

Progress report

Mucus and bicarbonate secretion in the stomach and their possible role in mucosal protection

How the gastric mucosa is able to withstand intraluminal acid, which can attain concentrations approaching 155 mM H^+ under conditions of maximal stimulation, remains an unanswered question. The concept of the gastric mucosa as a limiting barrier to ion diffusion developed from an appreciation that the concentration of electrolytes in the stomach varies with the rate of secretion. Thus gastric juice has a high Na^+ and a low H^+ concentration at low secretory rates, while at high secretory rates the reverse is found.¹ In addition it is well known that a reduction in H^+ and gain in Na^+ occurs after instillation of exogenous acid into the ligated stomach or gastric pouch.^{2,3} Teorell⁴ considered the gastric mucosa as a diffusion barrier and described events at the mucosal surface in terms of an exchange diffusion between H^+ in the lumen and Na^+ in the mucosa. In contrast, Hollander⁵ proposed a two component barrier consisting of the mucus layer lining the gastric mucosa together with the subjacent layer of epithelial cells and suggested that the reduction in H^+ concentration resulted from dilution and neutralisation by a Na^+ containing non-acid secretion or leakage of interstitial fluid.

The gastric mucosal barrier was systematically studied by Davenport,^{6,7} who considered it to be formed by the apical membrane of the surface epithelial cells together with the tight junctions linking adjacent cells. Competent tight junctions prevent diffusion of the gastric contents into the mucosa with loss of H^+ from the lumen and entry of Na^+ limited by this ion barrier. Numerous compounds which damage the stomach, including salicylates, alcohol, and bile salts, increase the permeability of the barrier inducing diffusion of acid into the mucosa with subsequent development of haemorrhage and mucosal erosion.^{8,7} Whether or not back-diffusion of acid is a normal physiological process is uncertain and, indeed, a recent review of the question concluded that there is no direct evidence that the disappearance of H^+ from the lumen of the stomach is caused by back-diffusion.⁹

Whereas Davenport ascribed to mucus the chief function of lubrication, Heatley¹⁰ suggested that mucus played a major role in protecting the gastric epithelium by providing an unstirred layer on the surface of the mucosa. He proposed that H^+ diffusing in from the gastric lumen is neutralised by HCO_3^- secreted from the mucosa. Thus the mucus gel could provide an unstirred layer which maintains HCO_3^- at the mucosal surface and prevents it from mixing with bulk HCl in the lumen of the stomach. Until now there has been a lack of appropriate data to enable assessment of these hypotheses on the nature of mucosal protection. However, recent work on two aspects of the mucosal barrier—namely, the structure of the mucus gel^{11,12,13} and the demonstration of an active HCO_3^- secretion by surface epithelial cells^{14,15,16}—has provided important new insights at the molecular

level into how these two secretions could function in mucosal protection. Taken in isolation, mucus and HCO_3^- secretions would be of limited effect against the luminal HCl but, as will become evident, when considered as a single system they could provide an effective means of protection.

In this report, we present our understanding of (1) the structure and properties of the mucus gel and (2) the mechanism of HCO_3^- secretion. In a third section, which of necessity is speculative, we discuss the possible role of mucus and HCO_3^- as a physiological mechanism for protection of the mucosal surface from acid and their possible implication in the pathogenesis of gastric mucosal damage.

Mucus structure and properties

Mucus is secreted to form a flexible gel adhering to the surface of the gastric mucosa. To understand how this is achieved it is necessary to know the structure of the gel. The molecules on which gel formation depends are glycoproteins, and these can be readily obtained in a soluble form by proteolysis^{17 18 19} or by reduction using thiol reagents.^{20 21} These techniques are successful because they break covalent peptide and disulphide bonds respectively within the gel matrix and produce degraded glycoproteins which are devoid of the gel-forming and viscous properties of the original secretion.^{22 23} However, it is possible to solubilise the gel to obtain undegraded glycoprotein by mild stirring or homogenisation in 0.2 M NaCl, and, by the latter method, all the glycoprotein in the gel is solubilised.²⁴ The resulting undegraded glycoprotein can then be separated from the contaminating non-covalently bound protein by gel-filtration; the very large molecular weight glycoprotein is excluded, whereas the lower molecular weight protein is retained.^{25 26} By following this procedure for pig gastric mucus, an undegraded glycoprotein is obtained which accounts for over 95% of the total glycosubstance in the gel and which possesses the viscous and gel-forming properties of the parent mucus secretion.^{11 12 13} This glycoprotein is still not pure, containing between 5–10% by weight of non-covalently bound extraneous protein, which is excluded with it on gel-filtration.²⁷ To enable accurate analysis of the glycoprotein, particularly with respect to its protein core, it is important that remaining free protein be completely removed. This can be

