Demonstration of a pH gradient across mucus adherent to rabbit gastric mucosa: evidence for a 'mucus-bicarbonate' barrier

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SUMMARY We examined the ability of the mucus layer adherent to isolated pieces of rabbit gastric mucosa to maintain a pH gradient across it. Using antimony microelectrodes, a stable pH gradient was detected from pH 2.31±0.04 on the luminal side to pH 7.26±0.15 (n=22) on the epithelial side of the mucus layer. The gradient was maintained for at least 60 minutes. A metabolic inhibitor, potassium cyanide, markedly reduced the tissues' ability to maintain this pH gradient, suggesting involvement of an active cellular process, probably that of bicarbonate secretion. These observations provide additional evidence in favour of a 'mucus-bicarbonate' barrier which may be of importance in protecting the underlying gastric mucosa.

The mechanisms by which gastric mucosa protects itself from high concentrations of intraluminal acid are uncertain. A hypothesis can be proposed which links the observations that surface epithelial cells secrete bicarbonate ions1–3 and that gastric mucus significantly retards diffusion of ions across it.4 According to this hypothesis bicarbonate secreted by the epithelium will diffuse slowly through the mucus towards the lumen, while hydrogen ions will diffuse slowly in the opposite direction creating a pH gradient across adherent mucus. This proposal of a 'mucus-bicarbonate' barrier, first postulated by Heatley,5 has never been confirmed experimentally and the aim of the present study was to examine this possibility directly in rabbit gastric mucosa.

Methods

pH was measured with antimony microelectrodes. These were prepared by the method of Cafish, Pucacco, and Carter6 and electrode tips of between 5 μ and 50 μ were prepared. The most useful size was found to be those with tip diameters of greater than 10 μ and these had a resistance of less than 106 Ω. Antimony electrodes, which develop a stable oxide (Sb2O3) at their surface in aqueous solution, develop a potential difference sensitive to hydrogen ion activity. Electrodes were calibrated using buffers over the pH range 2–8 and were found to be linear. With an 'indifferent' electrode (3M KCl in 3% agar) the slope of the relationship between pH and PD was between 55 and 61 mV per pH unit (mean 58±0.23, n=34). This is close to the theoretical value of 61 mV per pH unit at 37°C. All measurements were taken at 37°C, as the electrodes were found to be temperature sensitive. Electrodes were calibrated each day before each experiment. PD was recorded on a Servoscribe chart recorder via a Vibron electrometer. All electrical connections between electrodes, electrometer, and chart recorder were screened and kept as short as possible.

Male New Zealand white rabbits were killed by air embolus, the stomach removed, and the gastric mucosa gently washed with isotonic saline to remove food residue. Fundic and body mucosa were cut into pieces of approximately 1.5 cm² and bathed in glucose Ringer's solution bubbled with 95% oxygen 5% CO₂ at 37°C. Pieces of mucosa were mounted horizontally in small chambers with the serosal coat bathed in oxygenated glucose Ringer's solution and the mucosal surface bathed in HCl at pH 2. On average four tissue pieces were mounted from each rabbit. The tissues were kept at 37°C and the antimony microelectrode placed so that its tip was sited vertically in the acid solution immediately above the mucus layer adherent to the mucosa. The 'indifferent' electrode was placed in the mucus

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layer at the side of the tissue and the microelectrode slowly advanced through the mucus layer by a micromanipulator. The Figure illustrates the approximate relative sizes of the electrode tips, the epithelial layer, and an estimated mucus layer thickness.

A glass microelectrode filled with KCl and sensitive to PD but not to hydrogen ion activity was made with an automatic pipette puller and with a tip size of about 10 μ. With this electrode PD across the mucus was measured in a similar way to the pH measurements made with the antimony electrode. All results are given as the mean ± SEM.

Results

With the antimony electrode tip in the acid bathing the mucus the pH was 2.31 ± 0.04. Immediately adjacent to the mucus the pH rose to a mean of 2.85 ± 0.1. As the pH probe was slowly advanced through the mucus layer the pH rose to reach a maximum plateau value of between 6.1 and 8.65 with a mean of 7.26 (n = 22 tissue pairs, six rabbits). The gradient maintained across the mucus layer was thus between 3.9 and 6.45 pH units. The change in pH during passage through the mucus occurred in a smooth linear fashion and arrest of the progress of the electrode through the mucus produced a steady pH value being maintained for periods of up to an hour. In those instances where a plateau pH value of greater than 8 was achieved further, progression of the probe was followed by a fall in pH to about 7.4. Presumably the probe had damaged, and possibly entered, the mucosa at this point.

Using the glass microelectrode the PD measured across the mucus was less than 2 mV— that is, not significantly different from zero. Thus the potential difference measured by the antimony electrode could be safely attributed to the change in pH.

The pH gradient across mucus was maintained at a steady value for about an hour and then slowly deteriorated. Addition of potassium cyanide (10^{-8} M) markedly reduced the pH gradient to a mean of 2.6 ± 0.26 pH units (n = 9) within 15 minutes.

Discussion

The existence of a pH gradient across the mucus layer adherent to gastric mucosa was first postulated by Heatley. He suggested that hydrogen ions would not reach the epithelial cell surface because they were separated from it by a finite layer of alkaline mucus. Recent studies would lend support to such a concept. Thus alkaline secretion by in vitro and in vivo gastric mucus from amphibia and mammals has been demonstrated (Rees et al., unpublished observations) and this has been shown to be an active process which is stimulated by 'cytoprotective' prostaglandins and inhibited by damaging agents.

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**Figure** Diagrammatic representation of the approximate relative sizes of an electrode tip, the epithelial layer, and mucus layer. The value for mucus layer thickness is only an estimated approximation derived from the distance travelled by the electrode tip during pH determinations (see Discussion).
such as aspirin\textsuperscript{a} and bile salts.\textsuperscript{b} In addition, pig gastric mucus has been shown to delay the rate of diffusion of hydrogen ions across it,\textsuperscript{c} which would fit it well for a role in maintaining a pH gradient. In the present study this hypothesis was tested directly with antimony electrodes and it was clear that such a gradient does exist, the pH immediately adjacent to the epithelial cells being near neutral and, in many instance, alkaline. The inhibition of this gradient with the metabolic inhibitor potassium cyanide suggests that the gradient was dependent on an active process, presumably related to bicarbonate secretion.

The use of antimony electrodes has been criticised because they may be subject to variations in response over small pH ranges.\textsuperscript{10} However, in the present studies, the range of pH in which we were interested was large and the behaviour of these electrodes was efficient over this range. The size of the electrode tip used here, greater than 10 \(\mu\)m, is such that the tip was too large to enter individual epithelial cells and, if the tip did reach the epithelial layer, it was more likely to push the mucus in front of it than go into it. In those instances where the micro pH tip appeared to enter the epithelium the pH fell to a value of about 7 from the maximum value in the mucus of greater than 8. We thus believe that the pH gradient measured was present within the mucus layer rather than in the epithelium. This view was supported by the observation that a search for a pH gradient on the serosal surface of mucosa mounted upside down revealed virtually no gradient.

The thickness of the ‘mucus-bicarbonate’ layer is uncertain, but our crude indirect estimates, taken from the distance travelled by the microelectrode to reach the maximum pH value, provided a figure of 0.338 to 1.058 mm. However, these figures are likely to be overestimates, as there would be some element of pushing the mucus layer, in front of the tip, involved in their determination. In any event there does appear to be a pH gradient across the mucus lining the gastric mucosa and this could well serve a role in protecting the mucosa from intra luminal acid.

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