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Breakdown of the mucus layer by *H. pylori*

J. SAROSIEK, Z. NAMIOT, B. J. MARSHALL, D. A. PEURA, R. L. GUERRANT, D. HARLOW and R. W. McCALLUM

INTRODUCTION

Mucosal integrity in the gastrointestinal (GI) tract is preserved due to an equilibrium between exogenous and endogenous aggressive factors and protective mechanisms operating within pre-epithelial, epithelial and post-epithelial compartments. Since most aggressive factors operate within the lumen of the gastrointestinal tract, pre-epithelial defence seems to be a vanguard of mucosal protection and the target absorbing the major impetus of aggressive factors. The mucus layer, because of its ability to maintain a dynamic equilibrium between the rate of *de novo* synthesis and secretion, and luminal degradation due to proteolytic cleavage, is considered as a core component of pre-epithelial mucosal defence. Therefore, measurement of mucus components within gastric juice may provide direct information regarding the current status of the mucus layer, since it reflects the net results of mucosal secretory potential and degradative potency of luminal aggressive factors. Furthermore, the measurement of physical properties of the gastric juice may provide valuable information regarding the integrity of the pre-epithelial barrier.

BARRIER FUNCTION OF MUCUS

The GI tract mucosa is covered by an approximately $162 \pm 45\,\mu$m thick mucus layer[1] which provides a pH gradient that ensures the neutral pH at the luminal domain of the surface epithelium. In addition, the mucus layer remains a complete barrier for larger molecules such as pepsin, non-diffusible through the mucin polymer, whereas it maintains a concentration gradient for small molecules such as hydrogen ion and bicarbonate which diffuse at various rates through its unstirred layer[2–4]. Although the generation of a barrier for various chemical molecules seems to be a primary goal of the

mucus layer, it may also absorb major physical forces generated during grinding of food particles and subsequent aborad passage within the lumen.

It is generally believed that the mucus layer provides a unique biological niche for colonization by *Helicobacter pylori*, one of the most enigmatic microorganisms within the alimentary tract. *H. pylori* elaborates various factors that give it an advantage over other potential competing microorganisms and significantly benefit its own survival, multiplication and transmission. This Chapter reviews protective aspects of some of the biochemical and physical properties of the gastric mucus and the damaging potential of various chemically active components elaborated by *H. pylori* with emphasis on the interaction and impact of this organism on the function of the gastric mucosal barrier. Such insight is essential to understanding the pathogenesis of *H. pylori*-related gastroduodenal disease.

COMPOSITION AND PROTECTIVE QUALITY OF GASTRIC MUCUS

Composition of mucus

Alimentary tract mucus is a viscoelastic gel that covers the epithelium and is a complex mixture of mucus glycoprotein (mucin), non-mucin proteins, lipids and electrolytes. It is synthesized and stored in the form of secretory granules and subsequently secreted from the mucous cells stimulated by both physical and chemical (secretagogues) factors[2–4].

Mucus gel comprises inorganic and organic components. Inorganic components, predominantly bicarbonate, are imbedded into an architectural framework provided by the major organic constituent, mucus glycoprotein polymer.

Since mucus gel covers the surface of the epithelium, its organic composition is affected both by luminal and mucosal factors. Among luminal factors that may influence its composition are components of salivary secretion, food ingredients, and solubilized mucus components adsorbed secondarily to the surface of the mucus gel. Components originating within the gastric mucosa can be divided into the three broad categories[2–4].

1. *Secretory components:* mucus glycoprotein (mucin), secretory IgA, IgM, vitamin B_{12}-binding proteins, pepsinogens, pepsins and gastricsins.
2. *Transudatory components:* serum albumin, serum glycoproteins, lipoproteins, serum IgG, IgM and IgA.
3. *Exfoliatory components:* plasma membrane glycoproteins, phospholipids, glycosphingolipids, nucleic acids, integrins and ligands for integrins.

The approximate composition of the mucus gel, adhering to the plasma membranes of the surface epithelium, is 70% proteins, 14% sugars and 16% lipids[5,6]. Mucus glycoprotein, so-called mucin, is a major constituent and a leading determinant of the chemical composition and physical properties of mucus. This glycoprotein consists of 60–80% carbohydrates, 20–40% protein and 0.3–0.4% covalently bound fatty acids[2–4]. Mucin exists as a polymer,

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with an approximate molecular weight of 2×10^6, formed of subunits covalently bound to a linking protein. To its protein core rich in threonine, serine, glycine, and proline are linked carbohydrate chains composed of *N*-acetylglucosamine, *N*-acetylgalactosamine, galactose, fucose and sialic (*N*-acetylneuraminic) acid[3,4]. Controversy exists regarding the spatial organization of mucus molecules. The 'coiled thread' model, 'windmill' organization with 'bottle brush' shape of subunits rotated 120° along the linking protein are the most widely accepted three-dimensional configurations[2–4]. Only the last conformation, however, considers the modulatory role of lipids in the maintenance of viscous and the permselective properties of mucus[4].

