

Gastric Bicarbonate Secretion in Humans

EFFECT OF PENTAGASTRIN, BETHANECHOL, AND 11,16,16-TRIMETHYL PROSTAGLANDIN E₂

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ABSTRACT Although the stomach is mainly known for its ability to secrete hydrochloric acid, there is increasing evidence that the gastric mucosa also secretes bicarbonate. A simple method for simultaneous measurement of gastric HCO₃⁻ secretion and H⁺ secretion was developed from a two-component model of gastric secretion. The method, which is based upon gastric juice volume, H⁺ concentration, and osmolality, was validated both in vitro and in vivo. In 14 healthy human beings, basal gastric HCO₃⁻ secretion averaged 2.6 mmol/h (range, 0.7–8.7 mmol/h). Basal HCO₃⁻ secretion was ~50% of basal H⁺ secretion and there was a significant correlation between basal HCO₃⁻ and H⁺ secretion in individual subjects ($r = 0.79$). HCO₃⁻ was secreted in basal nonparietal secretion at a concentration of ~90 mmol/liter. Intravenous pentagastrin infusion markedly stimulated H⁺ secretion but did not increase HCO₃⁻ secretion. During pentagastrin infusion, the cholinergic agonist, bethanechol, significantly augmented H⁺ secretion (from 20.2 to 24.7 mmol/h) and increased HCO₃⁻ secretion (from 2.2 to 4.2 mmol/h). A prostaglandin E₂ analogue significantly reduced H⁺ secretion and increased HCO₃⁻ secretion during pentagastrin infusion. The reduction in net gastric juice H⁺ output following prostaglandin E₂ was due more to H⁺ secretory inhibition than to HCO₃⁻ secretory stimulation. We conclude that the healthy human stomach actively secretes HCO₃⁻ and that gastric HCO₃⁻ secretion can be influenced by cholinergic stimulation and by prostaglandin E₂.

INTRODUCTION

Although bicarbonate secretion probably plays an important role in protecting the gastric epithelium from

damage by luminal acid and pepsin (1), there is limited information on the amount of bicarbonate secreted by the stomach (2). Attempts to quantitate gastric HCO₃⁻ secretion have been hampered by concomitant H⁺ secretion, which converts HCO₃⁻ to CO₂. One approach to this problem has been to use agents that reduce H⁺ secretion, such as cimetidine (3). For quantitative purposes, it is assumed that the antisecretory agent blocks H⁺ secretion completely and that the antisecretory agent has no effect per se on HCO₃⁻ secretion. However, it is uncertain whether either of these assumptions is valid (2). A second method to avoid concomitant H⁺ secretion has been to measure the secretion from antral mucosa in vitro (4) or an antral pouch in vivo (5). These methods assume that no acid-secreting parietal cells are present in the antral preparation, an assumption which sometimes may not be true (5). Moreover, these techniques are not readily applicable to humans. A third approach has been to study gastric HCO₃⁻ secretion in patients with achlorhydria (6). It is uncertain, however, whether alkaline secretion rates in these patients with atrophic fundic gastritis are representative of HCO₃⁻ secretion rates in healthy individuals whose stomachs secrete H⁺.

A fourth method for estimating gastric HCO₃⁻ secretion is to measure the volume, H⁺ concentration, and chloride concentration of gastric juice and use a mathematical, two-component model of gastric secretion (7–10). However, the equations derived from a two-component model of gastric secretion have never been validated as a method for measuring gastric HCO₃⁻ secretion.

The present studies had two major purposes. First, equations were derived from a two-component model of gastric secretion to calculate gastric HCO₃⁻ and H⁺ secretion, nonparietal and parietal volume secretion, and the HCO₃⁻ concentration of nonparietal secretion utilizing measurements of gastric juice volume, H⁺

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concentration, and osmolality. Experiments designed to validate these derived equations were then carried out in vitro and in vivo. A second purpose was to utilize this method to calculate HCO_3^- and H^+ secretion rates, nonparietal and parietal flow rates, and the HCO_3^- concentration of nonparietal secretion in healthy humans. Subjects were studied basally, during intravenous pentagastrin infusion, during intravenous infusion of bethanechol (a cholinergic agonist), and after oral ingestion of a prostaglandin E_2 analogue.

METHODS

Studies were approved by a Human Research Studies Committee. Informed written consent was obtained from each subject.

Subjects and intubation. 14 healthy subjects (nine men and five women) participated in these studies. Their ages averaged 35 yr (range, 19–51 yr). Basal and peak acid output to 6 $\mu\text{g}/\text{kg}$ pentagastrin s.c. averaged 2.4 ± 0.6 and 31.2 ± 2.2 mmol/h, respectively. After an overnight fast, patients swallowed a nasogastric tube (H. W. Anderson Products, Inc., Oyster Bay, NY) which was positioned fluoroscopically in the gastric antrum. Gastric juice was collected by a Stedman pump which applied suction for 48 out of every 60 s (American Cystoscope Makers, Inc., Stamford, CT). The subjects were trained not to swallow their saliva and to collect saliva through two dental suction catheters.

Preliminary studies in three subjects indicated that simultaneous aspiration of alkaline duodenal juice through a second, nasoduodenal Anderson tube (that had been passed through the other nostril and positioned fluoroscopically in the second part of the duodenum) did not alter gastric juice volume, H^+ concentration, osmolality, calculated nonparietal volume secretion, or gastric bicarbonate secretion (compared with the use of a single nasogastric tube in the same subjects). For this reason and for patient comfort, only a single nasogastric tube was employed. Visible bile staining of gastric juice was seldom present. When gastric juice was noted to contain bile, the specimen was discarded and an additional 15-min collection performed. In a few instances, it was necessary to withdraw the nasogastric tube a few centimeters to obtain bile-free specimens because presumably, the tip of the tube had advanced past the pylorus after initial fluoroscopic placement.

