

Pepsin and the Esophagus

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Esophagitis results from excessive exposure of the esophagus to gastric juice through an ineffective or dysfunctional lower esophageal sphincter mechanism. A possible role of pepsin in damaging the esophageal mucosa with consequent esophagitis may be examined directly by testing pepsin under various conditions in experimental models of esophagitis. Since gastric juice contains both acid and pepsin, all experiments examine separately effects of perfusion of the esophagus by acid without and with pepsin in various combinations. Acid perfusion alone at concentrations represented by pH 1.3 or above does not produce esophagitis. The addition of pepsin to acid between pH 1 and 3.5 causes considerable acute esophageal damage. Outside the proteolytic range, i.e., higher than pH 3.5, pepsin does not damage the esophagus. The damage caused by acidified pepsin may be made much worse by the further addition of aspirin or other NSAIDs, presumably by further breaking down mucosal barriers.

INTRODUCTION

The esophagus maintains its integrity through many different mechanisms (Figure 1). The focus of this discussion, pepsin, looks at only part of the puzzle. A role for pepsin in the causation of esophagitis may be examined in several ways. Experimental evidence shows an important role for pepsin in acid medium in the causation of acute damage to perfused rabbit or cat esophagus [1-3]. Whether and how this may be translated to the chronic esophagitis seen clinically is not clear. Measurement of pepsin concentration and output in simple or complicated esophagitis (i.e., stricture, Barrett's) or in esophagitis resistant to treatment has

failed to show a difference from appropriate controls [4-7]. Furthermore, whether pepsin plays a part in delaying or preventing healing of clinical esophagitis is another question of great interest in designing or interpreting the results of treatment of erosive esophagitis.

INTEGRITY AND DEFENSE OF THE ESOPHAGEAL MUCOSA

Esophagitis results from an abnormal exposure to activated pepsin containing acid gastric contents due to a distortion of the physiological function of the LES [8]. Normal acid gastric contents refluxing into the esophagus are rapidly and efficiently

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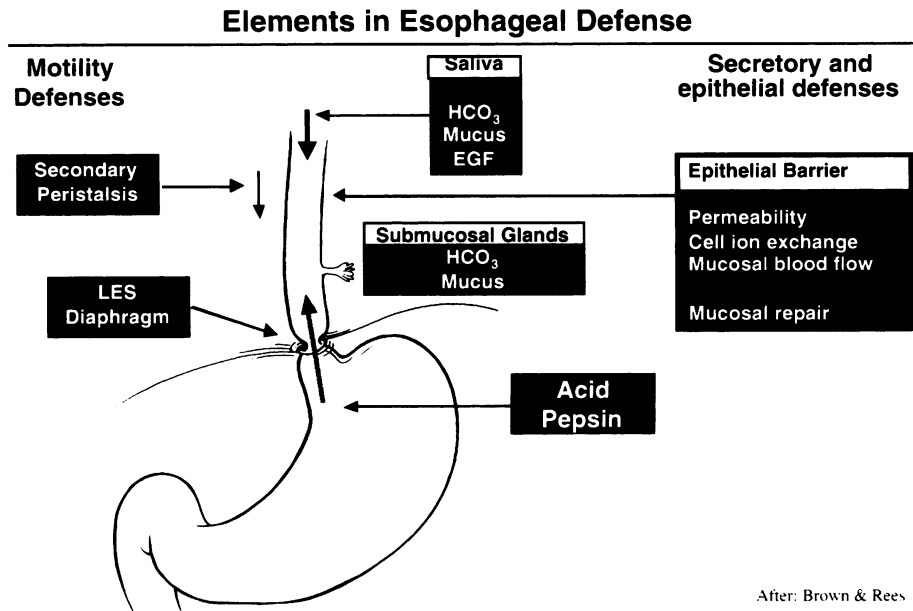


Figure 1. Model of the various mechanisms involved in the defense of the esophageal mucosa. (Adapted from Brown and Rees. Gut 1996).

cleared through a combination of reflex responses: propulsive orderly motility is initiated to empty the esophagus of the bolus, the esophagus secretes neutralizing HCO₃⁻ and an esophago-salivary reflex likewise initiates the flow of HCO₃⁻-rich saliva. It is not clear whether these reflexes are operative during sleep, when salivary flow ceases. Nocturnal acid reflux is presumably more damaging because of the loss of salivary neutralization.

