

Demonstration of a pH gradient across the mucus layer on the surface of human gastric mucosa *in vitro*

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SUMMARY In previous studies we have demonstrated a hydrogen ion concentration gradient across the mucus on rat and rabbit fundic mucosa, *in vivo* and *in vitro* respectively, observations which support the possibility of a 'mucus-bicarbonate' protective barrier. In the present studies we have demonstrated a similar gradient across the mucus on human gastric mucosa *in vitro*. The minimum mean hydrogen ion concentration at the mucus-epithelium interface was 1.1×10^{-4} mM (pH 6.96, n=10) when the mean luminal concentration was 5.6 mM (pH 2.25). Aspirin (10 mM) and N-acetyl cysteine (306 mM) (5%) increased the minimum intra-mucus hydrogen ion concentration and the gradient was overwhelmed by a luminal hydrogen ion concentration of 40 mM (pH 1.4). These results suggest that a hydrogen ion concentration gradient exists across the mucus on human gastric mucosa and that potential damaging agents may act by compromising one or other of the components of this 'mucus-alkaline', presumed 'mucus-bicarbonate', barrier.

The mechanisms by which the stomach protects itself from potentially damaging acid-peptic secretions are uncertain. Heatley¹ postulated that mucus on the surface of the mucosa provided an unstirred layer in which bicarbonate secreted by the surface epithelium neutralised hydrogen ions diffusing towards the epithelium from the lumen. Support for this hypothesis has come with the demonstration that fundic mucosa is capable of secreting bicarbonate by an active, energy-dependent, process^{2,3} and that mucus delays the transfer of small ions across it.⁴ Furthermore, our recent demonstration of a pH gradient across the mucus adherent to rabbit fundic mucosa *in vitro*⁵ and rat fundic mucosa *in vivo*⁶ provides direct evidence in favour of such a mechanism. The present studies were designed to examine the possibility that a similar gradient exists across human gastric mucus.

Methods

Gastric mucosa was obtained from gastrectomy specimens taken from 10 patients who had had premedication with pethidine, atropine, or

scopolamine, and anaesthetised with thiopentone, nitrous oxide, and halothane together with tubocurarine. The diagnosis was cancer of the stomach in six, gastric ulcer in three, and duodenal ulcer in one. Full thickness specimens were taken from the body of the stomach at least 3 cm away from the margin of any lesion, and placed in oxygenated bicarbonate-free, Ringer's lactate solution. The tissue was mounted horizontally in specially prepared chambers,⁵ within 30 minutes of resection, so that approximately 0.5 cm² of the luminal surface was bathed with 0.5 ml hydrochloric acid (HCl) 10 mM (pH 2.0) and the serosal surface with oxygenated bicarbonate-free Ringer's lactate solution maintained at 37°C. The hydrogen ion activity gradient across the mucus was measured using antimony microelectrodes manufactured as previously described.⁵ Microelectrodes had tip diameters of approximately 50 μm and these were slowly lowered into and through the mucus using a micro-manipulator which moved the electrode in 42.3 μ steps. Changes in hydrogen ion concentration were recorded in millivolts on a Servoscribe chart recorder *via* a Vibron voltmeter and converted to hydrogen ion concentrations using calibration curves obtained from standards covering the range 100 mM to 10⁻⁵ mM (pH 1 to 8). Calibration was carried out each day before each experiment. There was no significant change between the calibration curves before or after insertion into the mucus.

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The influence on the gradient of the mucolytic agent N-acetyl cysteine (306 mM; NAC, 5%) and of aspirin (10 mM) was investigated. The effect of a high acid concentration HCl, 40 mM (pH 1.4) on the luminal surface was also examined.

The effect of these manipulations of the luminal fluid on a constant hydrogen ion concentration reading was determined with the electrode held at some fixed site within the mucus layer. In addition, the effect of these agents on the minimum hydrogen ion concentration recordable was examined. Statistical analysis was performed using the Wilcoxon rank sum test and one way analysis of variance.

