>2 ml/kg) fluid volumes transiently increase transport in both the cat and guinea pig. It seems therefore advantageous to inject drugs or hormones in a small volume and to compare effects of intraluminal and intravenous administration. In addition, control of preoperative feeding seems as important in studies of the duodenum as of the stomach.

Properties of metabolism dependent duodenal HCO₃⁻ transport. Stimulants and inhibitors of transport in proximal bullfrog duodenum are summarized in Table 2. Basal transport of HCO₃⁻ (∼1.0 μeq·cm⁻²·h⁻¹) in this species is greater than that in gastric fundus (∼0.55 μeq·cm⁻²·h⁻¹) or antrum (∼0.45 μeq·cm⁻²·h⁻¹). The duodenum also shows some distinct differences from the stomach with regard to stimulation of HCO₃⁻ transport (26). DBCAMP but not DBCGMP stimulates transport in the duodenum, a situation opposite to their effects in gastric mucosa. Carbachol increased gastric HCO₃⁻ transport both in vivo and in vitro but had no effect in the duodenum. Gastric inhibitory peptide (GIP), a stimulant at concentrations as low as 10⁻¹⁰ M in the duodenum, was (weakly) inhibitory in the stomach. α-Adrenergic agonists inhibited transport in the stomach (18) but stimulated transport in the duodenum, whereas taurocholate (10⁻⁷ M), an inhibitor in the stomach (71), was without effect in the duodenum (unpublished data).

Stimulation of HCO₃⁻ transport by prostaglandins in duodenum (19) was in contrast to findings in the stomach (37) associated with a simultaneous rise in the transmucosal PD, suggesting different mechanisms of transport. Using the isolated proximal mucosa from bullfrog, Simon, Merhav, and Silen (81, 82) have recently studied ion requirements of duodenal HCO₃⁻ transport. They found basal transport to be independent of the presence of Cl⁻ in the bathing solutions but dependent on nutrient (but not luminal) Na⁺ and to be inhibited by ouabain. From these findings and studies of the transmucosal fluxes of Na⁺, Cl⁻, and HCO₃⁻ in short-circuited mucosae, they concluded that duodenal transport of HCO₃⁻ is electrogenic. In an interesting model, they (81, 82) propose that Na⁺ enters the cell by electroneutral cotransport of NaHCO₃ across the nutrient membrane and then recycles at this membrane due to the activity of an ouabain-sensitive Na⁺-K⁺-ATPase. Passive exit of HCO₃⁻ across the luminal membrane results in a net electrogenic HCO₃⁻ transport.

However, properties of duodenal epithelial HCO₃⁻ transport depend on both the “nature” of basal transport and the particular stimulant used to increase transport. In mucosae from frogs kept at ∼10°C or at room temperature and fed regularly, stimulation of HCO₃⁻ transport to high rates by PGE₂ was necessary to demonstrate inhibition by ouabain (22), suggesting that differences in pretreatment of animals affect endogenous prostaglandin levels and consequently the basal “drive” of the preparation. In experiments starting in Cl⁻-free conditions, nutrient administration of Cl⁻ decreased transport concentration dependently (22, 26). This indicates a nutrient membrane-transport mechanism for HCO₃⁻ that displays some affinity also for Cl⁻. In agreement with the findings of Simson et al. (81), basal transport was independent of luminal Cl⁻ (26). This was found also for prostaglandin-stimulated transport, while stimulation by glucagon required luminal Cl⁻. Stimulation by hormones (GIP and glucagon), in contrast to findings with prostaglandins or DBCAMP, was not associated with a rise in transmucosal PD. Furthermore, pretreatment with furosemide had no effect on basal or prostaglandin-stimulated transport but abolished stimulation by glucagon. These findings (26) suggest that duodenal mucosa, in addition to electrogenic transport of HCO₃⁻, transports this ion via Cl⁻–HCO₃⁻ exchange at the luminal membrane as in lower parts of the small intestine (55). Proposed pathways for HCO₃⁻ transport in duodenum are summarized in Fig. 2.