Measurements. All measurements were made at room temperature. Volumes of test solutions (in vitro studies) or gastric juice samples (in vivo studies) were measured in a graduated cylinder to the nearest 0.5 ml. The osmolality of each test solution, gastric juice sample, or venous plasma sample was measured in duplicate by freezing point depression (Advanced Digmatic Osmometer, model 3DII, Advanced Instruments, Inc., Needham Heights, MA) and expressed in milliosmoles per kilogram. Hydrogen ion concentration, $[\text{H}^+]^1$, was measured by in vitro titration to pH 7.00

with 0.1 N NaOH or by the glass electrode method of Moore and Scarlata (11) or by both methods. In 28 test solutions analyzed by both methods, the results were closely correlated ($r = 0.996$, slope = 0.979). Likewise, in 210 gastric juice specimens, there was a close correlation between $[\text{H}^+]$ with the two methods ($r = 0.972$, slope = 1.042). Unless otherwise stated, values for $[\text{H}^+]$ used in calculations were derived by the glass electrode method (11).

Equations. Two basic equations were used:

$$\begin{aligned} \text{measured acid output} \\ = \text{acid secreted} - \text{acid neutralized, and} \end{aligned} \quad (1)$$

$$\begin{aligned} \text{measured osmolar output} \\ = \text{osmoles secreted} - \text{osmoles neutralized.} \end{aligned} \quad (2)$$

Under usual circumstances, acid secretion (AS) exceeds bicarbonate secretion (BS). Thus, the amount of acid neutralized by bicarbonate equals the amount of bicarbonate secreted, and from Eq. 1:

$$\text{measured acid output} = \text{AO} = \text{AS} - \text{BS}^2 \quad (3)$$

$$\text{It is apparent that } \text{AO} = V_{\text{CJ}}[\text{H}_3\text{O}^+] \quad (4)$$

$$\text{and that } \text{AS} = V_{\text{P}}[\text{H}_3\text{O}^+], \quad (5)$$

where V_{CJ} is the volume of gastric juice sample, $[\text{H}_3\text{O}^+]$ is the hydrogen ion concentration of gastric juice sample, V_{P} is the volume of parietal (acid) secretion, and $[\text{H}_3\text{O}^+]$ is the hydrogen ion concentration of parietal secretion. Thus, substituting Eqs. 4 and 5 into Eq. 3:

$$V_{\text{CJ}}[\text{H}_3\text{O}^+] = V_{\text{P}}[\text{H}_3\text{O}^+] - \text{BS}. \quad (6)$$

From references 7, 10, 12, and 13 and from data that will be presented below, it is reasonable to assume that $[\text{H}_3\text{O}^+] = 160$ mmol/liter. Also, from the two-component model:

$$V_{\text{CJ}} = V_{\text{P}} + V_{\text{NP}}, \quad \text{or}$$

$$V_{\text{P}} = V_{\text{CJ}} - V_{\text{NP}}. \quad (7)$$

After substituting Eq. 7 into Eq. 6 and solving for V_{NP} , we obtain:

$$V_{\text{NP}} = \frac{V_{\text{CJ}}(160 - [\text{H}_3\text{O}^+]) - \text{BS}}{160}. \quad (8)$$

From Eq. 2, it can be determined that

$$V_{\text{CJ}} \text{OSM}_{\text{CJ}} = (V_{\text{P}} \text{OSM}_{\text{P}} + V_{\text{NP}} \text{OSM}_{\text{NP}}) - 2 \text{BS}, \quad (9)$$

where OSM_{CJ} is the osmolality of gastric juice sample, OSM_{P} is the osmolality of pure parietal secretion, and OSM_{NP} is the osmolality of nonparietal secretion. In Eq. 9, note that, for every millimole or milliosmole of HCO_3^- secreted, there is a loss of 2 mosmol since both HCO_3^- and an equal amount of H^+ are lost from the osmotic balance. Substituting Eq. 7 into 9 and rearranging to solve for V_{NP} :

plasma; PEG, polyethylene glycol; PGE, 11,16,16-trimethyl prostaglandin E_2 , V_{CJ} , volume of gastric juice sample; V_{NP} , volume of nonparietal secretion; V_{P} , volume of parietal (acid) secretion.

² When $\text{BS} > \text{AS}$, AO will be "negative" according to Eq. 3. This means that free bicarbonate is present in the gastric juice. Thus, when $[\text{H}_3\text{O}^+] = 0$ and AO is "zero" by Eq. 4, it is necessary to measure the concentration of HCO_3^- in gastric juice directly to correct for free bicarbonate (see below).

¹ Abbreviations used in this paper: AO, acid output; AS, acid secretion; $[\text{B}_{\text{NP}}]$, bicarbonate concentration of nonparietal secretion; BS, bicarbonate secretion; $[\text{H}^+]$, hydrogen ion concentration; $[\text{H}_3\text{O}^+]$, hydrogen ion concentration of gastric juice sample; $[\text{H}_3\text{O}^+]$, hydrogen ion concentration of parietal secretion; OSM_{CJ} , osmolality of gastric juice sample; OSM_{P} , osmolality of pure parietal secretion; OSM_{NP} , osmolality of nonparietal secretion; OSM_{PL} , osmolality of

$$V_{NP} = \frac{V_{GJ} (OSM_P - OSM_{GJ}) - 2 BS}{OSM_P - OSM_{NP}} \quad (10)$$

Since both Eqs. 8 and 10 are equal to V_{NP} , they equal each other:

$$\frac{V_{GJ} (160 - [H_{GJ}^+]) - BS}{160} = \frac{V_{GJ} (OSM_P - OSM_{GJ}) - 2 BS}{OSM_P - OSM_{NP}} \quad (11)$$