Protective function of mucus

The polymeric structure of mucin and its highly hydrophilic and expanded molecular configuration allow it to form a gel. This gel provides a viscoelastic, spinnable, and permselective layer, crucial for protection against exogenous or endogenous damaging luminal factors. In addition, mucus is the most physiological lubricant. It also agglutinates and aggregates microorganisms, binds bacterial toxins, and modifies the activity of pepsin[3,4,7–9]. Bicarbonate secreted by glandular mucosa is trapped in the mucus gel architectural network and helps the mucus layer maintain the pH gradient between the acidic gastric luminal milieu and the neutral epithelial cell surface[10].

Although one cannot overestimate the role of bicarbonate in the maintenance of the pH gradient within the mucus gel, mucin and non-mucin components also participate in the retardation of hydrogen ion diffusion. As we have demonstrated[11] the retardative capacity of purified gastric mucin was approximately 10-fold greater than control solutions. This ability of gastric mucin has also been confirmed by Bhaskar *et al.*[12] using viscous fingering methodology. A variety of factors, such as phospholipids, albumin, IgA and prostaglandins further enhance the protective physical properties (viscosity, retardation of hydrogen ion diffusion) of mucus[13–15]. These data support an active role of organic mucus components, mainly mucus glycoprotein, in the generation of a barrier to hydrogen ion diffusion. Both the viscosity and permselectivity of gastric mucus and mucin can be significantly compromised through interaction with damaging compounds such as acetylsalicylic acid, lysophosphatidylcholine (lysolecithin) or pepsin[11,16,17]. Various anti-ulcer drugs improve the physico-chemical properties of gastric mucus, therefore generating conditions favourable for the restoration *ad integrum* of the surface epithelium damaged during ulcerogenesis[18–21].

The luminal surface of the mucus gel is subject to continuous erosive activity from various agents and factors within the gastric luminal milieu. Pepsin, especially within the range of acidic pH, is the leading mucus-degrading factor. Since mucous cells actively secrete newly synthesized mucin after restoration of their intracellular mucin stores, equilibrium is maintained between the degradation and restoration of a mucus gel during physiological conditions. This balance, however, changes dynamically with the pace of continuously modifying stimuli and challengers and may reach a state of

disequilibrium if aggressive forces overcome protective factors. One factor known to affect the balance within the mucous barrier is *H. pylori.*

CLINICAL CONSEQUENCES OF *H. PYLORI* COLONIZATION

H. pylori, a spiral-shaped Gram-negative microorganism, 2.5–3.5 μm long and 0.5–1.0 μm wide with unipolar flagella[22–27], is one of the most intriguing microorganisms in the alimentary tract of humans. Its causative role in the development of active inflammatory changes within the gastroduodenal mucosa and association with duodenal (95%) and gastric (50–65%) ulcer has been established[28–31]. Relapse of ulcer disease is uncommon after eradication of *H. pylori*[32–34] and it has been suggested that one should attempt to eradicate the *H. pylori* in all patients with peptic ulcer disease. Even in NSAID users with concomitant *H. pylori* infection, eradication may prevent ulcer recurrence and complications[34,35].

Recent evidence suggests that prolonged colonization of the gastric mucosa by this microorganism may also lead to chronic atrophic gastritis and subsequently adenocarcinoma[36–38]. Thus further research into the mechanism of *H. pylori*-mediated mucosal damage is justified. It may be possible to prevent the progress of gastritis by early eradication of the infection; however, any potential effect on carcinogenesis will take years to evaluate.

INTERACTION BETWEEN MUCUS AND *H. pylori*

Mucus-related factors potentially affecting *H. pylori*

Mucus, covering the surface epithelium, due to its multiple components and structural diversity may serve both as a repellent and attractant for various exposed surface structures of *H. pylori.* Since exfoliated epithelial cells with specific *H. pylori* receptors are continuously shed into the mucus layer, one would expect that some receptor molecules would be exposed on the surface of a mucus gel. Therefore, initial docking of *H. pylori* on the surface of the mucus gel could potentially be mediated by gel-embedded membrane fragments with intact receptor molecules for the organism. This initial stage could allow *H. pylori* to contact and anchor within the mucus gel. The difference in viscosity and permselectivity of gastric mucus and purified mucus glycoprotein among individuals (unpublished data) could have a potential impact on both an early stage of *H. pylori* colonization and its survival during eradication regimens. In addition, chemical and physical modification of the mucus layer could potentially enhance the pharmacological effects of antimicrobial agents by allowing them to achieve a high concentration within the pre-epithelial and epithelial compartments. Increased concentrations of lysolecithin in patients with gastric ulcer exhibit a close relationship with the rate of luminal release of glyceroglucolipid[39], a molecule considered to be a receptor for *H. pylori* adhesion[40]. Such free receptor molecules may bind to *H. pylori* adhesins and potentially prevent an attachment of this microorganism to the surface epithelium. Interestingly,

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we also found that 4 weeks of therapy with ranitidine significantly enhanced secretion of glyceroglucolipid in patients with peptic ulcer[41]. Potential inhibition of *H. pylori* attachment by 'false receptors' released into the mucus gel could eliminate a direct impact on the metabolism and survival of the surface epithelium. Such a phenomenon could potentially contribute to the healing effects of this H_2 receptor antagonist in addition to inhibition of acid and pepsin secretions. These issues, however, still require further investigation.

