Intragastric pH regulates conversion from net acid to net alkaline secretion by the rat stomach

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Coskun, Tamer, Shaoyou Chu, and Marshall H. Montrose. Intragastric pH regulates conversion from net acid to net alkaline secretion by the rat stomach. Am J Physiol Gastrointest Liver Physiol 281: G870–G877, 2001.—Our previous report showed gastric mucosal surface pH was determined by alkali secretion at intragastric luminal pH 3 but by acid secretion at intragastric pH 5. Here, we question whether regulation of mucosal surface pH is due to the effect of luminal pH on net acid/base secretions of the whole stomach. Anesthetized rats with a gastric cannula were used, the stomach lumen was perfused with weakly buffered saline, and gastric secretion was detected in the gastric effluent with 1) a flow-through pH electrode and 2) a fluorescent pH-sensitive dye (Cl-NERF). During pH 5 luminal perfusion, both pH sensors reported the gastric effluent was acidic (pH 4.79). After perfusion was stopped transiently (stop-flow), net acid accumulation was observed in the effluent when perfusion was restarted (peak change to pH 4.1–4.3). During pH 3 luminal perfusion, both pH sensors reported gastric effluent was close to perfusate pH (3.0–3.1), but net alkali accumulation was detected at both pH sensors after stop-flow (peak pH 3.3). Buffering capacity of gastric effluents was used to calculate net acid/alkaline secretions. Omeprazole blocked acid secretion during pH 5 perfusion and amplified net alkali secretion during pH 3 perfusion. Pentagastrin elicited net acid secretion under both luminal pH conditions, an effect antagonized by somatostatin. We conclude that in the basal condition, the rat stomach was acid secretory at luminal pH 5 but alkaline secretory at luminal pH 3. 

THE STOMACH IS WIDELY KNOWN FOR ITS ROLE IN ACID SECRETION (18) AND THE RESPONSIVENESS OF THE REGULATORY CASCADES TO INTRALUMINAL pH. CHANGES IN INTRAGASTRIC pH ARE AN IMPORTANT SIGNAL REGULATING ACID SECRETION DURING A MEAL (5, 7, 8, 14, 21, 35, 36). Gastrin secretion is activated by the presence of food buffers and high luminal pH in the stomach (4, 9, 21, 35–37). Conversely, parietal gastrin concentrations decrease during fasting because of low pH values in the stomach lumen (9, 22, 38). In addition, somatostatin secretion is stimulated by a decrease in gastric luminal pH (4, 17, 22, 27–29, 37, 38). The reciprocal relationship between gastrin and somatostatin secretion, as seen in the fasted and fed states, is one of the major endocrine cycles regulating acid secretion (4, 37, 38).

Much less is known about the regulation of alkaline secretion by the stomach. Since the beginning of the last century, as originally shown by the Danish physiologist Schierbeck (1), it has been recognized that the stomach secretes alkali. Although Teorell (34) proposed that loss of acidity from the lumina was due to back-diffusion of hydrochloric acid into the tissue, Hollander (20) first differentiated the gastric secretions as parietal and nonparietal secretions. Later, this nonparietal secretion was identified as bicarbonate secretion (1, 23, 24). Investigators have indirectly or directly measured alkali secretion by the stomach, and different methods have been developed for this purpose (1, 6, 10, 13, 16, 20, 26). One report (25) suggests that alkali secretion is greater when intragastric pH is more acidic. This and numerous in vitro (11, 13, 32) and in vivo (10, 15, 16, 19, 24, 26, 30, 31) investigations helped to develop the concept that gastric alkali secretion protects the gastric mucosal surface from high proton concentration.

Gastrical alkali secretion has been studied much less than acid secretion. The major issue is that secreted alkali can be neutralized by acid in the lumen (1), making the alkali secretion difficult to detect and quantify. In most reports, acid and alkali secretion could not be studied in parallel because acid secretion had to be pharmacologically suppressed with H2 receptor antagonists (11, 12, 16, 26, 31, 32) or proton pump inhibitors (33) to “unmask” any alkali secretion.

