Effect of luminal pH on the output of bicarbonate and PGE₂ by the normal human stomach

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SUMMARY The gastric output of bicarbonate and prostaglandin E₂ has been calculated using a perfusion technique before and after instillation of 100 mM hydrochloric acid into the stomach of seven healthy volunteers. A significant increase in bicarbonate output occurred from $258 \pm 38 \mu\text{mol}/30 \text{ min}$ during the basal period to $531 \pm 86 \mu\text{mol}/30 \text{ min}$ after return of the intragastric pH to neutral ($p<0.05$). Prostaglandin E₂ output also increased significantly from $410 \pm 136 \text{ pmol}/30 \text{ min}$ to $1002 \pm 194 \text{ pmol}/30 \text{ min}$ ($p<0.05$). The changes were caused mainly by an increase in gastric secretory volume with only non-significant increases in concentrations of bicarbonate and prostaglandin E₂. The results suggest that mechanisms exist to adjust the rate of gastric bicarbonate secretion to the prevailing intraluminal pH and that this may occur through the release of prostaglandin E₂.

The existence of an alkaline secretion by gastric and duodenal mucosa of experimental animals and man has now been established. Evidence indicates that it is able to create a pH gradient across the adherent mucus gel and that this 'mucus-bicarbonate' barrier may constitute a first line defence against acid damage. In order for such a barrier to remain intact under varying intraluminal conditions the rate of bicarbonate secretion may alter according to luminal pH. Evidence from animal experiments both in vitro and in vivo indicates that this can occur and that the response may be due to endogenous prostaglandins. This study has examined whether gastric bicarbonate secretion in man can be influenced by luminal acid.

Methods

MEASUREMENT OF GASTRIC BICARBONATE SECRETION

This was according to a modification of a method previously described. After an overnight fast seven healthy volunteers (aged 22–54, mean 32 yr) swallowed a multilumen polyethylene tube which was positioned by fluoroscopy with its tip in the second or third part of the duodenum (Fig. 1). Ranitidine was administered (150 mg orally one hour before the study followed by 25 mg/h iv during perfusion) to maintain an intragastric pH between 6 and 7. Resting gastric contents were aspirated before perfusion and all saliva was removed with a dental sucker for the duration of the experiment. Under these conditions salivary contamination accounts for only 3% of measured gastric bicarbonate. The stomach was perfused with $^3\text{H}$-polyethylene glycol (12.5 $\mu\text{Ci}/l$ in saline at pH 7.4; 2 ml/min) and the duodenum with $^{14}\text{C}$-polyethylene glycol (12.5 $\mu\text{Ci}/l$, in saline at pH 7.4; 2 ml/min). Aspiration sites were

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Received for publication 19 March 1987.

Fig. 1 Diagram of the perfusion method of measurement of gastric bicarbonate secretion.
positioned in antrum and distal duodenum. Samples from the aspiration sites were collected by continuous suction at 10 min intervals for measurement of marker concentration. One millilitre samples were separated on ice and used for the measurement of prostaglandin E2 concentration. In the middle of each sampling period a separate 1 ml aliquot was aspirated using a closed cooled syringe for immediate determination of pH and pCO2.

**Analytical Methods**

Concentrations of 3H-PEG and 14C-PEG in the samples were measured by liquid scintillation counting (LKB Wallace 2000 Beta Counter, one ml aspirate with 10 ml PCS scintillator) with quenching correction by the external standards channels ratio method. The pH and pCO2 of the samples were measured immediately using a Corning 170 Blood Gas Analyser where pH was greater than 6 and a Radiometer Copenhagen pH meter 28 when the pH was strongly acidic using the same calibration buffers. Prostaglandin E2 concentrations were measured by radioimmunoassay after extraction into 2 ml ethyl ether, evaporation and resuspension into 1 ml radioimmunoassay buffer. Pasteur antiserum was used and the interassay coefficient of variation for standards was 6%. In these experiments assays of gastric samples at two fold dilutions gave similar results (103±9%, mean±SD, n=4). Additional experiments using exogenous PGE2 addition revealed a good correlation between amount added and amounts measured (measured calculated as 101±17% added, mean±SD, n=8) suggesting very low cross reactivity with potentially interfering substances. Furthermore the PGE2 antiserum used showed selective reactivity with material cochromatographing with PGE2.