If we let

$$\Delta H = 160 - [H_{GJ}^+];$$

$$\Delta OSM = OSM_P - OSM_{GJ};$$

and

$$K = OSM_P - OSM_{NP},$$

and we solve (11) for BS:

$$BS = \frac{V_{GJ} (160 \Delta OSM - K \Delta H)^3}{320 - K} \quad (12)$$

To calculate BS from Eq. 12, it is necessary to measure V_{GJ} , OSM_{GJ} , and $[H_{GJ}^+]$ and assign a value for OSM_{NP} and OSM_P . Several investigators have reported that OSM_{NP} is equal to the osmolality of plasma (OSM_{PL}), i.e., $OSM_{NP} = OSM_{PL}$ (9, 10, 13, 14). From references 10 and 15 and from data that will be presented below, it is reasonable to assume that $OSM_P = 1.06 OSM_{PL}$; i.e., OSM_P is 6% greater than OSM_{PL} . Thus, the terms ΔOSM and K in Eq. 12 can be estimated by measuring OSM_{PL} and OSM_{GJ} . Once BS is calculated from Eq. 12 (or Eq. 13), V_{NP} can be calculated from Eq. 8 or 10. Then, V_P can be calculated from Eq. 7. Finally, the bicarbonate concentration of the nonparietal secretion, $[B_{NP}]$, can be calculated from the equation

$$[B_{NP}] = BS/V_{NP} \quad (14)$$

Note that $[B_{NP}]$ is a calculated value in this method. Previous methods have sometimes assumed $[B_{NP}]$ to be the same as plasma (9, 10).

Statistics. Values are expressed as mean \pm 1 SE. BS, V_{NP} , V_P , and $[B_{NP}]$ were computed using a Hewlett-Packard 97 calculator (Hewlett-Packard, Palo Alto, CA) programmed with Eqs. 12, 8, 7, and 14, respectively. Statistical significance was tested by two-tailed paired *t* test or by two-tailed Wilcoxon's signed rank test with $P < 0.05$ considered significant. Pearson's correlation coefficient was used in the validation experiments.

RESULTS

In vitro validation. A pure "parietal" solution containing 160 mmol/liter HCl was prepared from a stock solution and the concentration of HCl confirmed by titration to pH 7.00 with 0.1 N NaOH. In addition to the "parietal" solution, 16 "nonparietal" HCO_3^- -containing solutions were prepared. These solutions contained from 6.0 to 88.1 mmol/liter of bicarbonate. Sufficient NaCl was added to the 16 solutions to make them approximately isotonic (osmolality 301 ± 2 mosM/

kg). Different volumes of one of the "nonparietal" solutions (V_{NP}) and the parietal solution (V_P) were mixed together in an open glass beaker at room temperature using gentle stirring. For simplicity, the final volume of the mixture was always 50 ml. V_{NP} , V_P , and the HCO_3^- concentration of the "nonparietal" solution were selected by one of us and were unknown to the other who carried out the measurements and calculations. From the $[H^+]$ and osmolality of the final 50-ml mixture, BS, V_{NP} , V_P , and $[B_{NP}]$ were calculated.

28 separate in vitro experiments were performed using varying mixtures of the "parietal" solution ($[H^+] = 160$ mmol/liter; $OSM_P = 307$ mosM/kg) and one of the 16 "nonparietal" solutions. There were close correlations between the calculated and actual values for all four parameters: BS ($r = 0.99$); V_{NP} ($r = 0.99$); V_P ($r = 1.00$); and $[B_{NP}]$ ($r = 0.96$). The lowest osmolality of a mixed sample, 238 mosM/kg, occurred when 30.0 ml "parietal" solution was added to 20.0 ml of a "nonparietal" solution containing 88.1 mmol/liter of bicarbonate.

In vivo validation. The purpose of these in vivo studies was to determine whether an intragastric infusion of an HCO_3^- -containing "nonparietal" solution, which simulates an increase in HCO_3^- secretion by the stomach, could be detected quantitatively by our method. To do this, an intragastric HCO_3^- infusion was superimposed on steady-state gastric secretion induced by a continuous intravenous infusion of 0.1–0.2 μ g/kg/h pentagastrin (Peptavlon, Ayerst Laboratories, New York) in eight subjects. These pentagastrin doses were submaximal in seven of the eight subjects (mean acid outputs were $60 \pm 7\%$ of the maximal acid response to 6.0 μ g/kg s.c. pentagastrin). In one subject, the dose of pentagastrin (0.2 μ g/kg/h) proved to be maximal. Thus, for the eight subjects, acid secretion during pentagastrin infusion ranged from 27 to 100% of maximal.

After basal gastric juice and saliva were collected for two 15-min periods, the intravenous infusion of pentagastrin was begun (volumetric infusion pump model 922; IMED Corp., San Diego, CA). Pentagastrin was infused for twelve 15-min periods. During the seventh 15-min period of intravenous pentagastrin infusion, one of eight HCO_3^- -containing test solutions was infused into the stomach (IMED infusion pump) through a small polyethylene catheter attached to the nasogastric tube. The HCO_3^- -containing solutions were approximately isotonic (osmolality, 293 ± 2 mosM/kg; plasma osmolality, 294 ± 2 mosM/kg). The HCO_3^- concentration of the solutions varied from 0 to 90.0 mmol/liter; the other electrolytes were Na^+ and Cl^- . The volume of the solution infused into the stomach varied from 12.0 to 56.0 ml. One of us selected the solution and volume to be infused; the other carried out measurements of V_{GJ} , $[H_{GJ}^+]$, and OSM_{GJ} without knowledge

³ For the situation in which $[H_{GJ}^+] = 0$, free bicarbonate needs to be considered and the expanded formula is: $BS = [160 V_{GJ} (\Delta OSM - K)] / 320 - K + V_{GJ} [B_{GJ}]$ (13), where $[B_{GJ}]$ is the concentration of bicarbonate in gastric juice.

of V_{NP} or $[B_{NP}]$. Infusion of the test solution into the stomach was started 2 min after the beginning of the seventh 15-min period of pentagastrin infusion and was stopped 3 min before the end of the period (i.e., the infusion lasted 10 min). The test solution also contained 2 g/dl polyethylene glycol (PEG) as a nonabsorbable marker in order to estimate completeness of recovery of gastric juice (16).