Table Comparison of glycoprotein preparations from pig gastric mucus

	Glycoprotein from gel (undegraded)	Proteolysis —pepsin etc.—→	Glycoprotein after proteolysis (degraded)
Carbohydrate content	81.5% by wt. galactose 33.5: N-acetylglucosamine 31.4: N-acetylgalactosamine 11.8: fucose 21.2: N-acetylneuraminic acid 2.1* ester sulphate 3.1% by wt. A and H blood group activity		84.8% by wt. Ratio of sugars and ester sulphate same as undegraded glycoprotein A and H blood group activity
Protein	12.9% by wt. thr 18.1 ser 15.8 pro 15.3†		9.6% by wt. thr 26.3 ser 24.0 pro 18.5
Mol. wt.	2×10^6 mercaptoethanol ↓ 5×10^5		5×10^5 mercaptoethanol ↓ no change

*mol/100 mol carbohydrate.

†mol/100 mol protein.

achieved by equilibrium centrifugation in a caesium chloride gradient which cleanly separates the lighter protein from the heavier glycoprotein on the basis of their different densities. Analysis of purified pig gastric mucus glycoprotein before and after proteolytic digestion is shown in the Table.

An important consequence of the analysis of purified glycoproteins from gastric mucus is an appreciation, in structural terms, of how the viscous and gel-forming properties of the undegraded glycoprotein from the mucus gel are lost on proteolytic digestion. Reference to the amino acid analysis shown in the Table indicates that about 25% more protein is present in the core of the undegraded glycoprotein than the proteolytically digested material, whereas the carbohydrate content, after allowing for changes in the total weight of the molecule due to loss of protein, is the same. This is explained by the fact that the protein core of the undegraded glycoprotein consists of two distinct parts, a glycosylated region where the carbohydrate chains are attached and a non-glycosylated region free of carbohydrate chains.^{13 22} The glycosylated region is rich in the amino acid residues, serine, threonine, and proline, and attached to the first two of these residues are large branched carbohydrate chains with an average of 15 sugar residues per chain.^{18 28 29} These chains are closely packed along the length of the core, forming a sheath of polysaccharide which protects it from proteolytic attack. In contrast, the non-glycosylated region of the protein core has an amino acid content more typical of a globular protein and is free of carbohydrate side chains, with the result that it is accessible to proteolytic digestion. It is this part of the core that is absent in the glycoprotein after proteolytic digestion—for example, with pepsin or trypsin (Table). However, the most striking difference between these two preparations is their molecular weight; 2×10^6 for undegraded glycoprotein compared with 5×10^5 for material obtained by proteolysis.²² Reduction with mercaptoethanol also splits the undegraded glycoprotein into four subunits of 5×10^5 molecular weight but has no effect on the glycoprotein obtained after proteolysis.²³

A diagrammatic representation of these findings is given in Fig. 1. The undegraded glycoprotein isolated directly from the gel is a polymer of four subunits, each of molecular weight 5×10^5 and joined by disulphide bridges linking their protein cores.^{13 23} The glycosylated region, which amounts to three-quarters of the protein of each subunit, is covered with a thick sheath of closely packed polysaccharide chains having an average of 15 sugar residues per chain and 160 chains per glycoprotein subunit. The other quarter of the protein core is non-glycosylated but contains the cysteine residues which form the disulphide bridges holding the subunits together.²² Proteolysis of this non-glycosylated region—for example, with pepsin—or reduction of the disulphide bridges will split the undegraded glycoprotein into its four subunits.

The polymeric structure above refers to the glycoprotein from pig gastric mucus where material is readily available. However, we have recently isolated the purified undegraded glycoprotein from human gastric mucus gel using the same methods.^{30 31} Human mucus glycoprotein has a molecular weight of 2×10^6 and is split into subunits in the order of 5×10^5 by proteolytic enzymes or mercaptoethanol. While detailed chemical analysis of the isolated glycoprotein has yet to be performed, these results in man demonstrate that the glycoprotein in the mucus gel also has a polymeric structure. Proteolysis thus solubilises the gel to produce degraded glycoprotein subunits in the lumen.