**Experimental Design**

After an equilibration period of two hours basal bicarbonate secretion was measured for 60 minutes. The gastric perfusate was then changed to 3H-PEG in 100 mM HCl (100 mM HCl plus 50 mM NaCl to maintain osmolality, at pH 1-6; 2 ml/min). The acidified marker was perfused for 30 minutes after which the original perfusate at pH 7-4 was recommenced. Aspiration continued throughout so that bicarbonate secretion during the pre and post acid periods could be calculated.

**Calculation of Results**

Effective gastric and duodenal volumes were calculated according to marker dilution and perfusion rate. Duodenogastric reflux was calculated according to the amount of 14C-PEG appearing in the gastric aspirate. Free bicarbonate concentration was calculated according to the Henderson-Hasselbalch equation and added to the CO2 bicarbonate (pCO2× solubility constant (0-031)) to give a figure for total bicarbonate concentration. Bicarbonate output was calculated as the product of volume and concentration and was corrected for refluxed bicarbonate. Prostaglandin E2 output likewise was the product of prostaglandin E2 concentration and volume. All outputs were expressed as mean±SEM per 30 minutes. Differences before and after acid perfusion were assessed by a paired t-test for significance.

**Results**

**Gastric pH and pCO2**

Mean basal gastric pH under these circumstances was 6-40±0-10. During acid perfusion (Fig. 2) gastric pH fell reaching a nadir after 30 minutes of 2-21±0-14 and thereafter gradually rose as the infused acid was cleared. The mean resting pCO2 was 35-9±3-1 mm Hg which increased to a maximum of 88-8±10-8 mm Hg during acid perfusion and fell again to levels close to basal as the pH was restored to near neutral.

**Bicarbonate Concentration**

Bicarbonate concentration in the gastric aspirate was 3-58±0-24 mmol/l. It was not possible to measure accurately bicarbonate concentration when the gastric pH fell below 5. At or below this pH, virtually all the bicarbonate present is in the form of CO2 which equilibrates rapidly with air within the gastric lumen. This process is rapid at high levels of pCO2 and thus causes inaccuracies of pCO2 measurement. Under basal conditions where pCO2 is similar to that occurring in plasma the losses are minimal. Furthermore high values of pCO2 are inaccurately measured by instruments calibrated for blood gas analysis. In these experiments, however, bicarbonate concentration can be measured once the pH returns to near neutral. Bicarbonate concentration in the aspirated samples was at its highest in the 30 minute period after acid instillation (4-61±0-28 mmol/l) although the difference from basal was not statistically significant (p=0-3).
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SECRETORY VOLUMES
Basal secretory volume was calculated as 28.6±5.6 ml/30 min which increased to 75.5±17.5 ml/30 min after acid perfusion (p<0.05) and thereafter declined to levels close to basal (32.6±7.7 ml/30 min) (Fig. 3). Marker recovery ranged between 32 and 86% (mean 62%) and was consistent for individuals.

BICARBONATE OUTPUT
Gastric bicarbonate output increased in the 'post acid' period from a basal of 258±38 µmol/30 min to a peak of 531±86 µmol/30 min after restoration of neutral pH (Fig. 4). Calculation of refluxed bicarbonate indicated that there was no alteration in duodenogastric reflux (basal refluxed bicarbonate 89±26 µmol/30 min compared with 55±22 µmol/min after acid).

PROSTAGLANDIN E$_2$ OUTPUT
Prostaglandin E$_2$ concentrations reached their highest in the 'post acid' period (20±5 pmol/ml compared with 14±4 pmol/ml in the basal period) although the difference failed to reach significance (p=0.2). When expressed as an output, however, there was a significant increase in prostaglandin E$_2$ output from a basal of 411±136 pmol/30 min to 1002±194 pmol/30 min (p<0.05) which declined as the pH was restored to normal (Fig. 5).