H. pylori-related factors potentially influencing mucus

H. pylori flourishes within a complex environment that is greatly influenced by various components of ingested food, the mucosal barrier constituents and factors elaborated by its own secretory potential. The organism's high metabolic activity and enormous mobility are presumably significant factors facilitating colonization. These factors may aid *H. pylori* in its continuous search for adhesion molecules. Short-range forces such as hydrogen bonding, ionic and hydrophobic binding may permit the microorganism initially to anchor within the mucus layer. Hydrophobic regions of *H. pylori*, recently described by two independent groups using hydrophobic interaction chromatography[42,43], may play an important role in such non-specific binding. Also strongly hydrophilic *H. pylori* surface structures have been described by salt aggregation testing, contact angle determination and adherence to sulphonated polystyrene[43]. Therefore, both hydrophilic and hydrophobic *H. pylori* membrane structures may play some role in adhesion of the microorganism to the mucus gel and in subsequent colonization of mucous cell membranes. Furthermore, dynamic changes in both the mucus layer and cell membrane milieu may favour hydrophilic or hydrophobic interaction at various stages of colonization.

The potential role of hydrophobic or lipophilic domains in *H. pylori* colonization has also been recently underscored by Goggin *et al.*[44,45] who demonstrated a decrease in hydrophobicity of the surface of the mucus layer in patients with *H. pylori.* This impairment in mucosal hydrophobicity normalized after eradication of the microorganism. Furthermore, we have recently found that the gastric juice of patients with dyspepsia, who are colonized with *H. pylori*, has a significantly higher ability to bind a hydrophobic probe when compared to dyspepsia patients without *H. pylori*[46]. Although our method measuring hydrophobicity is based on recording relative fluorescence generated by BIS-ANS bound to hydrophobic binding sites and is different from the method utilized by Goggin, both publications suggest excessive shedding of hydrophobic molecules from the mucus layer into the gastric lumen. This may be due to the enzymatic cleavage of mucus components by protease and phospholipase identified in some strains of *H. pylori*[47–52]. Therefore, perhaps *H. pylori* benefits from the presence of hydrophobic molecules within the mucus gel during the primary phase of colonization (docking in the mucus gel). However, mucus gel hydrophobic structures may impair the ability of *H. pylori* to attain its ultimate goal of colonization, an attachment to the cell surface receptor (secondary phase of

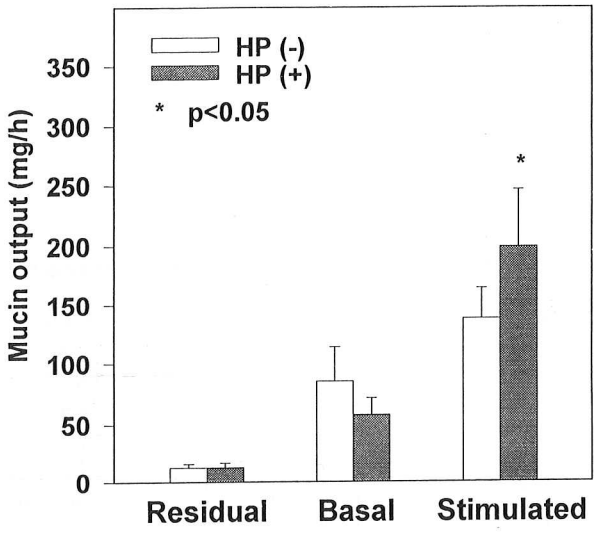
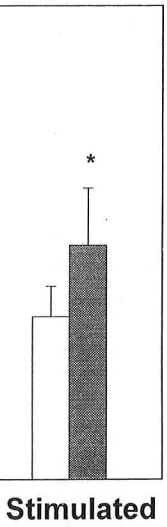


Fig. 1 Mucin output in gastric juice of patients with and without *H. pylori* colonization

colonization). Although excessive luminal release of hydrophobic molecules may benefit colonization, it would inevitably compromise the protective quality of the mucus gel as a barrier to hydrogen ion diffusion.