An example of an experiment using this protocol is shown in Fig. 1. In response to intravenous pentagastrin infusion, V_{GJ} , $[H^+]_{GJ}$, and OSM_{GJ} increased above basal levels and reached near steady values by the third 15-min period. In the seventh 15-min period, 25 ml of an HCO_3^- -containing solution was infused into the stomach. The solution contained 1.25 mmol HCO_3^- (50.0 mmol/liter) and had an osmolality of 300 mosM/kg. This simulated increase in HCO_3^- secretion in period 7 led to the expected increase in V_{GJ} and decrease in $[H^+]_{GJ}$ and OSM_{GJ} (Fig. 1). V_{GJ} , $[H^+]_{GJ}$, and OSM_{GJ} returned to near control values during the eighth through twelfth periods of pentagastrin infusion. Using the values for V_{GJ} , $[H^+]_{GJ}$, and OSM_{GJ} obtained in this experiment, it was possible to calculate BS, V_{NP} , V_P , and $[B_{NP}]$ for each 15-min period before, during, and after intragastric bicarbonate infusion. By comparing values in the seventh period with values before and after this period, changes in nonparietal alkaline secretion in period 7 were calculated and correlated

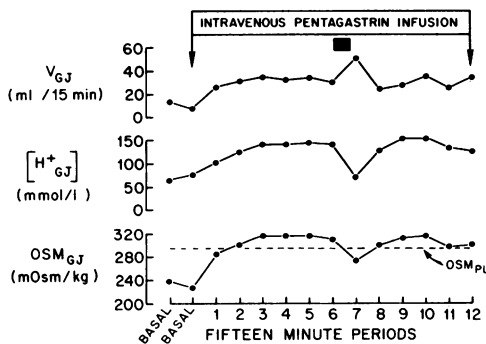


FIGURE 1 In vivo validation experiment in one subject. Volume of gastric juice (V_{GJ}), H^+ concentration of gastric juice ($[H^+]_{GJ}$), and osmolality of gastric juice (OSM_{GJ}) were measured for two 15-min periods (Basal). Then, intravenous pentagastrin ($0.1 \mu\text{g}/\text{kg}/\text{h}$) was infused for twelve 15-min periods. In response to pentagastrin, V_{GJ} , $[H^+]_{GJ}$, and OSM_{GJ} increased above basal values. Note that OSM_{GJ} increased above the osmolality of plasma (OSM_{PL}). During the seventh 15-min period of pentagastrin infusion, when secretion was nearly steady, a HCO_3^- -containing solution was infused into the stomach (solid bar). This was accompanied by an increase in V_{GJ} and a decrease in $[H^+]_{GJ}$ and OSM_{GJ} during period 7. These values returned to control values during the eighth through twelfth 15-min periods. From the changes in V_{GJ} , $[H^+]_{GJ}$, and OSM_{GJ} in period 7, the increase in simulated HCO_3^- secretion during period 7 could be calculated (Fig. 2).

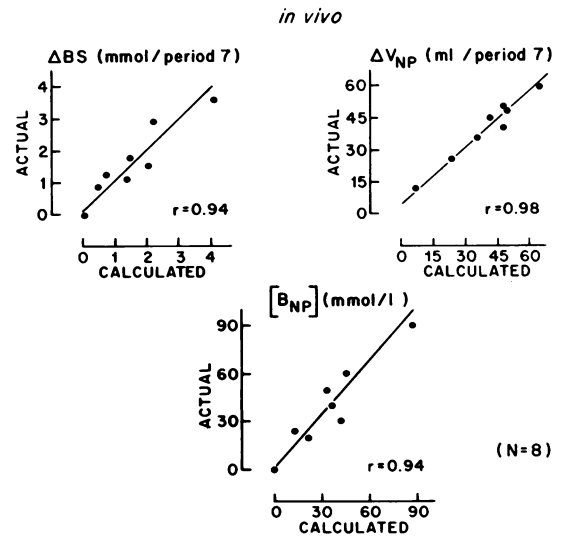


FIGURE 2 In vivo validation experiments in eight subjects. Comparison of calculated and actual values for changes in bicarbonate "secretion" during period 7 of pentagastrin infusion (ΔBS), change in nonparietal volume "secretion" during period 7 of pentagastrin infusion (ΔV_{NP}), and bicarbonate concentration of additional nonparietal "secretion" in period 7 ($[B_{NP}]$). The average of the calculated values for BS and V_{NP} during periods 2-6 and periods 8-12 were subtracted from calculated values for BS and V_{NP} in period 7 in each subject to obtain ΔBS and ΔV_{NP} (Fig. 1). Actual values for BS and V_{NP} represented the amounts of exogenous bicarbonate in millimoles and fluid in milliliters infused into the stomach during period 7 (corrected for percent polyethylene glycol recovery). Calculated $[B_{NP}]$ represented $\Delta BS/\Delta V_{NP}$ for period 7, whereas actual $[B_{NP}]$ represented the actual bicarbonate concentration of the fluid infused into the stomach in period 7.

with the amounts of fluid and HCO_3^- actually infused into the stomach during period 7.

Results from experiments in which eight healthy subjects were studied according to this protocol are shown in Fig. 2. There was a good correlation between calculated and actual values for (a) the increase in calculated HCO_3^- secretion during period 7; (b) the increase in V_{NP} during period 7; and (c) HCO_3^- concentration of the simulated secreted fluid during period 7. PEG recovery during period 7 averaged $93 \pm 3\%$.

Basal gastric secretion. Basal HCO_3^- secretion in 14 subjects averaged 2.6 ± 0.6 mmol/h, which was $\sim 50\%$ of basal H^+ secretion (4.9 ± 0.9 mmol/h). There was a significant correlation between basal HCO_3^- and H^+ secretion in individual subjects ($r = 0.79$, $P < 0.001$). Basal $[H^+]_{GJ}$ averaged 39.7 ± 7.3 mmol/liter.