**Table 2. Properties of HCO₃⁻ transport in bullfrog isolated proximal duodenum**

<table>
<thead>
<tr>
<th>Stimulants</th>
<th>DBcAMP (10⁻⁶ M), arachidonic acid (10⁻⁶ M), gastric inhibitory peptide (10⁻¹⁰ M), 3-isobutyl-1-methylxanthine (10⁻⁵ M), noradrenaline (10⁻⁴ M), pancreatic glucagon (10⁻³ M) * PGE₂ (10⁻³ M) * 16α-dimethyl PGF₂α (10⁻³ M) * PGF₁α (10⁻³ M) *</th>
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<tr>
<td>Inhibitors</td>
<td>Acetazolamide (5 × 10⁻³ M),* anoxia, CN⁻ (5 × 10⁻³ M), 2,4-dinitrophenol (10⁻⁵ M), indomethacin (5 × 10⁻⁵ M),* ouabain (10⁻⁷ M)</td>
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With the exception of ouabain, effects of agents were tested in spontaneously alkalinizing duodenum. An inhibitory effect with ouabain was only observed in prostaglandin-stimulated mucosa. For stimulants and inhibitors, the minimal concentration tested exerting a significant (P < 0.05) effect is provided. For agents without effect, the maximal concentration tested is shown. (Refs. 19, 26, 70, and unpublished data.) DBcAMP, dibutyryl cAMP; PGE₂, prostaglandin E₂; CN⁻, cyanide. * Agents have been tested and found to exert a similar response in mammalian duodenum in vivo.
and GIP (injected intravenously) produce similar responses to those found in vitro; there is thus a rise in HCO$_3^-$ transport but no change in PD (25, 29). Both indomethacin (29) and acetazolamide (1) inhibit alkalinization, whereas secretin (25) is without effect. Thus, although secretin is a stimulant of pancreatic HCO$_3^-$ transport, it is without effect on either gastric or duodenal HCO$_3^-$ transport in vitro and in vivo. These data strongly suggest that the in vitro amphibian mucosa provides a representative model for conditions pertaining to mammalian duodenum in vivo.

In a recent report (85), effects of cysteamine on a variety of enzyme activities in rat duodenal mucosal cells are described. Specific inhibition of brush-border alkaline phosphatase was demonstrated, and it is proposed that this enzyme via HCO$_3^-$-stimulated ATPase activity is part of the duodenal HCO$_3^-$ secretory mechanism. Cysteamine has been reported to inhibit duodenal HCO$_3^-$ transport in rats, and the duodenal ulcerogenic activity of this agent is well known (1).

Stimulation of Gastroduodenal HCO$_3^-$ Transport by Luminal Acid

It is well established that the rate of removal of luminal HCl either instilled or secreted into the stomach increases with increasing concentration of the acid. This has been interpreted as evidence for passive diffusion of H$^+$ along the chemical gradient into the mucosa (69,90). An alternative explanation would be that acid within the lumen of the stomach stimulates mucosal transport of HCO$_3^-$ or that a combination of these mechanisms occurred. Recent work has demonstrated that luminal acid stimulates HCO$_3^-$ transport (38). Thus instillation of 10 mM HCl into the lumen of the gastric remnant increased titratable alkalinity of fluid perfusing the (vagally denervated) dog fundic pouch (Fig. 3). Interestingly, this response was associated with an increase in plasma enteroglucagon (38, 39). Exposure of the lumen of cat duodenum, devoid of Brunner's glands, and pancreatic or bile HCO$_3^-$ to 10-25 mM HCl increased HCO$_3^-$ transport (29, 41). This response in the cat was associated with increased PGE$_2$ and decreased DNA appearance in the luminal perfusate (41). The reduction in DNA indicates that the rise in HCO$_3^-$ was not the result of cellular desquamation and further suggests that increased HCO$_3^-$ transport in response to luminal acid offered sufficient protection of the mucosal surface. Although acid-stimulated HCO$_3^-$ transport in control animals was sustained (cf. Fig. 4), the response was only transient in cats pretreated with indomethacin (41), suggesting that endogenous prostaglandin is involved in maintaining “steady-state” secretory rates.

Definitive evidence for humoral components in mediation of the response to luminal acid has been presented by Heylings et al. (47,48) in experiments where two frog fundic or duodenal mucosae were mounted in parallel with their nutrient (blood) surfaces facing the same buffered solution. Lowering the pH on the luminal (secretory) side of one mucosa caused an increase in HCO$_3^-$ transport by the other (not exposed to acid) tissue. Luminal acidification of one fundus transiently increased HCO$_3^-$ transport by a parallel fundus. A similar transient response occurred on unilateral acidification in pairs of duodenum, whereas fundic acidification did not affect a parallel duodenum, suggesting tissue-specific mediation of the fundic response. However, duodenal acidification caused a sustained increase in HCO$_3^-$ transport by a parallel fundus (J. R. Heylings, A. Garner, and G. Flemstrom, unpublished data). In addition to local (prostaglandin) and humoral stimuli, intrinsic nervous reflexes may also be involved (Fig. 5). This possibility, however, has not been tested.
HCO₅⁻ Transport in Regulation of Intraluminal Acidity