In previous studies, we used confocal microscopy to record the influence of gastric secretions on pH directly at the gastric mucosal surface. We demonstrated that changing gastric luminal pH from 3 to 5 caused a switch between net alkali and net acid secretion dominating control of surface pH, respectively (6). The results raised the controversial suggestion that at the fasted intragastric pH (pH 3), net alkali secretion occurred in the rat stomach, even in the absence of pharmacological inhibition of acid secretion. However, this conclusion was based on studies directly at the
gastric surface and could only tentatively be extrapolated to secretions by the whole stomach.

In this study, we directly address this controversy by reporting on the dynamic balance between acid and alkali secretion in the whole stomach. With in vivo perfusion of intact rat stomach, we use two independent measures of pH (via a fluorescent indicator and pH electrode) to compare gastric acid/base secretions in response to the values of luminal pH seen in the fasted and fed stomach.

METHODS

Animals. All experimental procedures were approved by the Animal Care and Use Committee of Indiana University. Male Sprague-Dawley rats (Harlan Sprague Dawley) were used for the experiments. Animals were housed individually in cages with raised mesh floors to prevent coprophagia. Animals were deprived of food for 18–20 h before experimental use but had free access to water at all times.

Surgery. For experiments, rats (250–300 g) were anesthetized with Inactin (100 mg/kg ip). A tracheal tube was inserted to facilitate breathing. The right jugular vein was cannulated with one or two catheters, and heparinized saline (150 mM NaCl and 3 IU/ml heparin) was continuously infused throughout the experiment at a total rate of 1 ml/h with a syringe pump (KDS 260). In all experiments, stomach distension was avoided, and results were not accepted if the stomach became distended during the experiment (e.g., due to perfusion blockage). Perfusates contained 150 mM NaCl, 4 mM HOMOPIPES, and 0.1 μM CI-NERF (a pH-sensitive fluorescent dye; Molecular Probes, Eugene, OR). Perfusates were titrated to either pH 5 or pH 3 before use to approximate the physiological luminal pH measured in fed or fasted rat stomach, respectively (6). Gastric perfusion was run continuously except for defined periods in which perfusion was stopped transiently for 10 min. This “stop-flow” interval allowed intragastric accumulation of acid/alkali secretions and thereby amplified observed changes in perfuse pH. These enhanced changes in perfuse pH were detected when perfusion was restarted and the gastric contents flowed past the downstream pH sensors (see Gastric acid/base secretion).

Gastric acid/base secretion. As shown schematically in Fig. 1, gastric perfusion effluent was sequentially routed past an inline reference and pH electrode (Microelectrodes, Bedford, NH) and into a flow-through cuvette placed in a fluorometer (Fluorolog-3, JY Horiba/EPX, Edison, NJ). A pH meter (Orion 720A, Orion Research, Beverly, MA) passed the pH-sensitive electrode signal to the host computer that controlled the fluorometer. After the animal stabilization period, data from both detectors were automatically and simultaneously recorded by the fluorometer host computer every 10 s. The pH electrode was calibrated by conventional pH standards flowed past the inline electrode at the same rates used during experimental measurements.

Optical measurement of pH was made with CI-NERF [negative log of acidic dissociation constant (pKₐ) = 4], a pH-sensitive fluorescent dye previously used to image pH near the gastric mucosal surface (6). Fluorescence emission at 540 nm was measured in response to alternating excitation wavelengths of 512 and 433 nm (both collected in a total time of 1–2 s). Ratiometric pH measurements with CI-NERF have not been previously reported, but the results shown in Fig. 2A demonstrate that fluorescence excitation ratios of 512/433 nm are a sensitive indicator of pH. Fluorescence ratios were a useful measure from pH 2 to 6 but were most sensitive between pH 3 and 5. The fluorescence intensity ratio was...
Fig. 2. Cl-NERF as on-line pH sensor. A: calibration curve of 512- to 433-nm fluorescence excitation ratio of Cl-NERF vs. pH. Cl-NERF (0.1 μM) added to perfusate solution was measured at the indicated pH values. B: comparison of pH electrode and Cl-NERF pH measurements during flow of solutions. Fresh perfusion solution was titrated to different pH values and then flowed through the experimental setup without the use of any animal (i.e., syringe pump connected directly to effluent tubing). Both pH electrode and Cl-NERF reported similar pH changes between pH 2 and pH 5, although Cl-NERF measurements became noisier at pH extremes.