Discussion
In order to provide effective mucosal protection the mucus-bicarbonate barrier needs to be maintained in a dynamic equilibrium with luminal acid and pepsin so that the pH gradient is preserved and the epithelium undamaged. The mucus gel layer, an important constituent of the barrier, is under a state of continued erosion by luminal pepsin and replenishment occurs by fresh production or secretion of preformed mucus. Bicarbonate secretion into the mucus gel is continually neutralised by luminal acid creating a pH gradient and steady state secre-
tion in able to maintain the gradient under most luminal conditions. Evidence from in vitro experiments indicates that under acidic conditions where luminal pH falls below 1.4, the gradient may be overwhelmed. It is likely, however, that in vivo secretion of bicarbonate is modulated by various neurohormonal control mechanisms which may increase bicarbonate output in accordance with increased requirements. One mechanism which may be of importance in maintaining the integrity of the mucus-bicarbonate barrier is a response of epithelial bicarbonate secretion to luminal acid. Experiments in dogs with Heidenhain pouches indicate that bicarbonate secretion by the pouch is increased dramatically by the instillation of acid into the main stomach. In addition in vitro experiments on isolated mucosa using a paired chamber with a common serosal side solution show that acidification of one mucosa is capable of increasing bicarbonate secretion by the other. In man a response of proximal duodenal bicarbonate secretion to luminal acid has been demonstrated and this has been shown to be attenuated in patients with duodenal ulcer but no studies to date have examined the effect of luminal pH on gastric bicarbonate secretion. Because this response may play an important role in the dynamics of gastric mucosal protection we have examined the effect of acid on bicarbonate output by the normal human stomach.

Our results indicate that acid perfusion produces an increase in gastric secretory volume. The secretion contains more bicarbonate than in the resting state and the output is significantly increased. The response represents a doubling of the basal rate even though in these experiments the luminal pH fell only to 2. It is possible that higher increments may be seen in the presence of lower pH levels which are frequently achieved in vivo under normal conditions. Although bicarbonate output could not be measured during acid perfusion the increase in volume occurred while the pH was low and that combined with the high PCO₂ levels suggests that bicarbonate secretion was actually increased during acid perfusion. Furthermore the increase in secretory volume may itself be important as mathematical analysis of a model of the mucus-bicarbonate barrier indicates volume flux as a vital component of the equilibrium. In these experiments the measured change in bicarbonate output observed occurred on return of the pH to near neutral. Although an apparently late response it is important to appreciate that return to neutrality occurred in the experimental situation due to aspiration of acid, which may be very different to normal gastric acid disposal which is likely to be slower.

There are a number of possible mechanisms for the response to intraluminal acid observed in this study. Gastric bicarbonate secretion may be stimulated by cholinergic agents, calcium, cGMP, E and F-type prostaglandins, CCK, and pancreatic glucagon. Similarly inhibition of bicarbonate secretion by indomethacin implies that endogenous prostaglandins are involved in regulating basal secretion. Prostaglandin E₂ analogues have been shown to increase gastric epithelial bicarbonate secretion in vivo. In high doses in man this effect can also be demonstrated. In lower doses, however, prostaglandin E₂ analogues do not stimulate bicarbonate secretion in man but do prevent the inhibition induced by aspirin or taurocholate indicating that endogenous prostaglandins are implicated in modulating basal secretion. Animal experiments have suggested that the response to luminal acid occurs via local release of prostaglandins. A recent study has reported that acid instillation into the human stomach increases luminal prostaglandin E₂ output and clearly the similar effect shown in our study may be responsible for the secretory response observed.

The increased output of prostaglandin E₂ observed in these experiments may be important both in relation to the bicarbonate response and to other mechanisms of mucosal protection. Prostaglandin E₂ is capable of increasing mucus gel thickness and release increasing mucosal blood flow affecting epithelial cell surface hydrophobicity and cellular restitution after injury, all of which are likely to be important in mucosal protection. The prostaglandin E₂ output rose in our studies as the pH fell and declined again as neutrality was restored indicating the close relationship between pH and secretory response by gastric mucosa.

In conclusion these experiments indicate the presence of an autoregulatory mechanism which may be capable of maintaining the gastric mucus-bicarbonate barrier and mucosal integrity under varying luminal acidity. Studies to evaluate the integrity of this response in patients with peptic ulcer disease would clearly be of interest.

The authors wish to thank Ms J Rostron for secretarial assistance and the Department of Medical Illustration for preparing the figures. Dr Crampton is in receipt of a North West Regional Health Authority Grant.

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Effect of luminal pH on the output of bicarbonate and PGE2 by the normal human stomach.

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*Gut* 1987 28: 1291-1295
doi: 10.1136/gut.28.10.1291

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