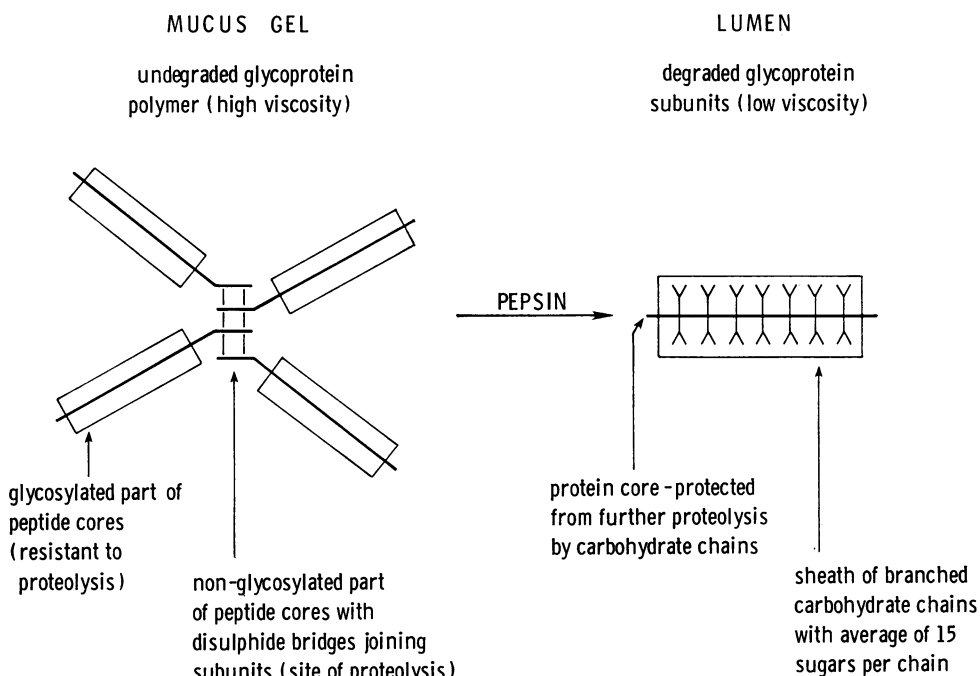


Fig. 1 Peptic hydrolysis of gastric mucus glycoprotein. A diagrammatic representation adapted from Allen (1978).¹³

It has already been established that the complex carbohydrate side chains of the glycoproteins from human and pig gastric mucus are very similar, if not the same,^{18 32} with both carrying the determinants for A and H blood group substance activity.^{28 29} Now it is clear that this close structural similarity extends to the polymeric structure of the undegraded glycoprotein forming the gel and, consequently, the biochemical and biophysical properties of pig gastric mucus can be applied to interpreting those of human gastric mucus.

To enable gel formation, the undegraded glycoprotein must exceed a threshold concentration of between 30–50 mg per ml both *in vivo* and *in vitro*.^{12 13} Preceding gel formation *in vitro*, the viscosity of the solution gradually increases with increasing glycoprotein concentration with a precipitous rise occurring when the concentration exceeds 20 mg per ml. Data from viscosity and sedimentation studies using the ultracentrifuge show that, in solution, the glycoprotein is highly hydrated with an expanded structure such that 1 g glycoprotein will occupy a solution volume of 40 ml compared with a volume of less than 1 ml occupied by 1 g of an average globular protein. At a concentration of about 20 mg per ml the expanded glycoprotein molecules are completely filling the solution and as the concentration increases further so their molecular domains overlap, the intermolecular non-covalent interactions increase, and the solution becomes increasingly viscous until it reaches the consistency of the mucus gel.¹²

Following on from this model of gel structure, where the glycoprotein molecules are permeating the whole of the matrix, it can now be appreciated that mucus gel provides an excellent unstirred layer retarding any mixing of the ions within its interstices with those in the bulk phase of the gastric

lumen. At the same time, the concentration of glycoprotein in mucus is still less than 5% of the total weight of the gel and it would seem unlikely to act as a physical barrier to the diffusion of small ions. Certainly ions such as H^+ and HCO_3^- can diffuse through the mucus gel,¹⁰ although at a slower rate than through an equivalent volume of solution.³³ Whether their rate of diffusion is the same as that for an equivalent volume of *unstirred* solution is difficult to measure because of the problems of creating such a layer of comparable thickness in the absence of mucus. This is further complicated by the charge distribution within the mucus gel, which may also influence the diffusion of ions. Once the threshold glycoprotein concentration for gel-formation has been reached, further increases in glycoprotein concentration will result in a still thicker gel. However, it would follow from the above, where 95% of the gel is composed of water, that a considerably thicker gel would be required before the passage of small ions through its matrix was prevented completely.