Basal V_{NP} averaged 24.7 ± 3.1 ml/h in these 14 subjects and basal V_P averaged 30.8 ± 5.8 ml/h. Thus, nonparietal secretion accounted for $\sim 45\%$ of the basal gastric juice output. There was also a significant cor-

relation between basal V_{NP} and basal V_P ($r = 0.75$, $P < 0.005$). The HCO_3^- concentration of basal nonparietal secretion averaged 99.0 ± 13.5 mmol/liter (median, 82.8 mmol/liter).

Effect of pentagastrin on gastric secretion. Mean calculated values for H^+ and HCO_3^- secretion, V_P and V_{NP} , and $[\text{B}_{NP}]$ before and during pentagastrin infusion in eight subjects who participated in the in vivo validation experiments are shown in Fig. 3. HCO_3^- secretion did not change significantly from basal rates during pentagastrin infusion, despite a large increase in H^+ secretion. Pentagastrin caused a small but significant increase in V_{NP} ($P < 0.01$) and a much larger increase in V_P ($P < 0.001$). $[\text{B}_{NP}]$ decreased to an average concentration of 61.9 mmol/liter during pentagastrin infusion ($P < 0.005$). Thus, pentagastrin increased V_{NP} and reduced $[\text{B}_{NP}]$ by approximately the same extent.

Fig. 4 shows the relationship between $[\text{H}_{GJ}^+]$ and OSM_{GJ} (expressed as a fraction of plasma osmolality) during pentagastrin infusion. Each point represents a separate gastric juice sample. A sample from a patient with pentagastrin-fast achlorhydria is shown as a triangle. When $[\text{H}_{GJ}^+]$ was zero after pentagastrin (achlorhydric patient), the osmolality of gastric juice was the same as that of plasma (i.e., $\text{OSM}_{GJ}/\text{OSM}_{PL} = 1.00$). Gastric juice was always hypotonic when $[\text{H}_{GJ}^+] > 0$, but < 120 mmol/liter. On the other hand, when $[\text{H}_{GJ}^+] > 127$ mmol/liter, gastric juice was always

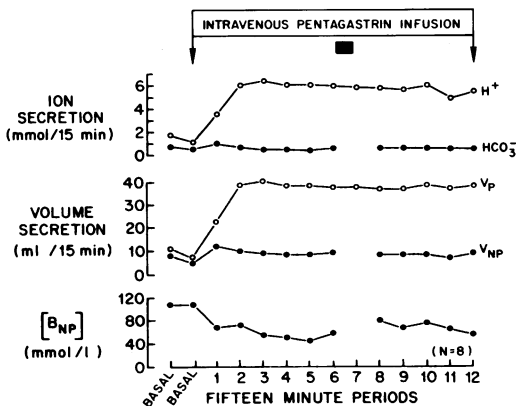


FIGURE 3 Effect of pentagastrin infusion on mean H^+ and HCO_3^- secretion, parietal and nonparietal volume secretion (V_P , V_{NP}), and bicarbonate concentration of nonparietal secretion ($[\text{B}_{NP}]$) in eight subjects in in vivo validation experiments. Mean values for HCO_3^- secretion, V_{NP} , and $[\text{B}_{NP}]$ in period 7 are not shown because these were varied (Fig. 3). Compared with basal values, intravenous pentagastrin ($0.1\text{--}0.2$ $\mu\text{g}/\text{kg}/\text{h}$) caused a significant increase in H^+ secretion, V_P , V_{NP} , and a significant decrease in $[\text{B}_{NP}]$. The change in HCO_3^- secretion during pentagastrin infusion was not statistically significant from basal rates.

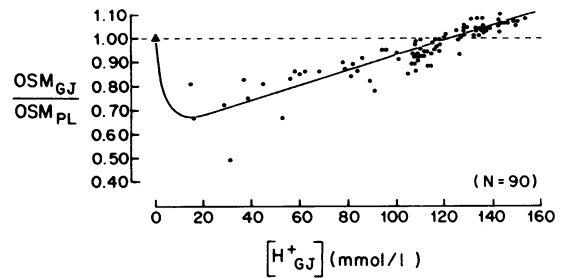


FIGURE 4 Relationship between H^+ concentration of gastric juice, $[\text{H}_{GJ}^+]$, and the osmolality of gastric juice expressed as a fraction of plasma osmolality, $\text{OSM}_{GJ}/\text{OSM}_{PL}$. Each point represents a separate sample of gastric juice obtained during intravenous pentagastrin infusion ($0.1\text{--}0.2$ $\mu\text{g}/\text{kg}/\text{h}$). The triangle represents an achlorhydric patient. At $[\text{H}_{GJ}^+] < 120$ mmol/liter, acid gastric juice was always hypotonic ($N = 61$), whereas at $[\text{H}_{GJ}^+] > 127$ mmol/liter gastric juice was always hypertonic ($N = 28$). The curve is hand-drawn and is not a mathematical fit of the experimental data. $[\text{H}_{GJ}^+]$ was measured by in vitro titration to pH 7.00. The curve was virtually identical when $[\text{H}_{GJ}^+]$ by glass electrode method (11) was used.

hypertonic to plasma. The osmolality of the 28 gastric juice samples with $[\text{H}_{GJ}^+] > 127$ mmol/liter averaged 1.05 OSM_{PL} (range, $1.01\text{--}1.10 \text{ OSM}_{PL}$). The highest observed $[\text{H}^+]$ in a sample of gastric juice was 154 mmol/liter. The osmolality of this sample was 312 mosM/kg (1.08 OSM_{PL}). The highest observed osmolality of a gastric juice sample was 320 mosM/kg (1.08 OSM_{PL} , $[\text{H}^+] = 146$ mmol/liter).