calibrated daily to solutions of known pH. Experiments compared the fidelity of response at the pH electrode vs. the Cl-NERF fluorescence ratio. When the pH of the perfusion solution was adjusted to different pH values and flowed by the two pH sensors (electrode and optical), measurements of pH by Cl-NERF reported changes in pH identical to those observed by pH electrode from pH 5 to 2 (Fig. 2B).

In some conditions, gastric effluent samples (85 μl) were collected by microcapillary tubes before and after a stop-flow period. Collected samples were measured with a blood gas analyzer (ABL 500, Radiometer Medical A/S, Copenhagen, Denmark) to determine the total CO₂ content (in mM) of the effluent.

Intravenous infusion of pentagastrin (16 μg·kg⁻¹·h⁻¹) or somatostatin (10 μg·kg⁻¹·h⁻¹) was used to stimulate or inhibit gastric acid secretion (3), respectively. Omeprazole (60 mg/kg ip) was used to block H,K-ATPase activity in some experiments (2, 33). In all cases, a stop-flow period was imposed 1 h after addition of the drugs.

Chemicals. The drugs used were thiobutabarbital sodium salt (Inactin, RBI, Natick, MA), pentagastrin and somatostatin (Sigma, St. Louis, MO), HOMOPIPES (Research Organic, Cleveland, OH), and omeprazole.

Pentagastrin was first dissolved in absolute ethanol and then diluted with saline to a desired concentration (final ethanol concentration <0.1%). Omeprazole was suspended at 28 mM in 0.5% (wt/vol) carboxymethylcellulose:water. Somatostatin (30 μM) was dissolved in 0.9% NaCl plus 1 mg/ml of BSA. Other agents were dissolved in distilled water. All agents were solubilized immediately before use. Routes of administration were intravenous infusion in a volume of 1 ml/h, intraperitoneal in a volume of 0.5 ml/100 g body wt, or intraluminal at a rate of 0.7 ml/min.

Statistics. Data are presented as means ± SE from 4–8 rats/group. Statistical comparisons between two groups were made with an unpaired two-tailed Student’s t-test. Statistical comparisons between groups used one-way ANOVA followed by Dunnett’s multiple comparison test. P < 0.05 was considered significant.

RESULTS

As shown schematically in Fig. 1, our goal was to perfuse the stomachs of anesthetized rats with solutions of either pH 5 or pH 3 (6) and monitor pH in the gastric effluent to measure net gastric acid/base secretion. Perfusion solutions were weakly buffered (4 mM HOMOPIPES, pKₐ = 4.32) and contained the pH-sensitive fluorescent dye Cl-NERF (0.1 μM). We used weakly buffered solutions to prevent sudden pH excursions during basal luminal perfusion. During our early trials we had observed that when the solution was not buffered, there were changes in gastric effluent pH under steady-state perfusion. We chose a concentration of buffer that did not affect the secreting capability of the stomach under basal and stimulated conditions (data not shown). We first compared the response of the electrode and optical pH sensors when measuring perfusion effluents from the gastric lumen in vivo, as described in METHODS.

Figure 3 shows recordings from a representative experiment in which pH was recorded in parallel by the two methods during luminal pH 5 perfusion. The two methods reported identical pH changes when gastric acid secretion was stimulated by pentagastrin and then blocked by somatostatin.

