Mechanisms of gastric mucosal protection: a role for the ‘mucus–bicarbonate’ barrier

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Introduction
It has long been recognized that gastric mucosa is capable of resisting acid peptic digestion, but the mechanisms responsible for this have not been adequately explained. An increased secretion of acid provides only a partial explanation, at best, for peptic ulcer formation, an observation which has focused attention on the mucosal components that prevent damage, the so called ‘mucosal barrier’. The nature of this barrier has proved difficult to elucidate, but recent developments are making us re-examine long-held concepts. Davenport proposed that the barrier to ionic diffusion resided within the surface epithelium, probably in the apical membrane and tight junctions between cells [1]. Damaging agents, such as aspirin and bile salts, are held to disrupt this barrier and the subsequent increase in acid back-diffusion leads to the release of histamine and other mediators of tissue inflammation, thus initiating the process leading to mucosal damage [1-3]. Increased permeability of the mucosal layer to hydrogen ions is seen as the vital initiating component in this hypothesis.

Many modern textbooks quote this mechanism with little reservation. However, doubt has been cast on its applicability to all types of mucosal damage [4-7], and attention has turned to a search for alternative explanations for mucosal protection.

The potential barrier role played by the extracellular layer of mucus has often attracted attention. Claude Bernard pictured it as being impermeable to gastric acid. The simple experiment performed on Tom’s gastric fistula in which mucosal erosions were produced by repeatedly wiping the mucus off the mucosa supported a role for the mucus layer [8]. However, the view that mucus acted as a physical barrier fell into disfavour when it was argued that it consists of over 95% of water and could hardly be expected to delay the diffusion of hydrogen ions across it. Indeed, Heatley seemed to confirm that mucus could act only as an unstirred layer of water on the epithelium when he demonstrated that diffusion of hydrogen ions through mucus was similar to that through water [9]. Hollander attempted to rekindle interest in mucus as an important element in his two-component barrier, which he proposed, consisted of mucus and epithelial renewal [10]. However, the consensus of opinion remained against the view that there was an extracellular component to mucosal protection and it has only been with the recent development of techniques for measuring secretion of bicarbonate by gastric mucosa that the importance of mucus as a juxta-epithelial unstirred zone, in which luminal acid and epithelial alkali may mix, has been recognized. These studies have allowed the concept of a ‘mucus–bicarbonate’ barrier to be developed [11, 12].

We will consider each of the elements of this postulated barrier in turn and then summarize the evidence in favour of their combined role in mucosal protection.

Mucus
Mucus forms a continuous layer over the gastric epithelium and, according to a recent study, is about 0.5 mm thick in the human stomach [13]. If this is confirmed the mucus layer may be as...
much as 10–20 times thicker than the surface epithelial cells (20–40 μm) and about half as thick as the gastric glands are deep (1.2 mm). The concentration of glycoprotein within this secreted mucus is 30–50 mg/ml, which is above the concentration at which the glycoprotein molecules are believed to occupy the whole solution volume and most of the solvent (water) is intramolecular [14]. Thus the mucus layer could theoretically provide an unstirred zone of considerable thickness in which mixing is impaired.

Unfortunately, measurement of mucus secretion remains unsatisfactory and studies of the rate of incorporation of labelled precursors or of the appearance of mucus fragments, such as hexosamine, in gastric juice provide only an indirect measure of mucus output. Clearly the important index of protective function would be the mucus layer thickness, but this has proved remarkably difficult to measure. Nevertheless, agents which are known to cause, or prevent, acute mucosal damage do influence indices of mucus synthesis in a way that would support a role for mucus in mucosal protection. Aspirin and other non-steroidal analgesics reduce the incorporation of radio-labelled precursors into glycoproteins, inhibit the activity of biosynthetic enzymes and, in one study, the mucus layer thickness appeared to be decreased by topical application of aspirin [15–18]. Topical and parenteral administration of the E₂ prostaglandins increase the output of mucus in the stomach of several species, including man [19–22], and recent measurements showed an increase in mucus gel thickness after topical application of 16,16-dimethyl PGE₂ [13].

Certain prostaglandins have been shown to protect the gastric mucosa against experimental damage by a variety of noxious agents including non-steroidal anti-inflammatory drugs, ethanol, sodium taurocholate, hydrochloric acid (0-6 mol/l), sodium hydroxide (0-2 mol/l) and even boiling water [23]. In other experiments repeated application of low concentrations of a damaging agent prevented damage by subsequent exposure to large concentrations of the same agent. Since this ‘adaptive cytoprotection’ may be abolished by pretreatment with indomethacin it seems possible that it is mediated by endogenous prostaglandin production [23]. These observations, although not conclusive, indicate that endogenous prostaglandins may be important in regulating mucus production and that this activity may be responsible in part for their ‘cytoprotective’ properties.