A detrimental impact of *H. pylori* on the mucous barrier has also been confirmed during our insight into the rate of secretion of mucin and protein within gastric juice in patients colonized by this organism. *H. pylori* positive individuals secreted an excessive amount of mucin (Fig. 1) and protein (Fig. 2) into the gastric juice especially after stimulation with pentagastrin. Such a phenomenon may result both from excessive degradation of the mucus components within the mucus gel and/or the augmented release of mucin depot from mucous cells due to their increased turnover accompanying inflammation. The former explanation is especially attractive, since we have found that the total output of all the components contributing to the viscosity of gastric juice in the same *H. pylori* colonized patients declined significantly (Fig. 3). These data confirm our earlier findings that there are differences in the viscosity of gastric mucus in patients with dyspepsia with and without *H. pylori* colonization[46]. Both groups of selected patients showed the same proteolytic profile of the gastric juice. However, in patients colonized by *H. pylori* the viscosity of mucus, isolated from the gastric juice, was significantly lower when compared to *H. pylori*-negative patients. It seems, therefore, that the gastric mucosal barrier in patients with *H. pylori* is physically compromised by bacterially-related factors. The decrease in the viscosity of gastric mucus may, at least partly, explain why gastroduodenal mucus gel thickness in *H. pylori*-positive patients with dyspepsia was significantly impaired as compared with *H. pylori*-negative dyspepsia patients. In those with confirmed *H. pylori* infection the thickness of the mucus layer



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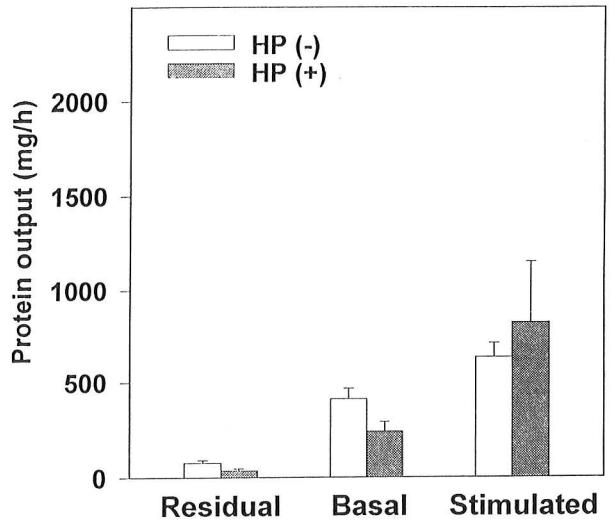


Fig. 2 Total protein output in gastric juice of patients with and without *H. pylori* colonization

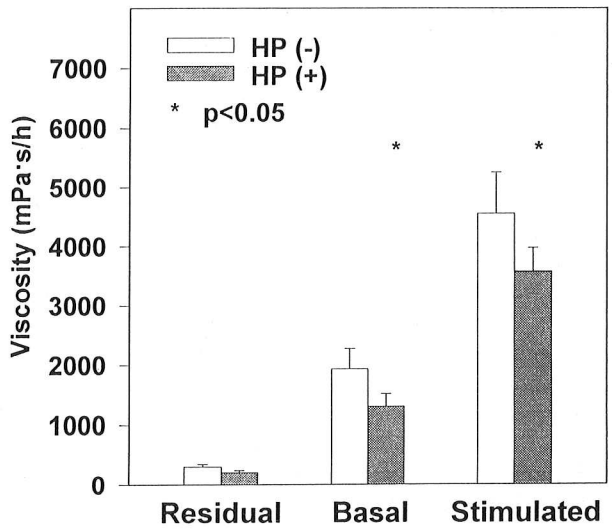


Fig. 3 Hourly output of gastric juice components contributing to its viscosity in patients with and without *H. pylori* colonization

(mean ± SD) was 0.093 ± 0.033 mm in duodenal, 0.085 ± 0.027 mm in antral, and 0.105 ± 0.033 mm in corpus mucosa. In those without concomitant *H. pylori* colonization the thickness of the mucus gel was 0.162 ± 0.045 mm; 0.175 ± 0.067 mm; 0.161 ± 0.064 mm in the duodenum, antrum and corpus

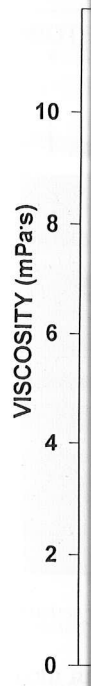
respectively. These differences were statistically significant[53].

Finally, a link between excessive concentrations of lysolecithin in the gastric juice and the presence of active phospholipase in patients colonized by *H. pylori* has also been clearly demonstrated[54]. We showed significantly elevated levels of lysolecithin in patients with gastric ulcer in 1983, when the *H. pylori* saga was still in its early stage of conception. The detrimental impact of lysolecithin on the gastric mucosal barrier may, at least partly, be related to its profound negative impact on viscosity and permeability to hydrogen ion of gastric mucin and its susceptibility to proteolytic cleavage by pepsin[17]. The net result of these effects is to reduce the protective quality of the mucus gel. How this relates to gastritis and peptic ulcer still remains to be determined.