Large changes in secretory status could be readily detected as pH changes during continuous perfusion (as in Fig. 3), but it was more difficult to be confident about baseline values of secretion before the addition of regulatory agonists. This was because the value of gastric effluent pH was too close to that of the original perfusate pH to reliably detect the pH change. Therefore, we developed a scheme to increase sensitivity for detecting lower secretory states. Our approach was to...
impose a transient halt in perfusion (stop-flow period) and then measure pH after restarting the perfusion. The stop-flow period allowed the stomach secretions to transiently accumulate in the lumen. The amplified pH change was subsequently detected by the pH sensors (downstream of the stomach) after perfusion was restarted. Figure 4 shows a representative experiment as an example of the response of the pH sensors during steady-state gastric perfusion at luminal pH 5 before stop-flow, during stop-flow, and after perfusion flow was restarted. Both optical and electrode techniques reported a transient acidic peak after stop-flow, qualitatively indicating net acid secretion under basal conditions. Because of the physical arrangement of the two flow-through pH sensors in the perfusion stream, there was a 1-min time delay between pH electrode and fluorescence measurements, which is evident in these fast time recordings. The ~200 μl volume of the fluorometer cuvette caused a relatively slow renewal of the cuvette chamber and blunted the magnitude of pH transients compared with the pH electrode that was simply inserted into the perfusion tubing inline. While perfusion flow was halted, the pH electrode reading significantly increased from pH 4.79 ± 0.02 (steady-state pH value during continuous luminal pH 5 perfusion) to pH 4.88 ± 0.01 (P = 0.0054). In contrast, Cl-NERF gave similar pH values during either perfusion (4.79 ± 0.04) or stop-flow (4.76 ± 0.02; P = 0.26). The small drift in pH electrode readings appeared to be a flow artifact that did not compromise the experimental observations but made it necessary for all measurements to be made under similar flow conditions. Despite these technical limitations, both the Cl-NERF ratio and the pH electrode could be used to sensitively detect the amplified pH changes revealed by stop-flow.

Comparison of gastric response to luminal pH 5 vs. luminal pH 3. During luminal pH 5 perfusion, the pH electrode reported a basal steady-state pH in the gastric effluent of 4.79 ± 0.02 (P = 0.00014 vs. fresh solution; n = 8 animals; Fig. 5). For comparison, the pH of fresh solution (without exposure to the stomach) was reported as 5.01 ± 0.01 when flowed past the pH electrode. The pH electrode reported a transient acidification after stop-flow (peak pH after stop-flow 4.08 ± 0.19; P = 0.0069 vs. basal steady-state pH). These results both suggest the presence of net acid secretion under these conditions, as is also suggested in the representative experiment shown in Fig. 4.

Results were qualitatively different during pH 3 perfusion. In this condition, the basal steady-state pH of the gastric effluent as reported by pH electrode was 2.96 ± 0.02 (n = 7 animals), which was not significantly different from that of the starting solution pH (2.99 ± 0.01; P = 0.212). Thus during continuous pH 3 perfusion, the pH electrode could not resolve any net acid/base secretion. After stop-flow, a transient pH alkalization of the gastric effluent was observed (peak pH value 3.33 ± 0.13; P = 0.00045 vs. basal steady-state pH), suggesting net alkali secretion. These results were corroborated by the Cl-NERF measurements shown in Fig. 6. Under pH 5 perfusion, the basal steady-state pH reported by Cl-NERF was significantly more acidic than the pH of fresh solution (4.79 ± 0.04 vs. 5.02 ± 0.02; P = 0.0015) and was correlated with a transient acidification after stop-flow (peak pH 4.35 ± 0.1; P = 0.00094 vs. basal steady-state pH). Under pH 3 perfusion, the basal steady-state pH reported by Cl-NERF was significantly more alkaline than the pH of fresh solution (pH 3.07 ± 0.02 vs. 2.98 ± 0.01; P = 0.011), and an alkaline peak was observed after stop-flow (3.26 ± 0.07; P = 0.0086 vs. basal steady-state pH). Thus Cl-NERF was able to resolve a significant alkaline secretion both during perfusion and after stop-flow. Both electrode and optical methods reported qualitatively different results in the stomach.