If the glycoprotein is to be capable of gel-formation it must be in the polymeric form of the undegraded glycoprotein (mol. wt. 2×10^6): in other words, the subunits alone will not form a gel at anything approaching the concentrations of glycoprotein found *in vivo* in the mucus.^{11, 13} The mucolytic action of proteolytic enzymes and thiol reducing agents is due to splitting of covalent bonds within the glycoprotein structure to produce subunits (mol. wt. 5×10^5). Therefore the polymeric configuration of four glycoprotein subunits joined together by disulphide bridges is an essential prerequisite to enable formation of the gel. Studies with both pig and human gastric mucus^{20, 31} show that pepsin will break down this polymeric structure and thus, *in vivo*, pepsin continuously erodes the gel, adhering to the surface, producing the soluble degraded glycoprotein subunits found in the lumen of the stomach.

The precise role of the other structural features of the glycoprotein in gel-formation is uncertain. This is particularly the case with the large carbohydrate chains which have been the subject of detailed analysis in mucus glycoproteins from a variety of sources including those from pathological conditions.^{34, 35} Clearly the carbohydrate chains will interact with water and, as they comprise over 80% by weight of the glycoprotein, their presence is compatible with the high degree of hydration which contributes to the special rheological properties of the molecule.¹² At the same time major changes can occur in the structure of the carbohydrate chains without apparently affecting gel-formation by the mucus secretion.¹³ Also, changes in conformation which result in contraction or expansion of the glycoprotein molecules in solution might be expected to alter the threshold concentrations for gel-formation.^{12, 13} The sharp increase in viscosity of mucus secretions at low salt concentrations (less than 10 mM) can be explained by expansion of the glycoprotein molecules.^{12, 36} So far studies with pig gastric mucus have brought to light no such factors which might act under physiological conditions and, importantly, the viscosity of the isolated glycoprotein is unaffected over a pH range between 1–8. It should be noted, however, that visual observations do suggest that changes in pH may affect the nature of the mucus gel *in vivo*.^{35, 37}

A clear and accepted role for gastric mucus is that of a lubricant protecting the underlying mucosal cells from mechanical abrasion.^{5, 38} Another role discussed in this article is that of providing a mixing barrier for the containment of HCO_3^- secretion and restriction of H^+ access to the mucosal surface.

In either of these cases the effectiveness of the surface mucus gel will depend on its depth. The layer of mucus gel is eroded because of the action of pepsin^{13 22} as well as by mechanical factors such as food. Therefore secretion of the glycoprotein will not only need to be at a sufficient concentration to exceed the threshold for gel-formation but also at a rate that will maintain an effective depth of gel on the surface despite erosion. Evidence for changes in the thickness of the surface gel under hormonal or neural influence and in pathological conditions has yet to be obtained. Several studies, using human gastric aspirates or experimental animals with gastric fistulae, have shown increases in luminal glycoprotein with different stimuli,^{34 39 40} for example, secretin^{40 41} and prostaglandins.^{42 43} However, increases in the amount of luminal mucus glycoprotein cannot necessarily be interpreted as an increase in secretion of mucus gel, as such a rise could be due solely to increased erosion by pepsin or other factors.⁴⁴ In the absence of a concomitant stimulation of secretion this would, in fact, result in a thinner layer of gel on the mucosal surface. A further problem arises in that many of the techniques used to measure mucus, including colorimetric estimation and viscosity measurement, are subject to considerable interference by contaminants, particularly protein, and in no case has the glycoprotein been purified.⁴⁴ Although the interpretation of such reported stimulatory effects on mucus secretion is uncertain, the inhibition of mucus biosynthesis by ulcerogenic agents such as non-steroidal anti-inflammatory drugs is well documented with *in vivo* studies⁴⁵ supported by *in vitro* experiments⁴⁶ on the isolated mucus glycoproteins. The mucus barrier is permeable to salicylates and such drugs accumulate in mucosa attaining concentrations in excess of those required to inhibit oxidative metabolism.⁴⁷ Thus, in addition to their other actions on the gastric mucosa, salicylates upset the dynamic balance between mucus secretion and erosion in favour of the latter and a thinner and therefore less effective mucus gel will result.