H. pylori appears to secrete glycosulphatase, which removes sulphate (SO_3^-) groups from the gastric mucus glycoprotein molecule. Sulphate groups within mucin enhance its protective quality by inhibiting the proteolytic activity of pepsin and interference with *H. pylori* binding to its epithelial receptor[55,56]. Therefore, desulphation may further diminish the protective quality of the mucus gel layer. Furthermore, *H. pylori* can elaborate toxins[57,58] and PAF-acether[59] which may in turn impair the rate of biosynthesis of mucus within mucous cells.

We have also demonstrated that ammonia, generated by *H. pylori* urease, diminishes the viscosity of the human gastric mucin, purified through equilibrium density-gradient centrifugation (Fig. 4). Ammonia significantly affected the ability of gastric mucin to withstand the higher shear rates, representing forces applied to the mucus layer *in vivo* during phase III of migrating motor complexes. Ammonia ion concentration during these measurements was maintained at levels comparable to the content of ammonia within the gastric compartment in patients colonized by *H. pylori*[60–62]. Changes in gastric mucin viscosity due to ammonia may partly explain its profound damaging effect on the gastric mucosa in an experimental setting[63,64] and offer insight to its pathogenetic effect on human gastric mucosa.

Since patients colonized by *H. pylori* exhibit a significantly higher proteolytic activity within gastric juice compared to healthy non-colonized individuals, one might wonder how *H. pylori* copes with the enormous destructive power of numerous gastric aspartic proteinase isozymes[65]. Furthermore, one should not underestimate the destructive potential of pepsin, which, although it requires low pH for maximal activity, still remains active when pH in *H. pylori* colonized areas drops below 4.0. Recently, we have demonstrated that gastric juice, aspirated from patients with *H. pylori*, inhibits proteolytic activity of pepsin in a dose-dependent fashion from 63% to 92% (Fig. 5). Interestingly, *H. pylori*-related pepsin inhibition was absent when *H. pylori* colonization was accompanied by severe atrophic changes and subsequent achlorhydria. So on the one hand *H. pylori* secretes its own protease, active at neutral pH, fully controlled by the microorganism and presumably helping to maintain an optimal viscosity of the mucus gel. On the other hand, in order to control a very strong endogenous proteinase such as pepsin, *H. pylori* secretes a pepsin inhibitor. Secretion of a pepsin



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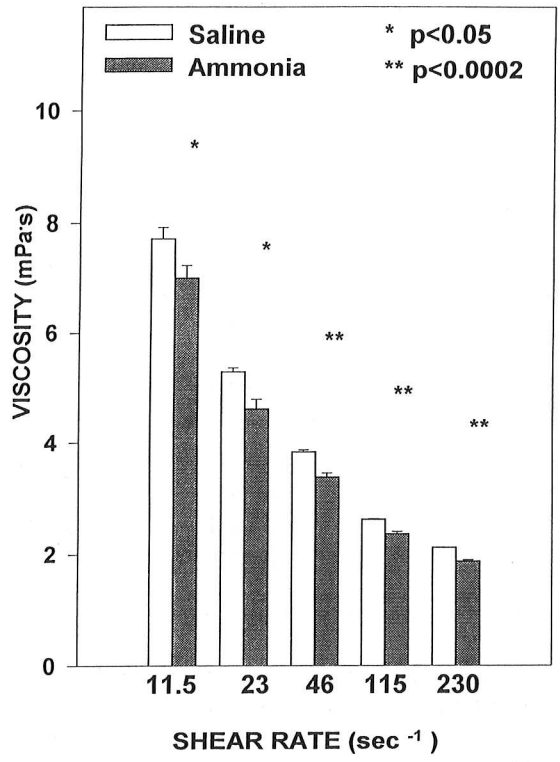

Fig. 4 Impact of ammonia on gastric mucin viscosity

inhibitor may protect *H. pylori* surface structures, presumably crucial for adhesion to cellular receptors. We cannot exclude, however, that human gastric mucosa could also potentially be the source of the pepsin inhibitor. Further investigations into this new and interesting area with respect to ulcer disease seem to be worthwhile.

H. pylori-induced quantitative and qualitative changes within the gastroduodenal mucus layer presumably provide optimal conditions for organisms residing in the mucus gel some distance from the surface epithelium and for those firmly attached to the mucosal cell membranes. We have recently shown that *H. pylori* exhibits either predominantly diffuse or predominantly focal type adherence to the surface of cultured human gastric epithelium isolated from patients with non-ulcer dyspepsia[66]. During physical contact between *H. pylori* and the surface of mucous cells, mucin granules were released and acted as a major repelling force on the surface of epithelium. Some *H. pylori* became entrapped by mucin granules. Final adhesion of *H. pylori* occurred only when cells were depleted of their mucin stores and reduced in size by approximately 40–50%[46,66]. Coaggregation where many *H. pylori* microorganisms bind to other *H. pylori* already attached to the epithelial cell surface in a focal pattern of adhesion was also seen[46,66].