![Fig. 4](image-url). Raw data from a representative experiment comparing pH of gastric effluent in response to a transient halt in perfusion. Stomach perfusion with pH 5 solution was transiently stopped (stop-flow) for 10 min to allow gastric secretions to accumulate in the stomach lumen. When perfusion was restarted, the accumulated secretions were emptied from the stomach and flowed by the electrode and Cl-NERF (optical) pH sensors. Accumulated secretions caused an exaggerated change in effluent pH, which rapidly peaked and in 5 min returned to its steady-state perfusion value before stop-flow. Acidic peak after stop-flow indicates net acid secretion under this condition. Both sensors reported qualitatively similar changes, and details are discussed in text.

![Fig. 5](image-url). Compiled time course of pH measurement reported by pH electrode during luminal perfusion with pH 5 and pH 3 solutions. Break between 300 and 900 s on x-axis represents the stop-flow period. As seen from the qualitative response to stop-flow, net acid secretion was observed under pH 5 perfusion and net alkali secretion under pH 3 perfusion.

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secretions at pH 5 vs. pH 3 and suggested the surprising result that alkali secretion dominated when the stomach lumen was perfused at pH 3.

**Blocking the H,K-ATPase.** Detection of gastric alkali secretion has often required pharmacological inhibition of acid secretion (1, 33); therefore, we asked if a H,K-ATPase inhibitor (omeprazole) would unmask greater net alkali secretion. During perfusion with luminal pH 5 (Fig. 7), the addition of omeprazole alkalinized the gastric effluent (pH 5.03 ± 0.03; \( P < 0.0025 \) vs. basal steady-state pH in preomeprazole state) and prevented the acidic peak observed after stop-flow (peak pH 5.34 ± 0.16). In the presence of omeprazole, the peak pH after stop-flow was not statistically different from the steady-state pH during continuous perfusion (\( P = 0.0983 \)). Thus although there was a trend towards alkalinization, stop-flow could not confirm or deny the presence of alkali secretion in the omeprazole-treated rats at luminal pH 5. At luminal pH 3 (Fig. 8), omeprazole significantly alkalinized steady-state pH during perfusion (3.08 ± 0.02; \( P = 0.00445 \) vs. basal steady-state pH in preomeprazole state), and a significant alkalinization was observed after stop-flow (peak pH 4.02 ± 0.27; \( P = 0.0229 \) vs. postomeprazole steady-state perfusion pH). These results are consistent with previous observations of net alkali secretion during suppression of acid secretion and confirm that elimination of acid secretion leads to greater net alkali secretion.

**Somatostatin blocks pentagastrin-stimulated gastric acid secretion.** During either pH 5 or pH 3 perfusion, pentagastrin stimulated gastric acid secretion, as reported by the pH values, either during perfusion or after stop-flow (Figs. 7 and 8). At pH 3 (Fig. 8), addition of pentagastrin converted the net alkali secretion observed in basal conditions to a net acidic secretion. Somatostatin inhibited the pentagastrin-stimulated gastric acid secretion during both pH 5 and pH 3 luminal perfusion (Figs. 7 and 8). The addition of somatostatin alone decreased net acid secretion at luminal pH 5 (Fig. 7) but did not modify the observed alkali secretion at luminal pH 3 (Fig. 8).