Moreover, the esophageal squamous mucosa represents a tight epithelium that resists penetration by H⁺ [9, 10] and probably, in part, under the influence of epidermal growth factor secreted by the salivary glands, desquamated epithelium is replaced, and damaged epithelium repaired.

If this continuously balanced mechanism is disrupted by one or more changes in the elements of the equation, esophagitis results; esophagitis, in turn, may have secondary consequences. It is not known

whether there is more than one initiating event, though it is clear that acid and pepsin are the major sustaining factors in esophagitis. However, not all reflux, much of which is physiological or very transient, results in esophagitis or even produces symptoms [1, 8]. The threshold for these consequences has not been defined.

There are a number of circumstances in which an excess of acid/peptic contents reflux into the esophagus. First, an excess of gastric contents may result from delayed gastric emptying, which may be physiological due to a large fatty meal, or pathological as in pyloric stenosis or in diabetic enteroneuropathy or other gastroparesis; the massive hypersecretion of Zollinger-Ellison syndrome may also promote reflux, which is more damaging because the refluxate is excessively acidic. Obesity, ascites, and pregnancy promote reflux because of increased abdominal pressure, the latter also reducing LES pressure by altered hormonal status. A low

LES pressure is normally present in infants.

In some adults, the LES may be intrinsically weak (< 10 mm Hg), and in most such cases, esophagitis results [4]. However, this defect accounts for no more than 30 percent of cases of esophagitis [4]. The LES may be rendered incompetent by an axial hiatal hernia, which often additionally functions as an intrathoracic reservoir of gastric contents at the thoracic pressure, so that the esophagus is not fully cleared of acid. Hiatal hernia is present in only half the cases of esophagitis. The LES can also be damaged by operations, such as a myotomy for achalasia. The reason why an LES with normal pressure relaxes inappropriately is unknown. This functional defect accounts for many of the remaining cases of reflux.

There are no good data in man, as opposed to experimental models, defining the conditions necessary to induce esophagitis. We do not know the necessary duration of exposure, the minimum composition of the refluxate, nor whether the

esophagus is more vulnerable at any particular time of day or night. In experimental models, acid without pepsin is much less ulcerogenic, and it may be assumed that pepsin is as important in the genesis of esophagitis [11] as it is in its continuance. This hypothesis is strongly supported by the efficacy of omeprazole.

EXPERIMENTAL ESOPHAGITIS

To delineate a possible role of pepsin we first examine experimental studies. It has been shown in several studies that acid perfusion alone of the esophagus or jejunum will not produce damage, whereas perfusion with added pepsin, or whole gastric juice will [3]. Figure 2 shows that acute esophageal damage does not occur with acid alone at pH 1.3 (approximately 50 meq/l) or above [3]. An esophageal mucosal barrier to hydrogen has been described in dogs, rabbits, and humans [9, 10]. This is a functional concept defined by the capacity of the esophageal mucosal

Histologic Esophagitis - 1 Hour
Scored 0 - 4
Pepsin $\mu\text{g} / \text{cc}$

mEq / l	pH	0	25	50	100	200
5	2.3			0		
10	2.0	0	.3	1.3		
HCl	25	1.6	.2	1.6	2.7	2.0
	1.6					2.1
	50	1.3	1.8	2.7	4	
	1.3					
	100	1.0	4		3	3.3
	1.0					

Figure 2. Damage levels to rabbit esophagus perfused for 1 hour by various combinations of acid and pepsin. Score 0-4.