Effect of bethanechol on gastric secretion during pentagastrin infusion. One day, after basal gastric secretion was measured, an intravenous infusion of bethanechol (Urecholine; Merck Sharp and Dohme Co., West Point, PA) plus pentagastrin was begun and continued for eight 15-min periods. The dose of bethanechol, 50 $\mu\text{g}/\text{kg}/\text{h}$, is a maximally tolerated dose in humans (15). The dose of pentagastrin was 0.1 $\mu\text{g}/\text{kg}/\text{h}$. On a separate day, the same dose of pentagastrin was infused without bethanechol as a control. As shown in Table I, bethanechol significantly increased saliva and V_{GJ} . The increase in V_{GJ} was due to significant stimulation of both parietal and nonparietal secretion. Bethanechol significantly stimulated both H^+ secretion (AS) and HCO_3^- secretion (BS), so that net acid output in gastric juice increased slightly but not significantly with bethanechol. The HCO_3^- concentration of nonparietal secretion $[\text{B}_{NP}]$ was significantly higher during bethanechol plus pentagastrin infusion than during infusion of pentagastrin alone (Table I).

Effect of prostaglandin E_2 on gastric secretion during pentagastrin infusion. The effect of an orally administered prostaglandin E_2 analogue (11,16,16-trimethyl PGE₂, RO21-6937; Hoffman-LaRoche Inc.,

TABLE I
Effect of 50 $\mu\text{g}/\text{kg}/\text{h}$ Intravenous Bethanechol on Salivary and Gastric Secretion during 0.1 $\mu\text{g}/\text{kg}/\text{h}$ Intravenous Pentagastrin Infusion in 10 Healthy Subjects

Parameter*	Pentagastrin	Pentagastrin + Bethanechol
Saliva (ml/2 h)	71 \pm 19	173 \pm 40†
V _{GJ} (ml/2 h)	335 \pm 41	408 \pm 27†
V _P (ml/2 h)	252 \pm 33	308 \pm 22†
V _{NP} (ml/2 h)	83 \pm 10	100 \pm 11†
AS (mmol/2 h)	40.3 \pm 5.3	49.3 \pm 3.5†
BS (mmol/2 h)	4.3 \pm 1.2	8.4 \pm 2.9†
AO (mmol/2 h)	36.0 \pm 4.9	40.9 \pm 2.4
[B _{NP}] (mmol/liter)	45.0 \pm 9.5	78.2 \pm 22.6†

* V_{GJ} is the volume of gastric juice output; V_P is the parietal volume secretion; V_{NP} is the nonparietal volume secretion; AS is the H⁺ secretion; BS is the HCO₃⁻ secretion; AO is the measured acid output in gastric juice; and [B_{NP}] is the HCO₃⁻ concentration of nonparietal secretion.

† P < 0.05 vs. pentagastrin alone.

Nutley, NJ) on pentagastrin-stimulated gastric secretion was evaluated in 10 subjects. Fasting subjects were intubated and then capsules containing 0 (placebo), 0.25, 0.75, 1.5, or 3.0 mg PGE₂ analogue were swallowed. Studies were performed in random order on separate days with at least 1 wk between studies. All subjects received all dosages, except that only five received the 3.0-mg dose. After allowing 75 min for emptying the drug from the stomach and for absorption, gastric contents were aspirated and pentagastrin (0.1 $\mu\text{g}/\text{kg}/\text{h}$) was infused intravenously for eight 15-min periods. Gastric juice and saliva were collected as described previously. Salivary output was unaffected by the prostaglandin compound.

As shown in Fig. 5 (upper left), there was a dose-related increase in HCO₃⁻ secretion in response to the PGE₂ analogue, with the peak response following 0.75 mg. With this dose, HCO₃⁻ secretion increased from 4.3 to 6.1 mmol/2 h (P < 0.05), and [B_{NP}] increased from 45.0 to 74.9 mmol/liter (P < 0.005, Fig. 5, lower right). PGE₂ did not stimulate nonparietal volume secretion; in fact, V_{NP} decreased significantly (Fig. 5, upper right). PGE₂ also decreased parietal volume secretion significantly. The 0.75-mg dose of PGE₂, for example, reduced pentagastrin-stimulated parietal secretion by 73.4 ml/2 h (P < 0.05, Fig. 5, lower left). Thus, this dose of prostaglandin reduced H⁺ secretion more than it increased HCO₃⁻ secretion (11.7 mmol/2 h vs. 1.8 mmol/2 h).

DISCUSSION

Validity of basic assumptions. One assumption of the two-component model of gastric secretion is that H⁺ is secreted at a concentration of 160 mmol/liter,

even at submaximal rates of acid secretion (7, 10, 12, 13). Thus, this model assumes that increases or decreases in H⁺ secretion result from increases or decreases in parietal volume secretion. In our in vivo validation experiments, which were carried out mainly during submaximally stimulated conditions, we found a close correlation between actual and calculated values for changes in nonparietal secretion when we assumed that [H_P⁺] = 160 mmol/liter and OSM_P = 1.06 OSM_{PL}. If H⁺ had been secreted at <160 mmol/liter under submaximal stimulatory conditions, it would have not have been possible to obtain the highly correlative data shown in Fig. 2. Thus, our in vivo validation studies indirectly support the assumptions that [H_P⁺] = 160 mmol/liter and OSM_P = 1.06 OSM_{PL} when acid output ranges from 27 to 100% of maximal. Our own experimental data from highly acid specimens of gastric juice also support these assumptions (Fig. 4). That the nonparietal component of gastric secretion is approximately isotonic to plasma is supported by several previous studies in animals and humans (9, 10, 13, 14) and by results in our patient with pentagastrin-fast achlorhydria and isotonic gastric juice (Fig. 4).