**Net proton secretion or consumption.** If the buffering capacity of gastric effluent is known, changes in pH can be converted to amounts of secreted acid/base equivalents. As shown in Fig. 9, titration of either fresh perfusate solution or gastric effluents produced identical buffering capacity curves. This demonstrates that gastric secretions do not alter the buffering capacity of the solution and permits reliable estimation of titratable acid/base equivalents added to the effluent. The small hump in buffering capacity values at pH 4 is a result of the presence of HOMOPIPES buffer in the solution (data not shown). Results were fit to an eighth-order polynomial (Fig. 9), and this equation was used to derive values of net protons produced (or consumed) in the lumen. Proton consumption is equivalent to net alkali secretion.

Fig. 6. Compiled time course of pH measurement reported by CINERF during luminal perfusion with pH 5 and pH 3 solutions. Break between 300 and 900 s on x-axis represents the stop-flow period. Net acid secretion was observed under pH 5 perfusion and net alkali secretion under pH 3 perfusion. Results from the optical pH sensor corroborate pH electrode measurements.

Fig. 7. Compiled values of gastric perfusate pH reported by the pH electrode during steady-state perfusion with pH 5 solutions under different treatments (open bars). Results are also shown for peak pH change after a 10-min stop-flow period (solid bars). Peak value was chosen to approximate the pH value of stomach contents directly after restarting the perfusion. Results are compared with the absence of treatment (basal) or after 60 min of exposure to pentagastrin (16 \( \mu g/kg \ h^{-1} \ iv \)), somatostatin (10 \( \mu g/kg \ h^{-1} \ iv \)), or omeprazole (60 mg/kg ip) as indicated. Dotted line, pH of fresh perfusate. Results are means ± SE; \( n = 5–8 \). **P < 0.05 compared with basal steady-state perfusion pH value. ***P < 0.05 compared with steady-state perfusion pH value of each group.

Fig. 8. Compiled values of gastric perfusate pH reported by the pH electrode during perfusion at luminal pH 3. All other conditions and analyses are identical to those described in Fig. 7. Values are means ± SE; \( n = 5–8 \).
CO\textsubscript{2} content is more difficult to explain if acid-back diffusion was the predominant mechanism leading to luminal alkalinization.

**DISCUSSION**

This report introduces the feasibility and benefits of combining optical and electrode measurements for the dynamic study of gastric secretions by the whole stomach in vivo. During continuous perfusion of the stomach lumen, we used two different pH measurement techniques to validate observed pH changes. Due to technical constraints, Cl-NERF pH measurements did not give exactly the same kinetic responses as pH electrode measurements. However, both measurements qualitatively and quantitatively reported similar pH changes. The consistency of results between Cl-NERF and the pH electrode supports the validity of our previous use of Cl-NERF in studies of gastric surface pH (6). Furthermore, the buffering capacity of gastric effluent remained the same as that of fresh perfusate, allowing accurate calculation of the amount of net proton secretion into the perfusate by the tissue. Previously, we demonstrated (6) that either acid secretion or alkali secretion could dominate control of pH in the microscopic regions adjacent to the gastric surface. We further showed that luminal pH of the stomach regulated the conversion between these two states. In both that study and the current work, we

With the use of the buffering curves shown in Fig. 9, the pH values shown in Fig. 6 were converted to net amounts of protons added to or removed from the lumen. Results are compiled in Table 1. When the pH 3 and pH 5 conditions are compared, the results suggest that the net amount of proton secretion stimulated by pentagastrin was larger at pH 5 than at pH 3. In addition, the net amount of alkali secretion during pH 3 perfusion was increased by inhibition of H,K-ATPase with omeprazole.