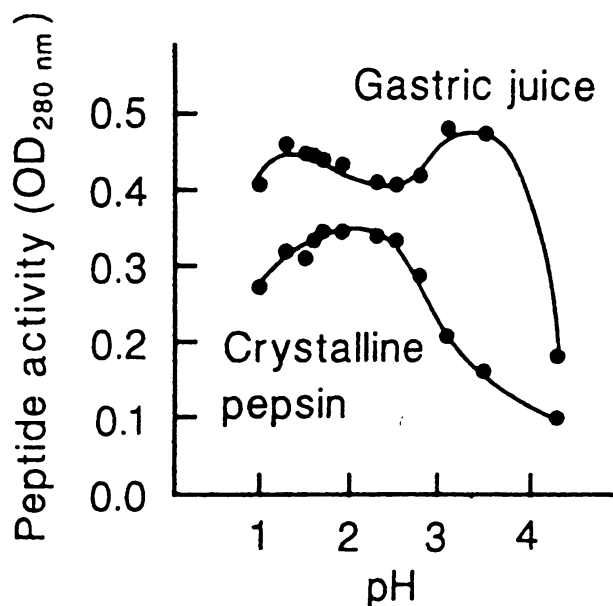


Figure 3. Proteolytic activity of pepsin and of gastric juice at various levels of pH [from Ref. 34].

to prevent the back-diffusion of hydrogen from the lumen and involves different mechanisms, including the hydrophilic lipid bilayers of cell membranes, the intercellular barriers or junctional complex, the intracellular buffer capacity for hydrogen ions, and the ability of cells to extrude hydrogen ions into the interstitial fluid (sodium-hydrogen antiport) [9].

However, the addition of pepsin at concentrations commonly found in human gastric juice leads to significant damage. At pH values of more than 3, the effect of pepsin is much diminished (Figure 3). However, gastric juice also contains other proteases, such as cathepsins B and D, which extend the proteolytic optimum to pH 4 (Figure 3). In another situation, injury of the gastric mucosa in experimental hemorrhagic shock can be prevented by a pepsin-binding analog of pepstatin [12, 13] suggesting an important role for pepsin in this process. The pepstatin experiments [12] suggest a role for intramucosal-activated pepsin, but it remains to

be shown whether the pH in the mucosa reaches the range of less than pH 3.3 required for pepsin action. Even if it did, such a degree of acidity would by itself be highly disruptive. "Normal" reflux may in fact be protective, as suggested by adaptation of esophageal mucosa exposed briefly to acid in developing resistance to subsequent acid/pepsin injury. This adaptation is not dependent on cell proliferation and may be mediated by NO and EGF [2].

Pepsin activity is dependent on acid. At pH 6.0 or below, pepsinogen is autocatalytically converted to pepsin which is optimally active at pH 1-3.3 (Figure 3). Pepsin is denatured at a pH of more than 7 and therefore remains present and activatable at pH 3.5 to 6.9 [11]. Since most agents directed to ulcer treatment do not elevate gastric pH > 7.0, pepsin is not destroyed.

If pepsin were to penetrate below the surface epithelium, a possible target of proteolysis would be collagen, especially type IV which is hydrolyzed by pepsin at

the non-helical ends of the molecules releasing monomers [14]. This would disrupt mucosae since cross-linking of collagen by carbohydrate moieties occur at the non-helical spacings [15]. Collagen IV makes up the basement membrane for surface epithelial as well as endothelial cells [16] and its disruption could quite readily be seen as leading to esophagitis and similar diffuse mucosal lesions. Once the surface epithelium is partly disrupted, the process then would allow further access of damaging acidified proteases (Figure 4).