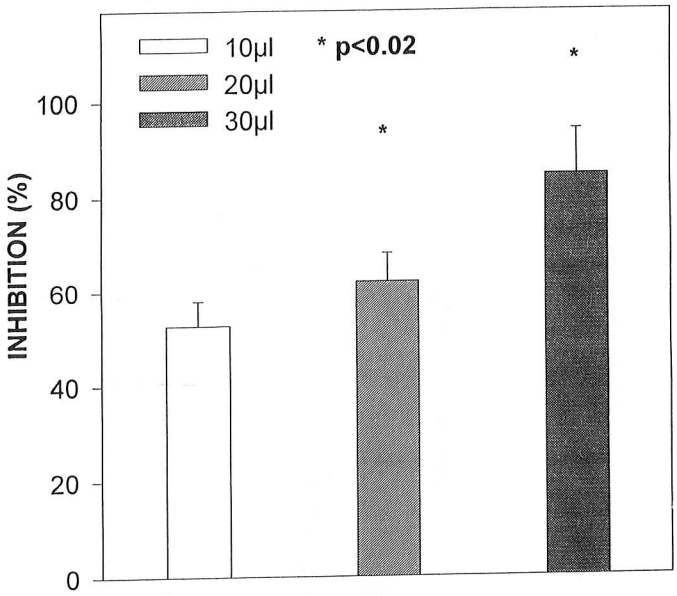

Fig. 5 Inhibitory effect of *H. pylori* (+) gastric juice on proteolytic activity of pepsin

Therefore, the mucus layer provides *H. pylori* with an ideal environment from which it initiates the pathogenic sequelae leading from mild and moderate inflammatory changes to severe gastritis accompanied by progressive atrophic changes, and ultimately the potential for adenocarcinoma.

In general, there are two categories of *H. pylori*-related biologically active factors in patients colonized by this microorganism (Fig. 6). One category includes substances directly released by the organism such as protease, glycosulphatase, phospholipase, urease, toxins and a possible pepsin inhibitor. The second category includes factors indirectly generated by bacterial activity such as ammonia (by urease) and lysolecithin (by phospholipase). Both ammonia and phospholipase are extensively generated *in vivo* and have a profound impact on the mucosal barrier. If the damaging potential of all these *H. pylori*-elaborated factors overlap with the aggressive power of luminal acid and pepsin, corrosion of the mucus gel resulting in a decline of the mucus thickness (Fig. 7) would inevitably occur.

Considering all the available data we would like to present a scheme by which *H. pylori* may mediate damage to the gastric mucosal barrier, especially to the mucus layer (Fig. 8). There are two types of *H. pylori*-related epithelial damage: (1) direct and (2) indirect. Both direct and indirect weakening of the mucosal barrier may generate the optimal conditions required for *H. pylori* colonization and replication. During direct contact between *H. pylori* and the cell, membrane structures are exposed to extremely high concentrations of potential cytotoxins, ammonia generated by *H. pylori* urease, proteases and phospholipases inevitably leading to cell damage. Injury may cause a total disruption of the mucosal barrier. This exaggerated damage may lead

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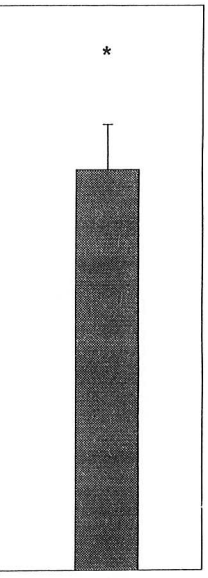

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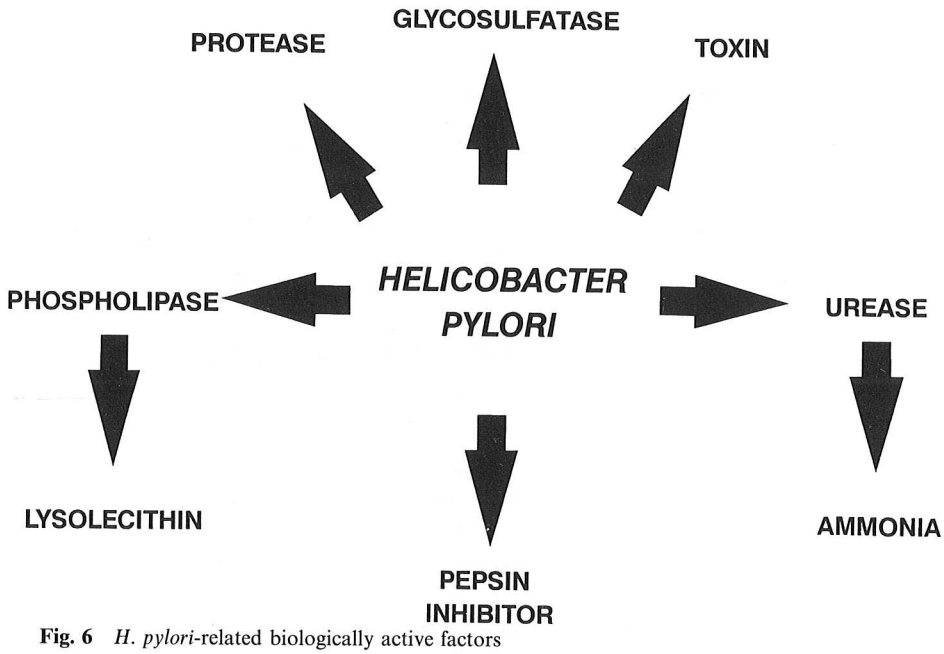