**Bicarbonate secretion as basis for net alkali secretion.** To determine whether the alkalinization was due to acid-back diffusion or bicarbonate secretion, total CO\textsubscript{2} content (in mM) of the gastric effluent was measured before and after the stop-flow period. The total CO\textsubscript{2} concentration during steady-state perfusion was 2.23 ± 0.06 mM and increased to 2.38 ± 0.09 mM (stop-flow caused the appearance of an additional 0.15 ± 0.03 mM CO\textsubscript{2}; \(P < 0.01\)). For comparison, we used the buffering capacity to calculate the change in hydrogen ion concentration under the same conditions. Stop-flow caused consumption of an additional 0.16 ± 0.03 mM protons (\(P < 0.01\)), a value indistinguishable from the amount of added CO\textsubscript{2}. Because any secreted bicarbonate would be converted to CO\textsubscript{2} (with obligatory consumption of a proton) at the prevailing pH of these experiments, these results strongly suggest that the alkalinization observed at pH 3 is quantitatively explained by bicarbonate secretion. The increased total

![Graph](image.png)  
**Fig. 9.** Comparison between buffering capacity of fresh perfusate solution (○) and gastric effluent (▼). Titration of fresh perfusate solution or gastric effluents produces identical buffering capacity curves. Line shown in the figure was fit to an 8th-order polynomial, and the resulting equation was used to calculate values of net protons produced or consumed in the stomach lumen. Solutions were manually titrated by repeated addition of known amounts of NaOH or HCl. Buffering capacity was calculated as amount of acid/base added and normalized to the final volume (titrants changed volume <10%) and the resultant pH change (a single bolus of titrant changed <0.2 pH units). Results are plotted vs. the midpoint of pH change in response to a single bolus of titrant. Each data point is a single measured value with results compiled from 17 separate effluents and 13 fresh perfusates.

**Table 1.** Net H\textsuperscript{+} amount/volume added or consumed by the stomach during luminal perfusions

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Condition</th>
<th>pH 5.0</th>
<th>pH 3.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>Steady-state perfusion</td>
<td>0.40 ± 0.05</td>
<td>0.10 ± 0.07</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>Stop-flow peak</td>
<td>1.87 ± 0.44†</td>
<td>−0.90 ± 0.17†</td>
</tr>
<tr>
<td>Pentagastrin</td>
<td>Steady-state perfusion</td>
<td>−0.00 ± 0.04*</td>
<td>−0.26 ± 0.07*</td>
</tr>
<tr>
<td>Pentagastrin + somatostatin</td>
<td>Stop-flow peak</td>
<td>3.71 ± 0.89*</td>
<td>1.31 ± 0.68*</td>
</tr>
<tr>
<td>Somatostatin</td>
<td>Steady-state perfusion</td>
<td>0.51 ± 0.07*</td>
<td>0.47 ± 0.05*</td>
</tr>
<tr>
<td>Somatostatin</td>
<td>Stop-flow peak</td>
<td>1.32 ± 0.39</td>
<td>1.95 ± 0.73</td>
</tr>
</tbody>
</table>

Values are means ± SE of H\textsuperscript{+} amount/volume added or consumed by the stomach during pH 5 and pH 3 luminal perfusions; \(n = 5\)–8 rats. †, added; †, consumed. Concentration of H\textsuperscript{+} (in mM) was calculated by applying the buffering capacity values shown in Fig. 9 to the difference in pH between the gastric effluent and that of fresh perfusate in individual experiments. For calculations, gastric effluent pH was measured under 2 different conditions for each experimental treatment: during continuous perfusion (steady-state perfusion) or at the peak change in pH after a 10-min stop-flow interval (stop-flow peak). Negative values (consumed protons) indicate net alkali secretion. Drugs were added as described in METHODS. *\(P < 0.05\) for experimental steady-state perfusion vs. basal steady-state perfusion values. †\(P < 0.05\) for stop-flow vs. steady-state perfusion values within an experimental group.
compared results when luminal pH approximated values found in the fasting (pH 3) or fed stomach (pH 5) of rats. The current work was designed to extend the previous measurements of pH regulation in microenvironments to the regulation of acid/base secretion by the whole stomach.