Another possible pathogenic role for pepsin via digestion of collagen might occur during the repair of chronic ulceration. Since new collagen is essential to the structural repair of the mucosa, including new blood vessels [16] the high susceptibility of both collagen III and IV to peptic digestion [14, 15] might delay ulcer healing. Since the repair of esophagitis is much more rapid with more profound suppression of acid by omeprazole, i.e., when pepsin is rendered inactive, the mechanism that promotes healing might involve removal of proteolysis rather than pH per se. Therefore, greater acid suppression is necessary to ensure healing of esophagitis.

No reliable non-toxic potent pepsin antagonist is available to test these possibilities independently of acid *in vivo*.

ROLE OF OTHER AGENTS

Injury in experimental esophagitis is promoted by other agents that damage the epithelium and so render it more permeable to acid and pepsin, thus causing mucosal disruption and esophagitis (Figure 4). Among the agents that may do so are aspirin [1] or similar compounds [17-20]. The clinical evidence for a contributory role of aspirin are the presence of esophagitis in 16 to 20 percent of healthy volunteers taking NSAIDs [17-18], and 25 percent incidence of esophagitis among patients taking NSAIDs compared to 15 percent in controls [19]. We [20] recently reported an unexpectedly high proportion (61 percent) of esophagitis patients taking NSAIDs chronically, mostly aspirin. Also esophagitis is a common feature among patients with refractory peptic ulcer due to ASA abuse [21].

Other agents that may cause primary damage to the esophageal epithelium include bile and locally acting medications

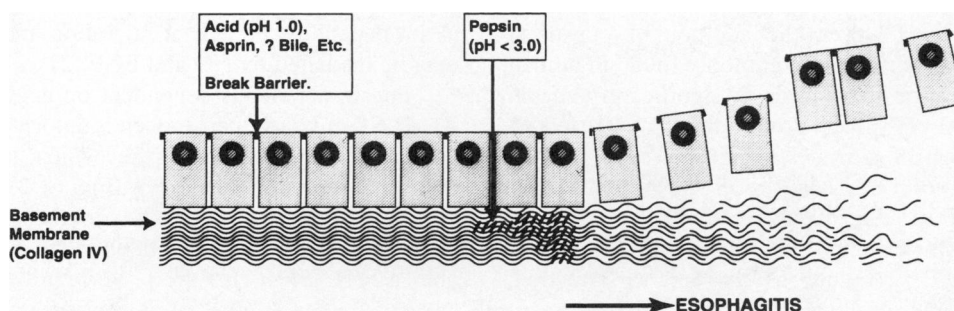


Figure 4. Schematic representation of esophageal mucosa showing possible mechanisms involving pepsin in causing or promoting esophagitis through proteolytic damage to the basement membrane.