Results

The mean luminal hydrogen ion concentration (HCl) bathing the mucosa was 5.6 mM (pH 2.25) (n=10). As the microelectrode was lowered through the mucus the hydrogen ion concentration fell steadily to a mean minimal value of 1.1×10^{-4} mM (range 7.9×10^{-6} – 5.9×10^{-3}) (pH 6.96; range 5.23 to 8.1). Any intermediate intra-mucus hydrogen ion concentration, obtained with the electrode held still at any point during passage through the mucus, could be maintained at a constant level for periods of up to two hours of observation. On reaching the minimum hydrogen ion concentration further progression of the electrode did not result in any decrease or, in some tissues, there was a small sudden increase which probably indicated that the electrode had entered the tissue.

When the hydrogen ion concentration of the luminal fluid was increased to 40 mM (pH 1.4) the concentration maintained at any point within the mucus layer was significantly increased within 10 minutes ($p < 0.05$) (Fig. 1).

306 mM NAC caused a rise in constant hydrogen ion concentrations obtained within the mucus layer in seven out of eight experiments (Fig. 2). This rise was significant after 25 minutes ($p < 0.05$).

Aspirin 10 mM increased constant hydrogen ion concentrations in eight out of nine experiments, the rise being significant after 20 minutes ($p < 0.05$) (Fig. 3).

The minimum hydrogen ion concentrations obtainable after 30 minutes in tissues bathed in aspirin (10 mM), NAC (306 mM) and HCl (40 mM) were all increased significantly (Table).

Discussion

The possibility that mucus adherent to the epithelial layer of the stomach could have a protective role

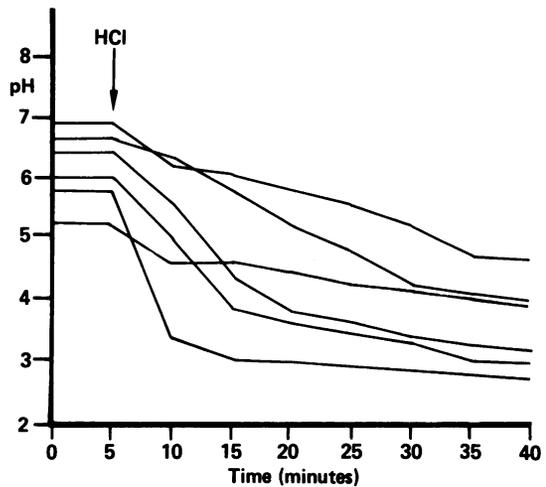


Fig. 1 The effect of HCl (40 mM, pH 1.4) applied to the luminal surface, on pH measured, with the electrode held steadily at a single point within the mucus layer, in each of six specimens of gastric mucosa.

against intraluminal acid has not received wide support. It has been difficult to envisage mucus resisting the diffusion of small ions through it. Nevertheless, it has been demonstrated that the diffusion of hydrogen ions through a layer of pig gastric mucus occurs four times more slowly than through an equivalent thickness of unstirred saline.⁴ Secondly, it is known that fundic mucosa can secrete bicarbonate,^{2,3} and that bicarbonate is present within mucus secretions.⁷ This combination of an unstirred layer with mucosal alkaline secretion

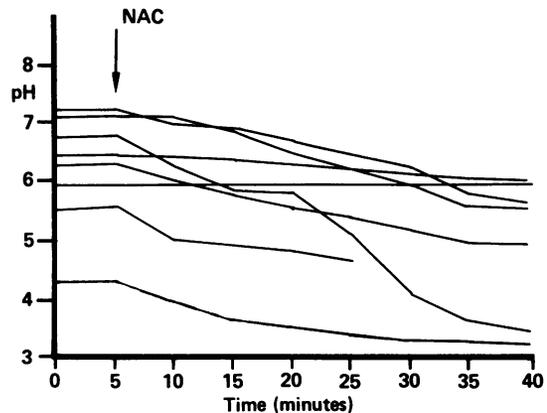


Fig. 2 The influence of NAC (306 mM, 5%) on pH measured at a single point in the mucus layer in each of eight specimens of gastric mucosa.