Fig. 6 *H. pylori*-related biologically active factors

to bacterial elimination or force *H. pylori* to move to surrounding less damaged areas to ensure its survival. Numerous *H. pylori*, however, reside in the mucus gel freely spread throughout the entire mucus layer. Damaging factors secreted by the organism into the surrounding milieu lead through proteolysis, desulphation and lipolysis to degradation of the mucus glycoprotein-lipid complex within the mucus gel resulting in a decrease of the mucus gel thickness. Quantitative changes of the mucus gel layer are accompanied by qualitative changes such as a decrease in viscosity, potentiation of permeability to hydrogen ion and impairment in hydrophobicity. Both quantitative and qualitative changes within the mucus gel would enhance back-diffusion of hydrogen ions leading to dissipation of the pH gradient in the mucus layer exposing the surface epithelium to an excessive amount of hydrogen ion. These effects in turn, may result in the metabolic impairment of mucus secreting surface epithelium leading to a decline in mucin secretion directly compromising the mucus layer thickness and facilitating any exposure of the mucous cells (affected by a direct impact of all *in situ* elaborated damaging factors by *H. pylori*) to luminal aggressive factors. Such a scenario would inevitably lead to the development of inflammation and perhaps, subsequently, ulcer.

FUTURE IMPLICATIONS

In summary: In patients with gastroduodenal colonization by *H. pylori* a variety of abnormalities within the mucus layer can be demonstrated. These

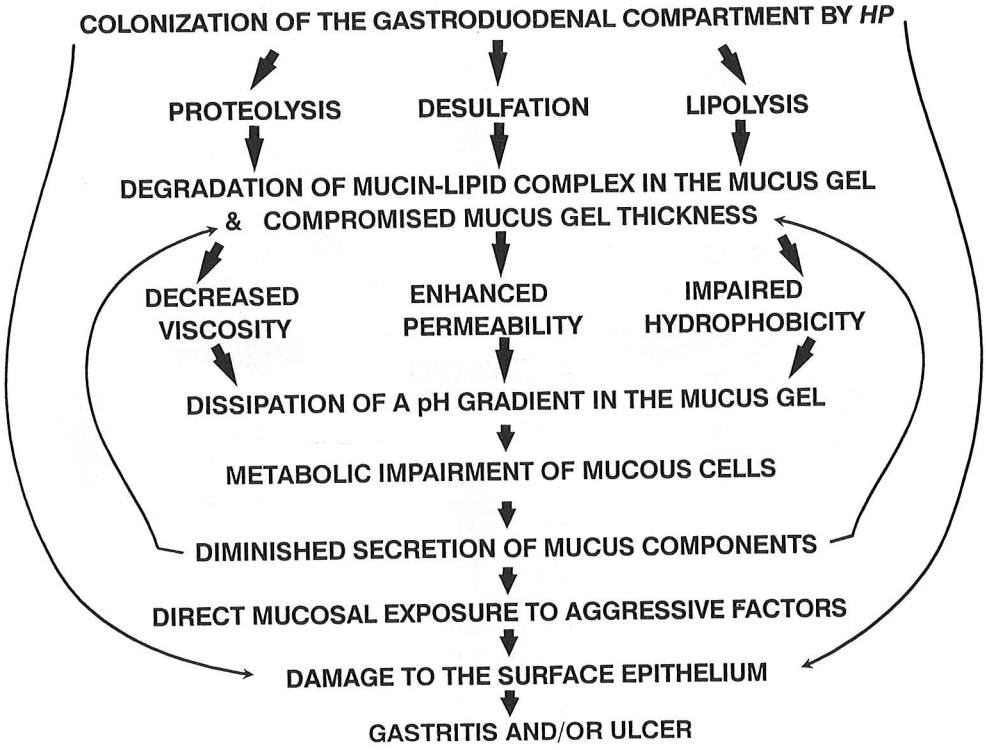

Fig. 7 Schematic outline of contribution of *H. pylori*-related damaging factors and luminal acid and pepsin in impairment of the mucous barrier

include (1) a decline of hydrophobicity with subsequent loss of hydrophobic components into the gastric juice; (2) a decrease of viscosity of gastric mucus accompanied by a significant reduction of the mucus layer thickness within the gastric body, antrum and proximal duodenum; and (3) a decline of gastric mucin viscosity. These changes within the mucus gel are accompanied by a decline of viscosity and an increase of hydrophobicity of gastric juice. The proteolytic activity of gastric juice depends upon the net result of a stimulatory impact on secretion of gastric acid and pepsin by the gastric mucosa and the content of pepsin inhibitor released into gastric milieu. The clinical importance of these interesting findings still needs to be defined. Targeting these abnormalities during therapy may not only facilitate healing of mucosal pathology but perhaps also eradication of the microorganisms.