Both passive diffusion of H⁺ (69, 90) and neutralization and dilution by nonparietal (52, 66) secretions have been proposed to account for the continuous removal of H⁺ secreted or instilled into the stomach. Mathematical treatment of data from experiments where only net acid (H⁺ minus HCO₅⁻) is measured probably cannot distinguish between the two processes (66). Therefore, to evaluate whether HCO₅⁻ transport is quantitatively sufficient to account for disappearance of acid, rates of this transport and H⁺ loss were compared in dogs with Heidenhain pouches (41). Acid disappearance calculated by linear regression analysis of the relation between acid output and secretory volume (on stimulation by histamine) was 157 ± 11 μeq/h. This method provides a good estimate of the acid loss occurring at high and medium rates of H⁺ secretion (Ref. 69, p. 69). Basal HCO₅⁻ transport after inhibition of H⁺ secretion in the same pouches was 53 ± 4 μeq/h, and instillation of 10 mM HCl into the remnant of the main stomach increased HCO₅⁻ transport in the pouch to 105 ± 16 μeq/h. These results indicate that, at high rates of H⁺ secretion, in addition to being neutralized by secreted HCO₅⁻, some intraluminal acid (approximately one-third) could be removed by diffusion. However, it should be noted that exogenous acid was not instilled into the HCO₅⁻-secreting pouch. Local mucosal mechanisms may increase HCO₅⁻ transport further in tissues directly exposed to acid.

These experiments were performed on dogs with intact mucosa. Davenport (11) has shown that several agents, including ethanol, sulphydryl reagents, and unionized forms of some weak acids, render gastric mucosa permeable to ions and larger molecules. It is very likely that, under conditions of increased permeability, H⁺ diffuse out from (and HCO₅⁻ into) the gastric lumen. Gastric nonparietal secretion has been postulated to have a defined volume (52, 66). It seems likely, however, that, due to the higher mobility of H⁺, neutralization of HCO₅⁻ occurs in close vicinity to the luminal surface of the epithelium. Reaction between H⁺ and HCO₅⁻ should reduce osmolarity of the combined secretion, and excess water probably moves along its chemical gradient into the mucosa (cf. Ref. 13). Gastric transport of HCO₅⁻ may therefore appear as a surface neutralization reaction as opposed to secretion with a defined volume. This contention would also be consistent with a HCO₅⁻-Cl⁻ exchange process.

Duodenal HCO₅⁻ transport stimulated by prostaglandins or luminal acid amounts to 0.3–0.4 μeq·cm⁻²·min⁻¹ in the cat and to ~0.5 μeq·cm⁻²·min⁻¹ in the guinea pig (25). These rates are similar to the rate of loss of H⁺ from instilled acid in the dog proximal and distal duodenum (~0.6 μeq·cm⁻²·min⁻¹). Direct titration (25, 29, 41) gives considerably higher values of duodenal HCO₅⁻ transport than estimates made on the basis of duodenal intraluminal CO₂ tension (44, 49). This difference probably reflects removal of some CO₂ by duodenal mucosa.
Proposed Protective Role of Gastroduodenal \( \text{HCO}_3^- \) Transport

The demonstration that gastric \( \text{HCO}_3^- \) transport is inhibited by several potential ulcerogens, including non-steroidal anti-inflammatory drugs (33, 34, 36, 70), ethanol (21), \( \alpha \)-adrenergic agonists (18), and acetazolamide (17, 30, 58), and stimulated by ulceroprotective agents (74, 76, 88) such as prostaglandins (8, 37, 39, 42, 57, 67) and glucagon (26) suggests that metabolism-dependent \( \text{HCO}_3^- \) transport is one component in mucosal protection against acid. Acetazolamide abolishes the ability of canine gastric mucosa to resist concentrations of luminal acid higher than 25 mM (92) and prevents the ulceroprotective action of \( \text{PGE}_2 \) in the rat (61). Sympathectomy prevents development of gastric stress ulcers in the dog (53). The inhibition of \( \text{HCO}_3^- \) transport by taurocholate in gastric (but not in duodenal) mucosa might relate to the gastric ulcerogenic activity of bile acids.