Our in vitro validation studies support the assumption that decreases in [H_{GJ}⁺] and OSM_{GJ} below [H_P⁺] and OSM_P are a direct result of neutralization and dilution by alkaline nonparietal secretion. Another mechanism (besides neutralization and dilution) by which [H_{GJ}⁺] and OSM_{GJ} could decrease in vivo is gastric ion absorption. However, the healthy stomach is highly impermeable to passive diffusion of ions from the lumen into the mucosa (17). It has even been suggested that the disappearance of H⁺ from the lumen by apparent dif-

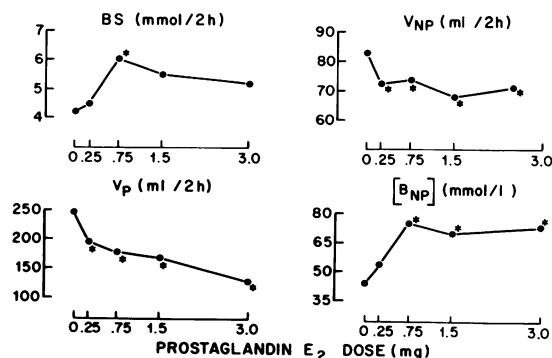


FIGURE 5 Effect of 11,16,16 trimethyl prostaglandin E₂ on mean HCO₃⁻ secretion (BS), nonparietal volume secretion (V_{NP}), parietal volume secretion (V_P), and HCO₃⁻ concentration of nonparietal secretion ([B_{NP}]) during intravenous pentagastrin infusion in 10 subjects. The prostaglandin was given orally 75 min before a 2-h intravenous pentagastrin infusion (0.1 $\mu\text{g}/\text{kg}/\text{h}$). The prostaglandin E₂ analogue caused a dose-related increase in BS and [B_{NP}] and a decrease in V_{NP} and V_P. Significant differences from control (0 dose) are shown by an asterisk (P < 0.05).

fusion into the mucosa may actually represent surface neutralization by bicarbonate secretion (10). Nevertheless, the rate of apparent absorption of H^+ by the stomach is $<0.1\%/min$ (18, 19), so that under the conditions of our experiments, in which secreted gastric juice was aspirated from the stomach continuously rather than remaining in contact with the gastric mucosa for a prolonged period, it is likely that absorption of H^+ and other ions by gastric mucosa was negligible.

Thus, our validation experiments indicate that it is possible to estimate gastric HCO_3^- secretion (BS) from measurements of gastric juice volume, H^+ concentration, and osmolality. In Fig. 6, a nomogram has been provided for determining BS once V_{GJ} , $[H^+_{GJ}]$, and OSM_{GJ} are known. This particular series of curves is applicable when plasma osmolality is 290 mosM/kg.

Physiology and pharmacology of gastric nonparietal volume secretion and bicarbonate secretion. The calculated basal nonparietal volume secretion rate averaged 25.8 ml/h (0.4 ml/min) in the 14 healthy subjects in this study. This rate agrees closely with nonparietal flow rates calculated by others (9, 10, 20). Under basal conditions, nonparietal volume secretion accounted for $\sim 45\%$ of total gastric volume secretion and parietal secretion for the remainder. We found that nonparietal volume secretion was not constant, as

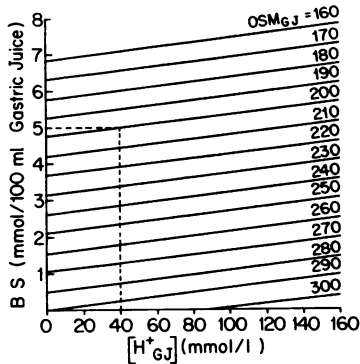


FIGURE 6 Nomogram for determining HCO_3^- secretion (BS) per 100 ml gastric juice from measurement of hydrogen ion concentration of gastric juice, $[H^+_{GJ}]$, and osmolality of gastric juice, OSM_{GJ} . Curves for OSM_{GJ} from 160 to 300 mosM/kg at 10 mosM/kg intervals are provided, assuming plasma osmolality = 290 mosM/kg. As an example (dotted lines), for a 50 ml sample of gastric juice collected over a given time period with a $[H^+_{GJ}] = 40.0$ mmol/liter and $OSM_{GJ} = 200$ mosM/kg, BS = 5.0 mmol/100 ml gastric juice, or 2.5 mmol HCO_3^- for this 50 ml sample of gastric juice. Note that if $[H^+_{GJ}] = 0$, it is also necessary to multiply the free HCO_3^- concentration of gastric juice, $[B_{GJ}]$, by V_{GJ} and to add this product to the value for BS derived from the nomogram. The author will provide nomograms for plasma osmolality values other than 290 mosM/kg upon request. The author will also provide nomograms for determining nonparietal volume and parietal volume secretion upon request.

had been assumed by Hollander (7) and by Makhlof et al. (9, 10), because it could be stimulated significantly by pentagastrin and bethanechol. Furthermore, pentagastrin-stimulated nonparietal volume secretion could be decreased significantly by the prostaglandin E_2 analogue. Thus, our data support Hunt's suggestion that both parietal and nonparietal volume secretion (and their ratios) can vary under different experimental conditions (8, 20).

Despite our agreement with previous studies regarding basal nonparietal volume secretion, our calculated values for basal HCO_3^- secretion are greater than those that have been estimated previously (3, 10, 21). Thus, we estimated that the healthy human stomach secreted ~ 2.6 mmol HCO_3^-/h . Previous two-component models, which automatically assumed that nonparietal secretion had a $[B_{NP}]$ equal to plasma (9, 10), would result in lower calculated secretion rates. For example, if $[B_{NP}]$ were assumed to equal 25 mmol/liter and basal $V_{NP} = 0.35$ ml/min (10), calculated basal HCO_3^- secretion would only be 0.5 mmol/h. Rees et al. recently attempted to calculate basal gastric HCO_3^- secretion directly during intravenous cimetidine infusion using pH and PCO_2 measurements (3). They estimated that basal HCO_3^- secretion was 0.3 to 0.4 mmol/h with a basal HCO_3^- concentration of only 2 to 20 mmol/liter. However, because pH of gastric juice was ~ 6 in those studies, it is almost certain that H^+ was still being secreted despite cimetidine. In that case, HCO_3^- secretion could be underestimated (2). Our estimate that basal HCO_3^- secretion is 50% of basal H^+ secretion and 8% of peak H^+ secretion is in good agreement with in vivo and in vitro studies by Garner and Flemstrom (2, 22).