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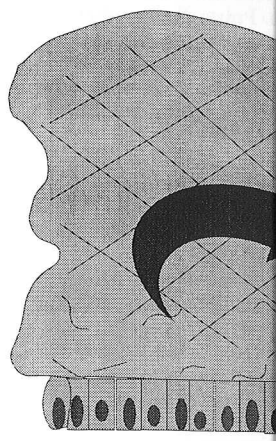

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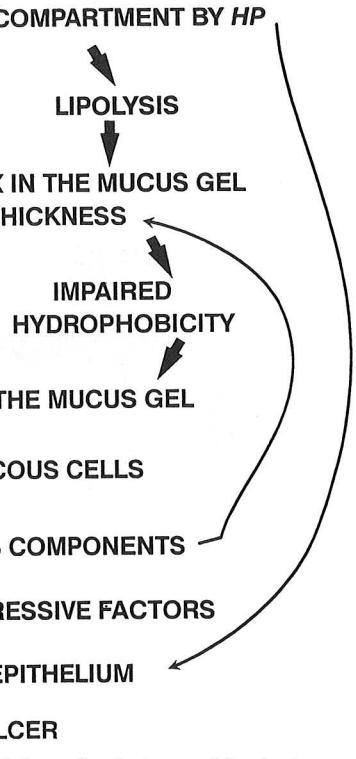

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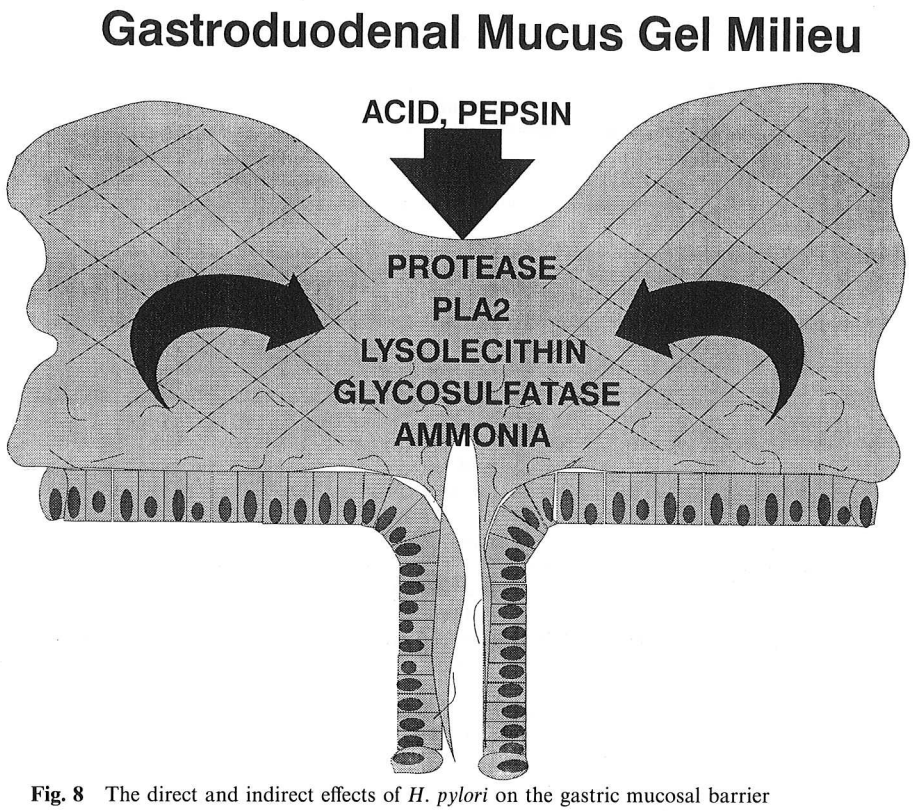

Fig. 8 The direct and indirect effects of *H. pylori* on the gastric mucosal barrier

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Decreased gastroduo *H. pylori* i

T. C. NORTHFIELD

INTRODUCTION

It is 80 years since ulcer is a product o factors in the lume concentrated on th has been easier to r as the most import opportunity and a remains difficult to bicity on endoscop can be applied to p be repeated followi since it reflects the a acid and pepsin.

MEASUREMENT

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