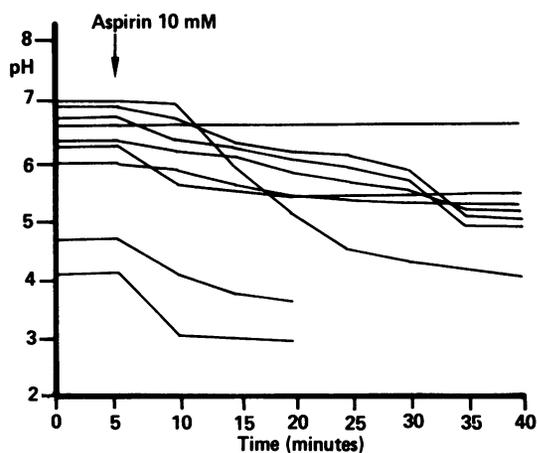


Fig. 3 The influence of aspirin (10 mM) on pH measured at a single point within the mucus layer in each of nine specimens of gastric mucosa.

provides the basis for a hypothetical protective barrier against luminal acid. Our recent demonstration of a pH gradient across mucus on rat and rabbit gastric mucosa^{5,6} supports this hypothesis. In the present studies we have demonstrated that human gastric mucus is also capable of maintaining a hydrogen ion concentration gradient across it when the luminal hydrogen ion concentration is 10 mM.

Although the gastric mucosae in these experiments were removed from patients with a variety of gastroduodenal diseases, presumably with abnormal mucosae, and despite the prior administration of a variety of anaesthetic drugs it is of interest that the results described here are strikingly similar to our previous data obtained *in vitro* in the rabbit and *in vivo* in the rat.^{5,6} Presumably these diseases and anaesthetic agents were insufficient to produce an obvious effect on the pH profile across the mucus layer.

The observation that 306 mM N-acetyl cysteine, a mucolytic agent, altered the gradient provides support for the importance of the mucus structure in the maintenance of this gradient. Indeed, it has been shown that the gastric mucus layer is reduced in thickness after treatment with NAC.⁸ The reduction in the gradient produced by 10 mM aspirin may be relevant to the known damaging effect of aspirin on the gastric mucosa. Aspirin has been demonstrated to have a number of actions any of which could affect a postulated 'mucus-bicarbonate' barrier. The precise mechanism by which it acted here is not known, but the effect could be due to inhibition of alkaline secretion,⁹ shedding of adherent mucus,¹⁰ exfoliation of epithelial cells,¹¹ and/or decreased synthesis of mucus.¹²

Our finding that an increased concentration of HCl will also impair the maintenance of the gradient is of interest. The human stomach can certainly secrete acid of 40 mM or more. However, under physiological conditions this concentration of acid is rarely maintained within the gastric lumen for any length of time. Such stimulated acid secretion would normally be buffered in part by ingested food and evacuated from the stomach.

The results demonstrated here confirm our previous observations that a hydrogen ion concentration gradient exists across the mucus on gastric mucosa^{5,6} and show that this gradient exists in human stomach too. The results with N-acetyl cysteine, aspirin, and HCl 40 mM solutions in human mucosa also confirm observations we initially made in the rat stomach *in vivo*.⁶ The finding that agents which damage the gastric mucosa, such as aspirin, can also affect the gradient, lends support to the proposal that a 'mucus-alkaline' barrier exists on the gastric mucosa. Although we have no direct evidence that human gastric mucosa secretes bicarbonate *in vitro*, by extrapolation from a number of other mammalian species in which this has been demonstrated, it seems highly likely that this 'barrier' is made up of mucus and bicarbonate.

Table Minimum hydrogen ion concentration (mM), measurable in adherent mucus of untreated stomachs (mean luminal H^+ concentration, 5.6 mM; pH 2.25) and after 35 minutes' treatment with NAC, aspirin, or HCl (40 mM; pH 1.4)

| | Untreated 'control' mucus n=10 | NAC 306 mM n=8 | Aspirin 10 mM n=8 | HCl 40 mM n=5 |
|-----------------|---|-------------------------|-------------------------|---------------------------|
| $[H^+]$ mM mean | 1.1×10^{-4} | 2×10^{-2} | 1.3×10^{-2} | 5.4×10^{-2} |
| range | 7.9×10^{-6} – 5.9×10^{-3} | 1×10^{-3} –0.5 | 6.3×10^{-4} –1 | 6.3×10^{-3} –0.5 |
| pH mean | 6.96 | 4.71 | 4.9 | 4.27 |
| range | 5.23–8.1 | 3.28–6 | 3.01–6.2 | 3.31–5.2 |
| | | p<0.001 | p<0.001 | p<0.001 |

p values are for differences between the untreated and each treated group. There was no significant difference between the three treatment groups.

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