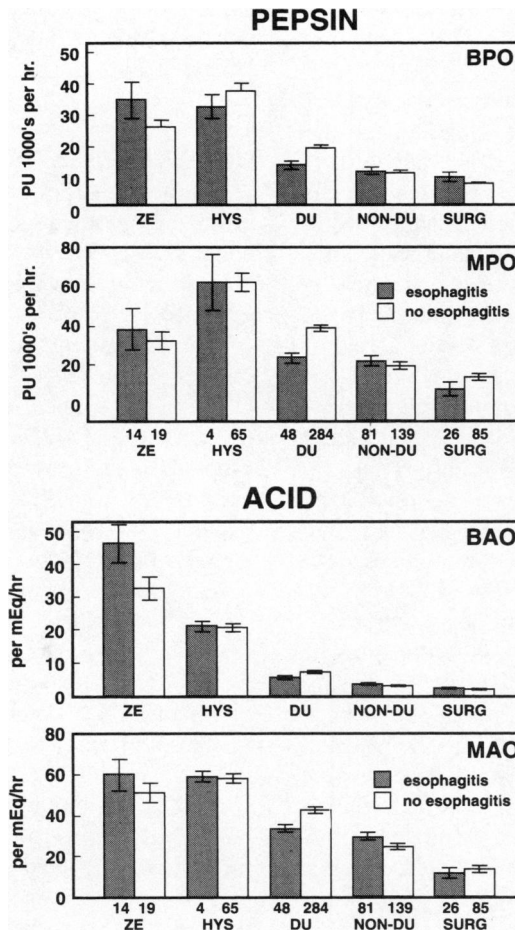


Figure 5. Basal and maximally stimulated pepsin and acid output in patients with esophagitis and in appropriate controls with the same background upper GI disease. ZES = Zollinger-Ellison syndrome; HYS = duodenal ulcer hypersecretors BAO > 15 mEq/hr; DU = duodenal ulcer, non-DU, and surg = patients after gastric surgery for peptic ulcer. Numbers below each column represent number of patients and controls, respectively [data from Ref. 4 with permission].

in pill form such as quinidine, especially in the formulation Quinaglute, tetracycline, ferrous sulfate and KCl, especially with underlying motility disorders leading to poor clearance.

Experimental data thus suggest an important primary as well as secondary role for pepsin in causing or promoting acute esophagitis.

CLINICAL ESOPHAGITIS

There is no direct evidence for a definitive role for pepsin in causing clinical esophagitis. Since there is no specific clinically useful antagonist to pepsin, direct proof of contribution of pepsin to persistence of esophagitis in man is lacking. Measurement of acid reflux (e.g., by pH probe and using pH 4.0 as threshold) is at best a surrogate marker of proteolytic