Bicarbonate secretion

A limited number of previous studies have demonstrated that, in the absence of acid secretion, HCO_3^- is present in gastric juice. For example, secretions from canine antral pouches contain about 8 mM HCO_3^- ⁴⁸ and a similar concentration occurs in vagotomised fundic pouch secretions in antrectomised animals.⁵ The presence of free HCO_3^- in human gastric juice aspirated under resting conditions has also been reported,^{49 50} but in other cases, data relating to composition of alkaline gastric secretion has been deduced indirectly from experiments in which net acid output was measured.^{51 52} It has generally been considered that the presence of HCO_3^- in gastric juice arises by diffusion from the blood and mucosal interstitium,⁴⁹ and this ion is readily demonstrable in the lumen when there is an increase in passive ion permeability after damage to the gastric mucosa⁷ or after raising mucosal interstitial fluid pressure.⁵³ Recent work has now provided evidence for an active, receptor mediated, HCO_3^- secretion by the gastric mucosa. Much of these data have been obtained from *in vitro* experiments using amphibian mucosa, although a number of *in vivo* mammalian studies have also been reported.

Inhibition of H^+ secretion and appearance of HCO_3^- in the lumen which accompanied intravenous administration of sodium thiocyanate in the cat

may result from SCN^- -induced leakage of HCO_3^- .¹ An alternative explanation of this finding, on the basis of the known inhibitory action of SCN^- on H^+ transport, is that it unmasks a simultaneous but quantitatively smaller HCO_3^- secretion. This explanation is supported experimentally by the observation that an alkalinisation of the luminal side of amphibian isolated fundic mucosa occurs in the absence of any increase in the passive conductance of the membrane after exposure of the tissue to SCN^- .¹⁴ The presence of HCO_3^- in human gastric juice after instillation of glycine buffer⁵⁴ and in achlohydric patients⁵⁵ also seems to support the proposal that alkaline secretion is normally masked by a higher acid output. Much of the controversy relating to the presence of a gastric alkaline secretion may therefore reflect the problem of measuring HCO_3^- in the presence of a higher rate of H^+ secretion.

Two main approaches have been used in the measurement of gastric HCO_3^- secretion—namely, pH-stat titration of net alkalinisation by antral and non-acid secreting fundic mucosa,¹⁵ or intragastric measurement of pH and pCO_2 .¹⁶ For *in vitro* experiments, amphibian mucosa is generally preferred due to the problems of providing sufficient oxygenation of the thicker mammalian tissue.⁵⁶ After removal of external muscle layers, the mucosa is mounted as a membrane between the two halves of a flux chamber. The serosal solution is buffered (pH 7.20) and the unbuffered luminal bathing solution maintained at pH 7.40 by continuous infusion of HCl, thereby enabling the rate of alkaline secretion to be determined. Unlike the isolated mucosal preparation, which can be adequately ventilated in order to remove CO_2 , intragastric titration of HCO_3^- with HCl *in vivo* can give rise to a number of potential problems, including CO_2 formation which will acidify the solution and necessitate use of a lowered (less than pH 7) endpoint. In some respects, this situation is comparable with determination of acid secretion by intragastric titration with NaHCO_3 and these limitations have been discussed previously.⁵⁷ However, titration with HCl at an endpoint of pH 6.0 has recently been reported for the measurement of net HCO_3^- secretion in canine Heidenhain pouch perfusates after inhibition of H^+ secretion by infusion of the histamine H_2 -receptor antagonist, cimetidine.⁵⁸ Measurement of pH and pCO_2 provides an alternative approach and also enables simultaneous determination of total acid and HCO_3^- output determined by applying the Henderson-Hasselbach equation.¹⁶ Neither of these methods is entirely satisfactory and much of the quantification and characterisation of gastric HCO_3^- transport has been performed using the isolated mucosal preparation.

Amphibian antral mucosa obtained from *Necturus* shows stable PD, electrical resistance and short-circuit current, and displays an active electrogenic Na^+ transport from mucosal to serosal side and an opposite but non-electrogenic Cl^- transport.⁵⁹ This tissue secretes alkali spontaneously at a steady basal rate with a mean value of 0.35 $\mu\text{eq per cm}^2$ per h,⁵⁹ A similar rate of alkaline secretion, amounting to 5–10% of maximal H^+ secretory rate, occurs in fundic mucosa from a variety of amphibia after specific inhibition of spontaneous acid secretion with SCN^- or the H_2 -receptor antagonists. In the isolated amphibian antrum⁵⁹ and also in the mammalian antrum *in vitro*,⁶⁰ active secretion accounts for about 60% of HCO_3^- appearance in the luminal solution, the remainder arising from passive permeation. In contrast, active transport alone accounts for alkaline secretion in fundic mucosa.¹⁵ Transport of HCO_3^- is unaffected by agents which influence H^+ secretion, including