An important question centres on the mechanism by which mucus may protect the mucosa. For example, can mucus delay the transfer of acid and proteolytic enzymes? It has seemed reasonable to accept the idea that large macromolecules would be delayed in their passage across the mucus either because of their physical size or because of electrostatic forces acting to impede movement through mucus. It has been difficult to imagine, however, that, since the mucus layer is predominantly water, it could delay transfer of small ions such as hydrogen ions across it. Indeed the experiments of Heatley suggested that hydrogen ions diffuse through a solution of mucus at the same rate as they do through unstirred water [9]. However, recent observations in our laboratories have indicated that the rate of transfer of hydrogen and sodium ions through mucus is delayed to a greater extent than the rate of diffusion through unstirred water, and this observation has been confirmed [24, 25]. Hydrogen ions traversed mucus at a rate three to four times slower than through a similar thickness of unstirred water. The discrepancy between these recent results and those of Heatley may be resolved by noting that the earlier experiments were performed with dilute solutions of mucus whereas the recent tests were performed with concentrations similar to those in the mucus layer on the gastric mucosa. Thus a layer of mucus could theoretically delay transfer of hydrogen ions across it both by its unstirred layer effect and by its additional delaying characteristic, presumably dependent in some way on its ability to sequester water in its interstices, rendering it unavailable for hydrogen ions.

Despite these observations the mucus layer alone is unlikely to be adequate to protect the mucosa from high luminal acid concentrations unless the layer was renewed at a rapid rate or some other factor contributed to its protective role.

Alkali secretion

The existence of a non-parietal, alkaline, gastric secretion has long been known but direct measurements of rates of alkali secretion have been feasible only during the last few years with the development of potent inhibitors of acid secretion and of accurate micro-autotitration techniques. Both fundic and antral gastric mucosa have now been shown to secrete bicarbonate, and this has renewed speculation about its role in gastric mucosal protection. The mechanisms of alkali secretion have been worked out predominantly in isolated amphibian mucosa [11, 26] but it has also been demonstrated in the rabbit antrum and fundus in vitro [27, 28] and in the guinea pig and Heidenhain pouch of the dog.
in vivo [29–31]. In addition we have recently confirmed its occurrence in normal man during acid inhibition with an \( \text{H}_2 \) antagonist [32]. In these studies the magnitude of alkalinization amounts at most to 5–10% of maximal acid output. Most experimental evidence suggests that the majority of this secretion is an active transport process inhibited by anoxia and metabolic inhibitors as well as by agents which uncouple oxidative phosphorylation [28, 33]. Carbonic anhydrase, present in high concentrations in surface epithelium [34], appears to be involved since inhibition of this enzyme with acetazolamide reduces alkali secretion [33]. It seems likely that the alkali ion is bicarbonate, probably secreted in exchange for chloride ions entering the cell from the luminal side [26] (Fig. 1). Its secretion is stimulated by cholinergic agonists via a mechanism probably involving the generation of intracellular cyclic GMP [33, 35]. \( \alpha \)-Adrenergic agonists inhibit secretion [36] and calcium on the serosal side of the epithelium stimulates secretion. The relevance of these observations to physiological control of alkali secretion is uncertain but two interesting observations suggests that the intraluminal pH may have a regulatory role. Firstly, in the canine Heidenhain pouch model acidification of the gastric remnant stimulated alkali secretion by the vagally denervated pouch [31]. Secondly, in an elegant study in which two isolated pieces of amphibian gastric mucosa were mounted in a specially designed chamber so that their serosal surfaces were bathed by a common buffer solution, acidification of the luminal surface of one mucosa induced alkali secretion in its partner [37]. These observations suggested that luminal acid causes the liberation of a humoral substance which stimulated alkali secretion. The nature of this mediator is uncertain but it is conceivable that it is a prostaglandin.

Certain prostaglandins (e.g. 16,16-dimethyl \( \text{PGE}_2 \) and \( \text{PGF}_{2\alpha} \)) stimulate alkali secretion by fundic and antral mucosa in vitro [38] and in vivo [30, 31]. Non-steroidal analgesics, which inhibit endogenous prostaglandin synthesis, inhibit bicarbonate secretion and this inhibition is prevented by pretreatment with 16,16-dimethyl \( \text{PGE}_2 \) [39, 40]. All these observations suggest the possibility that endogenous prostaglandins regulate gastric alkali secretion and implicate this action in their ‘cytoprotective’ activity.

It is clear that the amount of bicarbonate secreted is considerably smaller than the amount of acid in the lumen. It is therefore extremely unlikely that this alkali alone could protect the mucosa since it would be instantly neutralized by the much larger acid component.

‘Mucus–bicarbonate’ barrier

In isolation neither the mucus layer nor bicarbonate secretion is likely to protect effectively the gastric epithelium against damage by intraluminal contents. In combination, however, these secretions could produce an effective barrier with the mucus acting as an unstirred zone in which bicarbonate diffusing towards the lumen neutralizes the acid diffusing towards the epithelium. A gradient for hydrogen ion concentration across the mucus layer might be created in this way, as postulated by Heatley over 20 years ago [9] (Fig. 2). It seems likely that such a gradient would depend on several factors, including the rate of alkali secretion and its rate of diffusion through mucus, the rate of acid diffusion through mucus, and the thickness of the mucus layer, which is a function of its rate of renewal and loss into the lumen. It can be postulated that interference with one or more of these factors might reduce the pH gradient and interfere with the protective barrier created by these two secretions.