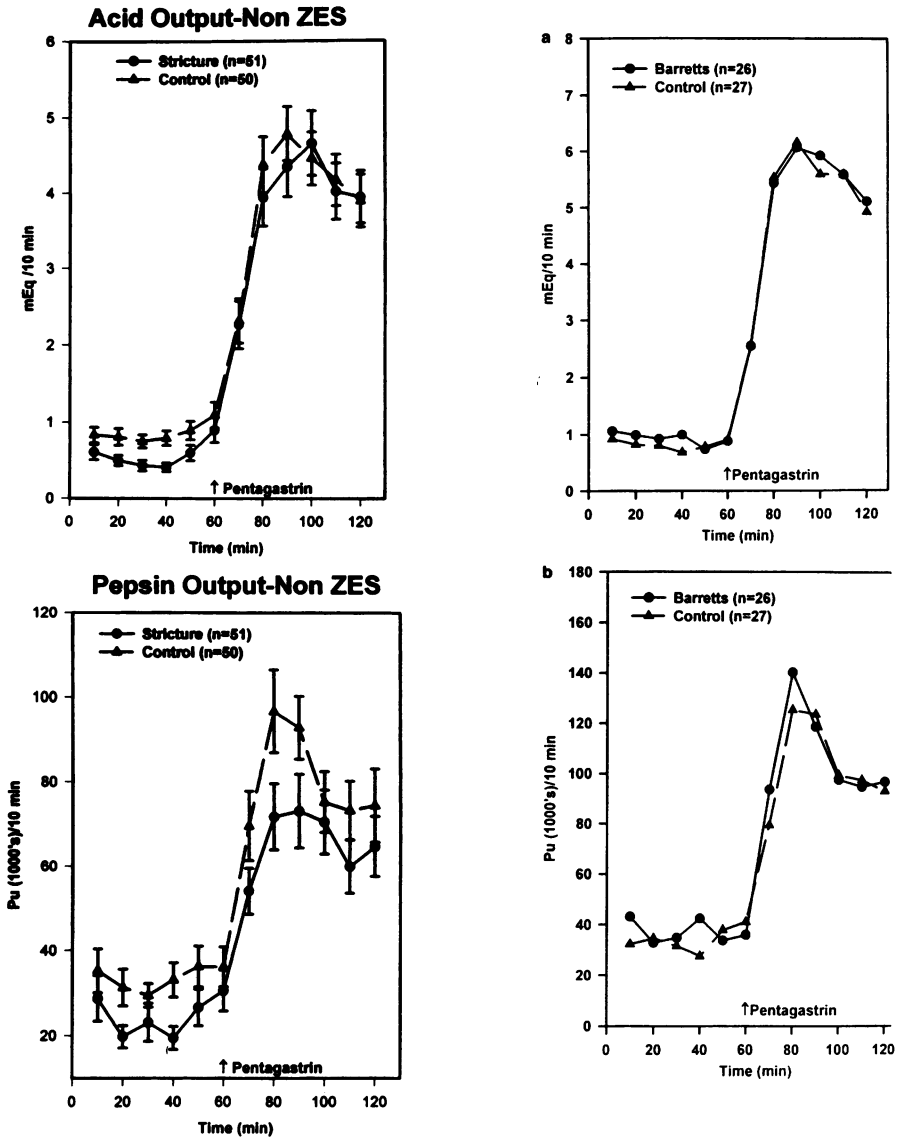


Figure 6 (left). Basal and pentagastrin stimulated acid and pepsin secretion in patients with Barrett's esophagus and in age, race and sex-matched controls with esophagitis. [From Ref. 6 with permission]. Figure 7 (right). Basal and pentagastrin stimulated acid and pepsin secretion in patients with esophageal stricture and age and sex-matched controls with esophagitis but without stricture. [From Ref. 7 with permission].

exposure. For pepsin exposure alone, the pH threshold would be approximately 3.3 (Figure 3). A rough estimate of non-pepsin contribution proteolytic activity may be obtained from the interval between pH 3.3 and 4.2. Moreover, since acid per se at pH of more than 2.5 causes no damage to the esophagus [3], it may be inferred that exposure between 2.5 and 4 is damaging solely by proteolysis. Most reports of 24-hr pH shows that longer exposure to pH of less than 4 is associated with increasingly severe esophagitis [8]. We, therefore, further examined the question by measuring acid and pepsin in gastric juice (the sole source of acid and pepsin in the refluxate) in esophagitis, both simple and complicated, to learn whether the composition of the refluxate was a factor added to exposure time. Because esophagitis is found in

patients with widely different secretory backgrounds from Zollinger-Ellison gastric hypersecretors at the one extreme to post-gastric surgery patients with low acid and pepsin secretion at the other, it was important to use appropriate controls [4]. Basal and maximal acid and pepsin outputs were higher in males than females [4], but the same or slightly lower than controls and patients with esophagitis (Figure 5). Moreover, composition of gastric juice — pH and acid and pepsin concentrations — was no different in fasting gastric juice in each subgroup between esophagitis patients and controls. However, patients with very low secretion (BAO < 0.1 meq/hr) rarely had esophagitis [4]. With acid (and pepsin) secretion present however, severity of esophagitis was unrelated to levels of acid output [4].