inhibitors such as SCN^- and H_2 -antagonists and stimulants such as histamine, gastrin and db-cAMP.⁶¹ The rate of HCO_3^- secretion is reduced or abolished by metabolic inhibitors (CN^- and dinitrophenol), anoxia (gassing with N_2), alpha adrenergic agonists and the carbonic anhydrase inhibitor acetazolamide.^{15 59 61} Secretion is stimulated by carbachol, db-cGMP, prostaglandins and by raising the external Ca^{++} concentration in the serosal side bathing solution from 1.8 to 7.2 mM.^{15 61 62} Furthermore, the actions of carbachol (stimulation) and noradrenaline (inhibition) are attenuated by atropine and phentolamine respectively. This sensitivity to inhibitors and stimulants indicates that HCO_3^- transport by the isolated mucosa is an active, receptor mediated process which involves a different stimulatory pathway from that controlling acid secretion.

Measurement of HCO_3^- output in the guinea-pig stomach *in vivo* has shown the presence of a constant basal rate of secretion of between 30 and 40 $\mu\text{eq per h}$.¹⁶ In spontaneously secreting preparations, the greater acid output converts HCO_3^- into CO_2 . A net alkaline secretion (total $\text{HCO}_3^- >$ total H^+) with the presence of free HCO_3^- in the lumen occurs after inhibition of acid secretion with H_2 -receptor antagonists. As found in the isolated mucosa, HCO_3^- secretion *in vivo* is stimulated by Ca^{2+} and carbachol, an action which is inhibited by atropine but is unaffected by H_2 -receptor antagonists.¹⁶ Cholinergic stimulation has also been demonstrated to induce an alkaline secretion by canine gastric mucosa and this response is accompanied by an increase in fundic and antral mucosal cyclic GMP levels.⁶³ The rate of HCO_3^- secretion by guinea-pig stomach is quantitatively sufficient to account for the continuous loss of H^+ ions from the gastric lumen reported previously.^{1 49} Removal of H^+ ions as a result of neutralisation could also account for the observation that a reduction in osmolarity occurs after intragastric instillation of isotonic HCl .⁶⁴ In the guinea-pig, it was suggested that HCO_3^- secretion is coupled to Na^+ co-ion as stimulation of HCO_3^- transport by carbachol was accompanied by an equivalent increase in Na^+ output. The net result of HCO_3^- and Na^+ secretion with subsequent neutralisation of acid would thus appear as an interdiffusion of H^+ and Na^+ , thereby providing an explanation which could account for the earlier hypotheses of both Teorell⁴ and Hollander.⁵

The surface epithelial cells of the gastric mucosa are responsible for mucus secretion and it is likely that these cells are also the major site of HCO_3^- secretion. Apart from some endocrine cells, *Necturus* antral mucosa, which secretes HCO_3^- , but not H^+ , is composed principally of surface epithelial cells. There is a close morphological similarity between this cell type in fundic and antral mucosa and the properties of HCO_3^- transport in tissues from these two regions of the amphibian stomach are almost identical.^{15 59} Furthermore, high concentrations of carbonic anhydrase and cGMP diesterase are present in the surface epithelial cells of gastric mucosa.^{65 66} In the isolated mucosa, the action of acetazolamide displays an asymmetric effect, inhibiting HCO_3^- secretion at a concentration of 10^{-4} M if applied to the secretory side of the membrane, whereas 10^{-2} M is required to produce the same effect when applied to the nutrient side.¹⁵ Mucus secretion is considered to be vesicular but whether HCO_3^- originates from these same vesicles or is a function of the luminal cytoplasmic membrane remains an intriguing question. Recent data obtained in isolated fundic mucosa have shown a marked reduction in HCO_3^- secretion in Cl^- free luminal side bathing solution,⁶⁷

suggesting that HCO_3^- may cross the apical membrane in exchange for Cl^- . An alternative model⁶⁸ proposes a $\text{HCO}_3^-/\text{Cl}^-$ exchange on the nutrient membrane of surface epithelial cells which provides the interior of these cells with HCO_3^- for intracellular neutralisation of H^+ diffusing from the luminal solution.