Our results show that in response to changes in luminal pH, the stomach converts from net acid to net alkali secretion. To balance this unusual finding, our results confirmed that more conventional aspects of the regulation of acid secretion remained intact. During intraluminal perfusion with pH 5 solution (to mimic the pH of the fed rat stomach), gastric acid secretion was observed. As expected from the known properties of the gastric H,K-ATPase, this acid secretion could be stimulated further with pentagastrin and inhibited by omeprazole or somatostatin. In contrast, during intraluminal perfusion with pH 3 solution (to mimic the pH of the fasted stomach), net alkaline secretion dominated over acid secretion. This was most evident when secreted alkali was allowed to accumulate in the gastric lumen during a stop-flow period, so that pH changes in the gastric effluent were more pronounced. Under both luminal pH 5 or pH 3 conditions, the accumulation of acid/base secretions in the lumen can always initiate feedback regulatory mechanisms in control of gastric secretions. These feedback mechanisms can be more noticeable, especially in pentagastrin-stimulated conditions when the luminal pH becomes very acidic. However, observing the same pH values before and shortly after the stop-flow period convinced us that the short period of stopping the perfusion may not trigger those feedback mechanisms. Nevertheless, further experiments related to these short-term regulatory mechanisms could be performed. Different experimental conditions and our preliminary observations showed us that the conversion of whole stomach from acid to alkali secretory status or vice versa did not occur in such a short period of time.

The observed gastric effluent pH changes at luminal pH 3 were not due to back-diffusion of acid. Total CO₂ measurements of gastric effluent confirmed that the pH changes in the gastric effluent were likely a result of bicarbonate secretion into the lumen. Pentagastrin, when added, was able to reverse the gastric secretions to net acid secretory, and omeprazole enhanced the net alkali secretion under basal conditions (presumably by blocking low basal acid secretion in these conditions). These results show the existence of steady-state gastric alkali secretion in the presence of lower intragastric pH and show that even in the absence of pharmacological suppression of H,K-ATPase, this alkali secretion is greater than acid secretion.

When pH changes were converted to amount of secreted protons, it was possible to quantitatively compare results between pH 3 and pH 5 perfusion. Exogenous pentagastrin stimulated net proton secretion during both pH 3 or pH 5 perfusion. However, the resulting acid secretion (measured as the peak pH change after stop-flow) was fourfold higher at luminal pH 5 compared with luminal secretion at pH 3. Even taking into account the amount of basal acid/base secretion under these two conditions or the alkali secretion unmasked by omeprazole, the results suggest that the ability of pentagastrin to stimulate acid secretion is limited at the lower intragastric pH. Conversely, in the presence of omeprazole, the net alkali secretion at pH 3 was sixfold greater than that at pH 5. This is consistent with an earlier report that gastric alkali secretion may be enhanced at lower intragastric pH (25). Somatostatin infusion antagonized the effect of pentagastrin on acid secretion, but somatostatin did not elicit significant net alkali secretion when added in either the presence or absence of pentagastrin. Most notably, at luminal pH 3, somatostatin alone had no effect on net alkali secretion. Because omeprazole unmasked greater alkali secretion under this same condition, the results suggest that somatostatin has an inhibitory effect on alkali secretion and/or (like pentagastrin) has limited ability to act at luminal pH 3. Further studies are needed to resolve these questions.

In conclusion, our results show that pH is a luminal signal regulating the dynamic physiological transition between acid and alkali secretion in the stomach. Although the intragastric proton concentration regulates this transition, other mediators such as gastrin and somatostatin likely play a role as downstream effectors. It will be important to determine the role of these and other endocrine and neurocrine regulators in the feedback mechanisms that control the transition between acid and alkali secretion. Our results are consistent with the previous observation that gastric surface pH can be regulated by either acid or alkali secretion during exposure to pH 5 or pH 3, respectively. The current report finds that net acid/base secretions measured in the microscopic space adjacent to the gastric surface mirrors net secretion observed at the level of the whole stomach. This strongly suggests that, at least under some circumstances, surface pH regulation is an extension and not an opponent of the dominant gastric secretions.

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REFERENCES
CONVERSION FROM ACID TO ALKALINE GASTRIC SECRETION