Recent work in our laboratories has directly demonstrated the existence of a pH gradient in the mucus layer, the pH at the epithelial surface being maintained at around 7.0 when the pH of the luminal bulk phase was 2.0. These observations have been made in rabbit fundic mucosa in vitro [41], rat fundic mucosa in vivo [42] and more recently confirmed on human fundic mucosa in vitro [43]. Furthermore, application of acetylsalicylic acid (10 mmol/l) to luminal fluid
FIG. 2. ‘Mucus–bicarbonate’ barrier. Diagrammatic representation of the gastric epithelium illustrating the interaction between hydrogen and bicarbonate ions within the mucus gel layer. Hydrogen ions diffusing from the lumen are neutralized by bicarbonate ions secreted by the surface cells, thus creating a pH gradient across the mucus interface.

considerably reduced the pH gradient across the mucus layer, probably by inhibition of bicarbonate and, possibly, mucus secretion [42]. Reduction of the mucus layer thickness, by application of N-acetylcysteine, also reduced the pH gradient. Finally, bathing the luminal surface with HCl at a pH lower than 1.4 overwhelmed the capacity of the mucosa to maintain the gradient [42]. All of these observations support the proposal that the combined ‘mucus–bicarbonate’ secretion of the surface epithelium serves as an effective extracellular barrier to luminal acid and that impairment of this barrier is liable to lead to mucosal damage. The observations place the barrier to luminal acid outside the epithelium rather than within it, although clearly its two components are entirely dependent on the metabolic integrity of the epithelium for their secretion.

**Pathophysiological considerations**

It is possible to suggest ways in which abnormalities in mucus or bicarbonate secretion can be implicated in a number of clinical circumstances. Non-steroidal anti-inflammatory drugs can cause acute erosions and have been implicated in the pathogenesis of chronic gastric ulceration when they are taken in large doses over prolonged periods [44]. That these drugs inhibit alkali and mucus secretion and reduce the pH gradient across the mucus layer may be relevant to these observations.

Ethanol is known to cause acute and chronic gastric mucosal damage and it too has been shown to interfere with each of the components of this barrier. It inhibits the incorporation of labelled N-acetylglucosamine into glycoprotein [17, 45] and decreases alkali secretion by amphibian gastric mucosa [26].

Severe stress frequently produces acute erosions or ulceration in animals and man and recent studies have implicated abnormalities in mucus and bicarbonate secretion in the pathogenesis of such lesions. Stress has a biphasic effect on mucus biosynthesis, glycoprotein output being reduced immediately after the onset of stress and subsequently increasing to a peak at days 3–6 [45]. This response may be due to an initial fall in mucus secretion followed by mucosal damage in which increased exfoliation of the mucus-containing surface cells occurs. The effect of stress on bicarbonate secretion has not been examined but it may be relevant that αadrenergic agonists inhibit bicarbonate secretion in amphibian gastric mucosa [36].

Refux of bile into the stomach has been held by some to be important in the pathogenesis of gastric ulceration [46] and animal studies have shown that bile salts alter the properties of mucus gel [47] and inhibit alkali secretion in concentrations commonly found in the gastric contents of patients with gastric ulcers [28, 48]. Furthermore, sodium taurocholate reduced the pH gradient across the mucus layer on rat fundic mucosa in vivo [42]. However, much of this evidence is inferential and further information must be obtained in patients with gastric ulcers before such a defect can be implicated in the pathogenesis of this condition.

Although abnormalities in the ‘mucus–bicarbonate’ barrier may be involved in the production of diffuse mucosal disease, such as acute and chronic gastritis, additional factors are
required to explain the pathogenesis of localized lesions such as acute and chronic gastric ulcers. The nature of possible local factors is uncertain but it is conceivable that a combination of some focal disturbance with a diffuse abnormality in the ‘barrier’, as may occur in chronic gastritis, could lead to local ulceration.

There are some implications for therapy in the mucus–bicarbonate hypothesis, since it may be possible to develop agents which might strengthen the barrier. A more rational approach to peptic ulcer therapy than antacids and antisecretory agents might follow. Since carbenoxolone has been shown to heal gastric ulcers interest has been displayed in its mechanism of action. This compound apparently increases mucus secretion [17, 49] but not alkaline secretion [50]. The observations that some prostaglandins are ‘cytoprotective’ and stimulate both mucus and bicarbonate secretion might follow. Since carbenoxolone has been shown to heal gastric ulcers interest has been displayed in its mechanism of action. This compound apparently increases mucus secretion [17, 49] but not alkaline secretion [50]. The observations that some prostaglandins are ‘cytoprotective’ and stimulate both mucus and bicarbonate secretion might follow. Since carbenoxolone has been shown to heal gastric ulcers interest has been displayed in its mechanism of action. This compound apparently increases mucus secretion [17, 49] but not alkaline secretion [50].

The recent demonstrations that the mucosa of the first part of the duodenum, in several species of experimental animal, also secretes bicarbonate by an active secretory process [52, 53] have extended the concept of mucosal protection by alkaline to this important region of the gut. The potential dividends from future studies of this process are considerable.

References

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