Mucosal protection

Consideration of the structure and properties of the mucus gel indicates that alone it is likely to provide little direct protection of the epithelial surface from acid, but does suggest its potential suitability as an unstirred layer for the surface neutralisation of H^+ ions by HCO_3^- . The rate of gastric HCO_3^- secretion in the guinea-pig amounts to about 5–10% of histamine stimulated (maximal) acid output. In these circumstances, therefore, only about one-twentieth of secreted acid can be neutralised by HCO_3^- . If this relatively small amount of alkali is to be sufficient to prevent acid reaching the mucosal cells then certain properties of the mucus gel are required. By providing an unstirred layer, the mucus gel would confine reaction between secreted HCO_3^- and H^+ entering the gel such that a pH gradient will occur from a low value on the luminal side to a pH approaching neutrality on the mucosal side (Fig. 2). For this to succeed, HCO_3^- must be secreted at a molarity approximately equal to that of H^+ entering the gel. The previously reported concentrations of HCO_3^- measured *in vivo*^{49 50} will have been diluted with fluid from the lumen and mucus gel and it is reasonable to assume that HCO_3^- is

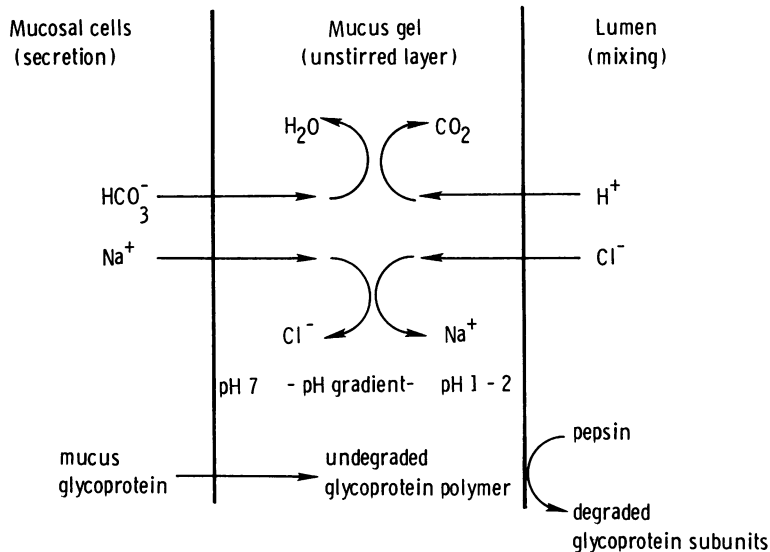


Fig. 2 A model for surface neutralisation within the unstirred layer of the gastric mucus gel. Reaction between H^+ diffusing into the gel from the lumen of the stomach and HCO_3^- secreted by the surface epithelial cells results in the formation of CO_2 and water. In the acid secreting stomach, HCO_3^- appears in the lumen as CO_2 and at high secretory rates some CO_2 could be reabsorbed and utilised by the parietal cells. Excess water may also diffuse into the mucosa. In the non-acid secreting stomach, the gel will become saturated with HCO_3^- and it will appear in the lumen in the form of the free ion.

actually secreted at a higher concentration. A high concentration of secreted HCO_3^- would be maintained by remaining within the relatively small volume of the unstirred layer. Also flux of H^+ across the unstirred layer must equal the HCO_3^- flux from the mucosal side. Calculations suggest that free diffusion of H^+ in an unstirred layer of solution is still a rapid process but whether the special properties of mucus further retard the passage of H^+ awaits experimental investigation. If H^+ passes through mucus at a relatively rapid rate, as might be expected for a purely unstirred solution, then the mucus layer and HCO_3^- would effectively resist a high luminal acid concentration for only a limited period of time.

In practice several factors will result in a more favourable balance of alkali to acid than 1:20. For instance, while acid output refers to conditions of maximal stimulation, that for HCO_3^- secretion is for basal output and recent evidence obtained with Heidenhain pouched dogs has shown that 10 mM HCl stimulates HCO_3^- output by a factor of 2.5 (A Garner and B C Hurst, unpublished observations). Further, during periods of non-acid secretion, a continuous basal output of HCO_3^- will saturate the mucus gel. A large amount of acid secreted in response to food will be buffered in the lumen of the stomach. Mucus itself could have a limited buffering capacity and acid is continuously removed as a result of gastric emptying. High intraluminal acid concentrations can occur, however, in the absence of food—for example, during histamine stimulation—without apparent damage to the mucosa, at least in the long term. Although, if such high concentrations of acid are present in the lumen of the stomach for prolonged periods and gastric emptying prevented, then ulceration will result as, for example, in the Shay ulcer model.