Measured basal acid output averaged 2.4 mmol/h in our healthy subjects, whereas mean basal H^+ secretion was calculated to be 4.9 mmol/h. Thus, basal HCO_3^- secretion reduced acid output in gastric juice to $\sim 50\%$ of the amount of hydrogen ions actually secreted. Stated in another way, basal H^+ secretion was $\sim 15\%$ of peak pentagastrin-stimulated H^+ secretion, whereas basal acid output was only 8% of peak acid output when assessed by conventional methods, which do not consider basal HCO_3^- secretion. Basal $[H^+_{GJ}]$ averaged ~ 40 mmol/liter, whereas $[H^+_{P}]$ was assumed to be 160 mmol/liter. Therefore, HCO_3^- secretion appears to play a major role in regulating basal gastric acidity.

There was a significant ($P < 0.001$) correlation between basal HCO_3^- secretion and basal H^+ secretion in the 14 subjects studied. Thus, subjects who secreted high amounts of acid basally tended also to secrete high amounts of HCO_3^- . Furthermore, this suggests that subjects with low basal H^+ outputs that use standard methods actually have low H^+ secretion rather than excessive basal HCO_3^- secretion. Because penta-

gastrin markedly increased H^+ secretion but had no effect on HCO_3^- secretion (Fig. 3), $[H_2O]$ increased during pentagastrin infusion and approached 160 mmol/liter in some subjects (Figs. 1 and 4).

In this study, the HCO_3^- concentration of basal gastric nonparietal secretion averaged 99.0 mmol/liter (median, 82.8 mmol/liter). This apparently high HCO_3^- concentration is in the range for pancreatic HCO_3^- secretion (75–130 mmol/liter) (23). A previous study in healthy humans studied at low rates of H^+ secretion estimated a basal HCO_3^- concentration of nonparietal secretion of only 13 mmol/liter (21). However, as emphasized by Flemstrom (2), under those experimental conditions, there may have been residual, but masked, H^+ secretion leading to falsely low estimates of $[B_{NP}]$. However, another study of peptic ulcer patients calculated from pH and PCO_2 a mean HCO_3^- concentration of gastric juice of 57.3 mmol/liter (range, 38.0–67.7 mmol/liter) when H^+ secretion had been blocked by intravenous secretin (24). Moreover, using a two-component model of gastric secretion, Fisher and Hunt calculated that the HCO_3^- concentration of nonparietal secretion could be as high as 107.8 mmol/liter (8).

In 1948, Hollander et al. measured the pH of gastric secretions from Heidenhain pouch dogs and found a median pH of 7.65 and some pH values as high as 9.22 (25). More recently, Turnberg and his associates (26–28) used microelectrodes and recorded the pH near the gastric mucosal surface to be as high as 8.65, 7.95, and 8.10 in rabbits, rats, and humans, respectively, when luminal pH was ~ 2.0 . As an example, a pH of 8.10 corresponds to a HCO_3^- concentration of 83 mmol/liter when $PCO_2 = 40$ mmHg. Furthermore, in our experience with 13 patients with pentagastrin-fast achlorhydria, maximal gastric juice pH ranged from 7.86 to 8.48 (Cowley, Y., and M. Feldman. Unpublished data.). Thus, current evidence suggests that both the HCO_3^- concentration and the pH of nonparietal gastric secretion are much higher than plasma.

Because the gastric lumen is negatively charged (-40 mV) with respect to the mucosa (17, 19), HCO_3^- appears to be secreted by the mucosa into gastric juice against both an electrical and a chemical gradient. This suggests that HCO_3^- secretion in humans is, at least in part, an active process. A similar conclusion has been reached in previous animal studies that measured HCO_3^- secretion in vitro by titration with HCl (and which, therefore, did not assess HCO_3^- concentration of secreted fluid) (2, 29). Because stimulation of HCO_3^- secretion is not accompanied by a change in potential difference, it has been suggested that active HCO_3^- secretion is an electroneutral process, which is possibly mediated by luminal Cl^-/HCO_3^- exchange (2). In support of this concept, fundic bicarbonate se-

cretion can be blocked in vitro by removing chloride from luminal fluid (2).

Pentagastrin caused a significant decrease in $[B_{NP}]$ and a significant increase in V_{NP} compared with basal values. This suggests that factors that control HCO_3^- concentration of nonparietal secretion differ from those that control nonparietal flow. Although $[B_{NP}]$ decreased during pentagastrin infusion, mean $[B_{NP}]$ remained significantly higher than plasma HCO_3^- concentration. Addition of bethanechol or 11,16,16-trimethyl PGE_2 to pentagastrin infusion caused a significant increase in $[B_{NP}]$ (Table I, Fig. 5). This suggests that cholinergic stimulation and prostaglandins may be factors that regulate active gastric HCO_3^- secretion in humans as well as animals (2).

Bethanechol caused a simultaneous and significant increase in pentagastrin-stimulated H^+ and HCO_3^- secretion (9.0 and 4.1 mmol/2 h, respectively). Although net acid output increased somewhat during bethanechol infusion, this was not a significant change (Table I). Thus, the significant increase in H^+ secretion during bethanechol infusion was masked somewhat by a concurrent stimulation of HCO_3^- secretion. The prostaglandin E_2 analogue also caused an increase in gastric HCO_3^- secretion while causing a much larger decrease in gastric H^+ secretion. Therefore, the significant reduction in net acid output in gastric juice due to the prostaglandin E_2 analogue was more due to inhibition of H^+ secretion than to stimulation of HCO_3^- secretion. It is possible that other prostaglandin compounds will affect the relationship between gastric HCO_3^- and H^+ secretion differently (30).

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