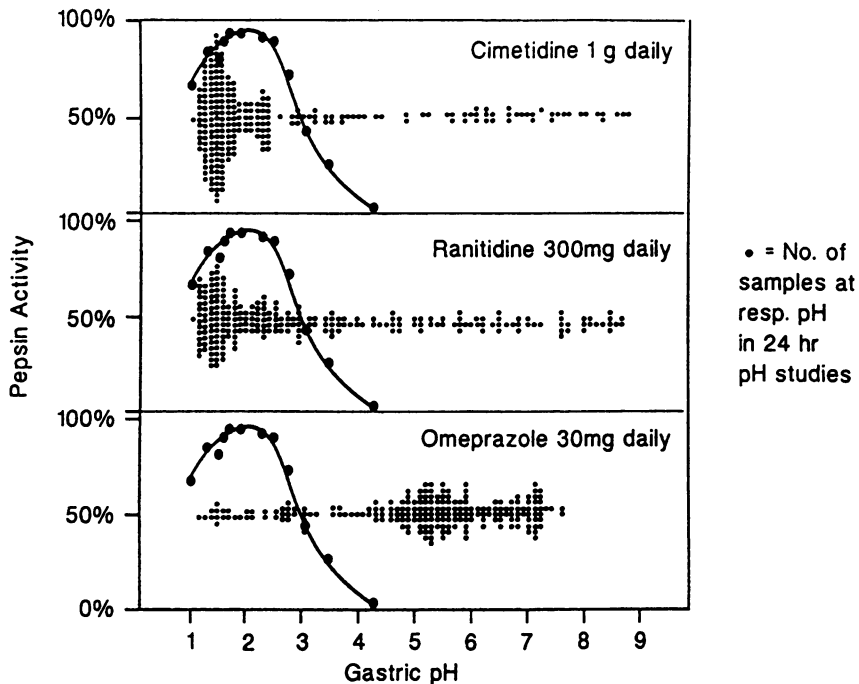


Figure 8. 24-hr intragastric pH values in 10 volunteers with three different acid suppressing drugs: cimetidine, ranitidine and omeprazole, respectively. Superimposed on each is the activity curve for pepsin, the optimum activity at pH 1.2 to 3.0. [From Ref. 24 with permission].

On further examining patients with treatment-resistant [5] or complicated esophagitis stricture [6] or Barrett's [7], and using patients with esophagitis who healed, or patients with esophagitis without stricture or Barrett's as appropriate age and sex-matched controls, there was no difference between patients and controls in basal or maximally stimulated acid or pepsin output, or concentrations of acid and pepsin in fasting gastric contents. Figures 6 and 7 illustrate the almost identical acid and pepsin outputs in the patients in the stricture and Barrett's studies.

The data derived from studying gastric secretion offer no direct evidence for a definitive role of altered pepsin secretion in esophagitis or its complications, showing only that in the virtual absence of acid (and pepsin), esophagitis rarely occurred.

EVIDENCE FROM THERAPY OF ESOPHAGITIS

Unlike the healing of peptic ulcer [22], healing of esophagitis requires more profound and prolonged 24-hr acid suppression [23]. Such suppression of acid secretion cannot be generally obtained with conventional "ulcer healing" doses of H₂ antagonists, and thus healing of esophagitis, especially grade II or greater, requires the more profound acid inhibition achievable only with proton pump inhibitors [25-28], and in adequate doses [29]. Moreover, maintenance of the healed state also requires daily treatment with proton pump inhibitor [29-31].

The principal difference between H₂ antagonists and proton pump inhibitors on gastric secretion is found in the degree to which pH is elevated. In normal volunteers, [32], the median pH for gastric juice over 24 hr was elevated to 1.8 by cimetidine (1 gm daily), to pH 2.2 by ranitidine (300 mg daily), but to pH 5.5 by omeprazole 30 mg per day, thus elevating pH out of the activity range of pepsin (pH < 3.3, Figure 8) or in fact of all acid proteases

found in gastric juice (Figure 2, Ref. 2) (pH < 4.2). Where 24 pH shows inadequate acid suppression by proton pump inhibitors esophagitis relapses [33]. Short of using a specific pepsin inhibitor, which is not currently possible, one cannot distinguish between the effect of acid suppression per se and the effect of acid suppression by elevation of pH to outside the range of peptic activity in promoting healing.

However, when combined with the evidence of acute experimental esophagitis models cited above, and the interpretation from Figure 8, there is enough circumstantial evidence to implicate acid active proteases, especially pepsin as the essential component in gastric refluxate responsible for causing and perpetuating the diffuse disorder of erosive esophagitis.

With persistence of the underlying pathophysiology that allows abnormal exposure of the esophagus to refluxed gastric juice, this conclusion leads to the clear therapeutic implication that requires such gastric contents to be above pH 4 in order to heal esophagitis and to prevent relapse. The proportion of the 24 hr necessary for the esophagus to be exposed to this degree of suppression appears to be in the order of 75 percent (16 hr/day) or more [23]. Prevention of excessive reflux may achieve the same goal, perhaps even without changing pH as in successful anti-reflux surgery. The problem of esophagitis is a multifactorial one as shown in Figure 1.

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