The depth of the mucus gel is an important consideration in terms of mucosal protection, as it must be sufficient to restrict interaction of luminal H^+ with secreted HCO_3^- to ensure neutralisation; a thicker gel would presumably increase this effectiveness. However, there will be a minimum depth of gel below which this interaction will not be contained, luminal acid entering the gel will overwhelm the HCO_3^- , complete neutralisation will not occur, and a fall of pH at the luminal membrane of the surface epithelial cells will result. If prolonged, this would lead to cell damage and the formation of mucosal erosions. Further verification of this model will have to await a more accurate knowledge of the rate of interaction of H^+ and HCO_3^- within the gel matrix and definitive measurement of the thickness of the mucus layer *in vivo* under different conditions. Recent experiments using the rabbit isolated gastric mucosa have demonstrated that a pH gradient across the gastric mucus layer can occur. By using pH-sensitive microelectrodes, mean pH on the epithelial side of the mucus layer was found to be 7.59 when the luminal pH was 2.36.³³

From the foregoing discussion, compounds that either inhibit or stimulate mucus or HCO_3^- production might be expected to have ulcerogenic or anti-ulcer properties respectively. The effects of different agents on HCO_3^- secretion is clearer than for their effects on the mucus gel. For example, while a wide variety of agents have been reported to change luminal mucus glycoprotein levels^{39,40} it is not possible to define whether this represents changes in the rate of secretion and/or erosion of the surface mucus gel. It is well known that salicylates damage the gastric mucosa both in man and experimental animals. In low concentrations, aspirin inhibits mucus biosynthesis^{45,46} and

reduces HCO_3^- transport in antral and fundic mucosa *in vitro* and in the guinea pig *in vivo*.⁴⁷⁻⁶⁹ At higher concentrations of aspirin, there is an increase in mucosal permeability and leakage of HCO_3^- into the lumen of the stomach.⁷⁻⁴⁷ Other non-steroidal anti-inflammatory agents known to damage the gastric mucosa, including indomethacin and fenclofenac,⁷⁰⁻⁷¹ also inhibit active HCO_3^- secretion.⁷² Acetazolamide, which inhibits antral and fundic HCO_3^- transport *in vitro*, damages the surface epithelium and decreases the ability of the mucosa to resist intraluminal acid.⁷³⁻⁷⁴ The bile salt, sodium taurocholate, which is known to be ulcerogenic, inhibits HCO_3^- secretion *in vitro*.⁷⁵ Finally, alpha adrenergic agonists reduce alkaline secretion in the isolated gastric mucosa,⁶¹ and this suggests a possible role in the pathogenesis of stress ulceration.

The ability of prostaglandins to inhibit ulceration in laboratory models was originally noted by Robert.⁷⁶ Inhibition of ulcer formation in the presence of exogenous acid or at prostaglandin doses below their antisecretory threshold and by prostaglandins devoid of antisecretory activity suggests that their anti-ulcer activity is mediated by a mechanism unrelated to inhibition of acid secretion.⁷⁷⁻⁷⁹ Some prostaglandins, including the synthetic analogue 16,16 dimethyl-PGE₂, have been reported to increase HCO_3^- secretion in amphibian isolated gastric mucosa and in the dog stomach *in vivo*.^{58-62, 80} This agent also prevents the inhibitory action of indomethacin on HCO_3^- transport *in vitro*.⁷² It is thus possible that some of the hitherto unexplained anti-ulcer actions of these agents are mediated *via* stimulation of HCO_3^- secretion.⁶² E-type prostaglandins have been reported to stimulate the production of soluble mucus but not mucus gel in the rat stomach and to increase the levels of bound sialic acid, a sugar characteristic of glycoprotein, in human gastric washouts,⁴²⁻⁴³ although it is not easy to relate the latter to changes in the surface mucus gel. On the basis of histological evidence and incorporation of radioactivity into the sugars of the total gastric mucosa, the anti-ulcer agent, carbenoxolone has also been reported to increase mucus production.⁸¹⁻⁸²

Protection of the gastric mucosal surface against acid and peptic digestion is a complex problem involving a balance between aggressive and defensive factors. The structure and physiochemical properties of mucus when considered together with the active secretion of HCO_3^- could provide the first-line defence. Other factors are clearly important. The rapidly regenerating, subjacent, epithelial cell layer would be a second line of defence. A first stage in this latter process would be the rupture of the surface membrane and explosive release of mucus as recently observed in a morphological study of the dog gastric mucosa.⁸³ As a consequence of this, large scale loss of surface membrane would be a major step in breakdown of the gastric mucosal barrier.⁸⁴ The special resistance of certain cell membranes is exemplified by the cells of the gastric glands which are not covered by mucus. Here HCl production is a membrane mediated process, although it is not known how their membranes withstand an environment of such low pH.

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