Due to smaller (2-20\%, even following stimulation) rates of \( \text{HCO}_3^- \) compared with \( \text{H}^+ \) transport, \( \text{HCO}_3^- \) would offer little protection if neutralization of \( \text{H}^+ \) occurred in the luminal bulk solution (18). Transport of \( \text{HCO}_3^- \) into a surface boundary of low turbulence would considerably enhance its protective effect. The thin layer of viscoelastic mucus adherent to the mucosa consists of a matrix of glycoprotein molecules, which form a gel joined by physical noncovalent interactions. Properties of this gel, recently reviewed by Allen (2) and Allen and Garner (3), seem appropriate to support \( \text{H}^+ \) and \( \text{HCO}_3^- \) gradients (Fig. 6). The gel is continuously formed by the mucus cells and degraded and solubilized at its luminal surface by pepsin. It is permeable to small ions but impermeable to pepsin. Prostaglandins stimulate gastric production of both mucus (7, 56) and \( \text{HCO}_3^- \), whereas the ulceroprotective drug carbenoxolone (7, 70) and the hormone secretin (26, 39, 58) increase only the former, indicating, at least in part, different stimulatory pathways for mucous secretion and \( \text{HCO}_3^- \) transport.

Teorell examined counterdiffusion of acid and base in a membrane model as early as 1936 (89) and also pointed out (91) that the profile of the ion gradients in a boundary depends on the amounts of acid and base available for diffusion, the mobilities of these and other ions present, its electrical charge, and the transboundary electrical potential. Later, Heatley (45), on the basis of studies on gastric mucus in vitro, proposed that counterdiffusion of \( \text{H}^+ \) and \( \text{HCO}_3^- \) occurs at the surface of gastric mucosa. Direct evidence for the occurrence of a pH gradient across the gastric mucous gel in the rabbit fundus in vitro was presented recently (93). With pH-sensitive micro-electrodes, it was shown that pH at the cell membrane is slightly alkaline (~7.3), in spite of that in the luminal bulk solution being acid (~2.3). Interestingly, \( \text{CN}^- \), an inhibitor of \( \text{HCO}_3^- \) transport, abolished the pH gradient. Similar findings have been made using acetylsalicylate in vivo (75).

Gastric mucosal resistance to acid is, however, probably multifactorial, and it cannot presently be ascertained whether \( \text{HCO}_3^- \) transport is sufficient to maintain a neutral pH at the luminal cell membrane with values of pH in the luminal bulk solution below 1.5-2.0. Metabolism-dependent \( \text{HCO}_3^- \) transport nevertheless seems sufficient for protection at intraluminal pH values that normally prevail in the stomach. A “second line” of defense may be provided by intracellular neutralization of \( \text{H}^+ \) in the surface epithelial cells by \( \text{HCO}_3^- \) transported into these cells by \( \text{Cl}^-\text{-HCO}_3^- \) exchange at the nutrient membrane (60). It is also possible that protein from cells exfoliated into the mucous gel buffers (6) and thus retarded access of \( \text{H}^+ \) to the mucosal surface. Exposure of dog gastric mucosa to 50 mM (but not 10 mM) HCl results in an increase in release of DNA into the lumen, indicating exfoliation of mucosal cells (40). Interestingly, exfoliation is inhibited by exogenous prostaglandins in both dogs (40) and humans (76).

Luminal membrane carbonic anhydrase activity, which is probably involved in gastric secretion of \( \text{HCO}_3^- \), is absent only in parietal and zymogen cells (46). Although less in the zymogen cell, the luminal (but not basal) membrane of both these cell types demonstrated \( \text{H}^+\text{-K}^+\text{-ATPase} \) activity (77). This activity has been shown to be part of the \( \text{H}^+ \) secretory process of the parietal cell (78), and ability to excrete \( \text{H}^+ \) might render these cells within the fundic glands less sensitive to acid.

In the duodenum, it would seem logical that pH at the epithelial surface is more important in protection than pH in the bulk solution. Pancreatic and bile secretion of \( \text{HCO}_3^- \) are clearly important in regulation of the latter, but, as demonstrated by Florey et al. in several mammalian species (31), duodenum devoid of pancreatic \( \text{HCO}_3^- \) possesses better ability than lower parts of the small intestine to resist luminal acid, suggesting a role for mucosal cell \( \text{HCO}_3^- \) transport in duodenal epithelial protection. It should also be noted that prostaglandins at concentrations too low to inhibit gastric \( \text{H}^+ \) secretion...
prevent ulceration and heal existing ulcers in experimental animals and humans (62, 74, 76). Prostaglandins are potent stimulants of duodenal epithelial HCO$_3^-$ transport (19, 25, 29) but inhibit pancreatic HCO$_3^-$ secretion (63). In contrast, secretin, which is a stimulant of pancreatic secretion but without effects on HCO$_3^-$ transport by duodenum, has proved unsuccessful in duodenal ulcer therapy.

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